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Board Invited Review

BOARD INVITED REVIEW

ASAS-NANP symposium: digestion kinetics in pigs: the next step in feed evaluation and a ready-to-use modeling exercise

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Abstract

Growing importance of upcycling agricultural by-products, food waste, and food processing by-products through livestock production strongly increased the variation in the nutritional quality of feed ingredients. Traditionally, feed ingredients are evaluated based on their measured extent of digestion. Awareness increases that in addition to the extent, the kinetics of digestion affects the metabolic fate of nutrients after absorption. Together with a growing body of evidence of complex interactions occurring within the lumen of the digestive tract, this urges the need of developing new approaches for feed evaluation. In a recently developed approach, we propose combining in vitro and in silico methods for feed ingredient evaluation. First steps in the development of such a systems were made by (1) evaluating in vitro the digestion potential of feed ingredients, regarding this as true ingredient properties and (2) predicting in silico the digestive processes like digesta transit, nutrient hydrolysis and absorption using dynamic, mechanistic modeling. This approach allows to evaluate to what extent the digestion potential of each ingredient is exploited in the digestive tract. Future efforts should focus on modeling digesta physicochemical properties and transit, applying in vitro digestion kinetic data of feed ingredients in mechanistic models, and generating reliable in vivo data on nutrient absorption kinetics across feed ingredients. The dynamic modeling approach is illustrated by a description of a modeling exercise that can be used for teaching purposes in digestive physiology or animal nutrition courses. A complete set of equations is provided as an on-line supplement, and can be built in modeling software that is freely available. Alternatively, the model can be constructed using any modeling software that enables the use of numerical integration methods.

Key words: digestion kinetics, feed evaluation, feed ingredients, modeling, swine

Introduction

In pig feed formulation, standardized ileal digestibility of amino acids (AA) and the (net) energy content of feed ingredients are the basis for optimizing diets that meet requirements at least-cost. The approach of assigning fixed feeding values to feed ingredients and matching these with nutrient requirements on a least-cost basis (see c.f., (NRC, 2012; CVB, 2016)) is very practical and has served its purpose well. With worldwide increasing fluctuations in the availability and nutritional quality of feed ingredients, maintaining accurate feed ingredient databases becomes laborious and expensive. The introduction of on-line and in-line approaches for feed ingredient evaluation based on

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Abbreviations

AA	amino acids
DDGS	dried distillers grains with solubles
SMART	Simulation and Modelling Assistant for Research and Training

(near) infrared spectroscopy has been a vast improvement in this respect. Several macronutrients can be well quantified in feed ingredients using near infrared spectroscopy (Prananto et al., 2020), and the potential to quantify variation in digestibility of macronutrients or energy from near infrared spectra of fecal samples has been identified as promising (Bastianelli et al., 2015; Nirea et al., 2018), particularly so for crude protein. The implied correlation of digestibility to complete near infrared spectra of feces rather than individual chemical entities, however, introduces risks for spurious associations. To minimize these risks, efforts in this area should focus on predicting indigestible or poorly digestible reference components in feces. This will, in analogy to the indigestible marker technique, contribute to accurate predictions of fecal output.

To accurately predict nutritional values when moving into an era of continuous changes in feed ingredient availability and composition/quality, we need to cross limitations of the current feed evaluation systems. These include: (1) the exclusive focus on the extent of digestion, hence ignoring the impact of variation in digestion kinetics; (2) potential interactions between ingredients and/or nutrients occurring within the lumen of the digestive tract; (3) interactions between macronutrients occurring postabsorption. The latter has been widely acknowledged and has been the main reason for the development of several dynamic growth models. Halas et al. (2018) provided a recent review on digestion and growth models in pigs. These dynamic models have the additional advantage of being response-systems, in contrast with the conventional, static, feed evaluation systems that consider nutrient requirements to be fixed, as discussed by Dijkstra et al. (2007).

The digestive processes in pigs have been modeled as well (Bastianelli et al., 1996; Strathe et al., 2008). To the best of our knowledge, however, existing models ignore variation in digestive processes that are related to the physicochemical properties of ingredients that nutrients are originating from. For example, they ignore that the rate of digestion and absorption of nutrients varies depending on botanic origin of the ingredient (often referred to as the ingredient matrix) and possibly also depending on feed processing conditions.

Here, we propose a combination of in vitro methods for feed ingredient evaluation and a subsequent in silico modeling approach as a next step in feed ingredient evaluation. In the second part of this manuscript, the dynamic mechanistic modeling approach is illustrated by a description of a modeling exercise that can be used for teaching purposes in digestive physiology or animal nutrition courses.

The Impact of Digestion Kinetics

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 AA in the small intestine. 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 associated with impaired intestinal health (Gilbert et al., 2018). Although the extent of enzymatic protein hydrolysis in the gastro-intestinal tract is important in relation to its nutritional value, the timing of AA absorption and thus the rate of protein hydrolysis is important as well (Yen et al., 2004; Van den Borne et al., 2007). For example, feeding slowly vs. rapidly digestible protein sources has been shown to affect the metabolic use of AA for protein deposition and extent of AA oxidation (Boirie et al., 1997; Dangin et al., 2001).

For the evaluation of starch in pig feed ingredients, apparent fecal starch digestion is usually assumed near complete. Some systems account for a difference in the energetic value of enzymatically digested vs. fermented starch (CVB, 2016). The net energy yield from fermented starch is then assumed to be about 70% of that of enzymatically digested starch. Recent research, however, has demonstrated that this difference is much smaller, if at all existent (Van Erp et al., 2020). After weaning, the digestive capacity for starch develops within a couple of weeks (White et al., 2008). Then, intestinal absorption of glucose is proportional to the rate of starch hydrolysis in the small intestine, which can be predicted from in vitro assays (Martens et al., 2018; Martens et al., 2019a). Virtually all of the starch escaping digestion is fermented, primarily in the colon of pigs, yielding short-chain fatty acids that are rapidly absorbed. The fermentation of starch, however, has been demonstrated to be initiated in the stomach (Martens et al., 2020), and the impact of differences in the rate of starch digestion on digesta properties, and thereby potentially on the digestion of other nutrients is large (Martens et al., 2019b).

Interactions Between Nutrients Within the Lumen of the Digestive Tract

The cell-wall matrix of feed ingredients in which the nutrients are embedded markedly influences the digestion kinetics of the macronutrients present. This matrix is dominated by cell wall architecture, greatly differing among feed ingredients. Apart from directly affecting nutrient degradation through physical hindrance, these cell-wall components also considerably affect physical properties of feed and digesta, thereby influencing digesta transport, nutrient hydrolysis, and absorption and ultimately affecting both the extent and rate of appearance of nutrients in the portal circulation. Examples of such interactions have been well documented in the literature. For example, addition of 10% oat β-glucans, to the diet of growing pigs strongly reduced phase separation in stomach emptying between solids and liquids, which coincided with reduced protein disappearance in the stomach but not in the small intestine (Schop et al., 2020). De Vries et al. (2016) found β-glucans from barley to reduce ileal digestibility of crude protein from dried distillers grains with solubles (DDGS) and rapeseed meal by 4 percentage units in growing pigs. Fermentation of fibers from rapeseed meal, but not DDGS, was increased by 6 percentage units in the presence of barley β-glucans, whereas addition of resistant starch to the diet reduced the fermentation of fibers from DDGS and rapeseed meal by more than 10 percentage units (De Vries et al., 2016). Addition of soluble arabinoxylans from wheat decreased small intestinal digestibility of meat proteins by 5 percentage units, likely by increasing digesta transit in the distal small intestine (Zhang et al., 2015). Exchanging 7% cornstarch for cellulose (w/w), reduced ileal protein digestibility by >15 percentage units, whereas exchanging cornstarch for guar gum reduced ileal protein digestibility only numerically by 6 percentage units (Owusu-Asiedu et al., 2006). Exchanging enzymatically

digestible starch for fermentable starch led to a nonsignificant decrease in ileal protein digestibility of 6 percentage units, and to a substantial influx of urea into the colon, leading to a drop in total tract nitrogen digestibility of 8% points (Van Erp et al., 2020). Although these interactions are often amplified by large experimental contrasts, these results illustrate that interactions between ingredients occurring within the lumen of the gastro-intestinal tract can be considerable. Quite likely, many of these interactions are associated with changes in digesta passage behavior. Modeling digesta passage behavior, affected by many factors, is important to increase our insight into these interactions, as illustrated by the modeling exercise, included at the end of this manuscript.

In Vitro Methods to Characterize the Potential Feeding Value of Ingredients

Accounting for nutrient degradation kinetics and physical properties of feed ingredients in future feed formulation approaches requires fractional rates of hydrolysis of nutrients and the interactions between nutrients to be quantified.

We propose to assign *in vitro digestion potential*, rather than best estimates of nutrient digestibility in vivo, as true properties of feed ingredients. Although not a commonly accepted approach (Stern et al., 1997), it is in analogy to several protein evaluation systems for ruminants, where potentially degradable fractions and fractional degradation rates of nutrients in the rumen for each feed ingredient are established (often using in sacco techniques), and subsequently combined with other characteristics, including fractional passage rates, in order to calculate ruminal degradation of nutrients (Van Duinkerken et al., 2011). The digestion potential, to be evaluated in vitro, should include the extent as well as the kinetics of macronutrient hydrolysis. The kinetics of nutrient hydrolysis can be studied using in vitro hydrolysis methods which simulate stomach, small intestinal, and large intestinal digestion. Wang and Zijlstra (2018) present an overview of current methods. Typically, such methods measure substrate disappearance after hydrolysis by enzymes (e.g., pepsin, trypsin, pancreatin, and peptidases) in a buffered system (set pH) over a period of time (Boisen and Fernández, 1997). Such systems, designed to mimic the extent of digestion, are generally poorly equipped to mimic digestion kinetics. Recently, in our lab, improved in vitro methods were developed for feed ingredient evaluation that target both the kinetics and the extent of digestion. Briefly, a dynamic stomach model was developed, mimicking a step-wise drop in pH (Martens et al., 2020). In the small intestine, these methods focus on the solubilization of proteins and appearance of AA and small peptides, or glucose, during incubation with digestive enzymes, rather than harvesting the insoluble residue at the end of incubation (Chen et al., 2019; Schop et al., 2019b).

In addition, physicochemical properties of feed ingredients and their contribution to the digesta matrix should be characterized and listed as feed ingredient property in feeding tables. Feed ingredient rheological properties have been recognized as being important previously (Lentle and Janssen, 2008), but have often been restricted to the measurement of viscosity of the supernatant of feed extracts or digesta, see Carré et al. (1994). Although such measurements provide useful information on the behavior of the liquid fraction of the digesta, contribution of the solid fractions is largely ignored. To increase our insight, physiochemical and rheological characterization of feed ingredients, feeds, and digesta are of great importance. It is interesting to note that quite drastic changes in rheological properties can be observed between ingestion and stomach contents, depending on technological treatment (e.g. extrusion, see Martens et al. (2019b)). This implies that assigning rheological properties to feed ingredients requires careful consideration and that predicting changes in rheological properties of digesta when moving from proximal to distal parts of the gastro-intestinal tract can be important. Preliminary studies in our lab on data published by Martens et al. (2019b) and Schop et al. (2019a, 2020) have demonstrated added value of rheological characterization of feed over simple measures of physical properties of feed ingredients like water binding capacity and viscosity, finding relations between several rheological properties and gastric retention time of digesta.

Modeling Digestion Kinetics, the Next Step in Feed Ingredient Evaluation

After appropriately characterizing the feed ingredient in terms of (macro)nutrient composition, digestion potential, and relevant physicochemical and rheological properties, prediction of the intestinal climate (e.g., pH, rheological properties) and digesta transit following the ingestion of a compound feed, determines the extent to which the digestion potential is exploited. It is this prediction that currently is the biggest scientific challenge, as the intestinal climate is governed by many factors, some of which are difficult to measure. A first step in the development of such a model has been recently completed (Schop, 2020). In the next section, a ready-to-use modeling exercise is provided, allowing students to translate the basic principles discussed above into a dynamic mechanistic model.

Modeling Exercise: Digestion Kinetics in Pigs

This modeling exercise provides a comprehensive teaching module for students in Animal Science with knowledge of Animal Nutrition but minimal background in modeling. In the exercise, mechanistic modeling techniques are applied. Mechanistic models describe the system under study and seek to describe causation. These models assume that a complex system, such as the gastro-intestinal tract, can be understood by examining the individual parts and the manner in which these parts interact. The dynamic behavior is described using differential equations, describing the changes in pool size (e.g., protein in the stomach) in time. Students can make use of a modeling tool for constructing dynamic simulation models especially aimed at educational use, viz. SMART, an acronym for Simulation and Modelling Assistant for Research and Training, developed at Wageningen University & Research (Wageningen, the Netherlands). It should be noted, however, that this exercise can be conducted with any software that provides tools to numerically solve a set of differential equations.

The process of model development can be divided into 4 phases: (1) building the model, (2) defining simulation experiments, (3) performing simulations, and (4) inspecting results. In SMART a clear distinction is made between these phases. Especially, the first 2 phases are clearly separated. Briefly, phase 1 involves studying the structure of the system of interest (e.g., digestion or growth), dividing it into key components and relations, translating the system into a set of mathematical equations, and preparing the model for use in simulation experiments. In phase 2, the user defines

parameter values, inputs, the choice of numerical integration algorithm with its settings, and which outputs to store. The user can define any number of experiments with one model, each experiment having its own settings. Phase 3 is the actual simulation process that generates series of outputs according to the model equations and experimental settings. Under phase 4, the results are interpreted using a variety of graphs and tables. When using this tool in teaching, a brief demonstration of the tool to be used and providing some background on the use of differential equations in modeling is advisable.

The aims of this modeling exercise are (1) to gain insight in modeling digestion kinetics, (2) to gain experience in dynamic modeling using differential equations, and (iii) to gain insight in representation of feed ingredient properties and their effect on digesta transport and hydrolysis kinetics.

For this exercise, it is assumed that SMART software will be used. Specific instructions on downloading and installing this software, as well as, some hints to get started quickly, are provided as an on-line supplemental material. In addition, all model equations, the completed model—as developed in SMART—and results of some key-simulations are provided as on-line supplemental material. Equations are presented in a format that allows direct copy-pasting into SMART software, thereby ignoring some mathematical conventions.

1. The system to be modeled

In the description of this modeling exercise, in particular in the formulas, we use the system of abbreviations denoted in Table 1. We follow the fate of a single protein meal in a pig of about 25 kg of body weight. The size of the meal is 100 g. For simplicity, we consider only soluble and insoluble proteins. In a typical pig diet, about 10% of the dietary protein is soluble, but solubility is highly variable among ingredients. Ingested soluble proteins are present in the *Qspstom* pool. Ingested insoluble proteins (in the *Qipstom* pool) are partly hydrolyzed in the stomach by pepsin in an acid environment, leading to solubilization of the protein (present in the *Qspstom* pool). Soluble proteins leave the stomach with the liquid phase, whereas insoluble proteins leave the stomach with the solid phase. Upon arrival in the small intestine, both insoluble and soluble proteins are considered to be degradable and end up in the degradable protein pool (*Qdpint*). In the small intestine, these proteins are further hydrolyzed by intestinal enzymes such as trypsin, chymotrypsin and peptidases into free AA, present in the *Qaaint* pool, which will be absorbed with high priority through the intestinal wall. Non-hydrolysed proteins as well as non-absorbed free AA leave the small intestine and enter the colon, after which their fate and nutritional value is not further considered here.

Table 1. General notation used in the model

Symbol	Description
Q	Quantity, used to denote state variables, g
F	Flux, g/hr
K	Fractional rate constant
ip	Insoluble protein
sp	Soluble protein
dp	Degradable protein
stom	Stomach
int	(Small) intestine
abs	Absorption
col	Colon

A complete flow chart of the model is presented in Figure 1 and includes the names of state variables and fluxes used in the Smart model. A full description of the model is given below.

2. Step 1. Gastric emptying (60 min)

Task Build a model simulating passage and hydrolysis of dietary insoluble and soluble proteins through the *stomach* into the small intestine. In this first step, *just the stomach pools* have to be included (in step 2, intestinal pools will be added). Note: specify each single flux in the 'Auxiliary variables' tab, *not* in the 'State variables' tab. In the flow chart (Figure 1), boxes represent state variables (=pools, in g); while arrows connecting the boxes represent fluxes (i.e., quantities per hour).

- Open the model "ASAS-NANP-2020 digestion model_starting_version" in SMART. In this model, the required constants and parameters, listed in Table 2, have already been added and names for variables and the fluxes are predefined.
- The complete protein meal is present at the start of the simulation in the *Qspstom* (soluble) and *Qipstom* (insoluble) pools. In SMART, this is defined as initial pool sizes in the state variable section. The default meal contains 90 g of insoluble and 10 g of soluble proteins. There are no further model inputs. When running a simulation, we just follow the fate of these proteins when moving through the system.
- Insoluble proteins can be either hydrolyzed to enter the soluble protein pool, or leave the stomach with the solid phase:
    - The hydrolysis rate of protein in the stomach (Fipstom_spstom, g/hr) depends on the concentration of insoluble proteins in the stomach. At low protein concentrations, the fractional rate of hydrolysis is high. At high concentrations, enzyme availability may be limiting hydrolysis, and the fractional hydrolysis rate decreases. This can be represented by standard enzyme kinetics, for example, a Michaelis–Menten equation:

$$Fipstom_spstom = Vmax_proteinhydrolysis_stomach * Qipstom/\left(1 + Cip_stom/Jip_stomach\right),$$

      In which Vmax_proteinhydrolysis_stomach is the maximum, fractional rate at which insoluble proteins are hydrolyzed by pepsin in the stomach; Cip_stom is the concentration of insoluble proteins in the stomach (g of ip/L, assume stomach volume to be 5 L); the Jip_stomach is an inhibition constant of protein hydrolysis in the stomach, representing its inhibition at higher concentrations of the substrate.

    - Pepsin activity is strongly dependent on pH. It preferentially cleaves at certain AA at particular positions in the peptide chain. Cleavage can be very specific at low pH (1.3), whereas the specificity is largely lost at pH > 2. Furthermore, pepsin activity is pH dependent because 2 aspartic groups composes the reactive site of the pepsin. It is assumed that pepsin thus behaves as a diacid, where the first deprotonated form is the active species, and the pH-dependent activity is governed by the 2 pKa values. Plotting the pepsin activity against pH shows a bell-shaped curve in the pH range

Table 2. Constants and parameters used in the model

Name	unit	Description	Default value
SolubleProteinMeal,	g	Size of the soluble protein meal, provided at time=0	10
InsolubleProteinMeal	g	Size of the insoluble protein meal, provided at time=0	90
Diet_intake_level	—	Level of feed intake, expressed as multiples of the energy requirements for maintenance	2.5
Diet_solubility	g/g	Dietary solubility, i.e., the proportion of soluble nutrients over total nutrients, typically varying between 0.1 and 0.3	0.1
Diet_viscosity	Pa s	Apparent dynamic viscosity of the diet, measured at 1/s shear rate	30
Mealduration	hr	Duration of the meal	0.5
Kpassage_colon	/hr	Fractional rate of passage of digesta from small intestine to colon (i.e., this is 1/mean retention time)	0.25
MRT_liquid_stomach_default	hr	Default mean retention time liquids in the stomach	1.6
MRT_solid_stomach_default	hr	Default mean retention time solids in the stomach	3.2
Kabsorption	/hr	Fractional rate of absorption of AA in the small intestine	10
Kproton1	—	Dissociation constant for the first proton	0.0251
Kproton2	—	Dissociation constant for the second proton	0.0032
Vmax_enzyme_stomach	g/g/hr	Maximum fractional rate of enzyme hydrolysis in the stomach, depending on enzyme secretion capacity	2
Vmax_proteinhydrolysis_intestine	g/g/hr	Maximum fractional rate of enzyme hydrolysis in the intestine, depending on enzyme secretion capacity	2
pH	—	pH in the stomach	3
Jip_stomach	g/L	Inhibition constant, representing the inhibition of higher concentrations of protein hydrolysis in the stomach at higher concentrations of the substrate (g IP/L)	4
Jdp_intestine	g/L	Inhibition constant, representing the inhibition of higher concentrations of protein hydrolysis in the small intestine at higher concentrations of the substrate (g DP/L)	2

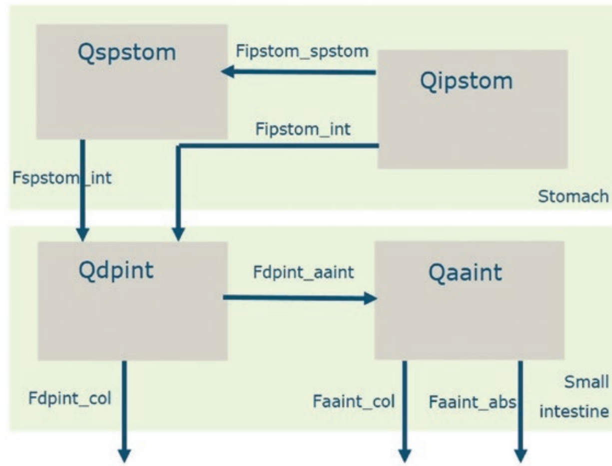

Figure 1. Flow chart of the model (steps 1 and 2).

0.1 to 6.0. It is therefore assumed that the maximum, fractional hydrolysis rate of protein in the stomach (Vmax_proteinhydrolysis_stomach) depends on pepsin secretions, and that pepsin activity depends on pH:

$$Vmax_proteinhydrolysis_stomach = Vmax_enzyme_stomach/(1 + (pow(10, -pH)/Kproton1) + (Kproton2/pow(10, -pH))),$$

In which the Vmax_enzyme_stomach is the maximum fractional rate of enzyme hydrolysis, depending on enzyme secretion capacity, and is a constant in the model; Kproton1 and Kproton2 represent the dissociation constants for the first and second protein, respectively.

- Insoluble proteins leave the stomach with the solid phase in a rate that is proportional to the pool size. It is assumed that while consuming the meal, the passage rate is 0. Hence, a variable, called "MealDuration" (set to 0.5 h), is defined as a constant. For representing this in SMART, tick the "Conditional" box for the variable Fipstom_int. When the condition "time<MealDuration" is true,

$$Fipstom_int = 0.\ Else\ (denoted\ by\ default)$$

$$Fipstom_int = Kpass_solid_stom * Qipstom$$

In which Kpass_solid_stom is the fractional passage rate of solids (/hr). This fractional passage rate is calculated as the inverse of the mean retention time of solids in the stomach, and is dependent on nutrient solubility and feeding level, as described by Schop (2020):

$$Kpass_solid_stom = 1/(MRT_solid_stomach_default + (-1.3 + 1.9158 * exp(-20.12 * exp(-1.7062 * Diet_intake_level))) + (0.87 * exp(-((Diet_solubility - 0.185)^2/(2 * 0.052^2))))),$$

In which MRT_solid_stomach_default is the default mean retention time of solids in the stomach, set to 3.1 hr; Diet_intake_level is the feeding level expressed as a factor times the maintenance energy requirement, and Diet_solubility is the proportion of soluble nutrients in the diet.

- Soluble proteins can be directly ingested or result from hydrolysis of insoluble proteins in the stomach (Fipstom_spstom, see above). They leave the stomach with the liquid phase, calculated in a similar way as for the digesta solids, with the exception that there is no delay during the meal. Hence:

$$Fspstom_int = Kpass_liquid_stom * Qspstom,$$

In which Kpass_liquid_stom is the fractional passage rate of liquids (/hr). This fractional passage rate is calculated as the inverse of the mean retention time of digesta liquids in the stomach, and is dependent on nutrient solubility, feeding level, and diet viscosity and is calculated based on the study by Schop (2020):

$$Kpass_liquid_stom = 1/(MRT_liquid_stomach_default + (-1.2 + 1.9158 * exp(-20.12 * exp(-1.7062 * Diet_intake_level))) + (0.87 * exp(-((Diet_solubility - 0.185)^2/(2 * 0.052^2)))) + (1.5 * 0.00174 * Diet_viscosity)),$$

In which MRT_liquid_stomach_default is the default mean retention time of solids in the stomach, set to 1.6 hr; Diet_intake_level is the feeding level expressed as a factor times the maintenance energy requirement, and Diet_solubility is the proportion of soluble nutrients in the diet, and Diet_viscosity is the dynamic viscosity of the diet in Pa s.

Check the model for syntax errors and compile it. Then, open the corresponding experiment or create a new experiment to run the model with different parameter values. In particular, try to explore the sensitivity of outflow kinetics of soluble and insoluble protein to changes in solubility of the protein, pH and diet viscosity. Use Euler's 4 fixed step length algorithm with a timestep of 0.001 hr and set the output control to 0.1 hr. Simulate for 12 hr. Note that you can you can change model parameters while running an experiment.

3. Step 2. Intestinal hydrolysis and absorption (60 min)

Task: Extend the model built in step 1 with a representation of small intestinal hydrolysis and absorption. You can use the model you built for step 1. State variables and names of fluxes are denoted in Figure 1.

- In the small intestine, degradable proteins (Q*dpint*) originate from inflow of insoluble and soluble proteins from the stomach (Fipstom_int and Fspstom_int, respectively).
- Degradable proteins can leave the small intestine after being hydrolysed to AA, or by passage to the colon.
    - In analogy to protein hydrolysis in the stomach, hydrolysis in the small intestine can be represented by a Michaelis–Menten equation:

    $$Fdpint_aaint = Vmax_proteinhydrolysis_intestine * Qdpint/(1 + Cdp_int/Jdp_intestine),$$

    In which Vmax_proteinhydrolysis_intestine is the maximum, fractional rate at which degradable proteins are hydrolyzed by enzymes in the small intestine; Cdp_int is the concentration of degradable proteins in the small intestine (g of dp/L, assume the small intestinal volume to be 8 L); the Jdp_intestine is an inhibition constant of

protein hydrolysis in the small intestine, representing its inhibition at higher concentrations of the substrate.

- Degradable proteins can leave the small intestine by passage to the colon:

$$Fdpint_col = Kpassage_colon * Qdpint,$$

In which Kpassage_colon is the fractional passage rate of digesta in the small intestine (i.e., 1/mean retention time). The mean retention time is set to 4 h.

- Amino acids in the small intestine (*Qaaint*) are assumed to originate exclusively from hydrolysis of degradable proteins (Fdpint_aaint, see above).
- Amino acids can leave the small intestine either by absorption or by passage to the colon.
  - Absorption of AA is considered to occur at a rate proportional to its pool size.

$$Faaint_abs = Kabsorption * Qaaint,$$

In which Kabsorption is a fractional absorption rate (arbitrarily set at 10/hr).

  - Amino acids can leave the small intestine by passage to the colon:

$$Faaint_col = Kpassage_colon * Qaaint,$$

In which Kpassage_colon is the fractional passage rate of digesta in the small intestine (i.e., 1/mean retention time). The mean retention time is set to 4 hr.

- Add a help variable to monitor the cumulative absorption of AA in the small intestine. To this end, specify a state variable Cum_aa_absorbed with AA absorption as the only input and no output, expressed as a proportion of the size of the protein meal:

$$Cum_aa_absorbed = Faaint_abs/\left(SolubleProteinMeal + InsolubleProteinMeal\right),$$

In which, the constants SolubleProteinMeal and InsolubleProteinMeal represent the size of the protein meals as represented by initial values of the state variables *Qspstom* and *Qipstom*, respectively. At the end of the simulation (i.e., after 12 hr) when the protein meals have moved through the intestinal tract, this variable represents the true protein digestibility.

Check the model for syntax errors and compile it. Then, open the corresponding experiment or create a new experiment to run the model with different parameter values. In particular, explore the sensitivity of AA absorption kinetics (time of peak absorption and its maximum rate) and the extent of digestion in response to the Vmax of protein hydrolysis in the small intestine. Vary kinetics by manipulating digesta passage rates in the stomach. Use Euler's 4 fixed step length algorithm with a timestep of 0.001 hr and set the output control to 0.1 hr. Simulate for 12 hr. Note that you can you can change model parameters while running an experiment. Results of some key simulations are included as on-line supplemental material. Briefly, these simulations illustrate the development of pool sizes of insoluble and soluble proteins in the stomach, resulting from fluxes of substrates and hydrolysis products over time. The influence of solubility of the dietary proteins, as well as that of stomach pH and viscosity properties on hydrolysis kinetics is illustrated. Finally, sensitivity of model predictions of the extent and kinetics of protein digestion to changes in some key parameters is presented.

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Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

Literature Cited

Bastianelli, D., L. Bonnal, Y. Jaguelin-Peyraud, and J. Noblet. 2015. Predicting feed digestibility from NIRS analysis of pig faeces. *Animal* **9**:781–786. doi:10.1017/S1751731114003097

Bastianelli, D., D. Sauvant, and A. Rérat. 1996. Mathematical modeling of digestion and nutrient absorption in pigs. *J. Anim. Sci.* **74**:1873–1887. doi:10.2527/1996.7481873x

Boirie, Y., M. Dangin, P. Gachon, M. P. Vasson, J. L. Maubois, and B. Beaufrère. 1997. Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc. Natl. Acad. Sci. USA.* **94**:14930–14935. doi:10.1073/pnas.94.26.14930

Boisen, S., and J. A. Fernández. 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. *Animal Feed Science and Technology* **68**(3–4):277–286 (Article). doi: 10.1016/S0377-8401(97)00058-8

Carré, B., J. Gomez, and J. Melcion. 1994. La viscosité des aliments destinés à l'aviculture. Utilisation pour prédire la consommation et l'excrétion d'eau. *INRA Prod. Animales* **7**:369–379.

Chen, H., P. A. Wierenga, W. H. Hendriks, and A. J. M. Jansman. 2019. In vitro protein digestion kinetics of protein sources for pigs. *Animal* **13**:1154–1164. doi:10.1017/S1751731118002811

CVB. 2016. CVB Veevoedertabel 2016; chemische samenstelling en nutritionele waarden van voedermiddelen. Federatie Nederlandse Diervoederketen. Available from http://www.cvbdiervoeding.nl. p 714.

Dangin, M., Y. Boirie, C. Garcia-Rodenas, P. Gachon, J. Fauquant, P. Callier, O. Ballèvre, and B. Beaufrère. 2001. The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am. J. Physiol. Endocrinol. Metab*. **280**:E340–E348. doi:10.1152/ajpendo.2001.280.2.E340

De Vries, S., W. J. Gerrits, M. A. Kabel, T. Vasanthan, and R. T. Zijlstra. 2016. β-Glucans and resistant starch alter the fermentation of recalcitrant fibers in growing pigs. *PLoS One* **11**:e0167624. doi:10.1371/journal.pone.0167624

Dijkstra, J., E. Kebreab, J. A. Mills, W. F. Pellikaan, S. López, A. Bannink, and J. France. 2007. Predicting the profile of nutrients available for absorption: from nutrient requirement to animal response and environmental impact. *Animal* **1**:99–111. doi:10.1017/S1751731107657760

Gilbert, M. S., N. Ijssennagger, A. K. Kies, and S. W. C. van Mil. 2018. Protein fermentation in the gut; implications for intestinal dysfunction in humans, pigs, and poultry. *Am. J. Physiol. Gastrointest. Liver Physiol*. **315**:G159–G170. doi:10.1152/ajpgi.00319.2017

Halas, V., W. J. J. Gerrits, and J. Van Milgen. 2018. *Chapter 14: models of feed utilization and growth in monogastric animals*. Wageningen, The Netherlands: Wageningen Academic Publishers.

Lentle, R. G., and P. W. Janssen. 2008. Physical characteristics of digesta and their influence on flow and mixing in the mammalian intestine: a review. *J. Comp. Physiol. B*. **178**:673–690. doi:10.1007/s00360-008-0264-x

Martens, B. M. J., E. M. A. M. Bruininx, W. J. J. Gerrits, and H. A. Schols. 2020. The importance of amylase action in the porcine stomach to starch digestion kinetics. *Animal Feed Science and Technology* 267. doi:10.1016/j.anifeedsci.2020.114546

Martens, B. M. J., T. Flécher, S. de Vries, H. A. Schols, E. M. A. M. Bruininx, and W. J. J. Gerrits. 2019a. Starch digestion kinetics and mechanisms of hydrolysing enzymes in growing pigs fed processed and native cereal-based diets. *Br. J. Nutr*. **121**:1124–1136. doi:10.1017/S0007114519000503

Martens, B. M. J., W. J. J. Gerrits, E. M. A. M. Bruininx, and H. A. Schols. 2018. Amylopectin structure and crystallinity explains variation in digestion kinetics of starches across botanic sources in an in vitro pig model. *J. Anim. Sci. Biotechnol*. **9**:91. doi:10.1186/s40104-018-0303-8

Martens, B. M. J., M. Noorloos, S. de Vries, H. A. Schols, E. M. A. M. Bruininx, and W. J. J. Gerrits. 2019b. Whole digesta properties as influenced by feed processing explain variation in gastrointestinal transit times in pigs. *Br. J. Nutr*. **122**:1242–1254. doi:10.1017/S0007114519002198

Nirea, K. G., M. Pérez de Nanclares, A. Skugor, N. K. Afseth, T. H. E. Meuwissen, J. Ø. Hansen, L. T. Mydland, and M. Øverland. 2018. Assessment of fecal near-infrared spectroscopy to predict feces chemical composition and apparent total-tract digestibility of nutrients in pigs. *J. Anim. Sci*. **96**:2826–2837. doi:10.1093/jas/sky182

NRC. 2012. *Nutrient requirements of swine*. 11th revised ed. Washington, DC: The National Academies Press.

Owusu-Asiedu, A., J. F. Patience, B. Laarveld, A. G. Van Kessel, P. H. Simmins, and R. T. Zijlstra. 2006. Effects of guar gum and cellulose on digesta passage rate, ileal microbial populations, energy and protein digestibility, and performance of grower pigs. *J. Anim. Sci*. **84**:843–852. doi:10.2527/2006.844843x

Prananto, J., B. Minasny, and T. Weaver. 2020. Near infrared (NIR) spectroscopy as a rapid and cost-effective method for nutrient analysis of plant leaf tissues. *Adv. Agron*. **164**:1–49. doi:10.1016/bs.agron.2020.06.001

Schop, T. A. 2020. *Modelling digestion kinetics in pigs. Predicting nutrient absorption based on diet and ingredient properties*. Wageningen: Wageningen University.

Schop, T. A., S. de Vries, W. J. J. Gerrits, and A. J. M. Jansman. 2019a. *In vitro enzymatic protein hydrolysis kinetics of feed ingredients, Modelling digestion kinetics in pigs*. Predicting nutrient absorption based on diet and ingredient properties, Wageningen; p. 57–73.

Schop, M., A. J. M. Jansman, S. De Vries, and W. J. J. Gerrits. 2019b. Increasing intake of dietary soluble nutrients affects digesta passage rate in the stomach of growing pigs. *Br. J. Nutr*. **121**(5):529–537. doi:10.1017/S0007114518003756

Schop, M., A. J. M. Jansman, S. de Vries, and W. J. J. Gerrits. 2020. 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* **14**:269–276. doi:10.1017/S1751731119001824

Stern, M. D., A. Bach, and S. Calsamiglia. 1997. Alternative techniques for measuring nutrient digestion in ruminants. *J. Anim. Sci*. **75**:2256–2276. doi:10.2527/1997.7582256x

Strathe, A. B., A. Danfær, and A. Chwalibog. 2008. A dynamic model of digestion and absorption in pigs. *Anim. Feed Sci. Technol*. **143**(1–4):328–371. doi:10.1016/j.anifeedsci.2007.05.018

Van den Borne, J. J., J. W. Schrama, M. J. Heetkamp, M. W. Verstegen, and W. J. Gerrits. 2007. Synchronising the availability of amino acids and glucose increases protein retention in pigs. *Animal* **1**:666–674. doi:10.1017/S1751731107736741

Van Duinkerken, G., M. C. Blok, A. Bannink, J. W. Cone, J. Dijkstra, A. M. Van Vuuren, and S. Tamminga. 2011. Update of the Dutch protein evaluation system for ruminants: the DVE/OEB2010 system. *Journal of Agricultural Science* **149**(3):351–367 (Article). doi: 10.1017/S0021859610000912

Van Erp, R. J. J., S. de Vries, T. A. T. G. van Kempen, L. A. Den Hartog, and W. J. J. Gerrits. 2020. Feed intake patterns nor growth rates of pigs are affected by dietary resistant starch, despite marked differences in digestion. *Animal* **14**:1402–1412. doi:10.1017/S1751731119002945

Wang, L. E., and R. T. Zijlstra. 2018. Prediction of bioavailable nutrients and energy. In: P. J. Moughan and W. H. Hendriks, editors, *Feed evaluation science*. Wageningen: Wageningen Academic Publishers.

White, G. A., F. J. Doucet, S. E. Hill, and J. Wiseman. 2008. Physicochemical changes to starch granules during micronisation and extrusion processing of wheat, and their implications for starch digestibility in the newly weaned piglet. *Animal* **2**:1312–1323. doi:10.1017/S1751731108002553

Yen, J. T., B. J. Kerr, R. A. Easter, and A. M. Parkhurst. 2004. Difference in rates of net portal absorption between crystalline and protein-bound lysine and threonine in growing pigs fed once daily. *J. Anim. Sci*. **82**:1079–1090. doi:10.2527/2004.8241079x

Zhang, D., B. A. Williams, D. Mikkelsen, X. Li, H. L. Keates, A. T. Lisle, H. M. Collins, G. B. Fincher, A. R. Bird, D. L. Topping, *et al*. 2015. Soluble arabinoxylan alters digesta flow and protein digestion of red meat-containing diets in pigs. *Nutrition* **31**:1141–1147. doi:10.1016/j.nut.2015.03.006