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Research Article

# In Silico Modeling of Food Digestion, Nutrient Bioavailability and Loss to Colon Linked to Microbiome Dysbiosis

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**Abstract**

A Mechanistic in silico Digestion Model (MDM) was used to model nutrient digestion, absorption, signals of hunger, satiety and appetite, and the delivery of unabsorbed nutrients to the colon. The model is based on many studies reported in the literature on nutrient hydrolysis by digestive enzymes and physiological studies that describe the regulation of digestion through neural and hormonal gut signaling by adapting transit rates through the mouth, stomach and small intestine, digestive fluid secretion, and absorption rates. Application the MDM gives mechanism-based predictions of digestion, bioavailability and prospective food intake. This publication focusses on protein digestion and unabsorbed proteinaceous and lipidic material reaching the colon. Particularly food-derived proteinaceous material reaching the colon is suspect because higher amounts have been shown to alter the composition of the gut microbiome (dysbiosis), promoting the growth of bacterial species that are capable of fermenting protein (protein putrefaction), leading to the release of harmful metabolites such as ammonia, amines, and sulfides. The MDM was used to predict the effects of a number of food and consumption parameters on the amount of proteinaceous material reaching the colon, enabling the design of strategies for reducing the risk of harmful protein putrefaction and microbiome dysbiosis.

**Keywords:** Computer Digestion Modeling; Digestive Physiology; Food Intake Regulation; Nutrient Bioavailability; Protein Putrefaction; Gut Microbiome Dysbiosis

## Introduction

The bioavailability of protein is reduced by incomplete digestion. For both animal-based and plant-based proteins, digestibility can be reduced by its natural molecular structure, isolation procedures and processing [1-3], and for less refined plant protein concentrates or whole plant materials also by the entrapment of the protein in cellular structures and the presence of protease inhibitors. Also the digestibility of well-digestible pure proteins, such as animal proteins, can be reduced by food processing conditions (e.g., UHT treated whey protein), such that the amount of protein exceeds the digestive and absorptive capacity of the small intestine, and by the presence of other materials in a whole meal, such as dietary fibers and antinutrients form vegetables.

Although some remaining unabsorbed proteins and peptides in the distal digestive tract are required as nitrogen source for the gut microbiota (for example the mucins, which due to their high glycosylation largely survive digestion in the small intestine [4]), and can deliver absorbable bioactive peptides and phytochemicals promoting antioxidant defense and reducing inflammation [5,6], an excess of incompletely digested and absorbed dietary proteins and peptides will be fermented by the gut microbiota, particularly in the colon. Too much unabsorbed protein entering the colon leads to dysbiosis and harmful metabolites by fermentation of protein (putrefaction) by the microbiome [7-9], which has been linked to systemic inflammation, metabolic diseases, cognitive decline, and colon cancer [10-18]. Moreover, the repeated presence of

higher amounts of proteinaceous materials (undigested protein and peptides) in the colon produces a competitive advantage for protein-fermenting microbes over carbohydrate-fermenting microbes, in this way changing the composition of the gut microbiome from a more healthy composition, able to ferment carbohydrates producing healthy metabolites such as short chain fatty acids, to a composition that favors protein putrefaction [19-21]. Moreover, reduced fermentation of carbohydrates results in an increased pH that favors the growth of pathogenic bacteria.

This is of particular important in relation to the current trend to increase protein intake to support weight loss, and to improve muscle strength by protein supplementation, for example through sport drinks and for countering sarcopenia. Protein putrefaction by the microbiome might limit the amount of protein that can be consumed safely. It has also been reported that dietary carbohydrate fibers can mitigate the effect of excess dietary protein in the colon [22,23,18].

In the literature, *in vivo* studies have been reported on the digestibility of proteins, measured by the amount of unabsorbed proteinaceous material in the ileum by sampling the composition of the ileal content through ileal stoma [24]. These studies can be performed in human with an ileal stoma, but these are typically patients suffering from gastro-intestinal diseases and show much variation due to variation in overall meal composition and different lifestyles [18]. Much insight has therefore been gained from animals with ileal stoma, particularly from pigs and rats [25].

The Mechanistic in-silico Digestion Model (MDM) [26,27] applied in this publication, has previously been used to model:

- the effect of the presence, formation and breakdown of solid material (cheese and flocculated casein formed at gastric pH and by pepsin hydrolysis) in the stomach on sensory scores of Fullness and Hunger,
- effects of the viscosity and nutrient composition on gastric emptying and the resulting effects on perceived fullness,
- the difference in gastric emptying rate, hormonal and glycemic effects (blood glucose and insulin excursions) between a bread and pasta meals with similar composition as used for *in vivo* studies [28,26,27],
- the effect of speed of consumption of a glucose and bread and pasta meals on the sensation of fullness, hunger and satiety [27].

## Method

The MDM used was based on the same model, using the same parameters as in previous publications [26,27]. In short, the model has been written in Pascal and is freely available from an open-source environment. It describes the alimentary tract as a sequence of well-mixed compartments the mouth, the stomach (divided into corpus, fundus and antrum), the duodenum (divided in a sections before and after the sphincter of Oddi), 3 jejunal segments and 6 ileal segments and a single colon, with physiologically controlled secretion of saliva, gastric juice, intestinal wall, bile and pancreatic fluid secretions, the detection of nutrients along the digestive tract and the release gut hormones that physiologically control the transits between these segments and digestive fluids release. The parameterization of the model is based on *in vivo* studies described in literature and *in vitro* digestion studies for the digestive enzymes.

Relevant to protein digestion, the model takes into account:

- the dynamic release of gastric fluid, containing pepsin and mucin,
- the pH-dependence of pepsin activity,
- protein buffering capacities: curves of pH versus gastric acid addition for mucin and the alimentary exogenous proteins entering the stomach (β-casein [29]; BSA [30]; β-lactoglobulin [31]; Gastric mucin [32]),
- control of pancreatic fluid secretion through the competition between the digestion by pancreatic proteases of Luminal CCK Releasing Protein and alimentary proteins,
- the formation of a protein gel under gastric conditions for β-casein,
- a simplified scheme of protein digestion by pepsin and pancreatic proteolytic enzymes to absorbable peptides and amino acids, kinetically described by Michaelis-Menten equation, including and inhibitor constants for predicting the effect of protease inhibitors,
- nutrients detected in the distal ileum and proximal colon illicit the ileal brake mechanism, signaled by neural signals and a number of gut hormones, predominantly PYY and GLP-1, limiting the intestinal transit rate, including the transfer of the ileal content to the colon [33], probably intended to optimize absorption and to restrict the amount of undigested nutrients entering the colon. The actual mechanism is however complex and not fully understood [34].

Each simulation run takes only a few tens of seconds, which allows a quick survey of variations in food compositions and amounts, food sequences such as pre-meals or repetitive small snack meals, and moreover the model can be adapted to different physiological conditions of the consumer, e.g., simulating the effect of variation in salvation, gastric juice release, gastric emptying and diabetic status.

## Simulations and Results

The MDM was used to model three different cases related to protein

digestion: A) Leucin blood levels after protein solutions, B) Cheese gastric digestion, effects on of fullness and hunger, C) Spill-over of protein to the colon due to incomplete protein digestion. Cases A and B are shown here to demonstrate the successful use of the model, giving support to the validity of modelling case C.

**Case A: Leucin blood levels after protein solutions**

Leucin promotes muscle protein growth, relevant for top sporters and the elderly [35,36], where higher peaks of blood Leucin are advantageous for muscle protein synthesis. Figure 1 shows how the balance of Leucine concentrations in the blood serum relates to the absorption of exogenous Leucine from the meal, release of endogenous Leucine from body tissues, and by injection into the blood ($R_a$: rate of appearance in blood plasma), together with the absorption of Leucine from the blood for protein synthesis and oxidation ($R_d$: rate of disappearance in blood plasma).

Attempting to simulate the results of *in vivo* measurements by experimental work from publications by the research group of Boirie et al. [37,38], in the simulation a tracer Leucine was added to protein (casein or whey), either as an amino acid separate from the protein, or included within the protein. The simulation results in Figure 2 and Figure 3 show tracer-leucine $R_a$ excursions that are very similar to the experimental excursions, showing immediate and rapid peaked absorption of free Leucine, a slightly delayed peaked release of Leucine incorporated into whey protein molecules because the release requires protein digestion, and a gradual and sustained release of Leucine incorporated in casein, due to flocculation and gelation of casein in the stomach, which delays gastric emptying.

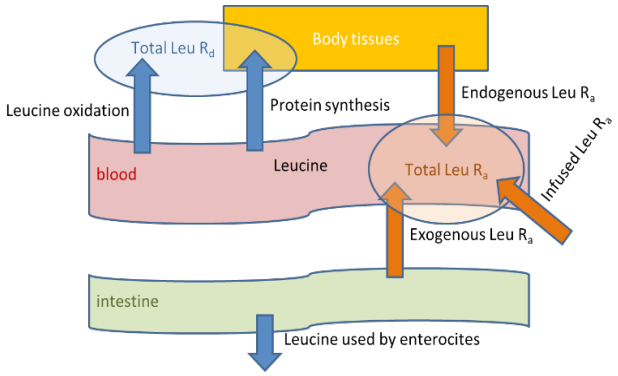


**Figure 1:** Balance of Leucine concentrations in the blood serum; $R_a$: rate of appearance in blood plasma, $R_d$: rate of disappearance in blood plasma.

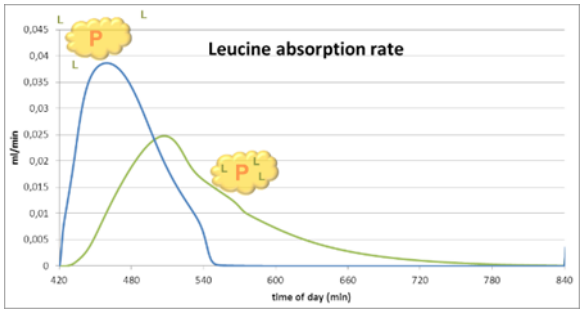


**Figure 2**: Simulated $R_a$ of tracer Leucine in blood plasma for tracer Leucine ingested separate from whey protein and tracer Leucine incorporated in whey protein, showing fast and sharply peaked absorption of tracer Leucine ingested separate from whey protein, and a broader delayed peak for tracer Leucine incorporated in whey protein.

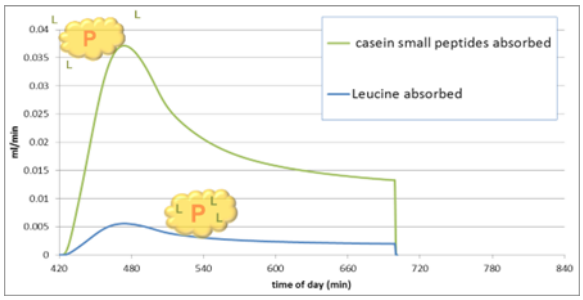


**Figure 3**: Simulated $R_a$ of tracer Leucine in blood plasma for tracer Leucine ingested separate from casein and tracer Leucine incorporated in casein, showing fast and sharply peaked absorption of tracer Leucine ingested separately from casein, and a gradual sustained release of tracer Leucine for tracer Leucine incorporated in casein.

**Case B. Cheese gastric digestion, effects on of fullness and hunger**

An in vivo human trial was conducted to measure the difference in texture (liquid versus semi-solid meals with similar nutrient composition) on gastric emptying and time-dependent CCK blood levels and sensory panel scorings of Fullness and Hunger [39]. The study showed that for the liquid meal the initial CCK blood levels are on the average more peaked compared to the semi-solid meal, tentatively explained by a less restricted initial emptying of the liquid meal, initially delivering more nutrients to the duodenum, where it induces CCK secretion. The semi-solid meal was retained longer in the stomach and prolongs the sensation of Fullness and

postpones the return of Hunger compared to the liquid meal, which was tentatively explained by a slower gastric emptying of the semi-solid meal because of its higher viscosity and the tendency to form boluses in the stomach. The difference between the two meal textures was modelled in the MDM by estimating the initial viscosities of both meals and the average sizes of boluses of the active meal in the stomach, including that lumps of semi-solids tend to sediment toward the antrum and are gradually broken up by gastric grinding and proteolytic erosion by pepsin at the surface, and the general knowledge that in the fed state lumps larger than 0.5 mm are retained in the stomach. In the simulations, "Hunger" was based on detected calories in the small intestine and "Fullness" was based on gastric tone, calculated from gastric distension and the modelled CCK concentration in the blood serum.

By adjusting the relevant parameters for gastric grinding and hydrolysis by pepsin, the simulated results (Figure 4) for Gastric volume and changes in the Visual Analogue Scales (VAS) in Fullness and Hunger are in close agreement with the experimental data [39] measured by a sensory panel. Also, the simulated blood CCK levels peaked more for the liquid meal compared to the semi-solid meal (not shown). This gives mechanistic support for the finding that semi-solids empty slower from the stomach than liquids with similar composition and that this leads to a more sustained sensation of Fullness and a delay in the recurrence of Hunger.

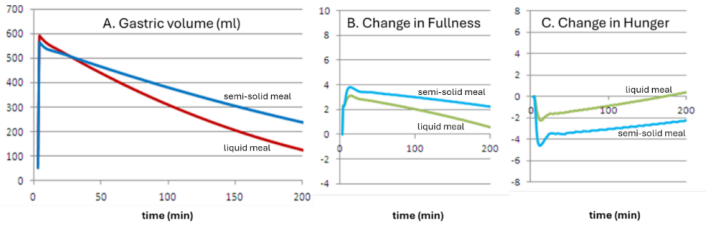


**Figure 4:** Simulated A: Gastric volume, B: Change in Fullness (VAS), and C: Change in Hunger (VAS) after liquid and semi-solid meals with similar compositions. The differences are caused by longer gastric retention of the semi-solid meal compared to the liquid meal.

### Case C. Spill-over of protein to the colon due to incomplete protein digestion

In healthy adults, relatively low doses of purified, well digestible proteins such as whey proteins or cooked egg protein, are almost completely absorbed, leaving approximately 5% of proteinaceous material to reach the colon [40]. However, from studies in piglets it is known that this amount increases in a roughly quadratic manner when the fodder is further enriched with additional protein [25].

The amount of protein and peptide material that enters the colon is difficult to obtain from *in vivo* studies, particularly in human subjects. Therefore, the MDM was used to predict this amount as amount of food-derived protein that has passed from the distal ileum to the colon, which is illustrated in the following for three different cases.

### Pasta meal: macronutrients reaching the colon

In this case the meal consisted of a pasta meal with the same composition as in ref. [27], based on the meal studies by Eelderink et al. [28]. The meal leads to a time-dependent variation in the total delivery of fat, proteins and carbohydrate to the colon, as shown in Figure 5.

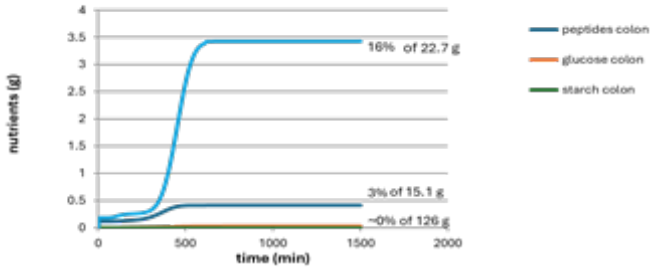


**Figure 5:** Simulated release of peptides, glucose and starch to the colon after digestion of 300 g of a pasta meal, containing 22.7 g highly digestible fat, 15.1 g highly digestible protein, and 126 g highly digestible starch. The figure shows that about 16% of the fat, 3% of the protein and almost no starch from the meal passes on to the colon (mostly in the form of bile micelles carrying fatty acids, peptides, glucose and maltose). The simulated values are in agreement with rough estimates for these values.

Figure 5 shows low initial values of fat and proteins entering the colon, before food-derived fat, protein and carbohydrate are starting to reach the colon after about 100-300 s. By variation of parameters in the model it could be established that these are related to fatty acids formed by lipolysis of phospholipids originating from bile phospholipids and digestion of endogenous proteins, in the model specifically mucin, which has a substantial contribution to endogenous protein losses to the colon [4], and which is only slowly digested by proteases of the gastrointestinal tract (such as pepsin and trypsin), because of its heavy glycosylation [41]. After about 500 s, all unabsorbed fat, protein and carbohydrate have entered the colon; their values remain constant because further processing in the colon is not yet included in the model.

### Comparing drink and pasta: the effect of the amount of protein in the meals

In this case the effect of the amount of protein in the meals on the amount food derived (exogenous) proteinaceous material delivered to the colon was calculated by the model. Except for the

protein, both 300g meals had the same composition as the pasta meal described in Figure 5, initially without protein, to which a varying amount of easily digestible protein (such as native whey) was added.

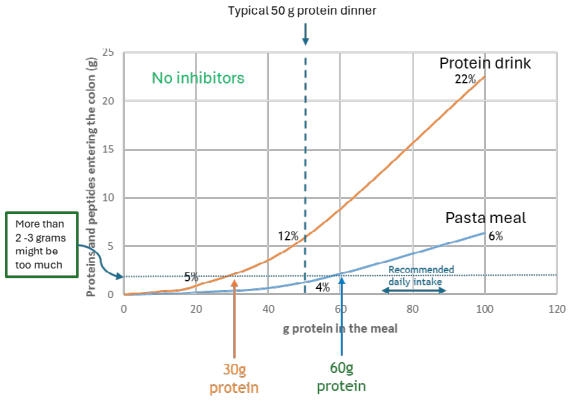


**Figure 6:** The effect of the amount of protein in the meal on the delivery of exogenous proteinaceous material delivered to the colon, comparing a liquid meal to a pasta meal with the same composition. Indicated is the typical recommended daily protein intake for a 80 kg person, which in reality depends on gender, age and physical activity [42]. The numbers near the curves correspond to the percentages of the ingested amounts of protein. More than about 2-3 grams of proteinaceous material delivered to the colon might be causing problems related to putrefaction by the microbiome in the colon.

The simulations shown in Figure 6 demonstrate that the amount of exogenous proteinaceous material delivered to the colon increases roughly parabolically with the protein content and does so faster for the liquid drink compared to the pasta meal, which corresponds to observations in pigs fed fodder enriched with caseins [25]. This is due to the slower simulated oral transit and gastric emptying for the pasta meals compared to the liquid drink. There is no exact value of the amount of proteinaceous material delivered to the colon that causes problems related to putrefaction by the microbiome in the colon, because this be highly dependent on the microbial composition of the microbiome and also the precise composition of the proteins (e.g., beef proteins contain substantially more sulfur-rich amino acids than plant-based proteins) and the accessibility of the remaining proteinaceous compounds for microbial fermentation [10]. However, to put the modelling results in perspective, based on the averaged fecal Nitrogen excreted on a daily protein intake of 100 g protein of 1.61 g [43], it seems reasonable to assume that a delivery to the colon exceeding about 2-3 grams of proteinaceous material may become harmful since roughly doubling the daily intake was demonstrated to cause a significant increase in inflammation/oxidative stress levels in overweight humans [44]. In view of Figure 6 this would correspond to about 30 g protein in the liquid drink and 60 g protein in the pasta meal.

**Effect of competitive pepsin and pancreatin inhibitors to 15.2 g protein snack – Comparing drink and pasta**

In this case the effect of a varying amount of competitive pepsin and pancreatin inhibitors in a protein snack containing 15.2 gram of protein (about 20 % of the Recommended Daily Intake) on exogenous protein reaching the colon are compared for liquid drinks and pasta meals with the same composition as in Figure 5. As an example, the properties of the Bowman-Birk inhibitor are taken into account.

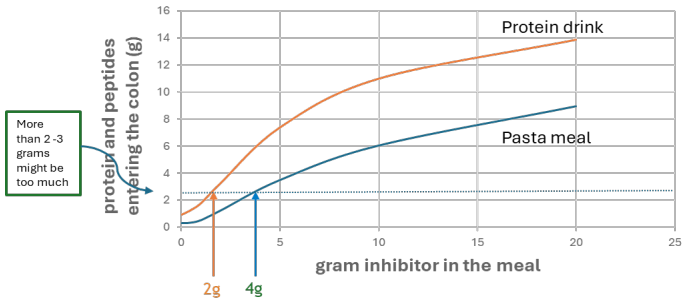


**Figure 7**: Exogenous protein reaching the colon for a varying amount of added Bowman-Birk inhibitor added to a liquid drink and to a pasta meal with the same compositions as in Figure 5, but now consumed in amount delivering 15.2 g of protein.

The results of the simulations, shown in Figure 7, demonstrate that the inhibitor strongly increases the amount of exogenous proteinaceous material delivered to the colon, exceeding the critical value of about 2-3 g at about 2 g inhibitor for the liquid snack and 4 g inhibitor for the pasta snack.

**Effect of added competitive pepsin and pancreatin inhibitors to a 50 g protein pasta meal, corresponding to a larger protein intake typical for dinner**

In this final case, the curve of exogenous proteinaceous material delivered to the colon for a variation in the presence of the Bowman-Birk inhibitor is calculated for an amount of pasta meal with the same composition as in Figure 5, delivering 50 g protein, which is about 70% of the Recommended Daily Intake for protein and typical for a dinner meal.

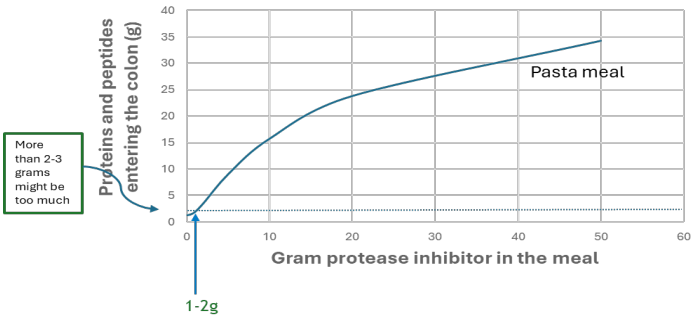


**Figure 8:** Same as Figure 7, but now for a typical dinner meal of pasta containing 50 g protein.

Figure 8 shows that for a pasta dinner meal containing 50 g of protein, the critical limit of 2-3 grams of proteinaceous material to the colon is now reached at about 1-2 g of the Bowman-Birk inhibitor.

## Discussion

The Mechanistic Digestion Model (MDM) was used to model aspects of protein digestion for three cases.

Two cases demonstrate the success of the model by reproducing *in vivo* experimental results. The first case concerns the rate of appearance ($R_a$) of tracer leucine in blood serum after protein drinks with unbound dissolved Leucine, and drinks in which tracer Leucine is incorporated within the molecular structure of whey protein, quickly emptying from the stomach, and casein, slowly emptying from the stomach. The second case concerns the effect of meal texture (liquid versus semi-solid meals with similar nutrient composition) on gastric emptying, time-dependent CCK blood levels, and sensory panel scorings for Fullness and Hunger.

Motivated by this success, as a third case, the MDM was used to predict the amount of unabsorbed nutrients (fat, carbohydrate and protein) that reach the colon, which is difficult to measure *in vivo* in humans. In particular, the delivery of exogenous proteinaceous material to the large intestine can lead to various diseases caused by microbial dysbiosis and the production of harmful metabolites. The modeling results show under which conditions (protein quantity per meal, liquid versus solid meal, and the presence of a protein digestion inhibitor), the predicted influx of exogenous proteinaceous material is large enough to cause dysbiosis of the microbiome and the formation of harmful metabolites. Specifically, the modeling results suggest that a slow and sustained entry of highly digestible protein into the small intestine may reduce the amount of exogenous protein entering the colon, which can be achieved by consuming protein-rich meals that forms solid clumps that contain the protein in the stomach.

Interesting extensions of the model for its application to optimize protein intake while avoiding harmful effects due to an excess of unabsorbed dietary proteinaceous material entering the colon could include the dynamics of the microbiome, the release of metabolites and their physiological effects, and the moderating effect on dysbiosis by soluble and insoluble fibers.

## Conclusion

The presented MDM combines knowledge from gastro-intestinal physiology with information obtained from *in vitro* digestion models and tissue and cell models. It enables a fast survey of the effects of a variety of food compositions and textures on the complex and mutually interactive processes involved in digestion. The MDM was shown to be able to predict *in-vivo* outcomes of blood serum excursions of Leucin produced by protein digestion and the effect of texture of protein-rich meals on gastric emptying and the sensations of Hunger and Fullness. The MDM was then used to predict the amount of unabsorbed nutrients (fat, carbohydrate and protein) that reaches the colon, which is linked to dysbiosis of the gut microbiome and the release of harmful metabolites by the gut microbiome, but difficult to measure *in vivo* in humans.

The MDM facilitates a fast surveys of product parameters, which is useful for the development of functional foods for enhanced protein intake and utilization, and can also be used in preparation of an *in vivo* intervention study based on the experimental results of *in vitro* digestion studies.

The MDM would benefit from input and collaboration between science fields, by further developing and refining the MDM and by connecting with *in vitro*, *in vivo*, and *ex vivo* (organ-on-a-chip) models.

## Disclosure Statement

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this work.

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