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ORIGINAL ARTICLE

Probiotic survival during a multi-layered tablet development as tested in a dynamic, computer-controlled *in vitro* model of the stomach and small intestine (TIM-1)

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Significance and Impact of the Study: Predictive GI *in vitro* models are very helpful and reliable tools for the development of new galenical formula containing probiotics, and in the current example helped to deliver >10-fold higher numbers of viable cells to the small intestine, presumably leading to improved functionality of the strains.

Keywords
Bifidobacterium, *Lactobacillus*, multi-layered tablet, probiotic, survival, TIM-1.

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Abstract

The aim of the research was to develop a galenical formulation for the combination of the three probiotic strains *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3 and *Bifidobacterium bifidum* MF 20/5 that would lead to the presence of a high amount of viable cells in the small intestine, the presumed site of action of these strains. This was tested in a validated, dynamic *in vitro* model of the stomach and small intestine (TIM-1), simulating human adults after intake of a meal. Experiments were performed both in the gastric compartment of the model, as well as in the complete system (stomach + small intestine). Survival of the strains in an unformulated probiotic powder after transit through the gastric compartment was 5·3% for the bifidobacteria and 1% for *L. gasseri*. After transit through the complete gastrointestinal tract, this dropped to 2% for bifidobacteria and 0·1% for *Lactobacillus*. After several rounds of optimization, an enteric-coated tablet was developed that increased the delivery of viable cells reaching the small intestine to 72% (gastric survival) for bifidobacteria, and 53% (gastric) for *L. gasseri*. Also survival in the small intestine increased by about an order of magnitude. The final galenical formulation was tested in two applications: adults and elderly, both of which have their own physiological parameters. These experiments corroborated the results obtained in the development phase of the project. In conclusion, the developed enteric coating led to a 20- to 40-fold increase in the delivery of viable cells to the small intestine.

Introduction

The consumption of fermented food, especially fermented milks, has a long tradition in several regions worldwide. Since the days of Metchnikoff, the idea that the bacteria that are responsible for fermentation are healthy has prevailed (Ozen and Dinleyici 2015; Calatayud and Suarez 2017). These bacteria have later been called probiotics (FAO/WHO 2001). Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' (FAO/WHO 2001; Hill *et al.* 2014). Today, probiotic bacteria in dairy products are successfully positioned in the food market but also a high diversity of dietary supplements, such as tablets, capsules,

sachets and other pharmaceutical (galenical) formulations containing probiotic bacteria is offered to the consumer.

Assuming the bacteria in question are alive, the definition focuses on two other important criteria: the administration in an adequate amount and the health benefit which is provided by the beneficial bacteria. There is no real consensus on what an adequate amount is, and this is likely to be strain dependent, and influenced by the survival of the strain during transit through the gastrointestinal tract, another feature that is strain dependent (Marteau *et al.* 1997; Campana *et al.* 2017). The postulated health benefits must be scientifically proven. Therefore, probiotics are the target of numerous scientific investigations and human studies. The beneficial effect of probiotics is also strain dependent and even bacterial strains of the same species may have different physiological effects. Therefore, the proof for health effects is only valid for the particular strain with which the clinical study has been performed (Azais-Braesco *et al.* 2010).

Probiotics may offer new therapeutic options in numerous areas such as inflammatory bowel disease, diarrhoea, lactose intolerance, paediatric atopic disease, allergic diseases, oral health, hypercholesteraemia, stimulation and regulation of the immune system, ageing and more. Moreover, probiotics can be combined with prebiotics for more efficiency (de Vrese and Schrezenmeir 2008; Martinez *et al.* 2011). The combination of the three strains, *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3 and *B. bifidum* MF 20/5, in a tablet has previously been shown to have clinical benefit in reducing the duration and severity, but not the incidence, of common cold episodes in a double-blind, randomized, controlled trial (de Vrese *et al.* 2005, 2006). The same cocktail of strains in a capsule showed a reduction in inflammatory cytokine profile in elderly (Spaiser *et al.* 2015). Moreover, the three strains in a capsule improved rhinoconjunctivitis-specific quality of life in individuals with seasonal allergies (Dennis-Wall *et al.* 2017). In these studies, however, survival of the probiotic strains during transit through the gastrointestinal (GI) tract was not reported.

The 'adequate amount' of a probiotic strain is the amount of the bacteria for which a health benefit is proven in a human study. This exact amount should be present in a food or dietary supplement product and delivered to the intestinal tract of the consumer when a positive health effect is claimed for such a product. Properties of the probiotic strain as well as the way of delivery (food matrix, formulation, etc.), or generally speaking the 'final product', are responsible for the delivery of an amount of viable cells to the site of action in the human intestinal tract. The strain should preferably feature a natural tolerance to gastric and bile acid, as well as sufficient resistance against digestive enzymes which enables the survival during the passage through stomach and upper intestinal tract. Where this is not the case, strategies to overcome killing by the human natural defence system (gastric acid, bile, digestive enzymes) can be applied, such as microencapsulation (Surono *et al.* 2018) or coating of tablets with an enteric coating that protects against gastric acid (Eiberger *et al.* 2011). A number of galenical (pharmaceutical) dosage forms such as drops, powders, granules, capsules and tablets are available to the consumer. In some cases, the number of colony forming units (CFUs) labelled on the pack of probiotic foods and dietary supplements is the number of viable bacteria contained in the product at the end of expiration date or consumed. However, it is more interesting to know how many of these bacteria are still alive at the site of action in the GI tract.

To evaluate this, microbiological analyses of faecal samples are a common way in clinical trials to investigate survival during passage through the entire GI tract. But these do not give insight into their survival during gastric and/or small intestinal transit. Moreover, cells surviving the upper GI tract may grow out again in the colon, leading to increased numbers of CFUs detected in faecal samples, giving an overestimation of survival. Besides, one can argue that it is more important to have viable cells in the upper GI tract, where the probiotics are thought to interact with the immune system. The use of a dynamic, computer-controlled *in vitro* model (TIM-1) to investigate the survival in the upper GI tract has been reported (Marteau *et al.* 1997). This model is highly validated and predictive for what happens with food (component)s, including probiotics, in the upper GI tract (Minekus *et al.* 1995; Minekus 2015). Survival of various probiotic species has been evaluated in this system, ranging from lactic acid bacteria and bifidobacteria (Marteau *et al.* 1997; Martinez *et al.* 2011), and bacilli (Hatanaka *et al.* 2012; Keller *et al.* 2017) to yeasts (Blanquet-Diot *et al.* 2012).

Such predictive *in vitro* models are a helpful tool in the development and evaluation of new galenical formulations containing probiotic bacteria. Changes in the composition of the formulation, for example adaptations of the composition or thickness of an enteric coating of a tablet, can be monitored and assessed. Furthermore, optimal conditions of intake can be defined using these predictive *in vitro* models. For instance, intake before, during or after a meal can influence the survival rate significantly, as human physiological conditions in the GI tract differ depending on the timing of administration, and therefore interact differently with, for example, an enteric coating that dissolves depending on the gastric pH. The pH prior to ingestion of a meal is different (*c.* 2) compared to during a meal (dynamic pH decline going from pH 5·5–7 to *c.* 2 during 3 h), which is again different 1 h after a meal

(between 2·5 and 4 depending on the age of the host). Moreover, age of the host influences physiological parameter, where, for example, gastric pH in elderly differs from that in adults (Murray and Barrie 2013). In addition, elderly also have a different GI transit (Brogna *et al.* 1999), and this may lead to difference in behaviour of the galenical form.

The aim of the current experiments was to develop an optimal enteric coating for a tablet containing the three probiotics *L. gasseri* PA 16/8, *B. longum* SP 07/3 and *B. bifidum* MF 20/5, making use of the validated TIM-1 system, simulating human adults. The effect of various types of coating and the thickness of the coating were evaluated on survival of the *Lactobacillus* and bifidobacteria strains, and compared to the unformulated probiotic powder product.

After selecting the optimal enteric coating, this coated tablet was used in two simulations to demonstrate the application of the developed enteric coating: in human adults and elderly. This was again tested in the validated, predictive *in vitro* model of the stomach small intestine (TIM-1), simulating the respective GI conditions in these two different age-populations.

Results and discussion

The TNO gastrointestinal model (TIM-1) is a validated system that simulates the successive dynamic physiological conditions in the stomach and the small intestine (SI). The model offers the possibility to simulate very closely the pH curves and the concentrations of enzymes in the stomach and SI, the concentrations of bile salts in the different parts of the gut, and the kinetics of transit of food or other materials through the stomach and intestine (Marteau *et al.* 1997; Minekus 2015). It has been extensively validated, also with respect to probiotic survival (Marteau *et al.* 1997) and coated tablets (Souliman *et al.* 2006; Souliman *et al.* 2007), and is used to predict the results of a clinical trial.

Development of the optimal enteric coating

Survival of the three probiotics (*L. gasseri* PA 16/8, *B. longum* SP 07/3 and *B. bifidum* MF 20/5) was evaluated both in the gastric compartment of the TIM-1 system to study the effect of gastric acidity and during transit through the complete TIM-1 system, to subsequently evaluate the effects of bile and pancreatic enzymes on survival. The three strains did not survive well when fed to the TIM-1 system as unformulated probiotic powder. Only 5·3% of the viable ingested bifidobacterial dose and 1% of the viable ingested *Lactobacillus* dose survived passage through the gastric compartment (Table 1). After passage through the complete TIM-1 system, the cumulative survival of bacteria from the unformulated probiotic powder was 2% for the bifidobacteria (Fig. 1a) and 0·1% for the *Lactobacillus* strain (Fig. 1b). To maximize the functionality of these probiotics during transit through the gastrointestinal tract, it is important to increase the survival of cells. Therefore, a three-layer tablet formulation was developed, with one layer containing vitamins, another layer minerals and trace elements, and the third layer the three probiotics, to provide protection to the viable bacteria and ensure their delivery to the site of action in the intestine. First, an uncoated version of this tablet (core) was tested in TIM-1 under the same conditions as the powder. Survival in the gastric compartment increased dramatically to 31·3 and 24% for bifidobacteria and *Lactobacillus*, respectively. However, it was observed visually that under the applied conditions, the uncoated tablet disintegrated to a large extent, and only *c.* 4·2–4·4% of the initial viable cells was retained in this uncoated tablet. Despite a higher delivery of viable cells to the SI, the conditions in the SI still led to a drastic

Table 1 Average survival of bifidobacteria and *Lactobacillus* from the unformulated powder, the core and the different coated tablets as percentage of intake during passage through the gastric compartment

	Powder		core		3% w.g.		5% w.g.		7% w.g.		Shellac aqueous 5% w.g.		HPMC:HPC aqueous 5% w.g.		HPMC:HPC ethanolic 5% w.g.		HPMC:HPC ethanolic 3% w.g.	
Sample	*Bif.*	*Lb.*	*Bif.*	*Lb.*	*Bif.*	*Lb.*	*Bif.*	*Lb.*	*Bif.*	*Lb.*	*Bif.*	*Lb.*	*Bif.*	*Lb.*	*Bif.*	*Lb.*	*Bif.*	*Lb.*
T0-60 min	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
T0-120 min	5·2	1·0	25·9	18·8	35·1	23·2	16·9	14·4	9·1	10·0	33·1	2·6	2·5	6·0	3·6	2·4	0·6	0·5
Grs	0·039	0·004	1·1	0·8	5·9	1·0	3·7	1·4	5·6	12·3	2·6	1·3	2·3	2·8	16·7	10·2	0·7	0·9
Tablet*	n.a.	n.a.	4·2	4·4	8·1	5·8	19·6	7·4	24·3	19·0	2·8	21·9	57·5	29·0	51·2	40·1	15·7	14·6
Total	5·3	1·0	31·3	24·0	49·1	29·9	40·2	23·1	39·0	41·3	38·4	25·8	62·3	37·8	71·5	52·7	17·0	15·9

Bif., *Bifidobacterium*; Lb., *Lactobacillus*; w.g. weight gain; n.a., not applicable; Grs, Gastric residue.
*Material retained in the (partially disintegrated) tablet after the incubation in the stomach.

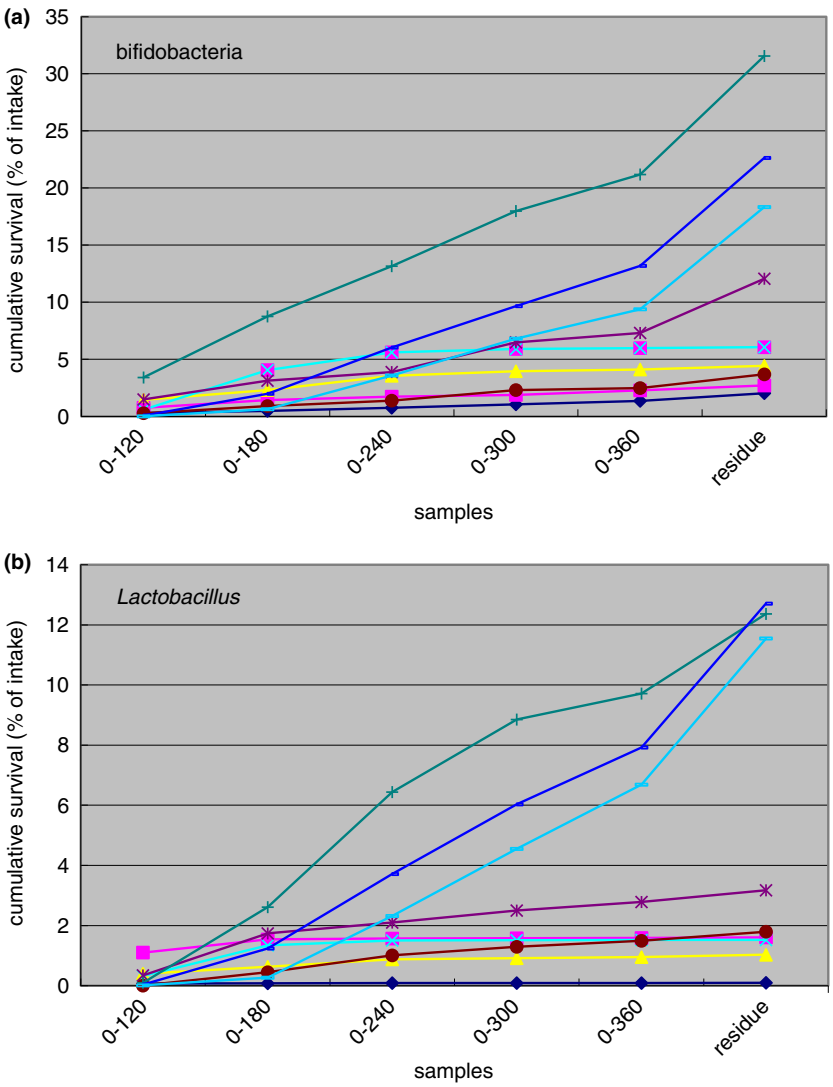

Figure 1 Cumulative survival (as percentage of intake) for the two genera during transit through the complete TIM-1 system simulating adults. (a) Survival of bifidobacteria; (b) survival of *Lactobacillus*. Legend: ♦ powder; ■ core tablet; ▲ 3% tablet; × 5% tablet; ✱ 7% tablet; ● Shellac-aq; + HPMC-HPC-aq; ▬ 3% HPMC-HPC-eth; ▬ 5% HPMC-HPC-eth. [Colour figure can be viewed at wileyonlinelibrary.com]

decline in survival, with final cumulative values of 2·6% (hardly better than the unformulated probiotic powder) and 1·6% for bifidobacteria and *Lactobacillus*, respectively. It is hypothesized that because cells were first exposed to a low gastric pH during gastric transit (because they were released from the tablet), they did not survive the second stress they encountered in the duodenum (high bile and pancreatic enzymes).

To prevent the tablet from disintegrating in the gastric compartment, an enteric coating was developed. At first, a mixture of hydroxypropyl-methylcellulose (HPMC) and hydroxypropylcellulose (HPC) was tested, at different thickness of the coating around the tablet, expressed as percentage increase in weight gain. At this stage in development, there was still some disintegration of the tablet occurring. And although with increasing weight gain of the enteric coating from 3 to 7%, it was shown that similar numbers of viable cells remained in the tablet during gastric passage (Table 1), SI survival (Fig. 1) increased from 4·2 to 6·1 to 11·9 for bifidobacteria in the 3, 5 and 7% weight-gain tablets, respectively, and from 1·0 to 1·5 to 3·2 for *Lactobacillus*. Thus, larger amounts of viable cells reached the SI than when the tablet was not coated.

Next, a Shellac/Solvent coating and HPMC:HPC coating were applied at 5% weight gain, as aqueous solutions. In both the gastric experiments (Table 1) and the experiments using the complete TIM-1 system (Fig. 1), HPMC:HPC coatings outperformed the Shellac/Solvent-coated tablets for both genera. For the Shellac/Solvent-coated tablets, there seemed to be a (selective) release of bifidobacteria from the coated tablet which is not understood, but because a HPMC:HPC coating was selected for further experiments, this was not investigated deeper. To see if an ethanolic solution of the HPMC:HPC polymers

formed an even more effective film, coatings were tested at 3 and 5% weight gain. The 5% weight-gain ethanolic HPMC:HPC coating led to the highest survival of all variables tested in the gastric compartment for *Lactobacillus*, primarily due to the fact that most cells were retained in the tablet (40%). Survival in the SI was similar for the aqueous and ethanolic solutions. But because the delivery of viable probiotics from the stomach to the SI was highest for the ethanolic HPMC:HPC product (71·5% for bifidobacteria and 52·7% or *Lactobacillus*), this was chosen as the product with the optimal enteric coating. The 3% weight-gain ethanolic solution resulted in much lower survival percentages, even lower than the HPMC/HPC aqueous solution (Table 1 and Fig. 1).

Testing of the optimal enteric coating in two different applications: adults and elderly

The selected product in the development phase was subsequently validated in applications for adults and elderly, each with their own physiological parameters (Fig. S2).

Once the development phase of the research was completed, the *in vitro* studies were validated using coated tablets manufactured at industrial scale, which led to even better survival results in the gastric compartment. Survival of both genera in the gastric compartment was close to 100% (and in some experiments even slightly higher; Table 2). The >100% survival is caused by the fact that for the TIM experiments, tablets were used that happened to contain (by chance) higher CFUs than the tablets used for the determination of initial counts. This variation in experiment-to-experiment counts was also observed – to a lesser extent – in the development phase and can be attributed to slight variations in the culturing conditions, batch-to-batch variation at manufacturing and/or standard error in the plate count method. Culture conditions have been shown to influence probiotic survival (Mills *et al.* 2011; Chen *et al.* 2017), and it has been shown to be able to precondition the cells to GI stress by incubating or growing them under, for example, low acid conditions (Mills *et al.* 2011; Chen *et al.* 2017). Lower stress during processing or preconditioning during cell culture leads to a hypothesized lower stress for the cell, and a higher ability to survive during gastrointestinal transit.

Next, survival in the complete TIM-1 system was tested and was shown to be 13·5% for bifidobacteria under conditions simulating elderly and 7·3% under conditions simulating adults. For *Lactobacillus*, the survival was 7·5% under elderly conditions, and 9·6% under adult conditions. This shows that the coated galenical formulation delivered almost all cells present in the tablet in viable form to the SI, and that thereby the enteric-coated tablet is much better than a sachet or stick with just the freeze-dried powder, which under similar conditions only showed an upper GI survival of 2% for the bifidobacteria and 0·1% for the *Lactobacillus*.

Overall, >10-fold higher numbers of viable cells were delivered from the stomach to the SI. When comparing data on the unformulated powder and the optimized tablet (5% ethanolic HPMC:HPC formulation), for bifidobacteria the increase is from 5·3% to 71·5% (13·5-fold), for *Lactobacillus* it is from 1·0% to 52·7% (50-fold). This was for the experimental products. If we compare the powder to the final BION3 tablets, then the fold-

Table 2 Average cumulative survival of bifidobacteria and *Lactobacillus* from the Bion3 tablets as percentage of intake during passage through the gastric compartment and the complete TIM-1 system for elderly (left) and adults (right)

	Elderly				Adults			
	Gastric		Complete		Gastric		Complete	
Sample	*Bif.*	*Lb.*	*Bif.*	*Lb.*	*Bif.*	*Lb.*	*Bif.*	*Lb.*
T0-60 min	0	0			0	0		
T0-120 min	25·5	22·3			38·0	15·1		
T0-180 min	50·8	64·1			84·3	58·8		
Grs	100+	100+			100+	93·5		
T0-60			0	0			0	0
T0-120			0·17	0·02			0·18	2·8
T0-180			1·5	0·49			1·1	3·7
T0-240			2·5	0·95			1·8	5·4
T0-300			2·9	1·6			3·6	6·9
T0-360			3·3	1·8			6·2	8·6
GDMIrs			13·5	7·5			7·3	9·6

Bif., *Bifidobacterium*; Lb., *Lactobacillus*; Grs, Gastric residue; GDMIrs, residue from the complete TIM-1 system; 100+, more viable cells than determined as the average in the tablet (see Table 1).

Table 3 Initial cell count added to TIM-1 (CFU per product, except for powder: CFU per g) as determined by microbiological cell count (average ± SD; $n = 6$)

Product	Lactobacilli	Bifidobacteria	Total count
Coating experiments			
Unformulated probiotic powder	$1.4 \times 10^9 \pm 3.1 \times 10^7$	$1.7 \times 10^8 \pm 8.3 \times 10^6$	$1.6 \times 10^9 \pm 4.1 \times 10^7$
Core tablet	$6.0 \times 10^8 \pm 4.3 \times 10^7$	$8.9 \times 10^8 \pm 7.8 \times 10^7$	$1.5 \times 10^9 \pm 8.9 \times 10^7$
HPMC/HPC 3% weight gain	$5.6 \times 10^7 \pm 3.6 \times 10^6$	$5.7 \times 10^7 \pm 3.1 \times 10^6$	$1.1 \times 10^8 \pm 3.7 \times 10^6$
HPMC/HPC 5% weight gain	$1.9 \times 10^8 \pm 4.9 \times 10^6$	$3.4 \times 10^7 \pm 2.6 \times 10^6$	$2.2 \times 10^8 \pm 5.4 \times 10^6$
HPMC/HPC 7% weight gain	$2.0 \times 10^8 \pm 6.2 \times 10^6$	$8.0 \times 10^7 \pm 2.8 \times 10^6$	$2.8 \times 10^8 \pm 6.3 \times 10^6$
Shellac/Solvent aqueous	$2.2 \times 10^7 \pm 6.9 \times 10^5$	$2.6 \times 10^7 \pm 8.1 \times 10^5$	$4.8 \times 10^7 \pm 1.4 \times 10^6$
HPMC:HPC-aqueous	$2.4 \times 10^7 \pm 7.5 \times 10^5$	$2.8 \times 10^7 \pm 8.9 \times 10^5$	$5.2 \times 10^7 \pm 1.6 \times 10^6$
HPMC:HPC-ethanolic 5% weight gain	$1.7 \times 10^7 \pm 3.5 \times 10^5$	$1.2 \times 10^7 \pm 1.1 \times 10^6$	$2.9 \times 10^7 \pm 1.1 \times 10^6$
HPMC:HPC-ethanolic 3% weight gain	$3.2 \times 10^8 \pm 6.4 \times 10^6$	$2.7 \times 10^8 \pm 6.2 \times 10^6$	$5.9 \times 10^8 \pm 1.2 \times 10^7$
Application experiments			
BION3 adult	$2.4 \times 10^8 \pm 7.5 \times 10^6$	$9.8 \times 10^8 \pm 1.1 \times 10^6$	$1.2 \times 10^9 \pm 9.8 \times 10^6$
BION3 senior	$2.2 \times 10^8 \pm 6.9 \times 10^6$	$1.2 \times 10^7 \pm 1.8 \times 10^5$	$2.3 \times 10^8 \pm 6.9 \times 10^6$

change is even higher, with all microbes surviving in the BION3 tablets. Also, survival in the SI increased by about an order of magnitude (10-fold). We realize that this fold-change is calculated with the per cent survival and not with the absolute numbers. However, we have ample previous evidence that increasing (or decreasing) the dose of ingested viable cells does not change the survival when expressed as percentage (K. Venema, unpubl. results).

In conclusion, in the process of coating development, TIM-1 was an essential tool to identify the appropriate formulation in terms of coating material and concentration (thickness) of the coating. The best coating selected, based on the experiments performed in the validated *in vitro* system, was shown to be efficacious in increasing survival of the probiotic strains. The developed product showed good results in terms of survival in both an adult and elderly setting. Predictive GI *in vitro* models, such as TIM-1, are therefore very helpful and reliable tools for the development of new galenical formula containing probiotics, and in the current example helped to deliver >10-fold higher numbers of viable cells to the small intestine, presumably leading to improved functionality of the strains.

Materials and methods

Products

Probiotic powder and tablets with the different coatings were provided by Merck Consumer Health (Darmstadt, Germany). Characteristics about the CFU content of the different products are provided in Table 3. Cells were extracted from the different formulations ($n = 6$) by scraping the probiotic layer from the tablet and resuspending in 300 ml of citrate buffer at pH 7·0.

TNO *in vitro* model of the stomach and small intestine (TIM-1)

Figure S1 shows the schematic of the *in vitro* model, which has been described extensively before (e.g. Hatanaka *et al.* 2012; Surono *et al.* 2018). The model was set-up and run according to the validated protocol for survival of probiotics (Marteau *et al.* 1997), with modifications for the physiological parameters for elderly (Brogna *et al.* 1999; Murray and Barrie 2013). The method has been described in brief in the Supplementary Online Material.

Sampling

In the gastric experiments, the gastric efflux was collected every hour for 3 h. In the complete TIM-1 experiments, the ileal efflux (Fig. S1-H) was collected every hour for 6 h. For each sample collected, the volume was measured and a 1 ml sample was taken for analysis. At the end of the experiments, the residue left in the system was collected and analysed as well.

Analysis

Serial 10-fold dilutions were prepared of the samples and these were plated on Rogosa for *Lactobacillus* and on Beeren's medium for the bifidobacteria to determine CFUs. Subsequently, the plates were incubated at 37°C for 3–4 days under anaerobic conditions. Cumulative survival as percentage of intake was calculated as the sum of surviving bacteria in the different efflux samples from TIM-1 divided by the amount of viable bacteria introduced in the model with the meal (see Table 3). The total of the 2 *Bifidobacterium* strains was analysed together as plating could not distinguish between the two strains.

Conflict of Interest

L.E. and S.C. are employees of Merck Consumer Health, the company that has commercialised the tablet with the enteric coating and the multiple vitamins, minerals and probiotics described in this research. K.V. has been a consultant for Merck Consumer Health, and does consulting for other companies in the area of gut microbiology. The research was carried out by the Centre of Healthy Eating & Food Innovation (HEFI) of Maastricht University – campus Venlo, as an independent research party, although L.E. and S.C. were involved in the discussion of the experimental set-up. This research has been made possible with the support of the Dutch Province of Limburg.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Schematic diagram of the dynamic, multi-compartmental TNO *in vitro* model of the stomach and small intestine (TIM-1). A. stomach compartment; B. pyloric sphincter; C. duodenum compartment; D. peristaltic valve; E. jejunum compartment; F. peristaltic valve; G. ileum compartment; H. ileo-caecal sphincter; I. stomach secretion; J. duodenum secretion; K. jejunum/ileum secretion; L. pre-filter; M. semi-permeable membrane; N. water absorption; P. pH electrodes; Q. level sensors; R. temperature sensor; S. pressure sensor. Reprinted from (Keller *et al.*,) with permission.

Figure S2. Curves mimicked in TIM-1 over time, representing the gastric (♦) and ileal delivery (▲) [both expressed as percentage of the ingested meal], and the gastric pH (■) for adults (A) and elderly (B).

