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The impact of meals on a probiotic during transit through a model of the human upper gastrointestinal tract

T.A. Tompkins[1], I. Mainville[2] and Y. Arcand[2]

[1]Institut Rosell Inc., 6100 avenue Royalmount, Montreal, H4P 2R2 Quebec, Canada; [2]Food Research and Development Centre, 3600 boulevard Casavant, Saint-Hyacinthe, J2S 8E3 Quebec, Canada; ttompkins@lallemand.com

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Abstract

Commercial literature on various probiotic products suggests that they can be taken before meals, during meals or after meals or even without meals. This has led to serious confusion for the industry and the consumer. The objective of our study was to examine the impact of the time of administration with respect to mealtime and the impact of the buffering capacity of the food on the survival of probiotic microbes during gastrointestinal transit. We used an *in vitro* Digestive System (IViDiS) model of the upper gastrointestinal tract to examine the survival of a commercial multi-strain probiotic, ProtecFlor®. This product, in a capsule form, contains four different microbes: two lactobacilli (*Lactobacillus helveticus* R0052 and *Lactobacillus rhamnosus* R0011), *Bifidobacterium longum* R0175 and *Saccharomyces cerevisiae boulardii*. Enumeration during and after transit of the stomach and duodenal models showed that survival of all the bacteria in the product was best when given with a meal or 30 minutes before a meal (cooked oatmeal with milk). Probiotics given 30 minutes after the meal did not survive in high numbers. Survival in milk with 1% milk fat and oatmeal-milk gruel were significantly better than apple juice or spring water. *S. boulardii* was not affected by time of meal or the buffering capacity of the meal. The protein content of the meal was probably not as important for the survival of the bacteria as the fat content. We conclude that ideally, non-enteric coated bacterial probiotic products should be taken with or just prior to a meal containing some fats.

Keywords: lactobacilli, *L. helveticus*, *Bifidobacterium*, intestinal model, survival

1. Introduction

Commercial probiotic preparations come in several formats. Traditionally, in the food industry, probiotics have been delivered as part of a whole fermented dairy product such as a yogurt, kefir or sweet acidophilus milk. In the nutritional supplement industries, they are often delivered in capsules or powders. According to FAO guidelines (FAO, 2002) it is considered essential that the probiotic microbes, no matter how they are delivered, survive the passage through the upper intestinal tract and arrive at their site of action. There are a few intestinal models such as the simulator of the human intestinal microbial ecosystem (SHIME) or the TNO upper intestinal model (TIM-1) which attempt to reproduce the gastrointestinal tract. These models have been used to examine the survival of primarily simple mono-strain lactobacilli-based probiotics; often they do not use commercially available probiotics, but rather only laboratory-grown versions (Alander *et al.,* 1999; Blanquet *et al.,* 2004; Kontula *et al.,* 1998). A single bioreactor simulating the upper gastrointestinal tract has been used to demonstrate that milk protein as a food matrix was important for the survival of commercial probiotics (Ritter *et al.,* 2009; Sumeri *et al.,* 2008). This model showed good correlation with piglet studies. Pitino *et al.* (2010) recently examined the survival of six commercial strains of *Lactobacillus rhamnosus* in their simulator. Other studies have examined the survival of probiotics by enumerating the microorganisms in human faecal samples (Brigidi *et al.,* 2003; Dommels *et al.,* 2009; Firmesse *et al.,* 2008; Tsai *et al.,* 2008), but since some probiotics are highly adhesive and selectively partition into the intestinal mucosa (Alemka

et al., 2010), faecal sampling alone may not be appropriate. Thus, without taking biopsy samples it is difficult to assess the survivability of probiotics. Few studies have examined the intestinal survival of probiotics after consumption in dairy products such as yogurts, kefirs (Mainville *et al.,* 2005; Reid *et al.,* 2005) and infant formulas (Botes *et al.,* 2008). Other groups have compared the faecal survival of commercial lactobacilli probiotic products when consumed with different dairy or non-dairy beverages or foods (Saxelin *et al.,* 2003, 2010; Varcoe *et al.,* 2002). Generally, the findings agree that dairy-based foods improve the gastrointestinal transit.

When it comes to probiotic supplements sold in capsules the commercial literature is often confusing in that sometimes the consumer is instructed to take the probiotics with meals, sometimes before or after meals, and occasionally on an empty stomach. In theory, the buffering of stomach pH by certain foods should improve the survival of probiotic microorganisms, but to what extent remains unknown. There exists a quantity of literature describing the survival of probiotic microbes as components of foods and in various microencapsulation matrices (Kailasapathy, 2002) but very little literature discusses the appropriate conditions for assuring the survival of probiotic microbes delivered in a capsule. To our knowledge there are no studies specifically examining the impact of probiotic delivery, in capsules, with respect to timing of a meal. Therefore, we examined the survival of a multi-strain, commercially-available probiotic when given 30 minutes before, during or 30 minutes after a meal of oatmeal-milk gruel. We also examined the impact on the type of meal/beverage by comparing the survival when taken with milk (1% w/w milk fat), apple juice or spring water. A multi-strain commercially-available probiotic, ProtecFlor®, was chosen because it contains two lactobacilli (*Lactobacillus helveticus* R0052, *L. rhamnosus* R0011), one bifidobacterium (*Bifidobacterium longum* R0175) and the probiotic yeast, *Saccharomyces cerevisiae boulardii*, making it possible to evaluate the survival of different probiotic microorganisms simultaneously.

2. Materials and methods

Probiotic preparation

ProtecFlor®, lot BC0097, was obtained from Institut Rosell-Lallemand Inc. (Montréal, Canada). Each hydroxypropyl methylcellulose white #1 capsule (total 5×10^9 cfu/capsule) contains *L. helveticus* R0052, *L. rhamnosus* R0011, *B. longum* R0175 and *S. boulardii* (cfu ratio 1:1:1:0.4). The microbes in this product were previously deposited in the Collection Nationale de Cultures de Micro-organismes (CNCM) at the Institut Pasteur (Paris, France) as I-1722, I-1720, I-3470 and I-1079, respectively. The bacteria in this product are not microencapsulated nor is the capsule enteric-coated which would render the capsules impermeable to the gastric acid.

Effect of time

Two capsules of ProtecFlor were added to the stomach vessel of the *in vitro* Digestive System (IViDiS) model 30 minutes before, during, or 30 minutes after a breakfast meal consisting of a portion of oatmeal (32 g of oat flakes – Robin Hood Minute Oats, Old Mill brand – cooked with 175 ml of water) and 250 ml of milk (Québon, 1% milk fat (MF); Agropur, St. Laurent, QC, Canada). When the probiotics were given 30 minutes before the breakfast meal, the capsules were added with 125 ml spring water. Each situation was repeated four times.

Effect of food/beverage

Two capsules of ProtecFlor were added, with 500 ml of either milk (Québon, 1% MF), oatmeal-milk gruel (32 g, cooked with 175 ml of water then added to 250 ml milk, 1% MF), 500 ml Oasis apple juice (Lassonde, Rougemont, QC, Canada; pH 3.5 and contained no preservatives) or 500 ml Eska spring water (Eska Water, St-Mathieu-d'Harricana, QC, Canada) to the IViDiS. Each situation was repeated four times.

Digestion in the IViDiS

Adapted from Mainville *et al. (*2005), the IViDiS, an in-house *in vitro* digestion system simulating the human upper gastrointestinal tract, was used to perform the digestions. A schematic diagram of the model is shown in Figure 1. The stomach and upper duodenum consist of jacketed vessels maintained at 37 °C and the lower duodenum is simulated using a tube (immersed in a water bath) with constrictions which, with a specific pumping profile, simulate peristaltic movement (Figure 1). The IViDiS can deliver a multi-service meal using a very realistic dynamic ingestion scheme (bite size, frequency and texture). A computer controls the flow rate of each food pump according to fully programmable 'profiles'. The same program controls the instantaneous flow rates of each enzyme solution, concentration of gaseous oxygen (proxy of the redox potential), pH, temperature, mixing and emptying of the stomach and of the duodenum. Five different solutions (enzymes, saliva, etc.) are injected in the stomach and three in the upper duodenum. HCl is used to control the pH in the stomach and a bicarbonate solution is used to neutralise the content entering the upper duodenum. Concentration of gaseous oxygen is monitored and controlled using air and nitrogen. Profiles of enzyme injection, pH and concentration of oxygen in the stomach and duodenum have been constructed from the Guyton and Hall Textbook of Medical Physiology (Hall, 2010) and are specific for each type of meal. For example, saliva flow rate in the pre-prandial phase is usually around 0.5 ml/min but rises when a meal is ingested. The flow rate of each pump may therefore vary from one test meal to another (e.g. apple juice vs. oatmeal breakfast). Structure, size, and

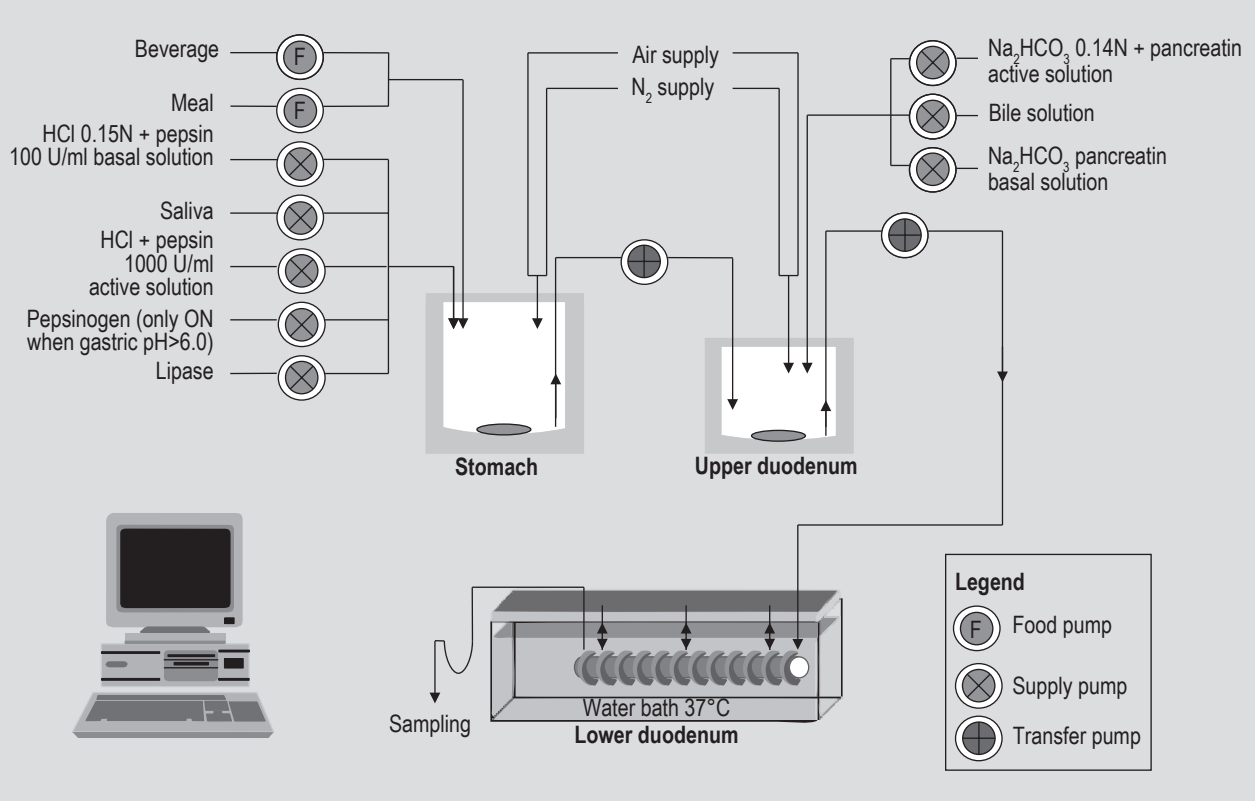

Figure 1. IViDiS model design and experimental set-up.

caloric content of the meal all have an impact on the final digestion profile. At the beginning of the digestion 40 ml of gastric juice was present in the stomach and 20 ml of duodenal juice was in the duodenum.

The digestions were allowed to take place for 2 to 3 hours, depending on the type of meal and on the eating habit modelled. All meals were ingested in five minutes. The events timing relative to the ingestion of the capsules (i.e. considering that the ProtecFlor capsules were introduced at zero minute) were as follows: for the experiments comparing beverages, the beverage was introduced at zero minute. For the oatmeal-milk gruel experiments, the timing relative to the ingestion of the ProtecFlor changed. For the capsules ingested during the meal, food was introduced at zero minute (as with the beverages). If the capsules were ingested 30 minutes after the meal, then the meal was introduced at t = -30 min. Finally, for the capsules ingested before the meal, the capsules were given with 125 ml of spring water at 0 minutes and the meal was introduced at t = +30 min.

Sampling for microbial analyses was begun 15 minutes after the probiotic arrived in the stomach reactor. The delay for sampling from the stomach vessel was due to the time required for complete disintegration of the sample and dissolution of the probiotic powder. Sampling from the duodenal reactor did not begin until 90 minutes after the introduction of the probiotic because there was insufficient volume arriving from the stomach vessel and exiting the reactor prior to this time to allow sampling.

Microbiological analyses

Microbiological analyses of *L. rhamnosus* R0011, *L. helveticus* R0052, *B. longum* R0175 and *S. boulardii* were performed using selective media. Total lactobacilli were enumerated on De Man, Rogosa and Sharpe agar using anaerobic conditions at 37 °C. *B. longum* was enumerated on RAF 5.1 agar using anaerobic conditions at 37 °C (Farnworth *et al.,* 2007; Roy, 2001). *S. boulardii* was enumerated on yeast extract peptone dextrose agar at 30 °C and aerobic conditions. Enumeration of each microbial component was done on the capsules prior to introduction into the IViDiS model. Beginning after 15 or 30 minutes in the stomach reactor, sample volumes of 2-5 ml were taken and 1 ml aliquots were diluted 1:10 into phosphate buffered saline (PBS). This solution was further diluted with PBS to give appropriate concentrations before plating on the appropriate agar. After 90 minutes there was sufficient volume in the duodenal reactor to begin sampling. Samples (2-5 ml) from the duodenal reactor were taken and similarly diluted in PBS and plated on agar. Enumeration was done on appropriate agar plates; triplicate plates were done for each dilution. Only plates with 25-250 colonies were included. The survival rate was evaluated by comparing the counts obtained in each reactor at specific time points to the

expected counts, assuming no mortality occurred. Expected counts were estimated using numerical approximation methods to solve mass balance differential equations for the volume and the theoretical bacterial concentrations in each reactor throughout the course of the study, in each vessel. In that way, we could follow the movement of the meal and microbes in the IViDiS, and knew the relative proportion of each solution in a vessel at any specific time.

Graphing and statistical analyses

Repeated measures ANOVA with Bonferroni post-test analysis were performed using Statistica 10 (Statsoft, Tulsa, OK, USA) to evaluate the effect of time and of all treatments (type of meal or capsule ingestion time). Differences were considered significant if $P<0.05$. 95% confidence intervals of the mean values for each treatment at each sampling time and location (stomach and duodenum) were used to plot graphs using MS Excel (Microsoft, Redmond, WA, USA).

3. Results

Timing of probiotic intake relative to oatmeal-milk gruel

Viable counts of lactobacilli, bifidobacteria and yeast released from the ProtecFlor capsules are presented as a function of time. Reported values are means of 4 repetitions. Disintegration of the hydroxypropyl methylcellulose capsules occurs within 5-10 minutes of being placed in the liquid then dissolution (i.e. complete dispersion of powder) occurs shortly thereafter, long before the first sampling in the stomach (30 minutes).

Figure 2A, 2B, and 2C show the survival of lactobacilli, *B. longum* and *S. boulardii* as a function of time through the IViDiS (stomach and duodenal vessels), respectively. In these figures, the time points of the X-axis (in minutes) represent the relative time of introduction of the probiotics to the stomach reactor conditions, and not the exposure of the meal which was introduced 30 minutes before, 30 minutes after or at the same time as the probiotic capsules. The survival of lactobacilli was affected more rapidly when the probiotics were given 30 minutes after the meal (Figure 2A). When given 30 minutes after a meal, a significant drop ($P<0.05$) in viable lactobacilli cell numbers was observed starting after 60 minutes in the stomach vessel. This coincided with a return to lower pH values (i.e. less than 4.0) as gastric acid solution was pumped into the vessel to simulate the normal gastric response to a meal. Similar to the lactobacilli, survival of *B. longum* in the stomach when given before or during the meal showed a better survival ($P<0.01$) then when given after the meal (Figure 2B). Lactobacilli and *B. longum* resisted significant losses in viability for more than 90 minutes in the stomach when taken before a gruel meal.

In the duodenum vessel, sampling did not begin until after 90 minutes when the volume exiting the system was sufficient to allow sampling to occur. Significantly lower numbers of lactobacilli and *B. longum* reached the duodenum when the capsules were given after the meal but once there, the percent survival remained constant. When the bacteria were given before a meal, there were additional losses in the duodenal vessel. However, this was not observed when the probiotics were given with a meal.

The probiotic yeast, *S. boulardii,* showed minimal loss of viability within the first 60 minutes ($P=0.03$) when given after the meal but thereafter there were no differences when compared to levels attained before or during meals. Survival was high for the entire 3 hour study period both in the stomach and duodenal vessels (Figure 2C).

Impact of type of beverage or gruel on probiotic survival

Figure 3A shows the pH in the stomach vessel when the probiotic capsules were given with spring water, apple juice, oatmeal-milk gruel and milk. The pH of the stomach increased briefly (i.e. 20 minutes) when the water was used as the beverage due to the slight buffering afforded by the saliva, the contents of the capsule and the high pH of this particular spring water which was approximately 8.0. The more acidic apple juice kept the pH of the stomach always below pH 4. When milk or oatmeal-milk gruel was taken with the capsules the buffering effect of these meals prolonged the higher pH (>pH 4.0) in the stomach vessel. The pH did not return below four until after 60 minutes. In the experiment, when ProtecFlor is given with water 30 minutes before the oatmeal breakfast, the pH curve is similar except that, a few minutes before the meal entry, the saliva flow rate increases to 2.5 ml/min instead of 0.5 ml/min simulating the impact of hunger (expectation of a meal, odour, etc.) before the meal. This flow rate is maintained during the meal entry and returns to unstimulated values afterwards. The increased entry of saliva in the stomach allows the pH before the meal to stay above 4 and therefore allows the survival of bacteria for a longer period since the entry of the meal further raises the pH. Figure 3B shows the emptying rate of the stomach vessel. The emptying was faster with the spring water but there was no difference in the emptying rates of the other substances. Due to the faster emptying rate, the experiment with spring water was terminated after 120 minutes rather than at 180 minutes for the other beverages. For the gruel or other beverages, approximately 50% of the meal or beverage had left the stomach vessel by 50 minutes, 75% had passed into the duodenum by 90 minutes and approximately 90% after 150 minutes.

Figures 4A, 4B, and 4C show the survival of the strains in ProtecFlor as a function of time in the IViDiS model

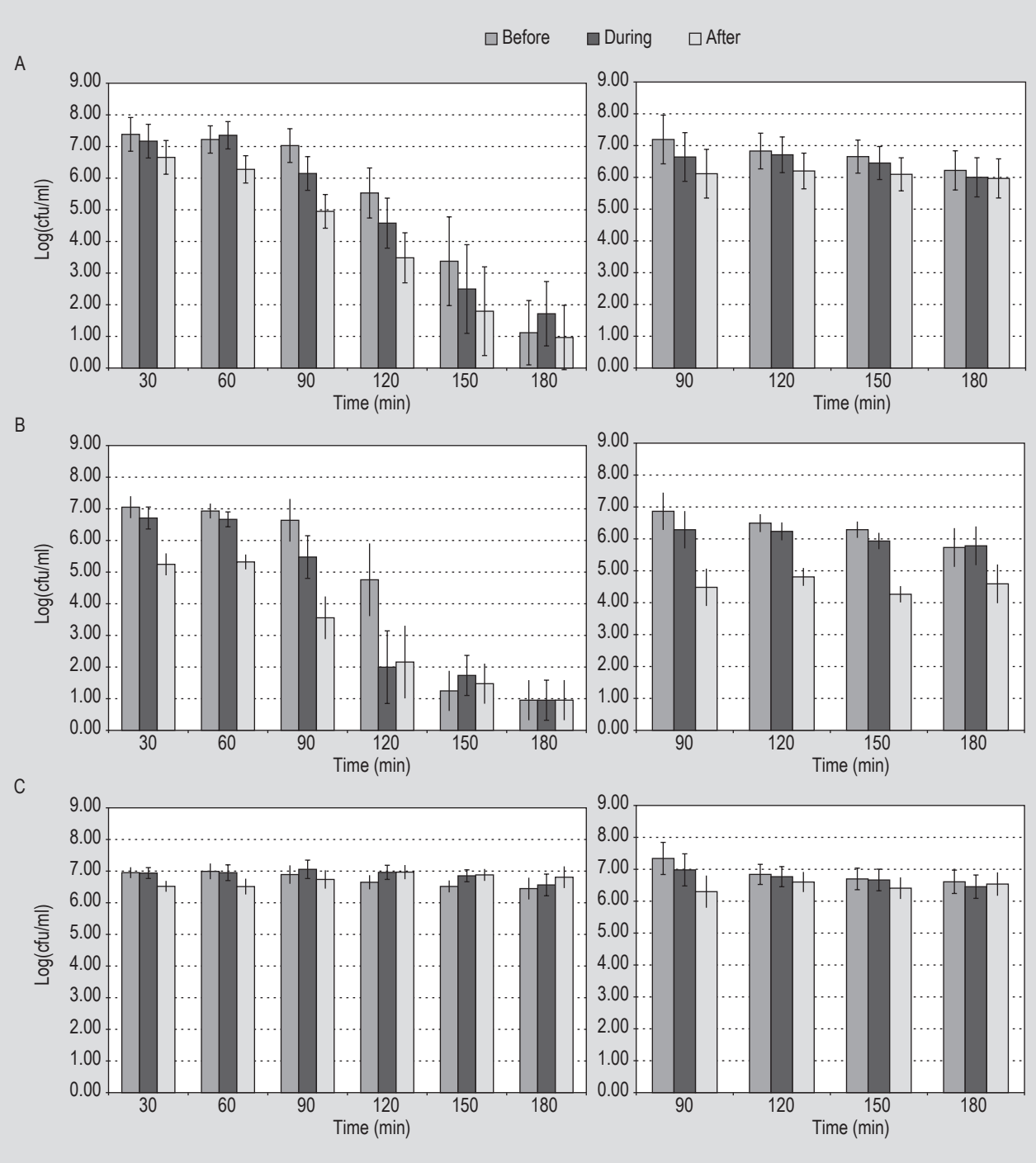

Figure 2. The survival of probiotic microorganisms as a function of time (min) through the stomach (left graph) and duodenal (right graph) vessels of the IViDiS model when given 30 minutes before, during, or 30 minutes after a meal of oatmeal-milk gruel. In each case, T=0 represents the time at which the probiotic arrived in the stomach reactor. (A) Lactobacilli survival; (B) *Bifidobacterium longum* survival; (C) *Saccharomyces cerevisiae boulardii* survival.

(stomach and duodenal vessels) when given with oatmeal-milk gruel or various beverages. In each case, T=0 represents the time at which the probiotic with the oatmeal-milk gruel or beverage arrived in the stomach vessel. With spring water and apple juice, the lactobacilli (Figure 4A) and *B. longum* (Figure 4B) showed a significant reduction of numbers even after 30 min. In milk, the bacterial counts remained high until 90 minutes. Bacterial survival in the oatmeal-milk gruel was intermediate between the milk and the spring water.

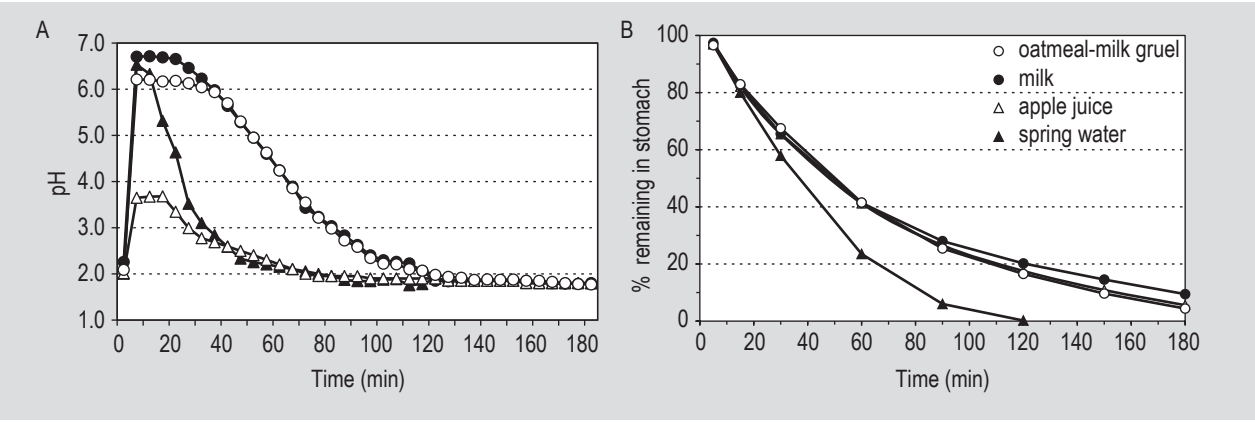

Figure 3. pH and emptying rates in the stomach vessel when given different beverages or an oatmeal-milk gruel with the probiotic. (A) pH values in the stomach vessel as a function of time. (B) Emptying rates of the stomach vessel with time.

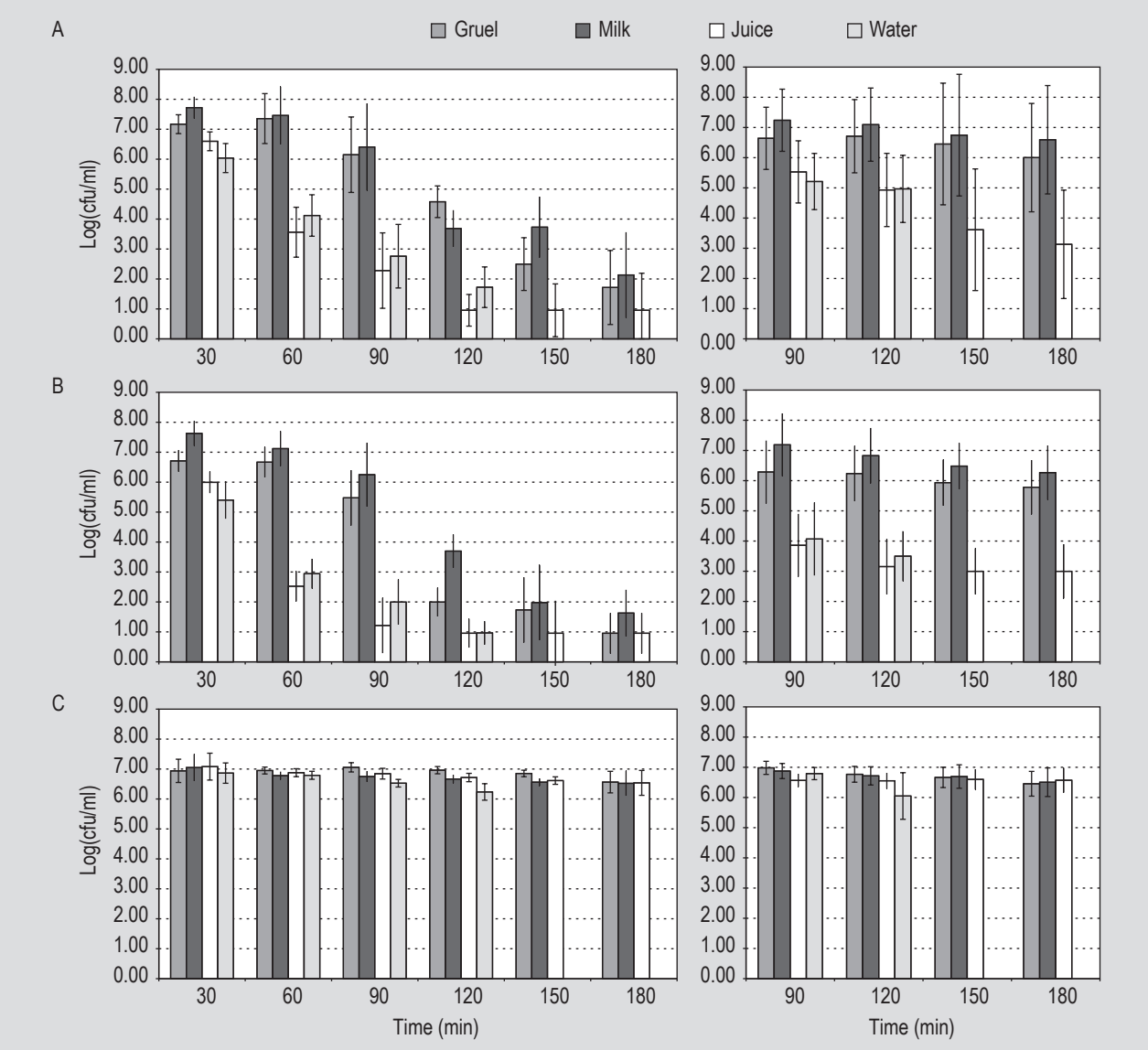

Figure 4. The survival of probiotic microorganisms as a function of time (min) through the stomach (left graph) and duodenal (right graph) vessels of the IViDiS model with oatmeal-milk gruel, 1% MF milk, apple juice or spring water. (A) Lactobacilli survival; (B) *Bifidobacterium longum* survival; (C) *Saccharomyces cerevisiae boulardii* survival.

Survival of the lactobacilli (Figure 4A) and *B. longum* (Figure 4B) in the duodenum remained significantly higher (all *P*-values between 0.07 and 0.0004) with milk and oatmeal-milk gruel rather than with the other beverages. With spring water and apple juice the bacterial counts were extremely low. While not statistically significant, slight but continued losses in bacterial survival in the duodenum were observed, even with milk and oatmeal-milk gruel.

S. boulardii was much less affected by pH and survived in any situation (Figure 4C). Survival of this strain remained high throughout the study period with milk, and apple juice. In the oatmeal-milk gruel, the percent survival began at 80% but quickly exceeded 120% (data not shown). In the duodenal vessel the yeast survival was independent of the meal or beverage consumed.

4. Discussion

The commercial literature on probiotic supplements is often confusing with respect to when they should be taken and very few clinical trials with probiotics actually describe the delivery of the probiotic capsules in detail. The literature on foods containing probiotic microbes suggests that buffering of stomach pH by certain foods improves the gastric survival (Saxelin *et al.,* 2003, 2010).

Viability of the bacteria in the commercial probiotic was superior when ProtecFlor capsules were given before the meal rather than during a meal [i.e. before>during>after] (*P*=0.0038 for lactobacilli – Figure 2A, and *P*=0.00046 for *B. longum* – Figure 2B). The bacteria survival, when given before a meal, can be explained by the fact that the pH in the stomach remained higher for a longer period of time after the probiotic capsules opened (pH data not shown) due to the important buffering effect of the spring water and the saliva (Figure 3A). The saliva secreted in response to the intake of the capsules, taken with water, raised the gastric pH and then when the meal entered the stomach, 30 minutes later, the gastric content was further buffered. When the capsules were given after the meal, the number of bacteria surviving stomach and duodenal passage was greatly reduced as the bacteria arrived about the same time as the pH of the system began to decrease. Also, in this case, the reduced numbers at the earliest time points may be due, in part, to incomplete rehydration and dispersion of the probiotic powders after capsule disintegration.

The type of meal significantly impacted the survival of the bacteria [i.e. 1% MF milk>oatmeal-milk gruel>apple juice/spring water] (Figures 4A and 4B). The protective effect of milk and oatmeal-milk gruel lasted long enough for a large percentage of bacteria to reach the duodenum before pH in the stomach became too harsh for survival.

The stomach content buffering may be due to the protein and/or fat content of the meal or beverage. The apple juice and spring water had no protein and no fat per 500 ml, whereas the milk contained 18 g of protein and 5 g of fat in 500 ml, as per the Nutrition Facts labels on the products. The oatmeal-milk gruel, which was made with 250 ml of 1% MF milk, contained about 14 g of protein and 5.5 g of fat. Varcoe *et al. (*2002) reported that skim milk was not any better than water for the survival of *Lactobacillus acidophilus*, and concluded protein buffering of the gastric acid was not sufficient for the stability of probiotics. Skim milk has nearly the same amount of protein as the milk used in this study (8.4 g /250 ml vs. 9.0 g /250 ml, respectively) but has significantly less fat content (0.4 g fat / 250 ml in skim milk compared to 2.5 g fat / 250 ml in 1% MF milk). This may suggest that the fat content was more important than the protein content for ensuring the survival of the probiotics through the stomach. However, the oatmeal-milk gruel had a comparable fat content but was not as protective as the milk alone. The hypothesis that fat is the protective agent would appear to be substantiated by the recent demonstration that two of the bacteria used in our study, *L. helveticus* I-1722 and *B. longum* I-3470 when microencapsulated in stearate, yielded nearly 100% survival when ingested in either dark chocolate or milk chocolate (Possemiers *et al.*, 2010), whereas half-skimmed milk results resembled the juice/water data in the current study. Both of these chocolate preparations contained approximately 2.7 g fat/13.5 g serving but the protein content was 0.37 g/serving and 0.52 g/serving for the dark and milk chocolates, respectively. These results also suggest that microencapsulation of probiotics in fats (Possemiers *et al.,* 2010) should be more beneficial for intestinal passage than entrapment in protein microgranules (Ding and Shah, 2007; Reid *et al.,* 2005). Reid *et al. (*2007) also showed that a milk-based matrix worked better than whey-protein encapsulation for improved long-term storage of the *L. rhamnosus* R0011 in fruit and vegetable juices, again suggesting a role for milk fats. Similarly, Saxelin *et al.* (2003) reported that *Lactobacillus* GG re-isolation from faeces was improved by the protective matrix of milk and dairy products as compared to powder or fruit juice. Dommels *et al. (*2009) demonstrated intestinal passage of viable probiotics when they gave healthy volunteers either *Lactobacillus reuteri* DSM 17838 or *L. rhamnosus* GG in a 'low-fat' spread with meals. While the spreads were deemed 'low fat' the fat content was still 5.6 g per 20 g serving.

The protective capacity of the fat from milk and oatmeal-milk gruel in the stomach greatly impacted the numbers of viable bacteria reaching the duodenum. It would appear that bile and pancreatic enzymes can have an additional negative impact on bacterial survival when foods or beverages with low concentrations of proteins or fat are the probiotic carrier.

The yeast survival (Figure 4C) was best in oatmeal-milk gruel but milk and apple juice were equally good. Yeast counts dropped off in spring water, again showing that it would be best not to take probiotics with water on an empty stomach. As in the previous study, some growth was observed for the yeast when given with the various meal/beverages except with spring water.

4. Conclusions

We have shown that the survival of non-microencapsulated probiotic bacteria through the stomach and duodenum is highly dependent on the time of ingestion and the protective capacity of the meal or beverage. Lactobacilli were somewhat less impacted by these factors than the bifidobacterium. Bacterial survival was best when provided within 30 minutes before or simultaneously with a meal or beverage that contained some fat content. As little as 1% (w/w) fat content proved superior to no fat content beverages for the preservation of the bacteria through upper GI passage. Protein content was not deemed to be as important for survival as fat content; however, additional studies should be performed to vary the protein/fat ratio over a broader range to further substantiate our observations. Survival was poorest when taken after a meal or when taken with spring water or apple juice. Probiotic yeast, *S. boulardii*, was not as influenced by these factors and even appeared to reproduce under most conditions tested. We conclude that probiotic capsules, when not enteric-coated, should be taken with a food or beverage with at least 1% w/w fat content to ensure the highest survival of viable microbes arriving in the small intestine.

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