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# Production and Characterization of Downgraded Maple Syrup-Based Synbiotic Containing *Bacillus velezensis* FZB42 for Animal Nutrition

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**Abstract:** The use of antibiotics to promote growth and prevent diarrhea in livestock production has raised concerns about the emergence of antibiotic-resistant bacteria. Probiotics, live microorganisms that confer health benefits, have been proposed as alternatives to antibiotics. In this study, we produced and characterized a downgraded maple syrup-based feed supplement containing *Bacillus velezensis* FZB42 as a potential synbiotic for animal nutrition. An optimized fermentation medium was developed through a central composite design to produce *B. velezensis* FZB42 at both the laboratory and pilot scale, reaching a concentration of $6.15 \pm 0.46 \times 10^9$ CFU/mL. Subsequently, *B. velezensis* FZB42 was incorporated into a protective whey permeate matrix and spray-dried, resulting in a 31.4% yield with a moisture content of 4.38%. The survival of *B. velezensis* FZB42 in a simulated gastrointestinal tract was evaluated using the TIM-1 system, revealing a survival rate of 16.05% after passage through the gastric, duodenal, jejunal, and ileal compartments. These findings highlight the possibility of *B. velezensis* FZB42 being an economically viable and possibly functional synbiotic supplement and effective alternative to antibiotic growth promoters in livestock production.

**Keywords:** fermentation; *Bacillus velezensis* FZB42; probiotic; maple syrup; TIM-1; in vitro digestion



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## 1. Introduction

In the field of animal nutrition, antibiotics have been traditionally employed to enhance growth rates and prevent diarrheal diseases, a practice supported by studies such as those by Cromwell [1] and Marshall and Levy [2]. However, the rising tide of antibiotic-resistant bacteria presents a formidable challenge, emerging as a critical global threat, as underscored by the World Health Organization [3]. In this context, probiotics, defined as "live microorganisms that, when administered in adequate amounts, confer health benefits to the host" [4], emerge as a promising and increasingly favored alternative. This shift towards probiotics, seen as a viable substitute for antimicrobial growth promoters, reflects a growing consensus in the scientific community about their potential to support animal health and productivity safely and effectively [5,6].

The burgeoning interest in probiotics has naturally led to an emphasis on their cost-effective industrial production, especially as functional foods enriched with these beneficial bacteria gain market traction. This has underscored the importance of innovating efficient media and bioprocessing techniques for probiotic cultivation [7]. In this context, researchers have explored various low-cost by-products from diverse industries as substrates for probiotic production, aiming to reduce costs and enhance sustainability. For instance, molasses from the sugarcane industry has been utilized for the mass production of *Lactobacillus*

*paracasei* [8]. Similarly, in the dairy industry, the by-product of ricotta cheese production, known as "scotta", has been used to cultivate high content of several live lactic acid bacteria species [9]. In a related line of research, dietary fiber extracted from peach pomace has been found to enhance the effectiveness of ingested probiotics as livestock growth enhancers [10], which is an example of what is known as a synbiotic effect, the synergic combination of a probiotic with a prebiotic, resulting largely from the increased viability of the probiotic cells.

Particularly, downgraded maple syrup has attracted attention due to its compositional similarity to regular maple syrup. The end of the season represents the period when the health benefits of the syrup are enhanced, but it is also the time when most flavor defects are encountered [11,12]. The careful analysis of maple products has revealed three factors that may have a positive impact on colonic microbiota in livestock. In addition to containing large amounts of sucrose, glucose, and fructose, which are all readily utilizable as carbon sources by probiotic strains, maple sap contains progressively more organic nitrogen as the season nears its end, primarily ureides, namely allantoic acid and allantoin [13]. These molecules have not yet been the focus of probiotic or prebiotic studies but appear to be utilizable as sole nitrogen sources by strains of bifidobacteria and appear to have a selective effect [13]. Subsequently, at least two prebiotic polysaccharides have been identified in syrup, specifically arabinogalactan and inulin [14,15]. Arabinogalactan is widely used to stimulate the growth of colonic microbiota. Furthermore, it is known that arabinogalactans can have several physiological effects in humans [16]. They have been recognized as constituents of foods with beneficial health effects [17]. Pectic polysaccharides, including arabinogalactans, can be effective in acting on the immune system, the coagulation system, and the digestive system [18]. Finally, maple products contain other compounds that can positively influence the colonic microbiota and thus growth, including organic acids, vitamins, mineral salts, flavonoids, and phenolic compounds [19,20]. All these compounds suggest strong potential for the use of downgraded maple syrup as a favorable and economical fermentation substrate for probiotic production [21].

Ensuring the survival of probiotics through the manufacturing process, shelf life, and gastrointestinal transit is critical for their effectiveness as nutritional supplements [22]. Commercially, probiotics are often formulated as powders for easy incorporation into foods and feeds, utilizing drying technologies like freeze drying, spray drying, vacuum drying, and fluid bed drying for production, with spray drying being favored due to its energy efficiency [23,24]. However, challenges persist in maintaining probiotic viability in products, necessitating protective measures against environmental, processing, and gastrointestinal stresses [25]. Whey permeate, for example, has been identified as a cost-effective and protective additive for probiotic formulations, particularly beneficial for piglet nutrition [26].

The selection of robust probiotic strains also plays a pivotal role in enhancing survival rates. *Bacillus* spp., known for their endospore-forming capabilities, offer exceptional stability and viability, making them suitable candidates for animal feed supplements [27].

Among the group of *Bacillus subtilis* species, phylogenetic and systematic updates frequently arise due to new analytical methods. *Bacillus velezensis*, originally isolated from the Vélez River in 2005, was initially considered a later heterotypic synonym of *Bacillus amyloliquefaciens* [28,29]. However, this designation was revised in 2016 based on DNA–DNA hybridization studies, distinguishing *B. velezensis* as a distinct species that is conspecific with *Bacillus amyloliquefaciens* subsp. *plantarum*, *Bacillus methylotrophicus*, and *Bacillus oryzicola* [30,31].

The strain *Bacillus velezensis* FZB42, formerly known as *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42, is known for its probiotic properties and potential in plant protection. Its genome is rich in biosynthesis clusters for antimicrobial compounds, including a variety of secondary metabolites such as polyketides (bacillaene, macrolactin, and difficidin) and lipopeptides (surfactin, bacillomycin D, and fengycin) [32–35]. Furthermore, its genome contains genes that encode enzymes such as amylase, xylanase, and phytase, which are valuable for the animal nutrition industry [36]. In fact, studies involving *Bacillus velezensis*

have shown positive outcomes in sows and piglets, as well as in fish, highlighting the broad applicability and potential of this species to improve animal health and productivity [37,38].

Given the challenges associated with in vivo probiotic survival studies, the development and application of sequential in vitro models, such as the TIM-1 system from TNO, offer a comprehensive approach to simulate and evaluate probiotic viability under gastrointestinal conditions [39,40].

The general objective of this study was to develop and characterize a novel synbiotic supplement designed to serve as a nutritional enhancer in animal feed. Leveraging its unique composition, downgraded maple syrup was used as the foundational component for this synbiotic formulation. *Bacillus velezensis* FZB42 was selected due to its probiotic properties, underpinning the development of this innovative supplement. This supplement could improve the intestinal microbiota in animals and enhance the immune response, thereby offering gains in the overall health and productivity of livestock and reducing the carriage of pathogenic and spoilage microorganisms in carcasses.

## 2. Materials and Methods

### *2.1. Strain and Preliminary Assays*

The *Bacillus velezensis* FZB42 strain was purchased from DSMZ (German Collection of Microorganisms and Cell Cultures). *B. velezensis* was long-term preserved at −80 °C in glycerol (40%) and activated by inoculation (1% *v*/*v* inoculum size) in Luria–Bertani Broth (LBB) (Fischer Scientific, Montréal, QC, Canada) under agitation at 37 °C for 24 h.

The fermentation media for preliminary tests were composed of Nutrient Broth (NB) (Fischer Scientific, Montréal, QC, Canada) (8 g/L) and downgraded maple syrup (5% equivalent). The fermentations were performed at 37 °C under stirring at 200 rpm.

The buddy maple syrup (BMS) and ropy maple syrup (RMS) used in this study were kindly provided by PPAQ (Producteurs et Productrices Acéricoles du Québec, Québec, QC, Canada) and stored at 4 °C before processing. The main sugars present in buddy maple syrup were sucrose (43.46% *w*/*w*), glucose (17.69% *w*/*w*), and fructose (11.33% *w*/*w*), and for ropy maple syrup, they were sucrose (53.35% *w*/*w*), glucose (10.00% *w*/*w*), and fructose (8.30% *w*/*w*), and the nitrogen source was yeast extract (Fischer Scientific, Montréal, QC, Canada).

Whey permeate was donated by a local industry (Québec, QC, Canada). It was obtained by drying deproteinated sweet whey and contained a maximum of 89% (*w*/*w*) lactose, 9% (*w*/*w*) ashes, and a minimum of 2% (*w*/*w*) proteins, as declared by the manufacturer.

### *2.2. Culture Conditions for Media Optimization*

The inoculum was prepared using LBB medium. *Bacillus velezensis* was incubated on a shaker in the laboratory at 150 rpm and 37 °C for 16 h. The volume of the inoculum constituted 5% (*v*/*v*) of the total volume of the cultivation medium, which was 50 mL. Subsequent to inoculation, the culture was incubated in Erlenmeyer flasks on a laboratory shaker set to 150 rpm and 37 °C for a duration of 96 h. All media were sterilized by autoclaving prior to inoculation to guarantee the purity of the culture. Due to the low sugar concentration, it was determined that the thermal treatment had no or a negligible effect on the sugar profile. Biomass was estimated by measuring the optical density at 600 nm.

Statistical analysis was performed using JMP Pro 16 software (SAS Institute, Cary, NC, USA). A custom design of the experiment platform was used to generate a central composite design (CCD) comprising two factors and three levels (Table 1). The fractional factorial design consisted of 4 factorial points, 4 face-centered axial points, and 4 central points. The following variables and levels were tested: buddy maple syrup (0.5, 12.75 and 25 g/L) and yeast extract (5, 10 and 15 g/L). Each condition was performed in triplicate. A standard least-squares model was fitted to the data, and the profiler was used to identify values that maximize biomass.

**Table 1.** Central composite design matrix for optimization of different variables to maximize biomass yield of *Bacillus velezensis* FZB42.

| Standard Order | Downgraded Maple Syrup (g/L) | Yeast Extract (g/L) |
|---|---|---|
| 1 | 12.75 | 15 |
| 2 | 12.75 | 10 |
| 3 | 25 | 15 |
| 4 | 12.75 | 10 |
| 5 | 0.5 | 15 |
| 6 | 12.75 | 5 |
| 7 | 0.5 | 10 |
| 8 | 0.5 | 5 |
| 9 | 25 | 5 |
| 10 | 25 | 10 |
| 11 | 12.75 | 10 |
| 12 | 12.75 | 10 |

### *2.3. B. velezensis Viability*

Viable counts were determined using the drop-plate method described by Herigstad, Hamilton, and Heersink [41]. Samples were serially diluted (1/10 from $1 \times 10^{-1}$ to $1 \times 10^{-7}$) in sterile 0.1% (*w*/*v*) peptone water (Difco Laboratories, Detroit, MI, USA), and 20 µL of each dilution was plated in duplicate on LB agar (LB broth with 1.5% agar) and incubated at 37 °C for 18 h. To determine the colony-forming units per gram (CFU/g), we selected the plates that displayed counts ranging from 300 to 30 CFU.

### *2.4. Scaled-Up Validation Experiment*

Scaled-up batch production in the optimized medium (3.87 g/L of downgraded maple syrup and 15 g/L of yeast extract) was carried out in a 30 L bioreactor (Biogenie, Quebec, QC, Canada) with a 20 L working volume. The bioreactor was equipped with accessories and connected to a computer equipped with the iFix 3.5, Intellution software (Intellution, Inc., Norwood, MA, USA) for the control of pH, temperature, air flow, agitation, and antifoam. The temperature was set at 37 °C, dissolved oxygen at 75% of saturation, and pH at 7.

### *2.5. Spray Drying*

Before spray drying, the *Bacillus velezensis* FZB42 culture was mixed with whey permeate in a 1:1 ratio and then stirred for 15 min, during which, the protective agent dissolved completely. Subsequently, the cultures were processed using a laboratory-scale spray dryer (Büchi B-290, Flawil, Switzerland), which featured a control panel; an electric heater; a peristaltic pump; dual fluid nozzles; a drying chamber; and a cyclone constructed from thick, transparent glass.

The air, warmed by the electric heater, was directed in parallel flow to the atomized liquid within the drying chamber at a rate of 38 $m^3$/h. A dual-nozzle peristaltic pump, with an internal diameter of the nozzle of 0.7 mm, propelled by compressed air at 8 bar and regulated by a flow meter, supplied the drying chamber. The resulting dried powder was gathered in a container attached to the base of the cyclone separator.

The dried formulate was transferred to hermetic plastic bags and stored at 4 °C. Three replicates were performed. Viable counts of *B. velezensis* were determined using the drop-plate method and expressed as log10 colony-forming units (CFU) per gram of powder.

Percentage yield was calculated with Equation (1).

$$\text{Yield (\%)} = (\text{Wd}/\text{Wl}) \times 100 \quad (1)$$

where Wd and Wl represent the weight after and before atomization expressed as g, respectively.

Powder moisture was determined in an air-circulation oven at 105 °C according to AOAC method no. 926.12 [42]. The values were expressed as g of water per 100 g of powder (*w*/*w*) (Equation (2)).

$$\text{Moisture content (\%)} = ((\text{Wi} - \text{Wf})/\text{Wi}) \times 100 \quad (2)$$

where Wf and Wi represent the weight after and before drying, expressed as g of water per 100 g of powder (*w*/*w*).

### 2.6. *B. velezensis* Survival during In Vitro Digestion of the Spray-Dried Product

The TIM-1 model (The TIM Company, Zeist, Netherlands) was used to assess the survival of the potential probiotic under porcine gastrointestinal pH and enzymatic conditions. The protocol was described by Minekus et al. [43] and was adapted to porcine conditions [44] as described in our previous publication [21] with some modifications. Briefly, the meal consisted of 149 g of dry synbiotic powder and 149 g of deionized water. Aliquots (0.5 mL) were removed from the gastric compartment at 0, 30, 60, 90, 120, and 180 min, 30, 60, 90, 120, and 180 min from the duodenal compartment, and 60, 180, and 240 min from the jejunal and ileal compartments. All samples were adjusted with compartment-specific solutions to ensure that they did not affect the digestion rate. Effluents were collected, weighed, and aliquoted at 60, 120, 180, 240, and 300 min. After digestion was complete, the chyme consisted of the remaining mixture within the duodenal, jejunal, and ileal sections. Digestion experiments were performed in duplicate. Viable counts of *B. velezensis* were determined using the drop-plate method. *B. velezensis* survival was expressed as decimal logarithm (log10) colony-forming units (CFU).

## 3. Results and Discussion

### 3.1. Preliminary Assays

The aim of these assays was to determine if *B. velezensis* FZB42 could grow in culture mediums supplemented with downgraded maple syrup. The bacterial growth was evident, as illustrated in Figure 1, despite the depletion of sucrose in the culture medium. The continued growth could be attributed to the consumption of other carbon sources, including the degradation products of sucrose, namely, glucose and fructose. Based on these results, we selected buddy maple syrup for subsequent experiments due to its greater availability.

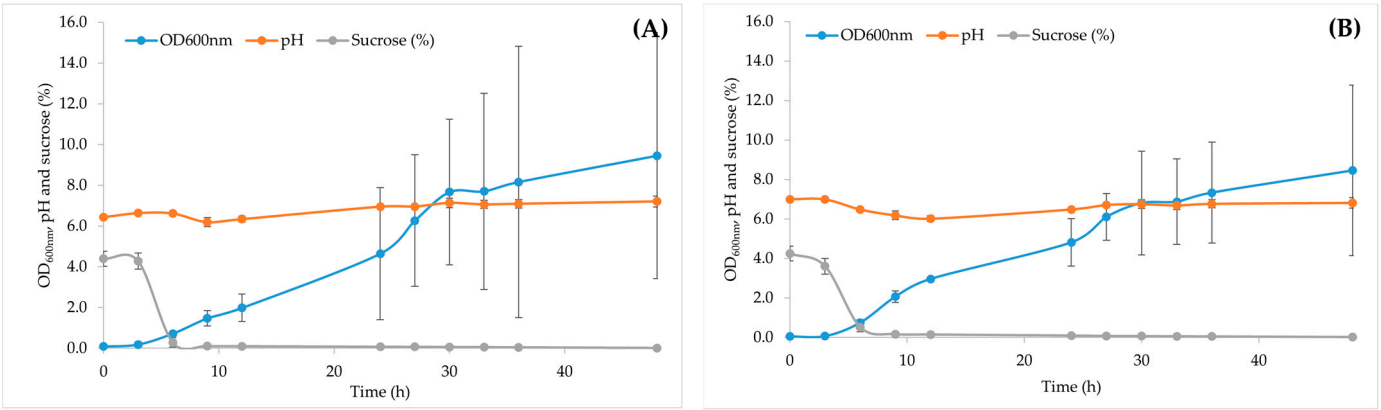


**Figure 1.** Changes in optical density, pH, and sucrose concentration in fermentation with ropy syrup (**A**) and with buddy syrup (**B**). Values are expressed as the mean ± standard deviation of replicate experiments ($n$ = 2).

### 3.2. Optimization of Nutrient Medium by Central Composite Design (CCD)

A central composite design was used to optimize biomass production by *B. velezensis* FZB42, and a standard least-squares model ($R^2$ = 0.86, $p$-value < 0.0001) revealed that quadratic effects were significant for both buddy maple syrup and yeast extract (Table 2).

**Table 2.** Effects tests of buddy maple syrup (BMS) and yeast extract (YE) on *B. velezensis* FZB42 growth.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| BMS (0.5, 25) | 1 | 1 | 0.02343750 | 10.4313 | 0.0179 |
| YE (5, 15) | 1 | 1 | 0.24200417 | 107.7090 | <0.0001 |
| BMS × BMS | 1 | 1 | 0.01269600 | 5.6506 | 0.0550 |
| BMS × YE | 1 | 1 | 0.00562500 | 2.5035 | 0.1647 |
| YE × YE | 1 | 1 | 0.01926667 | 8.5750 | 0.0263 |

The optimal concentrations to maximize biomass were 3.87 g/L of buddy maple syrup and 15 g/L of yeast extract (Figure 2).

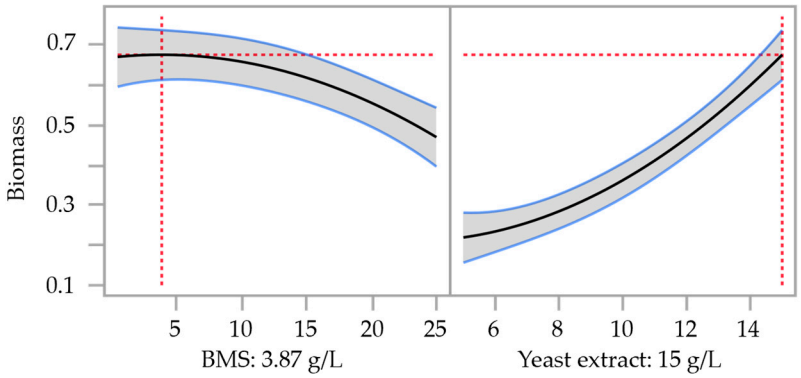


**Figure 2.** Optimization of the concentration of buddy maple syrup (BMS) and yeast extract for fermentation of *B. velezensis* FZB42.

The utilization of buddy syrup as a carbon source has proven to be an effective and cost-effective approach, showing potential as a feedstock for fermentation. Another study has demonstrated the feasibility of using buddy and ropy maple syrups as potential substrates for fermentation with *Saccharomyces cerevisiae* to produce ethanol [45]. Moreover, maple syrup has been used as a carbon source in the production of a nanobiomaterial, bacterial cellulose, by *Acetobacter xylinum* BPR 2001 [46]. These findings suggest that similar outcomes could be expected using downgraded maple syrup.

To enhance the cost-effectiveness of our media, the first method would be to substitute the nitrogen source, yeast extract, with an agricultural byproduct, such as corn steep liquor, brewer's spent grain, or wheat bran, for example, in a medium that uses cane molasses as a carbon source, where expensive nitrogen sources such as beef or yeast extract have been successfully replaced with steep corn liquor for the biomass production of the probiotic strain *Lactobacillus plantarum* [47].

### *3.3. Large-Scale Production of Fermented Downgraded Maple Syrup*

A large increase in viable count was observed when fermentations were scaled up from Erlenmeyer flasks to the pilot lab fermenter (Table 3). These results reflect expectations because the bioreactor, with its controlled conditions (pH control, mixing, and dissolved oxygen control), offered better media than Erlenmeyer flasks. As bacterial growth became active, there was a decrease in oxygen levels, which were automatically regulated within the bioreactor.

Moreover, the increase in viable count could be attributed to the bioreactor's axial rotating paddles, which offered enhanced mixing and oxygenation efficiency. The next research step to enhance the final viable count will be to study aeration, as it is acknowledged as a crucial factor that enhances the adaptation of microbial cells [48]. Furthermore, using a fed-batch strategy may be beneficial because of its well-known advantages such as higher productivity due to the continuous addition of nutrients and the substitution of

metabolic waste and an extended fermentation time [49]. No major technical obstacles to the large-scale production of *B. velezensis* FZB42 for industrial applications have appeared at this stage.

**Table 3.** Viable cell counts of *B. velezensis* FZB42 during production steps.

| Step | CFU/mL or g |
|---|---|
| Erlenmeyer fermentation | $7.75 \pm 0.35 \times 10^8$ |
| Bioreactor fermentation | $6.15 \pm 0.46 \times 10^9$ |
| Spray drying | $2.00 \pm 0.42 \times 10^8$ |
| TIM-1 [a] | $2.19 \pm 1.09 \times 10^9$ |

[a] Expressed as cells reaching the colon. Mean ± SD.

### *3.4. Spray Drying*

The yield of dry product obtained was 31.4 ± 1.06%. This yield is similar to other yields achieved in laboratory-scale atomization, with higher losses due to dust materials than in industrial ones [50]. The moisture content of the powder was 4.38 ± 0.55% humidity, which is below 5%, the upper limit for good product stability [51]. The final viable count for our product was $2.00 \pm 0.42 \times 10^8$ CFU/g. As reviewed by Luise [52], most probiotic products contain at least $1 \times 10^9$ CFU/g. Therefore, the optimization of our spray-drying conditions is necessary to enhance the viable count of our powder. Additionally, our choice of protective agent, whey permeate, may have inherent benefits. It has been reported to possess high nutritional value due to its elevated lactose concentration [53]. Furthermore, studies have demonstrated that whey permeate can positively impact growth performance in piglets weighing from 7 to 11 kg [26], although this effect gradually diminishes as pigs grow [54].

### *3.5. Survival Evaluation of the Dry Product with TIM-1*

The viable count of *B. velezensis* FZB42 during TIM-1 digestion is shown in Figure 3. The initial count in the gastric compartment was $9.00 \pm 0.70 \times 10^7$ CFU/mL. After 30 min in the gastric compartment, the population was affected by the pH drop and decreased to $1.68 \pm 0.10 \times 10^6$ CFU/mL, then continued to decrease slightly until the end of the gastric phase (180 min) and attained a population of $4.18 \pm 0.67 \times 10^5$ CFU/mL. In the duodenal compartment, the cell count after 30 min of digestion was $1.33 \pm 0.03 \times 10^5$ CFU/mL and then reached $8.25 \pm 1.77 \times 10^5$ CFU/mL after 60 min of digestion and remained stable until the end of the duodenal phase (180 min). Cell counts in the jejunal and ileal compartments were $2.55 \pm 0.14 \times 10^7$ CFU/mL and $3.28 \pm 0.17 \times 10^7$ CFU/mL and decreased after 240 min of digestion to reach $4.55 \pm 0.70 \times 10^6$ CFU/mL and $5.75 \pm 0.10 \times 10^6$ CFU/mL, respectively. The cumulative count of the entire effluent, representing the fraction that typically reaches the colon, cumulated to $2.19 \pm 1.09 \times 10^9$ CFU/mL. Since health benefits are realized when about $1 \times 10^6$ metabolically active probiotics are delivered to the colon [55], our results confirm that the product has the potential for probiotic activity. Also, based on the initial cell count, *B. velezensis* FZB42 showed a survival rate of 16.05 ± 6.83%.

Two commercial products were investigated with regard to the survival of *Bacillus* spp. in vitro, with the TIM-1 model simulating human conditions. *Bacillus coagulans* GBI-30, 6086, marketed as BC30, was administered as spores at a concentration of $2 \times 10^9$ CFU. When ingested with water, it exhibited a survival rate of 70%. In contrast, survival rates of 56% and 59% were observed when it was ingested with a 5% (*w*/*v*) lactose or fructose solution, respectively [56]. The authors concluded that this may suggest increased spore germination in the presence of these sugars, and since germinated cells are more sensitive to gastrointestinal conditions, their survival rate was lower. In 2019, the same strain was tested after being mixed with organic inulin (a prebiotic) before freeze drying, resulting in a powder with a spore concentration of $15 \times 10^9$ spores per gram. A complete breakfast meal was created, and 100 g of this meal was taken. Subsequently, $1 \times 10^9$ spores were added before introducing this meal into TIM-1. The survival rate was found to be 51%. The

effluents from TIM-1 were then introduced into TIM-2 (a colon model), where 93% of the cells were observed to have germinated after 24 h [57].

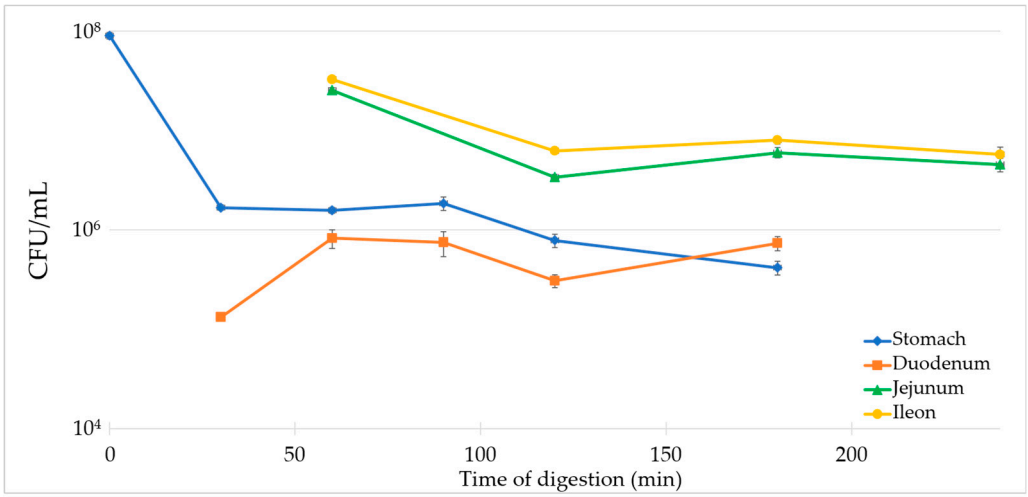


**Figure 3.** Viable cell counts of *B. velezensis* FZB42 during TIM-1 digestion. Stomach (diamond), duodenum (square), jejunum (triangle), and ileum (circle). All the values are the means of two independent experiments.

The other strain tested was *Bacillus velezensis* DSM 15544, previously known as *Bacillus subtilis* C-3102 and marketed as Calsporin®. It demonstrated a survival rate of approximately 74% during digestion [58]. The somewhat lower survival obtained in the present study may be due to differences in processing methods. Spray drying is harsher than freeze drying, and whey permeate, though an economical choice, may be less protective than organic inulin.

Various strategies can be used to enhance probiotic survival within the gastrointestinal tract, including tablet coating, microencapsulation, or the optimization of synbiotic effects [59]. However, it is imperative to bear in mind that these approaches must be both feasible on a large scale and economically viable for the livestock industry. Additionally, it is crucial to perform the validation of the final synbiotic product to ensure the viability of probiotic strains in the GIT in vivo, going beyond in vitro evaluations. This step is essential to confirm the effectiveness of the synbiotic formulation under real physiological conditions. Given the unique genome of this strain, which is rich in genes for the biosynthesis of antimicrobial compounds and digestibility-enhancing enzymes, we hypothesize an improvement in zootechnical performance and a reduction in pathogen infections.

## 4. Conclusions

This study of the use of downgraded maple syrup to produce a synbiotic containing *Bacillus velezensis* FZB42 for animal nutrition demonstrates that this strain utilizes this substrate efficiently as its main carbon source. Optimization using a central composite design suggested a cost-effective growth medium formulation for large-scale production, which gave promising results in a bioreactor. Spray drying with whey permeate as a protective agent yielded a stable powder that could have a synbiotic property. In a simulated gastrointestinal tract (TIM-1), *B. velezensis* FZB42 remained substantially viable. Overall, this study provides a proof of concept for *B. velezensis* FZB42 as an economically viable and commercially acceptable probiotic supplement for animal feed, offering a promising alternative to antibiotic growth promoters. These encouraging results warrant further development of the proposed product.

**Author Contributions:** Conceptualization, G.D., D.G. and I.F.; methodology, G.D., M.T., D.G., M.F. and I.F.; software, G.D.; validation, G.D., M.T., D.G., M.F. and I.F.; formal analysis, G.D. and M.T.;

investigation, G.D. and M.T.; resources, G.D., M.T., D.G., M.F. and I.F.; data curation, G.D.; writing—original draft preparation, G.D.; writing—review and editing, G.D., D.G., M.F. and I.F.; visualization, G.D.; supervision, D.G., M.F. and I.F.; project administration, I.F.; funding acquisition, I.F. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The data used to support the findings of this study can be made available by the corresponding author upon request.

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