J Biochem Technol (2026) 17(3): 43-47
https://doi.org/10.51847/pKAAzWnx2r
ISSN: 0974-2328

Resistance of Florentero Complex® (Candioli Srl) to Simulated Gastrointestinal Digestion

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Received: 18 May 2026 / Received in revised form: 28 June 2026, Accepted: 03 July 2026, Published online: 15 July 2026

Abstract

Gastrointestinal disorders and dysbiosis are frequent in veterinary practice. The maintenance of a stable intestinal microbiota is fundamental, requiring effective interventions to restore homeostasis. The product evaluated is a complementary feed formulated with a synergistic combination of a Multi-strain Probiotic System (*Saccharomyces cerevisiae* var. *boulardii* (DSM 34246), *Lactobacillus acidophilus* (D2/CSL), and *Enterococcus faecium* (DSM10663)), Vexantra® (*Malus domestica* and zinc), and a Prebiotic Matrix (fructooligosaccharides (FOS) and mannanoligosaccharides (MOS)). While *S. boulardii* is naturally antibiotic-resistant and reinforces the intestinal barrier, *L. acidophilus* contributes to microbial balance through organic acid production. Additionally, Vexantra® and prebiotics support mucosal integrity and beneficial bacterial growth. The clinical efficacy of oral probiotics depends on the "viability-activity" axis, requiring survival through the harsh gastric environment. This study aimed to evaluate the gastrointestinal resistance of this formulation using a standardized *in vitro* model simulating fed-state conditions (gastric phase at pH 4.8; intestinal phase at pH 7.5). The results demonstrated that the product matrix not only protected the microorganisms during simulated digestion but also promoted a significant numerical increase in their microbial load. All analyzed probiotic strains remained viable and showed higher counts in the digested product compared to the undigested one. These findings suggest that the formulation provides a protective environment that ensures the delivery of a functional microbial load to the intestine.

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Keywords: Probiotics, Digestion, Microbial viability, Vitro study

Introduction

In veterinary medicine, the maintenance of a stable and diverse intestinal microbiota is recognized as a fundamental pillar of overall health in companion animals. Gastrointestinal (GI) disorders, characterized by dysbiosis, are among the most frequent reasons for veterinary consultation in dogs and cats (Suchodolski, 2011). Florentero Complex® (Candioli srl) is a complementary feed formulated to address these imbalances through a synergistic combination of multi-strain probiotics, prebiotic fibers, and mucosal-protective nutraceuticals. This formulation integrates: (i) a Multi-strain Probiotic System (*Saccharomyces cerevisiae* var. *boulardii* (DSM 34246), *Lactobacillus acidophilus* (D2/CSL), and *Enterococcus faecium* (DSM10663)); (ii) Vexantra®, a specialized complex of *Malus domestica* and zinc; and (iii) a Prebiotic Matrix consisting of fructooligosaccharides (FOS) and mannanoligosaccharides (MOS). *Saccharomyces cerevisiae* var. *boulardii*, a probiotic yeast known by the proprietary name Canobios®, is naturally resistant to antibiotics. It has demonstrated significant efficacy in neutralizing enterotoxins, modulating mucosal immune responses, and reinforcing the integrity of the intestinal barrier, thereby contributing to the restoration of intestinal microbial balance (Kelesidis & Pothoulakis, 2012; Meineri *et al*., 2022; Bruni *et al*., 2023). The probiotic complex also includes *L. acidophilus*, a lactic acid–producing bacterium commonly used in both human and veterinary nutrition (EFSA, 2014; Marelli *et al*., 2020). This microorganism contributes to intestinal microbial balance through the production of organic acids and antimicrobial metabolites that lower luminal pH and inhibit the proliferation of potentially pathogenic bacteria. In addition, *L. acidophilus* supports digestive efficiency and may contribute to the modulation of intestinal inflammatory responses (Pascher *et al*., 2008). Another relevant functional component of the formulation is Vexantra®, a nutraceutical complex derived from *Malus domestica* (apple) and associated with zinc. This ingredient is specifically designed to support intestinal mucosal physiology and maintain epithelial barrier integrity. Zinc, in particular, is well-documented for its crucial role in stabilizing tight junction proteins and preserving the epithelial barrier during inflammatory insults (Skrovanek *et al*., 2014). Furthermore, apple-derived polyphenols provide antioxidant support, which synergistically contributes to mucosal

protection (Denis *et al*., 2013). Finally, the Prebiotic Matrix includes FOS and MOS, which provide selective fermentable substrates. These compounds are known to promote the growth of beneficial lactic acid bacteria while reducing the colonization of potentially pathogenic species (Grześkowiak *et al*., 2015). The clinical efficacy of any oral probiotic supplement is strictly dependent on the "viability-activity" axis. To exert a biological effect, microorganisms must survive the transit through the upper GI tract, resisting the bactericidal effects of gastric acid and the detergent action of bile salts (Bezkorovainy, 2001). While many studies focus on the concentration of probiotics at the time of manufacture, few evaluate their resilience within the specific delivery matrix (tablet or paste) (Govender *et al*., 2014). The objective of this study was to evaluate the gastrointestinal resistance of Florentero Complex® using a standardized *in vitro* digestion model. By simulating the fed-state conditions (pH 4.8 with pepsin followed by an intestinal phase at pH 7.5), we aimed to verify whether the product's matrix protects the viability of *S. boulardii*, *E. faecium*, and *L. acidophilus*. Proving this resistance is a fundamental prerequisite for ensuring that a functional microbial load reaches the lower intestine, providing the scientific basis for subsequent *in vivo* clinical trials.

Materials and Methods

Product Preparation and Digestion Simulation

The product under study is Florentero Complex® in tablet form. It was stored in a cool and dry place away from heat and light sources. The ingredient composition of the product is detailed below in **(Table 1)**.

Table 1. Ingredients (in %) of Florentero Complex®, Candioli srl.

Ingredients	%
Fructooligosaccharides (Profeed®P95)	39.0000
Microcrystalline cellulose 2	28.4800
Mannanoligosaccharides	5.5600
Yeast Product (Epicorpets)	5.3680
***Saccharomyces cerevisiae* var. *boulardii* (DSM 34246)**	4.5000
Prosolv SMCC 90	4.0000
***Lactobacillus acidophilus* D2/CSL**	2.8000
Optimizor Uranus	2.0000
BacNutra	2.0000
Vexantra	1.5300
Compritol E ato	1.0000
***Enterococcus faecium* DSM10663/NCIMB 10415 (35 mld/g)**	0.8000
Magnesium Stearate	0.6700
Colloidal silica E551b (for tablets)	0.6673
Dicalcium phosphate dihydrate	0.3900
Vitamin B1 hydrochloride	0.2800
Magnesium Sulfate heptahydrate	0.2777
Sodium chloride	0.1670
Potassium Sulfate	0.1670
Vitamin B6 hydrochloride	0.1070
Soy Lecithin	0.1000
Vitamin B2 80%	0.0830
Vitamin B12	0.0420
Corn starch	0.0100
Vitamin E acetate 50% coated	0.0010
Total	**100.0000**

The product was ground under sterile conditions, resuspended in sterile physiological solution (H2O + 0.9% NaCl), kept at room temperature for half an hour, diluted, and plated on selective media to assess the baseline load. To evaluate gastrointestinal resilience, the formulation was subjected to simulated digestion under fed-state conditions. The gastric phase was simulated by incubating the product at 37°C with agitation for 2 h in Simulated Gastric Fluid (SGF). The SGF consisted of a buffer adjusted to pH 4.8 (to replicate postprandial conditions) containing 0.03 M NaCl, 5% BSA, 6% oil and fats, and pepsin. Subsequently, the intestinal phase was simulated by adding pancreatin and 0.05 M KH2PO4, adjusting the pH to 7.5. The sample was then incubated at 37°C with agitation for an additional 2 h. At the end of the entire digestive process, the samples were diluted and plated on selective media.

Media Preparation and Enumeration

The digested and undigested Florentero Complex® samples were diluted, plated on selective media for the specific microorganisms of interest, and incubated under dedicated growth conditions **(Table 2)**. The probiotic strains included in the formulation were *Saccharomyces cerevisiae*var. *boulardii* DSM 34246, *Enterococcus faecium* DSM 10663/NCIMB 10415, and *Lactobacillus acidophilus* D2/CSL (CECT 4529), obtained from their respective official culture collection repositories.

Table 2. Selective media and growth conditions used for the isolation of each microorganism.

Microorganisms	Selective media	Growth condition
S. cerevisiae* var. *boulardii	SDA + chloramphenicol	37°C
E. faecium	SBA	37°C
L. acidophilus	MRSA	37°C

For the selective quantification of the microorganisms, sample dilutions were processed as follows:

- **For *S. cerevisiae* var. *boulardii* (DSM 34246):** Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol was used. This medium features an acidic pH and high dextrose levels that promote yeast/mold growth while inhibiting most bacteria; chloramphenicol further prevents bacterial contaminants.

- **For *E. faecium* DSM10663/NCIMB 10415:** Slanetz & Bartley Agar (SBA), a highly selective medium for enterococci, was utilized.

- **For *L. acidophilus* D2/CSL:** MRS Agar (MRSA), an enriched and selective medium for lactobacilli, was employed.

All culture plates were evaluated and counted after 24–48 hours of incubation.

Statistical Analysis

Plate counts were converted to CFU/kg and log10-transformed prior to analysis. Results are expressed as mean ± standard deviation of three independent digestion runs. Digested and undigested samples were compared by paired Student's t-test; normality of the differences was assumed. Statistical significance was set at $p < 0.05$. Analyses were performed with: R version 4.6.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

Results and Discussion

The gastrointestinal resistance of the three target microorganisms was examined by comparing the microbial counts of the product subjected to simulated digestion under fed-state conditions with the undigested product baseline. The results demonstrate that Florentero Complex®, due to its distinctive formulation and the presence of functional prebiotic ingredients, not only protects the microorganisms during simulated gastrointestinal transit but also promotes a significant numerical increase under fed-state conditions. Specifically, the simulated digestive phase triggered an increase in the microbial load, indicating that the product matrix and its specific components support the viability and growth capacity of probiotic microorganisms during transit. The microbial counts for both the undigested and digested states are detailed in **(Table 3)**. Following simulated gastrointestinal digestion, all probiotic microorganisms remained fully viable. Moreover, for all analyzed strains, the final microbial count was higher than in the undigested product, highlighting a favorable shielding and nurturing effect of the product matrix and its ingredients.

Table 3. Microbial counts of the undigested and digested product.

Microorganisms	Undigested product (CFU/kg)	Digested product (CFU/kg)	Log_{10} Differential
S. cerevisiae* var. *boulardii	2.20×1011	4.68×1011	+0.33
E. faecium	8.26×1010	3.36×1013	+2.61
L. acidophilus	2.51×1011	3.94×1013	+2.20

Values are expressed as CFU per kg of product.

The distinct numerical increase in microbial load observed during simulated digestion can be attributed to the synergistic interaction between the multi-strain probiotic system and the protective matrix of Florentero Complex®. Specifically, the combination of *L. acidophilus*, which promotes microbial balance through the production of organic acids, and *S. boulardii*, which is naturally resistant to antibiotics and reinforces the intestinal barrier, creates an environment conducive to eubiosis (Bruni *et al*., 2023). This effect is further enhanced by the Prebiotic Matrix (FOS and MOS), which provides selective fermentable substrates that promote the growth of beneficial bacteria while inhibiting potential pathogens (Grześkowiak *et al*., 2015). Furthermore, Vexantra® provides a dual-action support system: the zinc component is essential for stabilizing tight junction proteins and maintaining epithelial barrier integrity (Skrovanek *et al*., 2014), while the *Malus domestica* polyphenols offer crucial antioxidant support during inflammatory stress (Denis *et al*., 2013). Beyond these functional ingredients, the survival of probiotic microorganisms during gastric transit is fundamentally supported by the overall formulation structure. This architecture includes lipid components, such as mono- and diglycerides of fatty acids and vegetable oils (e.g., Compritol® and soy lecithin), alongside a carbohydrate

matrix including starches **(Table 1)**. Together, these elements create a protective microenvironment that helps reduce direct exposure of the microorganisms to harsh gastric acidity (Charteris *et al.*, 1998). Additionally, the presence of yeast cell wall components (from *S. boulardii* and yeast products) may contribute to a physical shielding effect. This structural protection, combined with a high initial probiotic count, ensures that an adequate number of viable microorganisms reach the intestine to exert their biological effect (Corcoran *et al*., 2005). From a technological standpoint, the inclusion of lecithin and an optimized structural matrix (incorporating Prosolv® and Microcrystalline cellulose) improves the organization and stability of the formulation. These excipients reinforce the protective effect on probiotic viability during gastrointestinal transit. Consequently, the product's gastro-resistance is achieved through a synergistic combination of formulation design and an optimized dosage strategy, transforming the gastrointestinal transit from a phase of potential loss into an active period of microbial recovery and proliferation. These results are entirely consistent with the hypothesis that the matrix of Florentero Complex® and its functional ingredients contribute to maintaining probiotic viability and promoting their recovery. From an application perspective, this supports the rationale that the product can effectively deliver live and cultivable microorganisms to the intestine even under fed-state conditions. Although these in vitro findings provide valuable preliminary evidence regarding the structural protection and survival of the probiotic strains during simulated transit, this model cannot fully replicate the complex physiological dynamics of the live gastrointestinal tract. Therefore, further prospective in vivo clinical studies in target companion animals (dogs and cats) are required to validate these results, confirming microbial survival, intestinal mucosal colonization, and functional health benefits under actual physiological and pathological conditions.

Conclusion

This study evaluated the behavior of the product Florentero Complex® during simulated gastrointestinal digestion under fed-state conditions, with particular attention to the viability of the three probiotic microorganisms contained in the formulation. The results demonstrate that Florentero Complex® not only preserves the viability of *S. cerevisiae* var. *boulardii*, *E. faecium*, and *L. acidophilus*, but also promotes an increase in their microbial load during the digestive phase. This effect suggests an active role of the product matrix and its ingredients in supporting the survival and growth of microorganisms in the gastrointestinal environment. Overall, the *in vitro* results indicate that Florentero Complex® can deliver a substantial and functionally relevant amount of viable probiotic microorganisms to the intestine, supporting its nutraceutical efficacy *in vivo*.

Acknowledgments: None

Conflict of interest: None

Financial support: Candioli Pharma S.r.l., covered the APC of the Journal.

Ethics statement: This study was performed entirely *in vitro* using simulated digestive fluids and bacterial/yeast cultures. No live animals or human subjects were used; therefore, institutional ethical approval reference numbers are not applicable.

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