STUDY WITH A PROBIOTIC PREPARATION COMPOSED OF KLUYVEROMYCES MARXIANUS CECT 13203 AND LACTOBACILLUS RHAMNOSUS CECT 30579 IN THE DIGESTIVE HEALTH OF A HEALTHY POPULATION WITH DIGESTIVE SYMPTOMS

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SUMMARY

Digestive health plays an essential role in overall well-being.
Probiotics have emerged as a promising therapeutic alternative to restore intestinal microbial balance.
This study evaluated the effects of a probiotic preparation composed of *Kluyveromyces marxianus* CECT 13203 and *Lactobacillus rhamnosus* CECT 30579 (Bioithas Digest ®) in a healthy population with mild to moderate digestive symptoms.
A randomized, open-label clinical trial was designed with a duration of 30 days, comparing an experimental group that received the probiotic with a control group that received no intervention.
Differences were observed in digestive symptom scores after 30 days of intervention, with clinically relevant and statistically significant improvement in the group that received the probiotic treatment compared to the values of the group without intervention in the symptoms postprandial pain, epigastric pain, abdominal bloating, retrosternal discomfort, heartburn, abdominal cramping pain, loss of appetite and excessive flatulence.
A large majority of participants (over 85%) in the probiotic group completed the intervention period without presenting symptoms.
When participants were classified as “responders” (they experienced a decrease in at least one category of severity in digestive symptoms), statistically significant differences were identified in favor of the group treated with probiotic in the symptoms of postprandial pain,

epigastric pain, abdominal bloating, heartburn, crampy abdominal pain, loss of appetite and excessive flatulence.
This study suggests that the administration of *Bioithas Digest* ® may be effective in relieving digestive symptoms in individuals with mild to moderate digestive discomfort, improving their quality of life.

KEYWORDS :
- *Probiotics*
- *Kluyveromyces marxianus*
- *Lactobacillus rhamnosus*
- *intestinal microbiota*

1. INTRODUCTION

Digestive health is essential for optimal functioning of the body, as it not only facilitates the correct absorption of nutrients, but also plays a fundamental role in regulating the immune system and protecting against pathogens.
A key component in maintaining proper digestive function is the intestinal microbiota, a complex ecosystem composed of trillions of microorganisms that inhabit the gastrointestinal tract (1).
This microbiota, composed mainly of bacteria, fungi, viruses and other microorganisms, contributes to the digestion of food, the production of beneficial metabolites, such as short-chain fatty acids, and the modulation of the immune response.
An imbalance in the composition and function of the intestinal microbiota, known as dysbiosis, has been associated with a series of digestive, metabolic disorders and even systemic diseases (2).
Therefore, maintaining a balanced gut microbiome is essential not only for digestive health, but also for the overall well-being of the individual.
On the other hand, functional digestive disorders are increasingly common, which has generated a growing interest in the use of treatments that can restore intestinal balance and alleviate these symptoms (3).
In this context, probiotics (4) have emerged as a promising therapeutic strategy, due to their ability to regulate intestinal microbiota and improve digestive health.
The aim of the present study is to evaluate the effects of a probiotic preparation composed of *Kluyveromyces marxianus* CECT 13203 and *Lactobacillus rhamnosus* CECT 30579 (Bioithas Digest ®) on the digestive health of a healthy population with mild to moderate digestive symptoms.
The aim is to determine whether the administration of this probiotic preparation can improve digestive function by reducing symptoms and thereby contribute to a better quality of life in individuals who experience digestive discomfort without serious underlying pathologies.

2. MATERIAL AND METHODS

2.1 Clinical Trial Design.

The present study is a randomized, open-label clinical trial designed to evaluate the effect of intervention with a nutritional preparation compared to a control group without intervention.
The duration of treatment and follow-up will be 30 days.
Data related to digestive health will be collected at two times: at the

time of inclusion of participants, coinciding with the start of treatment, and again at the end of the 30 days of intervention.

2.2. Participant Selection Criteria.

2.2.1. Inclusion Criteria.

- Men and women between 18 and 65 years old.
- Presence of digestive symptoms (e.g., bloating, gas, heartburn, abdominal discomfort, etc.) without a formal diagnosis of digestive disease.
- Good general health, without significant comorbidities.
- Ability and willingness to sign informed consent.
- Availability to continue treatment and attend follow-up visits.

2.2.2. Exclusion Criteria

- Previous diagnosis of chronic gastrointestinal diseases (irritable bowel syndrome, inflammatory bowel disease, gastric ulcer, celiac disease, or other digestive diseases at the discretion of the researcher that contraindicate participation in the study).
- Recent use of treatments that affect the intestinal microbiota (in the last 4 weeks): Antibiotics, probiotics, prebiotics or other digestive supplements.
- Presence of serious systemic diseases (cardiovascular, renal, hepatic, or other severe pathologies at the discretion of the researcher).
- Pregnant or breastfeeding women.
- History of allergic reactions or intolerance to the ingredients of the product under study.
- Participation in another clinical trial in the last 3 months.

2.3. Randomization and Intervention.

Participants were randomized in a 1:1 ratio to be assigned to one of two study groups, using a previously generated randomization list.
The experimental group received one daily capsule of the probiotic supplement. *Bioithas Digest* ® for 30 days which contained the *Lactobacillus strains rhamnosus* CECT 30579 1×10 9 CFU and *Kluyveromyces marxianus* CECT 13203 1×10 8 CFU.
The control group did not receive any type of intervention during the study, serving only as a comparison group to evaluate the specific effects of the treatment applied to the experimental group.

2.4. Instruments for assessing digestive symptoms and main outcomes.

Structured Digestive Symptoms Assessment Questionnaire Assessment of Gastrointestinal Symptoms Scale (SAGIS) (5).
Symptoms assessed included: postprandial pain, epigastric pain, abdominal bloating, early satiety, retrosternal discomfort, dysphagia, heartburn, gastroesophageal reflux, colicky abdominal pain, nausea, vomiting, loss of appetite, diarrhea, urgency to defecate, incontinence, pain or discomfort before or during defecation, excessive flatulence, and constipation.
Responses to each of the questionnaire questions were recorded using a 5-point Likert-type scale, ranging from "no problem" to "very serious".

The possible categories were: “no problem” (1), “mild” (2) (the symptom is perceived, but can be ignored if attention is not paid), “moderate” (3) (the symptom cannot be ignored, although it does not interfere with daily activities), “severe” (4) (the symptom affects the normal development of daily activities) and “very severe” (5) (the symptom significantly impacts the performance of daily activities).
The main outcome considered in the study was the following:

- Proportion of healthy volunteers showing improvement in digestive symptoms at the end of the intervention period, comparing the changes recorded between the experimental group and the control group. Clinical improvement will be established as the step , from the initial visit to the end-of-study visit, to a category of lesser severity on the SAGIS scale.

2.5. Statistical Analysis.

The analysis of the results was carried out according to protocol, considering only the data from the participants who completed the clinical study.
Descriptive clinical and demographic variables of healthy volunteers were summarized in a table to provide an overview of the baseline characteristics of the participants.
No comparisons were made using statistical tests for these variables; instead, homogeneity between groups was assessed by considering the magnitude of the differences observed.
This approach allows us to identify potential baseline differences that may influence the results of the analysis.
For the evaluation of the main outcome, the Likert-type scale score corresponding to each digestive symptom was used, treating it as an ordinal variable in its conceptualization, but statistically analyzed as a continuous variable. The Wilcoxon test was used to determine the existence of significant differences ($p<0.05$) in the main variable after 30 days of intervention with respect to the initial values.
A table was presented detailing the proportion of patients assigned to each category for each symptom in which significant differences were identified.
Finally, participants were categorized as responders or non-responders for each digestive symptom assessed, depending on whether or not, after the intervention period, they experienced a decrease of at least one category of severity to a lesser degree.
The responder rate for each digestive symptom was compared using Fisher's exact test.

3. RESULTS

3.1 Characteristics of the Included Population.

A total of 40 participants were included in the study, with 20 assigned to each study group.
The distribution in each intervention group was balanced in terms of their baseline clinical and demographic characteristics, as can be seen in Table 1. Likewise, there were no losses to follow-up or withdrawals during the study period, with data from all participants being analyzed at the end of the study.
Differences were observed in the scores of digestive symptoms after 30 days of intervention, with clinically relevant and statistically significant improvement in the group that received the probiotic treatment compared to the values of the group without intervention in the symptoms: Postprandial pain ($p = 0.027$), epigastric pain ($p = 0.008$), abdominal

dbloating (p = 0.015), retrosternal discomfort (p = 0.020), heartburn (p = 0.008), colicky abdominal pain (p = 0.027), loss of appetite (p = 0.011) and excessive flatulence (p = 0.017).

TABLE 1: CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF INCLUDED SUBJECTS

VARIABLES DESCRIPTIVAS	PROBIÓTICO (N=20)	CONTROL (N=20)
AGE (years)	39.6 (10.8)	39.5 (14.3)
SEX (♀)	8/20 (40%)	11/20 (55%)
WEIGHT (kg)	77.5 (11.45)	68.7 (13.5)
HEIGHT (m)	1.72 (0.09)	1.68 (0.08)
BMI (kg/m 2)	25.8 (2.7)	24.1 (3.3%)
ALLERGIES	0 (0%)	0 (0%)
INTOLERANCES	0 (0%)	0 (0%)
BACKGROUND • **CARDIOVASCULAR** • **RESPIRATORY** • **METABOLIC**	2/20 (10%) 1 1 0	3/20 (15%) 0 2 1

Table 2 presents a summary of the severity scores of the different digestive symptoms in the participants of the probiotic group that showed significant differences with respect to the initial values.

The results show a significant reduction in the severity of these digestive symptoms after 30 days of treatment with the probiotic, highlighting that a majority of participants (greater than 85%) in the probiotic group completed the intervention period without presenting symptoms.

When classifying participants as "responders," defined as those who experienced a decrease in at least one severity category of digestive symptoms, statistically significant differences were identified in favor of the probiotic-treated group in the following symptoms: postprandial pain, epigastric pain, abdominal bloating, heartburn, crampy abdominal pain, loss of appetite, and excessive flatulence (Table 3).

4. DISCUSSION

The results of this clinical trial indicate that consumption of the investigated probiotic product improves digestive symptoms overall.

A higher percentage of cases with response to treatment was observed in the experimental group compared to the control group.

Furthermore, for several specific digestive symptoms, improvement reached both clinical and statistical significance (Tables 2 and 3).

In recent years, several studies have been published that support the efficacy of specific probiotic strains in improving occasional digestive symptoms, which affect a high percentage of the general healthy population (6).

In this regard, a previous clinical trial evaluated the effect of a probiotic mixture that included the same strain of *L. rhamnosus* used in the present study (7).

TABLE 2: CHANGES IN THE CATEGORIZATION OF DIGESTIVE SYMPTOMS WITH SIGNIFICANT DIFFERENCES IN THE GROUP OF PARTICIPANTS TREATED WITH THE PROBIOTIC

DIGESTIVE SYMPTOMS	CATEGORIES	CONTROL *Basal*	CONTROL *30 Days*	PROBIOTIC *Basal*	PROBIOTIC *30 Days*
POSTPANDRIAL PAIN	No problem	10 (50%)	8 (40%)	10 (50%)	17 (85%)
	Mild	5 (25%)	9 (45%)	6 (30%)	3 (15%)
	Moderate	5 (25%)	3 (15%)	3 (15%)	0 (0%)
	Serious	0 (0%)	0 (0%)	1 (5%)	0 (0%)
	Very serious	0 (0%)	0 (0%)	0 (0%)	0 (0%)
EPIGATRIC PAIN	No problem	13 (65%)	13 (65%)	13 (65%)	17 (85%)
	Mild	4 (20%)	5 (25%)	4 (20%)	3 (15%)
	Moderate	3 (15%)	2 (10%)	3 (15%)	0 (0%)
	Serious	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Very serious	0 (0%)	0 (0%)	0 (0%)	0 (0%)
ABDOMINAL SWELLING	No problem	10 (50%)	10 (50%)	10 (50%)	17 (85%)
	Mild	5 (25%)	6 (30%)	6 (30%)	3 (15%)
	Moderate	5 (25%)	4 (20%)	3 (15%)	0 (0%)
	Serious	0 (0%)	0 (0%)	1 (5%)	0 (0%)
	Very serious	0 (0%)	0 (0%)	0 (0%)	0 (0%)
RETROSTERNAL DISCOMFORT	No problem	13 (65%)	10 (50%)	13 (65%)	17 (85%)
	Mild	4 (20%)	6 (30%)	4 (20%)	3 (15%)
	Moderate	3 (15%)	3 (15%)	3 (15%)	0 (0%)
	Serious	0 (0%)	1 (5%)	0 (0%)	0 (0%)
	Very serious	0 (0%)	0 (0%)	0 (0%)	0 (0%)
PYROSIS	No problem	13 (65%)	13 (65%)	13 (65%)	17 (85%)
	Mild	4 (20%)	5 (25%)	4 (20%)	3 (15%)
	Moderate	3 (15%)	2 (10%)	3 (15%)	0 (0%)
	Serious	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Very serious	0 (0%)	0 (0%)	0 (0%)	0 (0%)
COLICKY ABDOMINAL PAIN	No problem	10 (50%)	11 (55%)	10 (50%)	17 (85%)
	Mild	5 (25%)	5 (25%)	6 (30%)	3 (15%)
	Moderate	5 (25%)	4 (20%)	3 (15%)	0 (0%)
	Serious	0 (0%)	0 (0%)	1 (5%)	0 (0%)
	Very serious	0 (0%)	0 (0%)	0 (0%)	0 (0%)
LOSS APPETITE	No problem	13 (65%)	14 (70%)	13 (65%)	18 (90%)
	Mild	4 (20%)	3 (15%)	4 (20%)	2 (5%)
	Moderate	3 (15%)	3 (15%)	3 (15%)	2 (5%)
	Serious	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Very serious	0 (0%)	0 (0%)	0 (0%)	0 (0%)
EXCESSIVE FLATULENCE	No problem	10 (50%)	10 (50%)	10 (50%)	17 (85%)
	Mild	5 (25%)	7 (35%)	6 (30%)	3 (15%)
	Moderate	5 (25%)	3 (15%)	3 (15%)	0 (0%)
	Serious	0 (0%)	0 (0%)	1 (5%)	0 (0%)
	Very serious	0 (0%)	0 (0%)	0 (0%)	0 (0%)

TABLE 3: RATE OF RESPONDERS FOR DIGESTIVE SYMPTOMS WITH SIGNIFICANT DIFFERENCES IN FAVOR OF THE PROBIOTIC GROUP

DIGESTIVE SYMPTOMS	CONTROL	PROBIOTIC	P value
POSTPANDRIAL PAIN	3/20 (15%)	10/20 (50%)	0.040
EPIGATRIC PAIN	1/20 (5%)	8/20 (40%)	0.044
ABDOMINAL SWELLING	1/20 (5%)	10/20 (50%)	0.030
PYROSIS	1/20 (5%)	7/20 (35%)	0.044
COLICKY ABDOMINAL PAIN	2/20 (10%)	10/20 (50%)	0.014
LOSS OF APPETITE	1/20 (5%)	7/20 (35%)	0.044

The results showed a statistically favorable response rate in the group receiving the probiotic compared to the group without intervention, particularly in terms of the reduction of heartburn, abdominal pain and general symptoms.
Consequently, administration of the product proved to be effective in reducing digestive symptoms.
The probiotic strain mixture used in the present study included *Kluyveromyces marxianus* CECT 13203, the first *non-Saccharomyces* yeast approved as a probiotic for human consumption (8).
Kluyveromyces marxianus has been shown to be effective in improving digestive

Asymptoms and has therapeutic potential in various conditions such as intestinal diseases, halitosis, lactose intolerance and side effects resulting from the use of antibiotics.

In patients with irritable bowel syndrome (IBS), administration of a fermented milk containing *Kluyveromyces marxianus* and other probiotic species resulted in significant improvement of symptoms (9).

In subjects with halitosis, probably caused by a bacterial imbalance, the administration of *Kluyveromyces marxianus* for two weeks eliminated halitosis in 91% of patients.

The mechanism of action of the probiotic is attributed to the restoration of the intestinal microbiota, without a direct effect at the oral level (10).

In any case, Kluyveromyces marxianus is a particularly interesting probiotic because not only has its capacity to improve digestive symptoms been shown, but its immunomodulatory potential has also been demonstrated (11).

only has its capacity to improve digestive symptoms been shown, but its immunomodulatory potential has also been demonstrated (11).

The other probiotic strain in the experimental product is Lactobacillus rhamnosus CECT 30579, which has already been evaluated in combination with other components for the treatment of digestive symptoms, showing good results.

Furthermore, another factor that supports the selection of both probiotic strains is that they have been previously used in elderly and immunocompromised patients, without causing significant side effects and showing excellent tolerance in patients (7,9,10).

A longer treatment period is likely to benefit a greater number of patients.

However, although this is an open study, the results are promising, as various outcomes evaluated, such as digestive symptoms and general symptoms, show a significant improvement in the group of subjects who sreceived the probiotic compared to the control group.

6. CONCLUSIONS

The results of the present study indicate a beneficial effect on digestive symptoms in the population evaluated. This evidence supports the use of the probiotic preparation Bioithas Digest in healthy subjects with sporadic digestive symptoms and opens the possibility of exploring its application in other clinical conditions. Thus, it is suggested that this treatment could become an effective option with a wider use in clinical practice.

CONFLICTS OF INTEREST:

The authors declare none.

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