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Research Article

Effect of the Intake of Probiotics Isolated from Human Milk in People with Gastritis and Irritable Bowel Syndrome

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ABSTRACT

Background and objective: The gut microbiota is critical to human health, and can be affected by multiple factors including diet, ethnicity, use of antibiotics, geographic location, bad habits such as cigarette smoking, alcohol or junk products consumption. The gut microbiota have various functions such as vitamin production and other essential products, but an imbalance of it, could trigger various conditions associated with diseases to metabolic syndrome, as well as, gastrointestinal problems. The objective of this work was to evaluate the effect of the intake of lactic bacteria isolated from human milk, in patients with gastritis problems and irritable colon syndrome.

Methods: 10 Mexican patients with gastritis,10 with irritable bowel syndrome and 10 healthy as control or placebos, were selected from Guadalajara, Mexico. For 3 months, the volunteers ingested capsules with *L. fermentum* LH01, *L. reuteri* LH03, and *L. plantarum* LH05 (10^9 CFU/g). All participants underwent clinical studies and nutritional evaluation. Stool samples were collected from the thirty volunteers, to determine their microbial profile and lactic acid bacteria content by identifying the strains with the Maldi-Tof analyzer.

Results and conclusions: Due to constipation problem laxative use was a common factor among people with irritable bowel syndrome. Were identified a strain of *Salmonella spp*, in stool of the gastritis and irritable bowel syndrome groups. A diversity of bacteria was isolated in the stool, especially species of *Bacillus subtilis* and *Enterococcus faecium*. The intake of human milk probiotics favored the health of sick patients by 85%, an improvement in the evacuations of these people was observed from the second month after having ingested the probiotics. Decrease of disinflammation of the intestines and an improvement in the general health of the patients was observed. *L. fermentum* LH01, *L. reuteri* LH03, and *L. plantarum* LH05 showed significant probiotic potential to improve the problems, such as, the gastritis and irritable bowel syndrome.

Keywords: Gut microbiota; Constipation; Gastritis; Irritable bowel syndrome; Probiotics; Human milk

INTRODUCTION

The presence of microorganisms in our intestinal tract have many benefits, because thanks to them we degrade some food and drugs that we can not to digest by ourselves, also permit to balance other immunological functions against infections, it means, it significantly influences in intestinal homeostasis [1]. Part of the intestinal microbiota comes from the moment of birth, cesarean or vaginal section. Later it is enriched with the bacteria that the mother brings to the infant through breast milk, representing many benefits until we are adults, although it differs from individual to another due to lifestyle, diet, exercise, age, so there isn't an universal microbiota, however, it is important to maintain a good host-guest relationship to prevent

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various diseases that have to do above all with metabolic issues [1-3].

The human milk is consider a synbiotic food, thanks to the composition of oligosaccharides and commensal bacteria (*Lactobacillus plantarum*, *Lactobacillus fermentum*, *Pediococcus pentosaceus*, and *Lactobacillus brevis*, among other species), contained in this food, achieving an intimate microbial interplay among maternal diet, colonization and personalization of milk microbiota, and the gastrointestinal microbes in breastfed infants. Indeed, understanding the probiotic nature of mother's milk is an important step in understanding how all probiotic foods affect beneficially to health and diseases throughout our life [4].

Even the role they carry the microbiota out within our body is incomparable to that of another person, because they carry out various functions that are reflected in health benefits such as the maintenance of the structural integrity of the intestinal mucosa barrier, immunomodulation and protection against pathogens among other gastrointestinal problems [2,3]. Recent study have proved that absence of a microbiota with probiotic potential in our intestine can favor the presence of chronic degenerative diseases such as obesity, metabolic syndrome, type 2 diabetes, irritable bowel syndrome and dysbiosis [5,6].

Although most of these species are bacteria, there may also be amoebas, yeasts, viruses, and parasitic. The composition of species depend to the type of diet, competition for substrates and their resistance to intestinal conditions, in addition to a combination of environmental and genetic factors of the host [7,8]. It has also been seen that physical activity induces compositional and functional changes in the intestinal microbiota of obese people, being these changes independent of diet [9,10].

Inflammatory bowel disease is associated with altered intestinal microbial community structure and functionality, which may contribute to inflammation and complications such as colon cancer and liver disease, in addition, the intestinal microbiota changes can affect the quality of life of people in social and work matters [11,12]. The ulcerative colitis is the first disease that causes intestinal inflammation. The second is a combination of symptoms as constipation, abdominal pain, and diarrhea [13]. Irritable bowel syndrome is a common condition but its treatment is not simple [14,15]. Among the measures that have been taken have been to try to change the intestinal microbiota, however the results have not always been positive [16].

It has been experienced with dietary fiber has the ability to improve gastrointestinal diseases, but it has been seen that it does not present any relevant effects, since it is known to favor the presence of certain microorganisms such as *Bifidobacterium* and *Lactobacillus spp.* in fecal matter, but the intestinal microbiota remains the same and people continue with their diseases [17].

Probiotics have been used successfully in patients with constipation and irritable bowel syndrome, such as *B. animalis subsp. lactis*, *B. bifidum*, *B. longum subsp. infantis*, *Lactobacillus paracasei*, *L. rhamnosus*, *L. plantarum*, *L. acidophilus*, *Propionibacterium freudenreichii*, *Streptococcus salivarius*, among others. The effects of probiotics depend on the strain and the probiotic formulations available with both single-strain or multi-strain, in addition, probiotic dose and form of administration [18,19].

Due to the need to find new alternatives to help people with gastritis and irritable bowel syndrome, this work aimed to evaluate the effect of the ingestion of lactic bacteria isolated from human milk with probiotic potential, to improve the quality of life and the health of people with gastrointestinal problems.

MATERIALS AND METHODS

Selection of patients with irritable colon syndrome

This project was approved by Ethics Committee of the Hospital Civil de Guadalajara "Fray Antonio Alcalde" on October 20, 2020, number: HCG/CEI-1228/20. Participants were selected according available resources, between 20 to 55 years old, indistinct sex, that they didn't have chronic degenerative diseases and that, according to the Bristol scale, they are diagnosed with irritable bowel. Groups of 10 people were selected: first group healthy volunteers as control or placebos, second group with gastritis and third group with irritable bowel syndrome. The patients (30 people) were informed of the study and the guidelines for informative consent and confidentiality were followed in accordance with the provisions of the Federal Health Law on Health Research [20], title 2, chapter I, article 17, section I, Research of minimal risk, always taking care of the integrity of the patient, and taking into account the ethical criteria established by the Research Ethics Committee of the HCFAA of Guadalajara, as well as the safeguarding of the patients personal information, as stipulated by the Federal Protection Law of Personal Data. Finally, each participant filled out a form to learn about their eating habits, type of food, according National Health and Nutrition Survey (ENSANUT for its acronym in Spanish) 2018 for adults over 20 years of age, number of times they consume certain products, physical activity, use of laxatives and antibiotics [21].

Production of the lactic acid bacteria *L. fermentum* LH01, *L. reuteri* LH03, and *L. plantarum* LH05

The strains *L. fermentum* LH01, *L. reuteri* LH03, and *L. plantarum* LH05, were isolated from human milk and characterized in genus and species by molecular biology techniques and are stored at -80°C. Actually the strains are part of the stock of the Industrial Microbiology Laboratory Research Area, Universidad de Guadalajara.

For their propagation, the strains was reactivated in sterile MRS broth and later cultivated in Petri dishes on MRS agar. Subsequently, an inoculum of each bacteria was prepared in 15 mL of sterile MRS broth, plus 10% Nutraflora® (Ingredion). The flasks were incubated at 37°C, without shaking for 24 hours. Once the concentration of cells in the inoculums (109 CFU/mL) has been obtained, 1% of the inoculum of each bacterium was transferred to a 1 L flask containing a total volume of 600 mL of sterile MRS broth. The flasks were incubated at 37°C for 24 hours, to obtain the biomass necessary

for the treatments. After 24 h of incubation, the flasks were removed and in sterile falcon tubes they were centrifuged at 3,500 rpm for 15 minutes. Once the tubes have been centrifuged, the supernatant was discarded; the biomass obtained from the culture of each of the *lactobacilli* (*L. fermentum* LH01, *L. reuteri* LH03, and *L. plantarum* LH05) was collected. At the end, 3 mL of sterile 0.85% saline solution was added to recover the cells and 1.5 g of Nutraflora® will be added as a stabilizer.

Preparation of *lactobacilli* capsules

To prepare the capsules of probiotics, the biomass obtained of each strain was frozen with liquid nitrogen in falcon tubes and they were lyophilized for a period of 48 h. Subsequently, a sample of the lyophilisate was taken and a viability analysis of the strains was performed, taking 0.1 g of biomass diluted in 1 mL of 0.85% saline solution, diluting up to 1:10,000. In MRS agar (sterile) was realized by triplicate for each of the dilutions, the plate casting technique, homogenizing 1 mL of each of the dilutions. The Petri dishes were incubated for 36 h, at 37°C in an anaerobic environment during 24 h. Once the incubation time has passed, the boxes were removed from the incubator and the CFU/mL was be counted.

Taking the number of cells per gram of biomass of each strain (109 CFU/g), a mixture was made with each of them and the capsules (size #00), were filled with a Profiler® brand encapsulator, with encapsulation capacity of 100 capsules. The equipment was sanitized with 70% alcohol and we were work within a sterile area for filling the capsules. 60 capsules were placed in the dry amber bottle for later use.

In vivo study in patients with gastritis and irritable colon syndrome

The 30 patients were summoned to taken a blood sample for to perform a hematic biometry. A stool sample was taken for to assess the presence of pathogenic bacteria and parasites, at the beginning and final of treatment. The patients were also goes at the nutritional counseling, and were taken into account the eating habits of the patients, BMI, weight, height, among others factors. Patients were monitored for 3 months, and instructed to take 2 capsules per day, (morning and night) before meals. At the end of 90 days, a survey was realized to measure the satisfaction of treatment and to give their personal testimony, as well as, they were given a copy of their laboratory and nutritional results.

Statistical analysis

The data were organized in Excel and averaged presented with standard deviations. An ANOVA with post hoc test (GraphPad prism V6.01; GraphPad Software, Inc.) were performed and the mean values were compared to determine the presence of a significant difference ($p<0.05$).

RESULTS AND DISCUSSION

Results obtained before the ingestion of probiotic bacteria isolated from human milk

Figure 1 show the physical activity realized by participants, were we seeing that people with Gastritis and irritable bowel syndrome problems hardly exercise. 80% of people with irritable bowel syndrome don't exercise. It is known that changes in the gut microbiota can cause fatigue-related problems [22]. Exercising regularly brings many health benefits, however, when intensive physical activity is performed, energy sources such as glucose or glycogen are reduced, and it has also been seen that some reactive oxygen species and peroxides of lipids can accumulate damaging organs and causing tiredness or fatigue [23]. Some studies have shown that the consumption of dairy products rich in bacteria and yeasts with probiotic potential, such as kefir, can contribute to recovery after intensive physical activity. One study showed that in mice fed a high-fat diet with physical activity, it provided them with greater grip strength compared to mice that did not exercise. This result ensures that the long-term consumption of this type of products benefits muscle strength even when physical exercise is not performed, also showed that percentage of bacterial phyla at 12 weeks increase. A correlation between total exercise distance and the ΔCt Bacteroidetes: ΔCt Firmicutes ratio from qPCR demonstrated a significant inverse correlation [24].

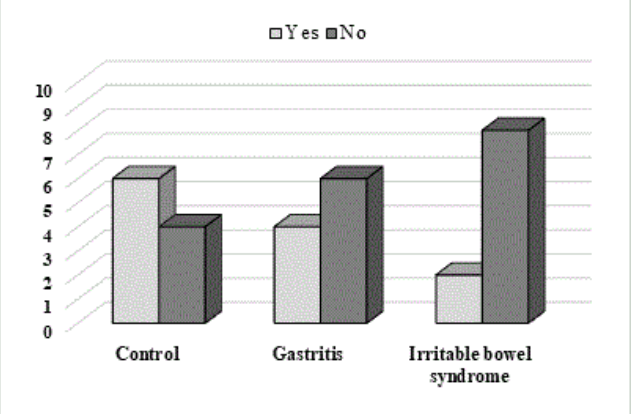

Figure 1: Physical Activity of patients.

Although it has not been tested directly in humans, there are various researches that indicate physical exercise has important effects on the gut microbiota in animal models, such as changes in metabolism, behavior and immunity [25-27]. It would also be important to be able to test it in people with obesity problems, since it has been proven that exercise training in mice protects the intestinal barrier and the inflammatory tissue responses caused by obesity, thus showing that this may be due to some mechanism regulated by the intestinal microbiota [28].

The use of laxatives in the participants was graphed in Figure 2, where it is shown that this type of product is frequently used by people with intestinal problems. 70% of patients with irritable bowel syndrome and 50% of people with gastritis use laxatives in order to evacuate. Irritable bowel syndrome and constipation they are usually related, so many people with these conditions turn to laxatives to relieve constipation. Osmotic laxatives can

determine electrolyte disturbances or abdominal complaints such as abdominal pain and intestinal bloating, that is important to understand exactly how much these treatments can help the condition [29]. Irritable bowel syndrome, where constipation represents the predominant complaint, associated with recurrent abdominal pain, abdominal distention and abdominal distention, has a strong personal, health and social impact, affecting the quality of life of patients who suffer from them [30].

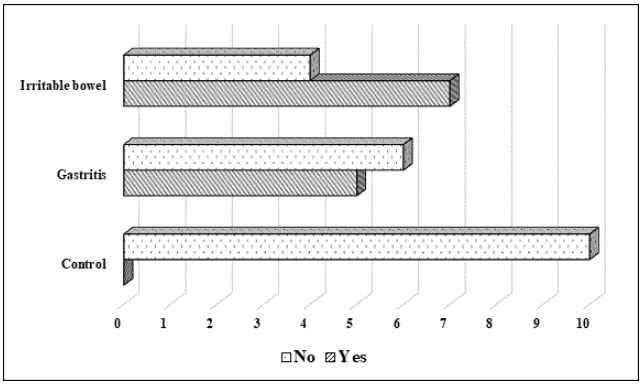

Figure 2: Use of laxatives.

On the other hand, gastritis produces inflammation of the stomach lining, generally caused by infection or acute infestation by viruses, bacteria or parasites, without considering the bacterial *Helicobacter pylori*, which causes most stomach ulcers. Another cause of gastritis is the consumption of drugs [31]. In our study, 80% of people with Gastritis and Irritable Bowel Syndrome declared that they consumed antibiotics at least 4 times a year. Some studies indicate that the continuous use of antibiotics can cause changes in the intestinal microbiota, reducing its diversity, and thus losing certain advantages that are had with their presence, such as the metabolism of glucose and bile acids in people with metabolic syndrome [32]. This may be due to several factors, such as the chemical characteristics of the antibiotic such as class and pharmacological properties, added to the host's own habits such as diet, lifestyle, which produce changes in the composition of the intestinal microbiota both in the short and long-term [33,34]. In the intestinal microbiota there is a great variety of microorganisms, it must be taken into account that each time a pharmacological treatment is carried out, many unwanted microorganisms die, but the beneficial ones too, the contact that humans have with these beneficial microbes are much more frequent than with pathogenic organisms, therefore it must be assessed whether the consumption of antibiotics is really necessary [35].

In figure 3 it showed the frequency of use of some foods during their diet. According to the results, the most frequently consumed products are flours and cereals, followed by meat, dairy products and soda. The control group is out for the main consumption of vegetables, dairy products and meat. Soda consumption was more common in people with gastritis, while eating sweets by people with syndrome irritable bowel problems. Coffee consumption is reduced in people with gastritis and irritable bowel syndrome, compared to healthy people who 80% consumed coffee at least once a day. Normally the effects observed by the consumption of coffee are the gastroesophageal reflux, in addition to stimulating the contraction of the gallbladder and increases the motor activity of the colon even minutes after drinking it [36]. However, dietary fiber present in coffee has been shown favor the development of intestinal microbiota, as it is rapidly metabolized in the form of short chain fatty acids, increasing the concentration of Bacteroides [37].

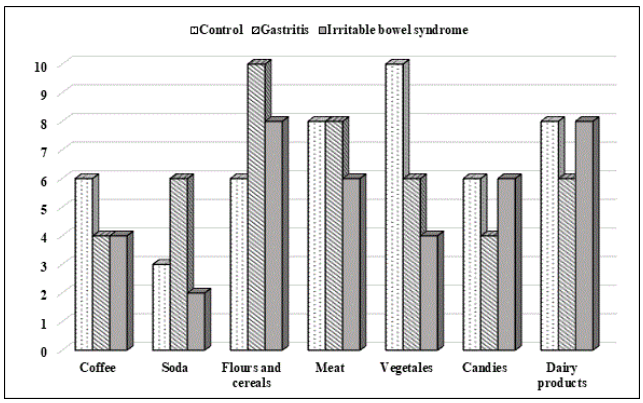

Figure 3: Kind of products consume in diet.

In Mexico, approximately 22% of the population suffers of constipation [38]. In countries like Japan, this disorder affects women more than men, and specifically affects those of older age [39]. Diet influences the colonization of bacteria in the intestine; one of the reasons is that people who usually eat junk food suffer from gastrointestinal diseases, while people with a good diet do not present any disorder. It is important to note that the quality of the food must be considered, to avoid gastrointestinal diseases [40]. It is recommended to have a proper diet and supplement it with probiotics for help repair the gastrointestinal damage. Therefore, in this case it was concluded that it is better to add foods rich in fiber to the diet and thus avoid the use of laxatives [41]. The diet regulates both the composition and the functional capacity of the intestinal microbiota, which intervenes in various biochemical processes in humans. All the biomolecules that we obtain from food and the metabolites formed are important pieces to modulate the host's immune response, intervening in the exchange of mutual benefits between human health and the intestinal microbiota [42].

Figure 4, show to identify of microorganisms analyzed in faeces. The microbiological results obtained in the stools of control group and groups with gastritis and irritable bowel syndrome, showed that the bacteria with the highest incidence were *Bacillus subtilis* and *Enterococcus faecium*, in the case of the gastritis and irritable bowel syndrome group, to presence of *Enterococcus durans* and *Candida glabrata* were found. Some genera of *Staphylococus* were identified in the 3 groups (figure 4), and also observed the incidence of *Micrococcus luteus*, *Pediococcus pentosaceus* and *Lactobacillus plantarum*, but only in the control group. The strains found in stool correspond to bacteria typical of phyla such as Bacteroides sp. and Enterobacteriaceae, in addition to some *Lactobacillus*. Various studies have shown that there are differences in the composition of the intestinal microbiota in constipated patients compared to healthy controls [43]. Simren et al. carried out a microbiological study in stool of

patients with irritable bowel syndrome with predominantly constipation, observing in their results a significant increase in Bacteroides sp. and Enterobacteriaceae [44].

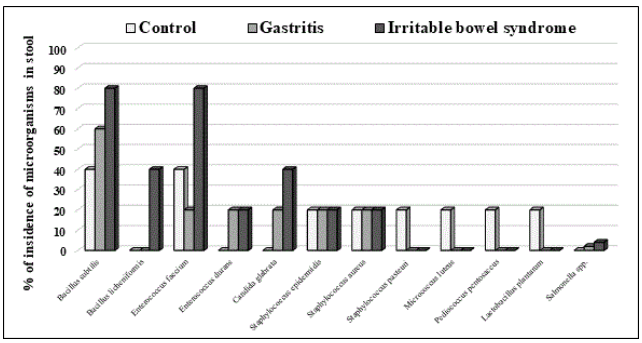

Figure 4: Microorganisms identified by Maldi-Tof analysis in stool.

It is important to note that 20 and 40% of patients with gastritis and irritable bowel syndrome, respectively, were found to have asymptomatic Salmonellosis, due to the incidence of a strain of *Salmonella* spp., founded in their stool (Figure 4). According to nutritional and clinical studies, these patients had the habit of eating outside the home, mainly street food. It is well known that foods that are stored at room temperature favor the growth of *Salmonella*. Also, the food handlers prominently play a role in disseminating typhoid bacilli through different food products and water. Poor personal hygiene and inadequate food handling can potentiate the transmission of *Salmonella typhi* [45].

On the other hand, the stool study showed that 30% of patients with irritable bowel syndrome had parasites, mainly *Endolimax nana* and *Entamoeba hystolitica*, this was confirmed with the interview conducted during the clinical study of the patients, who expressed not having the habit of deworming, only 20% of all participants, including the control group, if they dewormed every 6 months. Digestive parasitosis is caused by protozoa and helminths that mainly affect the intestine. These can be the cause of diarrhea and recurrent gastrointestinal illnesses, as well as, abdominal pain, bloating and nausea [46]. The living conditions of patients also intervene in the problem of intestinal parasitosis, leading to conflictive situations for health centers in Mexico. Education campaigns can be carried out to improve and prevent parasitosis, but the socio-economic reality such as poverty and different levels, can affect so that the campaigns are effective and convince the population to deworming for their health.

Results obtained after the ingestion of probiotic bacteria isolated from human milk

In the figure 5, show the count of bacteria acid lactic (CFU/g stool), in patients with gastritis and irritable bowel syndrome, after the consumption of human milk probiotics. We can observe that one month after the ingestion of probiotics, patients with gastrointestinal affectations increased the content of lactic bacteria in the stool, which demonstrates the colonization capacity of the probiotics consumed. The control patients or placebos, none exceeded 3,000 CFU/g of lactic acid bacteria in the stool, while the sick patients, all are above this figure, they even exceeded the amount of lactic bacteria in their stool of zero bacteria up to 1.2 × 105 CFU/g of stool.

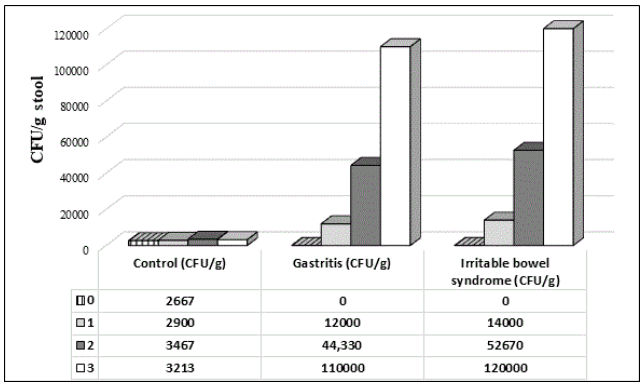

Figure 5: Count of bacteria acid lactic (CFU/g stool), in patients with gastritis and irritable bowel syndrome, after the consumption of human milk probiotics.

On the other hand, at the 3 months after treatment, people who initially had *Salmonella* spp. Detected in their stool, was no longer found, so we consider that the mixture of *L. fermentum* LH01, *L. reuteri* LH03, and *L. plantarum* LH05, favored the health of the host and an antimicrobial activity was produced against pathogen. In addition, we were able to verify, according to the clinical studies carried out, that patients with gastritis and mainly those with irritable bowel syndrome, improved their health condition by 85%, after 3 months of treatment with probiotic of human milk. Intake of these probiotics increased the number of evacuations in some patients up to 2 times a day and decrease intestinal inflation.

Antibiotics are common treatment for infections caused by pathogenic bacteria be difficult to treat, so probiotics have been researched as a possible way to prevent or reduce the severity of an intestinal bacterial infection. For ejemplo, a study realized whit *Lactobacillus fermentum*, showed that this bacterium was capable of inhibiting the growth of *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*. The growth of *L. fermentum* was similar to pathogenic bacterial strains, proving that it can be a natural combatant to bacterial infections in the intestine [47].

Buckley et al. demonstrated that after a 28 day supplementation with *L. reuteri* DSM17648 (2 × 1010 cells per day), at single-blinded, placebo controlled study, has the potential to suppress *H. pylori* infection, and may lead to an improvement of *H. pylori* associated gastro intestinal symptoms. They observed a reduction of *H. pylori* load in 66.7% of the subjects and no side effects were reported in either study phase [48].

Has been reported the preventive effects of *Lactobacillus plantarum* ZS2058 and *L. rhamnosus* GG against *Salmonella* spp. in a murine model. Were observed anti-infection and inflammatory responses in the mice feeded whit *Lactobacillus plantarum* ZS2058 and *L. rhamnosus* GG. *Lactobacillus plantarum* ZS2058 was more efficiently to reduce the pathogenicity of *Salmonella*; meanwhile, *L. rhamnosus* LGG more strongly alleviated gut inflammation [49].

Various studies promote the use of probiotics to improve gastrointestinal problems, but there are no studies that have

shown the efficacy of lactic acid bacteria of human milk origin, to help improve gastritis and irritable bowel syndrome, as it is demonstrates in this work.

CONCLUSION

An improvement regarding to inflammation and constipation was noted in the patients, specially with irritable bowel syndrome, from the second week of treatment with the probiotic bacteria's. Inflammation decreased to the point of disappearing, as well as, discomfort such as burning and pain in the patients with gastritis, which significantly increased the comfort and quality of life of the sick patients. The strains *L. fermentum* LH01, *L. reuteri* LH03, and *L. plantarum* LH05, isolated from human milk, showed efficacy and favored the health and quality of life of patients with gastritis and irritable bowel syndrome, however, and for probable applications against chronic degenerative diseases.

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AUTHOR'S CONTRIBUTION

Blanca R. Aguilar Uscanga was responsible for project design and writing the majority of the manuscript, Josue R. Solis Pacheco, Ariana Rodríguez Arreola and José A. Velarde Ruiz Velasco, were responsible for collecting and analyzing data. Jessica G. Solis Aguilar, Jésus A. Amézcua López, Manuel Loera Parra, were responsible for collecting and analyzing data and writing and preparing the manuscript for submission.

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