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Effect of probiotics on human blood urea levels in patients with chronic renal failure

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Abstract

Introduction: **Patients with chronic kidney disease (CKD) show an increase in bowel aerobic bacteria that produce uremic toxins and decreased anaerobic bacteria as bifidobacteria and lactobacillus. The latter can be used as probiotics. The probiotic with greater availability in Mexico, is the *lactobacillus casei shirota* (LcS), currently there is no known LcS specified dose that produces a benefit to the patient with CKD.**

Objective: **To determine the effectiveness of two different LcS doses in achieving a decrease in urea concentrations of at least 10% in patients with KDOQI stage 3 and stage 4 CKD.**

Metodology: **A simple randomized, controlled clinical trial. Outpatients treated at the National Institute of Medical Sciences and Nutrition Salvador Zubirán in México D.F. Patients were provided the LcS, as follows: Group A: 8 x 10^9 colony-forming units (CFU) and Group B: 16 x 10^9 CFU. Patients were followed-up for eight weeks, and baseline and final samples were obtained to calculate the basal and final concentrations, respectively, of blood urea and serum creatinine (CrS). During the follow-up, both groups consumed a diet of 30 kcal/kg/weight and 0.8 g/kg/weight of protein, and a food diary was made to assess both the adherence to the diet and LcS.**

Results: **Thirty patients with CKD were evaluated. When analyzing the percentage change between the different doses, a decrease > 10% was found in the blood urea concentrations for patients treated with the 16 x 10^9 dose, which was significant with respect to the baseline measurement.**

Conclusion: **There was a > 10% decrease in the serum urea concentrations with *LcS* in patients with stage 3 and 4 CRF.**

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EFECTO DE LACTOBACILLUS CASEI SHIROTA SOBRE CONCENTRACIONES DE UREA EN LA ENFERMEDAD RENAL CRÓNICA

Resumen

Introducción: **Los pacientes con enfermedad renal crónica (ERC) muestran un aumento a nivel intestinal de bacterias aeróbicas que generan toxinas urémicas y disminución de bacterias anaeróbicas como bifidobacterias y lactobacilos. Estas últimas se pueden utilizar como probióticos. El probiótico con mayor disponibilidad en México, es el *lactobacillus casei shirota* (LcS), actualmente no se conoce que dosis de LcS puede generar un beneficio para el paciente con ERC.**

Objetivo: **Determinar el efecto de 2 dosis diferentes de LcS para disminuir al menos 10% las concentraciones de urea en pacientes con ERC estadios KDOQI 3 y 4.**

Métodos: **Ensayo clínico controlado con asignación aleatoria en el cual se incluyeron pacientes ambulatorios con ERC del Instituto Nacional de Ciencias Médica y Nutrición Salvador Zubiran. Se asignó a los pacientes a uno de los dos grupos, grupo A: 8 x 10^9 unidades formadoras de colonias (UFC) y grupo B: 16 x 10^9 UFC. El seguimiento fue de ocho semanas, obteniendose una muestra de sangre basal y otra final para conocer concentraciones de urea y creatinina. Ambos grupos consumieron una dieta de 30 kcal/kg/peso y 0,8 g/kg/peso de proteína, se realizó un diario de alimentación para evaluar el cumplimiento de la dieta y del tratamiento del LcS.**

Resultados: **Se evaluaron 30 pacientes. Al analizar el porcentaje de cambio entre las diferentes dosis se encontró una disminución mayor al 10% en urea sanguínea en pacientes con la dosis de 16 x 10^9 con respecto a su medición basal.**

Conclusión: **Existe una disminución > 10% de la concentración sérica de urea con el *LcS* en pacientes con ERC 3 y 4.**

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Palabras clave: *Urea. Toxinas urémicas. Dosis. Probióticos. Lactobacillus casei Shirota.*

Introduction

Background

Currently, rising evidence exists that certain food can exert a beneficial effect on specific functions of humans in addition to their beneficial nutritional value. This benefit may lead to a positive impact on human health by preventing or treating diseases.[1] Thus, the concept of "functional food" has arisen, and it is defined as a product, modified food or nutritional ingredient that can exert beneficial health effects other than its traditional nutritional value.[2] Probiotics, prebiotics and symbiotics have obtained a relevant role in the field of functional foods.

The concept of probiotics was introduced at the beginning of XX century with Metchnikoff's studies.[3] Now the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) define probiotics as "the living organisms that provide health benefits in the host when consumed in the appropriate quantity."[4]

The attributed protective effect of probiotic microorganisms in improving the host resistance to pathogens.[5-7]

Various *in vitro* and *in vivo* studies of different pathological states suggest numerous health effects promoted by probiotics.[5,7-13] For a beneficial effect in the host, it is necessary to ingest appropriate quantities of probiotic microorganisms or sufficient colony-forming units (CFU).[16] This approach achieves a modification and equilibrium in the ecosystem of billions of microorganisms residing in the human gut, which reflects a good healthy state.

To understand the probiotics' effect on renal disease better, it is necessary to take into consideration that patients with renal disease usually have impaired intestinal microbiome. It is suggested that almost two thirds of individuals with uremia have abnormalities in the gastrointestinal mucosa and a disequilibrium in the intestinal ecosystem.[17] The majority of these changes happen at the ileum level and in the colon, where the microbiome plays an important role. The increase in aerobic bacteria, such as *Escherichia coli,* results in an intestinal microbiome disequilibrium. These bacteria generate toxic substances, called uremic toxins, and decrease the anaerobic bacteria, such as bifidobacteria and lactobacillus.[18]

In chronic renal disease (CRD), there are higher urea concentrations and, consequently, increased ammonium. Thus, there is an increase in pH that promotes the growth of aerobic bacteria in the gastrointestinal tract and the subsequent production of uremic toxins. Conversely, bifidobacteria ferment carbohydrates and produce acetic and lactic acid to acidify the intestine. In that way, these bacteria prevent the growth of aerobic microorganisms and normalize the altered intestinal microbiome in CRF patients.[19]

Evidence exists that patients with uremia have a deteriorated intestinal barrier that is mainly due to the disequilibrium of intestinal microbiome caused by the increase of pathogens.[20-22]

One of the requirements for the use of probiotics as adjuvants to remove urea or uremic toxins is the capacity of microorganisms to use metabolites as substrates. Thus, probiotics help intestinal microbiome decrease the bacteria producing uremic toxins. Urease is the enzyme responsible for hydrolyzing urea into ammonium and carbon dioxide, but only certain microorganisms can synthesize urease. In uremic patients, it has been shown that at high plasma urea concentrations, the fecal urease activity is increased. Thus, the increase in colon bacterial urease is considered a beneficial factor for uremic patients.[23] However, ammonium can be converted into nitrates by other microorganisms or return to the liver by diffusion, where it can be metabolized again into urea.

Probiotic dose in CRF

There have been several studies performed on CRF patients using different types of probiotics at different doses with the aim to reduce some uremic toxins. Simenhoff et al.[24] and Dunn et al.[25] have shown a decrease in dimethylamine (DMA) and nitrodimethylamine (NDMA) concentrations after using *L. acidophilus* in CRF patients with dialysis. Simenhoff's study was a double-blind trial with 30 patients on hemodialysis.[24] This researcher showed that 8 patients who were supplemented with *lactobacillus acidophilus* had lower dimethylamine and nitrodimethylamine concentrations, which are two of the uremic toxins produced in the small intestine. In the case of DMA, concentrations decreased from 224 ± 47 to 154 ± 47 μg/dL, while NDMA decreased approximately 31% ($p < 0.001$). Dunn et al. observed a significant decrease of 42% in the mean concentrations of DMA for patients supplemented with the probiotic ($p = 0.001$).[25]

Among the most relevant clinical studies, which are used as background for the present work, are the studies by Takayama[26] and Taki.[19] Both studies tested the probiotic *Bifidobacterium longum* in hemodialysis patients and reported a decrease in the toxin indoxyl sulfate. Takayama[26] observed a decrease in indoxyl sulfate from 4.9 mg/dL to 3.5 mg/dL ($p < 0.005$). Two years later, Taki et al.[19] studied 27 patients over 12 weeks using different probiotic doses. From the first to the fourth week, these researchers supplemented a dose of 3×10^9 CFU, while from the fifth to the eighth week, a dose of 6×10^9 CFU was used, and from the ninth to the twelfth week, a 12×10^9 CFU dose was provided. These authors found that the most effective bifidobacteria dose was a 6×10^9 CFU dose and that these microorganisms were able to reduce the indoxyl sulfate concentrations from 164.4 ± 15 mmol/L to 149.6 ± 15.5 mmol/L ($p < 0.05$). These studies were performed in hemodialysis patients, which is a situa-

tion that could imply an important bias in the results, as it is unknown whether the decrease in uremic toxins was due to the dialysis process itself or due to the significant effect of the probiotics on the decreased urea.

Lactobacillus casei Shirota (LcS)

In the traditional classification system, *Lactobacillus casei* is a gram (+) bacteria, and it belongs to the subgenus *Streptobacterium*. This subgenus includes homofermentative organisms that can grow at 15° C and up to a maximum temperature of 41° C. This strain's guanine-cytosine content is 45-47%, and it produces L-lactic acid as its principal metabolic product from glucose, sucrose, lactose, fructose and maltose. In Mexico LsC is one of the probiotics with the greatest economic and material availability, and it is used for the production of fermented dairy products.

Human and animal studies have shown that administering LcS has beneficial effects, such as the following:

In humans:

- Beneficial modulation of intestinal flora;[27]
- Improved fecal consistency;[27]
- Infection protection;[27]
- Immune activity modulation;[27]
- Prophylactic effects on cancer development;[28]
- Immunomodulatory effects;[29]
- Salmonella typhimurium inhibition;[30]
- Normal maintenance of ammonia concentrations and intestinal microbiome changes in patients with hepatocellular damage at stage Child-Pugh B, with or without ascites.[31]

In animals:

- Immune and cellular response modifications of type II collagen, thereby reducing arthritis development in rats;[32]
- Decreased action of triglycerides and plasmatic cholesterol in rats;[33]
- Growth inhibition of tumor cells in the thoracic cavity of mice.[34]

Methods

The present study is a controlled, simple randomized clinical trial without blinding.

Participants

CRF patients were recruited through the external consultation of the Nephrology Department at the National Institute of Medical Sciences and Nutrition Salvador Zubirán (Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran; INCMNSZ). Before the consultation, the records of the candidate patients were reviewed, and an invitation to participate in the study was made on the consultation day. To fulfill the study's objectives, CRF patients in stage 3 or stage 4, as reflected by the glomerular filtration rate (GFR) based on the MDRD (modification of diet in renal disease) formula, were considered. These patients were selected because stages 3 and 4 have more metabolic alterations without taking into account replacement therapy. Therefore, outpatients who fulfilled the inclusion criteria were invited to participate in this study.

Inclusion criteria

- Nephrology outpatients with stage 3 or 4 CRF (glomerular filtration rate from 59 to 15 mL/min/1.73 m^2 calculated by MDRD).
- Age between 18 and 65 years.
- Either sex.
- Literate patients.
- Mexico City residents.
- Signed informed consent.

Exclusion criteria

- Patients under replacement therapy.
- Patients with a diagnosis of diabetes mellitus.
- Patients with lupus erythematosus.
- Patients who had a renal transplant.
- Intolerance to whole milk and dairy products.

The evaluated interventions were two different doses of *LcS* that were included in a fermented dairy drink product. Two groups were formed as follows: group A received a fermented dairy drink in an 80-mL bottle with 8 x 10^9 CFU of LcS, and group B received two 80-mL bottles of the fermented dairy drink for a total of 16 x 10^9 CFU of LcS. The size, color, flavor and physical aspects of the bottles were the same for both groups. Patients visited INCMNSZ every 15 days to obtain 15 or 30 bottles of LcS (depending on the assigned group) during the two months.

Objectives

The objective was to determine the LcS dose needed to achieve a greater than 10% decrease in the blood urea concentration in stage 3 and stage 4 CRF patients. Therefore, the hypothesis was that the administration of a fermented dairy product containing 16 x 10^9 CFU of LcS could decrease the blood urea concentrations by at least in 10% in stage 3 and stage 4 CRF patients.

Measurements

All patients were attended at the Metabolic Unit of INCMNSZ.

This study was conducted according to the principles of the Declaration of Helsinki of the World Medical Association and was approved by The Ethical Committee of the INCMNSZ.

All participants gave written informed consent Once a potential patient was identified, the patient and a relative received an explanation of the nature and objectives of the present study. The patients had the opportunity to read the informed consent and resolve all of their doubts. A free decision to enter the study and the policy of no reprisal against the patient for a denial of participation were highlighted. Once agreeing to enter the study, the patient was asked to sign the informed consent. The informed consent was also signed by a responsible relative of the patient, by the researcher and by two witnesses. After the consent was received, the intervention, evaluation and biochemical measurement components of the study were started.

In the first visit, general information was obtained about the patient's underlying disease, comorbidities and type and dose of the medications taken. All patients gave a baseline blood sample in the fasting state for a determination of the serum creatinine and urea concentrations. After the blood draw, the patients were randomized to received a LcS dose. The subjects participated in a follow-up at two weeks to monitor their adherence to the diet and the consumption of the LcS dairy drink and the final evaluation after the two months of treatment. Blood samples were obtained at the end of the intervention period to determine the final blood creatinine and urea concentrations.

All patients followed an isocaloric (30 kcal/kg ideal weight) and isoproteic (0.8 g/kg ideal weight) diet during the two-month intervention. These diets were designed to ensure a good protein and energetic supply that would not directly affect the biochemical concentrations under study. A nutritionist specialized in renal disease calculated and explained these diets to each patient.

Each patient had previously received a daily food consumption diary to record food and lactobacillus (dairy drink) consumption during the 15 days before the follow-up visit. The dietary record aimed to monitor the adherence of each patient to the LcS and dietetic treatment. The specialized nutritionist trained each patient to correctly report the food and its quantity in the diary. The nutritionist explained how to record the day, the timetable of each meal, the dish name for each meal, the ingredients of each dish and the quantities to maintain a record that was as accurate as possible.

Nutripac 1.5® software was used to analyze the food diaries. The software assessed the quantity of the macronutrients consumed daily for each patient and calculated their mean during the 60 days of the LcS consumption. This approach allowed for the obtainment of their mean energy, protein, carbohydrate and lipid consumption. Adherence was assessed by the percentage of overall adequacy. A good diet and LcS consumption adherence was considered when the percentage was not outside the ± 10% (meaning between 90 and 110%) of the recommended diet for energy and each macronutrient in grams. Similarly, a low adherence to the fermented dairy drink was considered when the consumption of the total number of bottles was outside the ± 10% of the recommended consumption (depending on the assigned dose).

Sample

Given that this was an exploratory study, the sample size was obtained at convenience. A total of 34 INCMNSZ outpatients who fulfilled the inclusion criteria were assessed.

A simple randomization was performed by using a table with random numbers and assigning even numbers to group A (8×10^9 CFU of LcS) and odd numbers to group B (16×10^9 CFU of LcS).

Statistical methods

Descriptive statistics based on the measurement levels of the variables was used, supporting the proportion measures, central tendency and dispersion. A paired t-test was used to compare dependent samples (baseline and final), and a t-test was used to compare independent samples (group A *vs.* group B). For categorical variables, a χ^2 test was used. A $p < 0.05$ was considered significant. The SPSS 16 statistical program was used to perform the data analysis.

Results

Participant flow diagram

A total of 36 patients were invited to participate in the study. Three of them did not fulfill the inclusion criteria, two of them refused to participate, one did not tolerate the fermented dairy product and one claimed personal reasons for not being able to attend the follow-up. A total of 32 patients were included and begun the protocol (fig. 1).

Baseline samples were obtained from a total of 30 patients, which included 14 women and 16 men. Table I shows the assessed variables. A medications registry was evaluated based on each drug's activity, thereby allowing the grouping of them into seven different types of medications, as follows: lipid lowering, in which only statins and fibrates were registered; antihypertensives (calcium channel blockers, beta-blockers and angiotensin-converting enzyme inhibitors were registered); diuretics; calcium carbonate; xanthines in-

hibitors; and supplements, among which only iron and complex B vitamin was registered. Table I also shows the number of patients who used these medications at baseline.

During the follow-up, the adherence to the lactobacillus supplementation and the nutritional plan provided to the patients was evaluated. Sixty nutrition diaries per patient were analyzed. The data were used to estimate the mean energy, protein, carbohydrate and lipid consumption for each patient. From these data, a population mean was obtained, and it was compared with the mean recommended by the nutritionist to obtain the percentage of adequacy. The differences between the recommended energy quantity and the consumed energy during the follow-up were 2,058 ± 197.1 kcal. *vs* 2,071 ± 230.4 kcal. respectively. No statistically significant differences were found, and an adequacy percentage of 101% was observed, reflecting a good adherence to the consumed calories.

When the macronutrient consumption during the follow-up was compared in all patients, no significant differences were found between the recommended quantities in grams and ingested grams. The adequacy percentage between the recommendation and the real consumption was 106% (56.3 g *vs.* 60.14 g, respectively), 102% and 100.1% for proteins, lipids and carbohydrates, respectively. Although these percentages are greater than the recommended values, they are within the range of ± 10% and are thereby considered to indicate good adherence.

No significant differences were found between groups A and B for energy and macronutrient consumption after an eight-week follow-up. Energy (kcal): 2,087 ± 62.92 *vs* 2,057 ± 58.87 grams of protein: 61.97 ± 6.29 *vs* 58.31 ± 4.38 grams of carbohydrates: 333.5 ± 8.03 *vs* 325.3 ± 8.31 and grams of lipids: 58.18 ± 2.52 *vs* 58.74 ± 1.74, respectively.

The mean adherence to the LcS treatment was 97% for group A and 98% for group B with an overall adherence of 98%.

When the final data were obtained from all patients who fulfilled the eight-week follow-up, the baseline and final measurements for the variables under study were compared in the entire population. No significant differences were obtained with the exception of weight and BMI (body mass index) and blood urea (table II).

The effects of LcS on the urea concentrations (fig. 2) and on different variables (table III) were analyzed in groups A and B.

An analysis of the percentage change obtained during the eight-week follow-up was performed. Patients who consumed a dose of 16 x 10^9 CFU showed a greater percentage change when compared with those who consumed only a dose of 8 x 10^9 CFU, which was a difference of -10.98% *vs.* -3.37%, respectively (p = 0.309). Table IV shows the percentage change of the different studied variables.

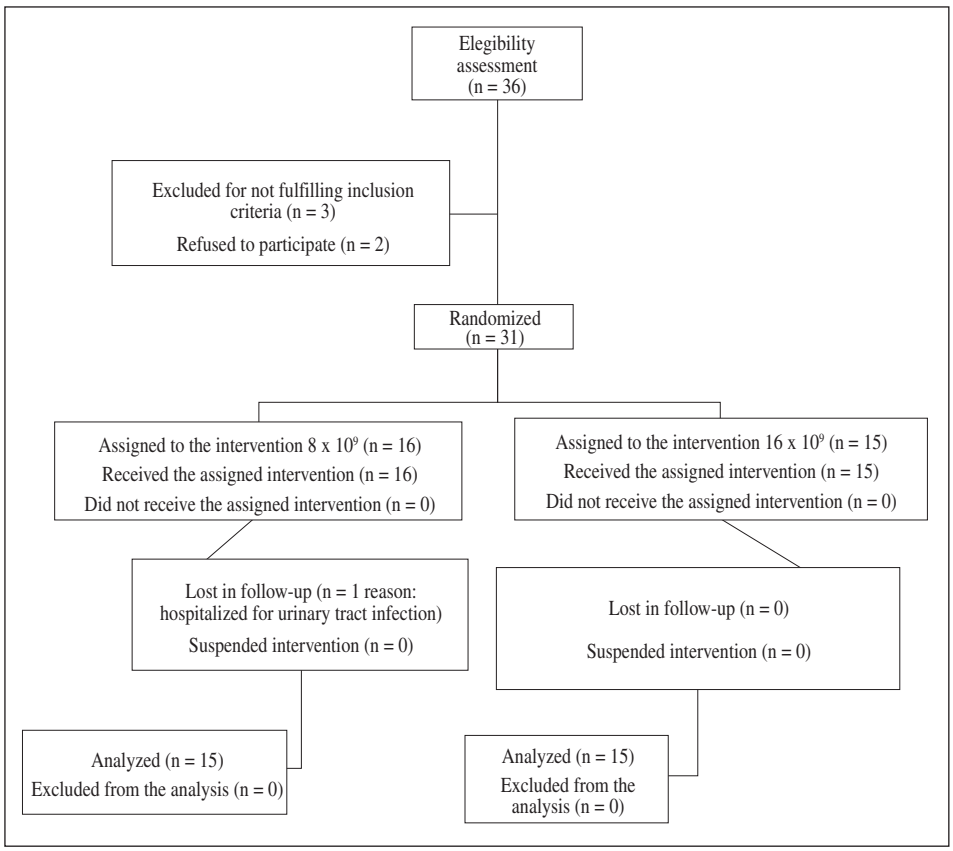

Fig. 1.—Subjects flow diagram through follow-up.

Table I
Baseline characterisics of the population according to the assigned dose

	Total sample n = 30 x ± SD	*Group A dose 8 x 10^9 CFU n = 15 x ± SD*	*Group B dose 16 x 10^9 CFU n = 15 x ± SD*	*p*
Age (years)	41.47 ± 15.35	43.8 ± 14.44	39.13 ± 16.36	n.s.
Weight (kg)	70.68 ± 12.11	69.66 ± 12.8	71.7 ± 12.39	n.s.
BMI (kg/m^2)	26.23 ± 3.36	25.52 ± 3.15	26.93 ± 3.51	n.s.
Height (cm)	163 ± 0.94	164 ± 0.99	162 ± 0.92	n.s.
Sex (F/M)	14/16	7/8	7/8	n.s.
Urea (mg/dL)	81.66 ± 26.39	82.13 ± 32.96	81.20 ± 18.86	n.s.
Creatinine (mg/dL)	2.48 ± 0.89	2.44 ± 0.79	2.52 ± 1.01	n.s.
GFR MDRD (mL/min/1.73 m^2)	30.7 ± 11.77	30.66 ± 12.18	30.74 ± 11.71	n.s.
Medications				n.s.
Statins n (%)	18 (60%)	8 (50%)	10 (71%)	n.s.
Fibrates n (%)	17 (56%)	9 (56%)	8 (57%)	n.s.
Antihypertensives n (%)	27 (90%)	15 (93%)	12 (85%)	n.s.
Diuretics n (%)	22 (73%)	10 (62%)	12 (85%)	n.s.
Calcium carbonate n (%)	12 (40%)	5 (33%)	7 (71%)	n.s.
Xanthines inhibitors n (%)	13 (43%)	7 (43%)	6 (42%)	n.s.
Vitamin and mineral supplements n (%)	19 (63%)	9 (56%)	10 (71%)	n.s.

Table II
Variables measured at baseline and at the end of the follow-up

Parameters	*Baseline measurement n = 30 X ± SD*	*Final measurement n = 30 X ± SD*	*p*
Weight (kg)	70.38 ± 12.11	69.81 ± 12.01	0.013
BMI (kg/m^2)	26.23 ± 3.36	25.90 ± 3.36	0.008
Sex (F/M)	14/16	14/16	n.s.
Urea (mg/dL)	81.66 ± 26.39	73.23 ± 19.49	0.031
Creatinine (mg/dL)	2.48 ± 0.89	2.47 ± 1.04	n.s.
GFR MDRD (mL/min/1.73 m^2)	30.7 ± 11.77	31.86 ± 12.34	n.s.

Discussion

It has been shown that patients with renal diseases have intestinal microbiome alterations. Approximately two thirds of uremic individuals show abnormalities in the gastrointestinal mucosa and a disequilibrium in the intestinal ecosystem.[6] The majority of these changes occur at the level of the ileum and in the colon, where microbiome play an important role. An intestinal microbiome disequilibrium is due to an increase of aerobic bacteria, such as *Escherichia coli*. These bacteria are able to generate toxic substances, known as uremic toxins, that subsequently decrease anaerobic bacteria, such as bifidobacteria and lactobacillus.[7] The majority of the produced fecal ammonium comes from urea hydrolysis by intestinal bacteria. In CRF, there are greater urea concentrations and, consequently, increased ammonium. Thus, there is an increase in pH that promotes the growth of aerobic bacteria in the gastrointestinal tract and the subsequent production of uremic toxins. Bifidobacteria (used as probiotics) ferment carbohydrates and produce acetic and lactic acids to acidify the intestine. Hence, these bacteria prevent the growth of aerobic microorganisms, and they normalize the altered intestinal microbiome in CRF patients.[15,35]

In the present study, an eight-week intervention with LcS was evaluated in 30 patients with stage 3 or 4 CRF. This study is one of the few in this new field of research concerning probiotics and their effect on renal diseases, specifically in patients without replacement therapy. The problem of previous studies,[16,17,25,26] where dialysis also occurred, is the difficulty in evaluating the actual probiotic effect without the dialysis interference. However, the importance of this type of study in CRF patients lies in the benefits that could be obtained if symptoms promoted by the increase of uremic toxins were decreased. Notably, the present study is an exploratory study based on the previous studies by Simenhoff,[24] Taki,[19] Takayama,[26] Dunn[25] and, specifically, Torre and Vargas.[31] The latter study evaluated the effect of LcS on the ammonium concentrations in patients with chronic liver disease. These researchers demonstrated that LcS had a positive effect on decreasing ammonium levels in these patients because ammonium is a urea precursor for which intestinal bacteria are notably involved. LcS was chosen as a good probiotic to be tested in CRF patients with the main objective of establishing a recommended dose for these patients. In fact, only a few reports exist concerning an acceptable dose for each case. This

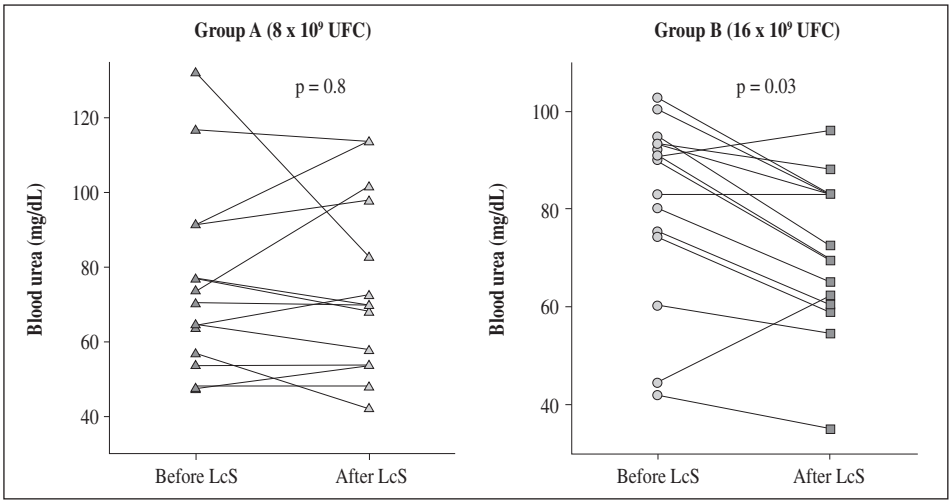

Fig. 2.—The effect of the LcS treatment on the serum urea concentrations.

Table III
Variables assessed at baseline and at the end of the intervention for each group

Parameters	*Group A dose 8 x 10⁹ CFU*			*Group B dose 16 x 10⁹ CFU*		
	Baseline measurement n = 15 X ± SD	*Final measurement n = 15 X ± SD*	*p*	*Baseline measurement n = 15 X ± SD*	*Final measurement n = 15 X ± SD*	*p*
Weight (kg)	69.6 ± 12.18	68.64 ± 12.44	0.038	71.7 ± 12.39	70.98 ± 12.51	n.s.
BMI (kg/m²)	25.52 ± 3.15	25.13 ± 3.03	0.19	26.93 ± 3.52	26.66 ± 3.41	n.s.
Sex (F/M)	7/8	7/8		7/8	7/8	
Urea (mg/dL)	82.13 ± 32.96	75.52 ± 23.06	n.s.	81.20 ± 18.86	70.95 ± 15.62	0.003
Creatinine (mg/dL)	2.44 ± 0.79	2.40 ± 0.76	n.s.	2.52 ± 1.01	2.53 ± 1.29	n.s.
GFR MDRD (mL/min/1.73 m²)	30.66 ± 12.18	31.22 ± 12.44	n.s.	30.74 ± 11.77	31.86 ± 12.34	n.s.

Table IV
Percentage change according to the assigned dose

Variables	*Group A (8 x 10⁹ CFU) (n = 15) X ± SD*	*Group B (16 x 10⁹ CFU) (n = 15) X ± SD*	*p*
Weight %	-1.53 ± 2.27	-1.04 ± 2.86	n.s.
BMI %	-1.51 ± 2.24	0.91 ± 2.76	n.s.
Urea %	-3.37 ± 22.43	-10.98 ± 16.45	n.s.
Creatinine %	0.51 ± 12.62	-2.05 ± 10.76	n.s.
GFR MDRD %	3.28 ± 15.90	4.34 ± 13.01	n.s.

scarcity is observed because the effects of these microorganisms still require further study. Additionally, each strain can function differently, which also hinders the research on dose determinations and the effects of probiotic bacteria.

In the present study, the evaluated doses were the following: 8 x 10^9 and 16 x 10^9 CFU. Although a dose of 24 x 10^9 CFU was used in the study performed by Torre and Vargas,[17] it is important to consider that the results of the present study are the first part of future studies where the effect of LcS on clinical and biochemical parameters and on different toxins will be assessed in CRF patients. The fermented dairy LcS product contains an important quantity of carbohydrates, and the majority of people with CRF also suffer from diabetes mellitus. For these reasons, it was suggested that larger doses could affect the patients' glycemic control. Moreover, the objective was to have a greater external validation in future studies. In the present study, patients with diabetes mellitus were not included. The study population was small, and patients with diabetes mellitus suffer from major comorbidities that could introduce possible confounders when evaluating the dose effect specifically on CRF patients.

When all the population under study was used to assess the LcS effect on the serum urea concentrations, the decrease in this toxin was confirmed. This result coincides with the one reported by Torre and Vargas,[31] where a decrease in serum ammonium concentrations was observed in patients with hepatic cirrhosis. The main difference was the level of decrease. In the case of hepatic patients, the level of decrease was 45%, while the decrease for the renal patients was only 10.98%. This finding could be due to the dose used because, as

mentioned before, Torre and Vargas[31] used a dose of 24 x 10^9 CFU in all patients.

The greatest decrease, of almost 11%, in the patients' serum urea concentrations was observed after the 16 x 10^9 dose. Comparing these results with other corresponding studies that used different types of probiotics, the present LcS results do not seem encouraging. In a study performed by Simenhoff,[24] *lactobacillus acidophilus* was used, and a 67% decrease in the dimethylamine (DMA) concentrations and a 31% decrease in the nitrodimethylamine (NDMA) concentrations (toxins generated in CRF) was achieved for dialysis patients. Nevertheless, it is important to emphasize two points that are directly involved in the decrease of DMA and NDMA. First, both toxins are directly produced in the intestine in a way that the lactobacillus used can have a direct effect on the toxin. Urea is a toxin that comes not only from amino acid oxidation by intestinal bacteria but also from various reactions in the urea cycle where intestinal bacteria are not present. Second, hemodialysis patients receive an additional intervention for the elimination of toxins generated by the CRF. The present study evaluated only patients in stage 3 and 4 such that replacement therapy would not cause any confounding effect.

A tool that could provide a greater credibility to the effect of any probiotic microorganism under study in not only the present study but also in the previous studies is the intestinal bacteria count by fecal microbiology. In this way, the change in intestinal microbiome during the intervention could be assessed, verifying the bacterial overgrowth of the intervention microorganism. This approach could ensure that the effect corresponds to the concrete microorganism and not to another mechanism. This method could serve as a tool in future research.

In other studies, the reduction in uremic toxins with the use of probiotic bacteria was greater than the reduction observed in the present LcS study. These studies with lower doses have found a decrease percentage similar to the one observed by Simenhoff in dialysis patients.[24] Similar to Simenhoff, Dunn[25] used *lactobacillus acidophilus* at a dose of 3 x 10^9 CFU and obtained a 42% decrease in the DMA toxin. Alternatively, Taki[19] and Takayama[26] used *bifidobacterium longum*, and they found a decrease in the indoxyl sulfate toxin, which has also the advantage of being produced directly in the intestine. Takayama[26] observed a 28% reduction using a dose of 3 x 10^9 CFU. Taki[19] is the only investigator who obtained results similar to the ones obtained in this study with LcS. This researcher used three different doses over 12 weeks and observed that a dose of 6 x 10^9 CFU had a greater effect on indoxyl sulfate, achieving a reduction of 9.2%. The variance found in the doses used and the differences in the percentage decrease of toxins suggest the need for further investigation on the effects of probiotic bacteria, their adequate dose and the time by which they must be used. To date, there has been little consistency among the studies with renal patients, which might suggest that each probiotic bacteria is different and specific.

Concerning the evaluated nutritional treatment, we observed a great adherence from all participants in our study, which positively influenced the results. A good diet adherence from all patients in both the 8 x 10^9 and 16 x 10^9 CFU groups permitted a greater homogeneity in variables that could influence the results, mainly including the serum urea concentration, which is significantly affected by protein consumption.

Adherence to the LcS treatment was high, reaching 98%. However, the applied methodology in the present clinical trial was not the best possible. For future studies, an intestinal bacteria quantification to verify the adherence to the probiotic consumption is recommended.

Based on the results obtained to date, it can be concluded that a further investigation on the effects and adequate doses is necessary to prevent and help minimize the production of uremic toxins in CRF patients. In the present study, the 16 x 10^9 CFU dose showed better results, reaching a decrease of almost 11% for the serum urea concentration. This decrease was significant with respect to the baseline value for the urea concentrations. However, it is necessary to assess larger doses to determine whether they have a greater effect on the reduction of urea. Additionally, it is necessary to assess different toxins to determine if a greater reduction could be obtained that could yield a positive impact on uremic symptoms and on complications caused by CRF-generated toxins. The study's sample size was also small, which could account for the lack of differences between the baseline and final values with the evaluated doses.

Conclusions

In patients with stage 3 and stage 4 CRF, there is a greater than 10% decrease in the serum urea concentrations after a conventional dietetic treatment with LcS. A LcS dose of 6 x 10^9 CFU resulted in a greater decrease of the blood urea level.

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