

Short-term effect of a synbiotic in salivary viscosity and buffering capacity; a quasi-experimental study.

Efecto a corto plazo de un simbiótico en la viscosidad y la capacidad tampón salival; Un estudio cuasi experimental.

Yolanda Hernández.¹

Briana Medina.¹

Edith Mendoza¹

Luis Octavio Sánchez-Vargas.²

Diana Alvarado.³

Saray Aranda-Romo.²

Affiliations:

¹Pediatric Dentistry Clinic, School of Dentistry Universidad Autónoma de San Luis Potosí, San Luis, S.L.P., Mexico.

²Laboratory of Biochemical, Microbiology and Pathology, School of Dentistry Universidad Autónoma de San Luis Potosí, San Luis, S.L.P., Mexico.

³Health Sciences School, Universidad del Valle de México campus San Luis Potosí, San Luis, S.L.P., Mexico.

Corresponding author: Saray Aranda-Romo. Laboratory of Biochemical, Microbiology, Pathology, and Diagnostic Clinic, School of Dentistry Universidad Autónoma de San Luis Potosí. Avenida Dr. Manuel Nava No. 2, Zona Universitaria Poniente, San Luis Potosí, S.L.P. México. **Phone:** (52-444) 8262300. **Ext:** 5137 **E-mail:** sarayaranda@fest.uaslp.mx

Receipt : 08/30/2019 **Revised:** 09/19/2019
Acceptance: 04/24/2020

Cite as:

Hernández Y, Medina B, Mendoza E, Sánchez-Vargas L, Alvarado D & Aranda-Romo S.

Short-term effect of a synbiotic in salivary viscosity and buffering capacity; a quasi-experimental study.

J Oral Res 2020; 9(2):98-103

Doi:10.17126/joralres.2020.014

Abstract: Evaluate the effect of a synbiotic on salivary viscosity and buffer capacity. **Materials and Methods:** A follow-up one-week study was performed on 24 healthy volunteers in San Luis Potosí, Mexico, during July 2017. Volunteers must have had active tooth decay at the moment of study. All 24 patients were given a Lactiv® probiotic package, advising not to modify usual oral hygiene practices, and were followed up during 6 days. Primary output variable was salivary viscosity while the secondary was salivary buffer capacity. Salivary viscosity was assessed by using an Ostwald Pipette and buffer capacity with bromocresol purple. **Results:** A total of 8 male patients (33.3%) and 16 females (66.6%) patients were included, with an average age of 10.92 years. All the volunteers completed the study. Comparisons between pre- and post-treatment showed a decrease in salivary viscosity, while buffer capacity was showed to increase. **Conclusion:** The use of a synbiotic during a short period of time lowered the viscosity of saliva and increased salivary buffer capacity.

Keywords: Synbiotics; probiotics; saliva; viscosity, dental caries.

Abstract: Evaluar el efecto de un sinbiótico sobre la viscosidad salival y la capacidad de tampón de la saliva. **Materiales and Métodos:** Se realizó un estudio de seguimiento de una semana en 24 voluntarios sanos en San Luis Potosí, México, durante julio de 2017. Los voluntarios cursaban caries dental activa en el momento del estudio. Los 24 pacientes recibieron un paquete de probióticos Lactiv®, fueron aconsejados a no modificar las prácticas habituales de higiene oral, y fueron seguidos durante 6 días. La variable primaria fue la viscosidad salival mientras que la secundaria fue la capacidad tampón. La viscosidad salival se evaluó usando una pipeta Ostwald y capacidad tampón con bromocresol púrpura. **Resultados:** Se incluyeron un total de 8 pacientes varones (33,3%) y 16 mujeres (66,6%), con una edad promedio de 10,92 años. Todos los voluntarios completaron el estudio. Las comparaciones entre el pretratamiento y el postratamiento mostraron una disminución de la viscosidad salival, mientras que se demostró que la capacidad del tampón aumentó. **Conclusión:** El uso de un sinbiótico durante un corto período de tiempo mostró un efecto sobre la disminución de la viscosidad y el aumento de la capacidad del tampón salival.

Palabra Clave: Sinbióticos; probióticos; saliva; viscosidad; caries dental.

INTRODUCTION.

Childhood tooth decay is an important public health problem worldwide, particularly in developing countries, where prevalence is above 95%.¹ An imbalance in the oral microbiome (also known as dysbiosis) promotes selection and an increase in acid-producing bacteria (*Streptococcus mutans*, *Lactobacillus* spp) and fungus such as *Candida albicans*, which will in turn promote tooth enamel demineralization.² Micro environmental physicochemical factors are strong determinants that favour bacterial growth (of both commensals and pathogens), amongst which stand out:

- a) salivary viscosity,
- b) salivary pH,
- c) presence of proline and mucin-rich glycoproteins (MUC7 and MUC5B), and which differ among individuals according to diet, hydration level and oral hygiene techniques.³

A relation between salivary viscosity and tooth decay incidence has been described, which proposes that an increase in salivary viscosity contributes to the formation of a more adhesive biofilm, decreasing the elimination of acid-producing bacteria.⁴ On the other hand, the saliva's capacity to neutralize acids is crucial to keep an oral microenvironment equilibrium free of tooth decay.⁵ Bacteriotherapy has recently been introduced through use of probiotics, prebiotics and synbiotics.⁶

Probiotics are defined as "alive microorganisms" that, when administered in adequate amounts, provide beneficial effects on the host's health, while a prebiotic is considered and defined as a selectively fermentable ingredient, that allows specific changes on microbiota composition and activity, triggering positive effects in health; thus, a synbiotic is the combination of a pro- and a prebiotic.⁷ A clear conclusion has not been established yet about the effects that synbiotics have on tooth decay prevention. Bafna *et al.*,⁸ evaluated the effect that a yoghurt has on *Streptococcus mutans* counts on a high-risk tooth decay group, finding that yoghurt consumption decreased bacterial levels.

Nozari *et al.*,⁹ used *Bifidobacterium lactis* on a 6-12 years old group during 15 days, however, no significant reduction on bacterial counts was observed. It is important to consider the evaluation with different probiotic strains as well as considering the use of synbiotic in order to decrease *Streptococcus mutans* concentrations, considering changes on the micro-environment that maintains a balanced healthy oral microbioma. Based on

the above, our aim was to assess the effect of a synbiotic on salivary viscosity and buffer capacity.

MATERIALS AND METHODS.

Study design and population

A quasi-experimental study, with no-control group where treatment or intervention was administered to patients on a consecutively way and for convenience, was performed. This study was approved by the Bioethics and Research Committee of the Stomatology School, UASLP (CEI-FE-043016). (Figure 1)

Twenty-four individuals that presented to the dental clinic during July 2017 ranging in age between 5 and 15 years old, healthy, with no allergies and with a tooth decay diagnosis were included. Diagnosis was made through a clinical epidemiologic examination by using the DMFT index, according to WHO criteria. Patients under antibiotic, anticonvulsant, antihistaminic, diuretic, or analgesic treatment, as well as those currently taking probiotics or with any chronic-degenerative pathology were excluded. Informed consent was signed by parents/guardians of the volunteers.

The study was performed during 6 consecutive days in the Odontopediatry Clinics at the Stomatology School, San Luis Potosí Autonomous University (UASLP), in San Luis Potosí, México.

Intervention

Lactiv® (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Bifidobacterium infantis*, and *Streptococcus thermophilus*, Naturex laboratorios.) was administered to all 24 volunteers. Directions about probiotic preparation and administration were given to every patient as follows: package content should be diluted in 50ml of water and taken during the morning before any meal during 6 days.

On day 7.5ml of non-stimulated saliva was collected in the morning between 9:00 and 12:00 in a clean 50ml glass flask. Instructions about teeth brushing were given and no restriction regarding diet was included.

Measurements

The primary outcome variable was salivary viscosity while the secondary was buffer salivary capacity. Two assessments were made: basal measurement on day 0, before which an informed consent was signed, DMFT or DMF index was registered, first saliva collection was taken and directions about treatment were given. From every sample, 5ml of saliva were taken and delivered into glass flasks.

Relative salivary viscosity was assessed by using an Ostwald Pipette, previously calibrated with distilled water at a constant temperature of 37°C.

Running time through glass column was measured for each saliva sample, and average time was used to calculate relative viscosity with the equation:

$$\text{Relative Viscosity} = \frac{(\text{Running time in 5ml of saliva})}{(\text{Running time in 5ml of water})}$$

According to results obtained through this equation, saliva could be classified as: high viscosity (≥ 1.5) or low viscosity (< 1.5).

To determine buffer capacity, a drop of bromocresol purple was mixed into each flask. Secondly, 50 μl of hydrochloric acid was added to each flask until the colour of the solution changed into yellow.

According to the total volume of hydrochloric acid added, salivary buffer capacity was established. A low buffer capacity was considered if a volume between 0 to 150 μl was added, medium capacity from 600 to 900 μl , while a high capacity was considered if more than 1000 μl was added. Final evaluation was made at day 7 by repeating these procedures.

Statistical Analysis

Descriptive statistics were used to analyse the data, by using measures of key trends for continuous variables, while for nominal variables, percentages were used.

Normality was tested by using D'Agostino-Pearson testing and according to results, Wilcoxon signed-rank

test was used to evaluate the efficiency of synbiotic before and after treatment. A p -value < 0.05 was considered as statistically significant.

RESULTS.

Study population included a total of 24 patients. Regarding demographic characteristics, 66.6% ($n=16$) were female and 33.3%⁸ were male, with an average age of 10.92 years.

Oral hygiene practices are shown in Table 1, most of the studied population uses tooth brush and mouth rinse, while dental floss is only used by 12.5% of the patients. After intervention, a statistically significant decrease in salivary viscosity was observed ($p < 0.0001$).

In Figure 2, comparison between pre-and post-treatment with the synbiotic is shown, regarding salivary viscosity variable, where an important improvement is observed. Regarding salivary buffering capacity, it was observed that 58% ($n=14$) showed a low capacity, 41.7% ($n=10$) an intermediate capacity and none of the patients showed a high or very high capacity.

After treatment with synbiotic, all of the studied individuals showed an improvement, from low capacity to medium, high or very high (20.8%, 50% and 29.2% respectively).

Patients who produced high viscosity saliva (54%) had a low buffer capacity before treatment, and afterwards, only 2% remained with these salivary features (low buffer capacity and high viscosity). No adverse secondary effects were detected or reported during the study.

Figure 1. Flow diagram of the study design.

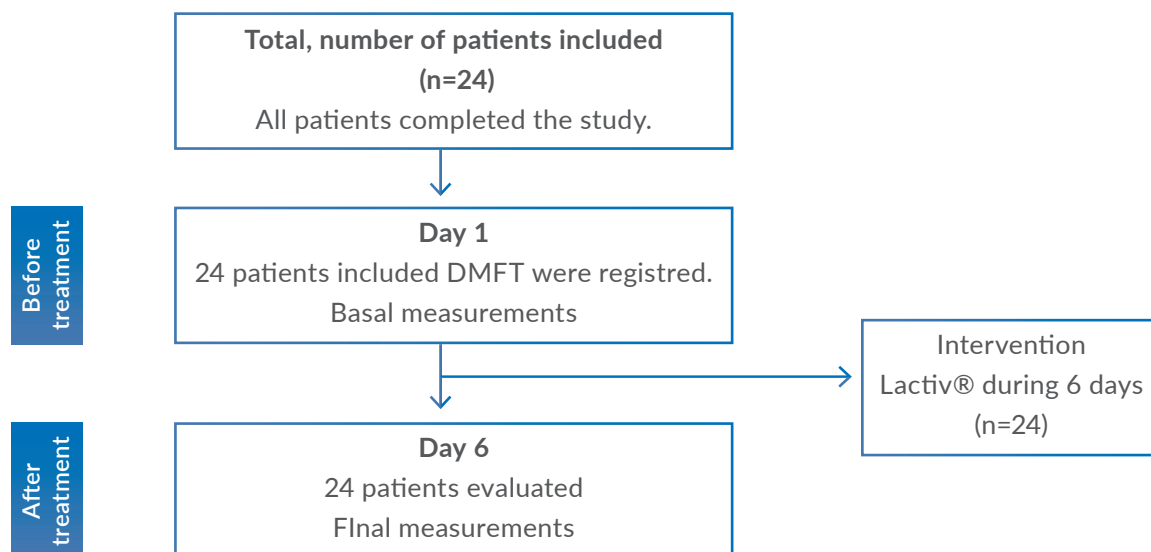


Figure 2. Comparison in viscosity of saliva in children with active caries before and after treatment with a synbiotic.



Median plus range ***: $p=0.0003$. Wilcoxon matched-pairs signed rank test. **iViscosity**: Initial viscosity. **fViscosity**: Final viscosity.

Table 1. Oral hygiene practices in the evaluated population..

Oral hygiene practices	Yes (%)	No (%)	Total (%)
Toothbrush	17 (70.8)	7 (29.2)	24 (100)
Mouthwash	11 (41.7)	13 (58.3)	24 (100)
Floss	3 (12.5)	21 (87.5)	24 (100)

DISCUSSION.

A synbiotic used during a short period of time (6 days) decreased viscosity and increased salivary buffering capacity in children with active tooth decay. High salivary viscosity is a risk factor to develop caries, since bacteria elimination is decreased, thus increasing number of pathogenic bacteria, driving dysbiosis.¹⁰

Salivary viscosity is a feature due to the presence of MUC5B mucin, proteins and glycoproteins, determined by specific host factors like salivary flow, salivary pH and a low intake of liquids.¹¹

A correlation between high salivary viscosity and acidic pH has been observed, which improves growth of acid-producing bacteria, like *Streptococcus mutans*, one of the main bacteria linked to caries.¹⁰

However, assessment of salivary viscosity should be performed under strict protocols since there is a strong

influence of several factors like circadian cycle, feeding regime, coffee intake, stress and smoking habits, thus, a standardization of sample taking was implemented.¹²

Additionally, to preserve physicochemical properties, the saliva samples were kept on ice, and no more than 2 hours elapsed between taking and processing it. On the other hand, salivary viscosity measurements were made through a validated method using a Ostwald pipette.¹³

A decrease in salivary viscosity due to *Lactobacillus* and *Bifidobacterium* administration has been described. This action is due to a modification of proteins that are part of biofilm composition, which improves adhesion and co-adhesion, eliminating pathogens and restoring microbiota equilibrium.¹⁴

In this work, an increase in salivary buffering capacity from low to medium or high was observed after administering the synbiotic. The microbioma of children

with active tooth decay contains high concentrations of acid-producing bacteria, which is highly related to enamel demineralization and a low salivary buffering capacity.

Although the exact molecular mechanisms of action of a synbiotic are largely unknown, proposed mechanisms include:

- a) enhancement of colonization of hard and soft tissues in the oral cavity,
- b) modulation of the immune response and
- c) antagonism of pathogens either by the production of antimicrobial compounds or through competition for mucosal or binding sites.

The enhancement of the colonization in non-shedding surfaces is a unique feature of the mouth such as tooth surfaces and is thought to be mediated by the interaction of microorganism-associated molecular patterns (MAMPs) with specific receptors. The direct antagonist effects of synbiotics on potentially pathogenic species are possibly mediated by competition for nutrients or adherence, and via direct antimicrobial activity.

It is believed that improvements in the physicochemical micro environmental resulting in oral biofilm composition changes, favour an increase in the production of bicarbonate ions, thus increasing pH and salivary buffer capacity.⁶ On the other hand, the inulin-type prebiotic is resistant to salivary enzymes and have no direct effect on the oral microbioma.

However, specific commensal bacterial growth is promoted in the colon; therefore, fructans stimulate the growth of Bifidobacterium species that produce, at low doses, a bifidogenic effect, increasing colonic bacteria density and improving conditions in the whole gastrointestinal tract from mouth to anus.¹⁵

The quasi experimental design in our study allowed us to show how an intervention using a synbiotic in children with active caries was able to improve salivary composition. Future studies should include a placebo group or another experimental group that could help confirm efficacy of synbiotic on salivary composition.

CONCLUSION.

Daily administration of a synbiotic during six days decreases salivary viscosity and increases the buffering capacity of saliva.

Conflict of interests: Authors have no conflict of interest with this report.

Ethics approval: This study was approved by the Bioethics and Research Committee of the Stomatology School, UASLP (CEI-FE-043016).

Funding: Financial support for this study was provided by Fondo de apoyo a la investigación FAI C17-FAI-06-53.53 and own resources.

Authors' contributions: All authors contributed to this manuscript.

Acknowledgements: The authors wish to thank Q.F.B Virginia Flores Gutiérrez for her cooperation in the laboratory experiments.

REFERENCES.

1. Villavicencio J, Arango MC, Ordonez A, Contreras A, Villegas LM. Early childhood caries, salivary and microbiological aspects among 3- to 4-year-old children in Cali, Colombia. *Eur Arch Paediatr Dent.* 2018;19:347-52.
2. Marsh PD. In Sickness and in Health-What Does the Oral Microbiome Mean to Us? An Ecological Perspective. *Adv Dent Res.* 2018;291:60-5.
3. Animireddy D, Reddy Bekkem VT, Vallala P, Kotha SB, Ankireddy S, Mohammad N. Evaluation of pH, buffering capacity, viscosity and flow rate levels of saliva in caries-free, minimal caries and nursing caries children: An in vivo study. *Contemp Clin Dent.* 2014;5:324-8.
4. Kaur A, Kwatra KS, Kamboj P. Evaluation of non-microbial salivary caries activity parameters and salivary biochemical indicators in predicting dental caries. *J Indian Soc Pedod Prev Dent.* 2012;30:212-7.
5. Pyati SA, Naveen Kumar R, Kumar V, Praveen Kumar NH, Parveen Reddy KM. Salivary Flow Rate, pH, Buffering Capacity, Total Protein, Oxidative Stress and Antioxidant Capacity in Children with and without Dental Caries. *J Clin Pediatr Dent.* 2018;7:357-65.
6. Caglar E, Kargul B, Tanboga I. Bacteriotherapy and probiotics' role on oral health. *Oral Dis.* 2005;11:131-7.
7. De Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. *Adv Biochem Eng Biotechnol.* 2008;111:1-66.
8. Bafna HP, Ajithkrishnan CG, Kalantharakath T, Singh RP, Kalyan P, Vathar JB, Patel HR. Effect of Short-term Consumption of Amul Probiotic Yogurt Containing *Lactobacillus acidophilus* La5 and *Bifidobacterium Lactis* Bb12 on Salivary *Streptococcus mutans* Count in High Caries Risk Individuals. *Int J Appl Basic Med Res.* 2018;8:111-5.
9. Nozari A, Motamedifar M, Seifi N, Hatamizargaran Z, Ranjbar MA. The Effect of Iranian Customary Used Probiotic Yogurt on the Children's Salivary Cariogenic Microflora. *J Dent Shiraz Iran.* 2015;162:81-6.
10. Aminabadi NA, Najafpour E, Razavi Rohani Z, Sighari Deljavan A, Ghojzadeh M, Jamali Z. Linear reciprocal interaction between dental caries and salivary characteristics. *J Oral Sci.* 2013;554:337-32.
11. Ueno M, Takeuchi S, Takehara S, Kawaguchi Y. Saliva viscosity as a potential risk factor for oral malodor. *Acta Odontol Scand.* 2014;728:1005-9.
12. Nieuw Amerongen AV, Ligtenberg AJM, Veerman ECI. Implications for diagnostics in the biochemistry and physiology of saliva. *Ann N Y Acad Sci.* 2007;1098:1-6.
13. Ortega Pantaleón ME, Calzado Suárez M, Pérez Marqués M. Evaluación del flujo y viscosidad salival y su relación con el índice de caries. *Medisan.* 1998;2:33-9.
14. Twetman S, Keller MK. Probiotics for caries prevention and control. *Adv Dent Res.* 2012; 24:98-102.
15. Kelly G. Inulin-type prebiotics--a review: part 1. *Altern Med Rev J Clin Ther.* 2008;13:315-29.
16. Haukioja A, Loimaranta V, Tenovuo J. Probiotic bacteria affect the composition of salivary pellicle and streptococcal adhesion in vitro. *Oral Microbiol Immunol.* 2008;23:336-43.
17. Dassi E, Ferretti P, Covello G, Bertorelli R, Denti MA, et al. The short-term impact of probiotic consumption on the oral cavity microbiome. *Sci Rep.* 2018;8:10476.