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ARTÍCULO ORIGINAL

Safety of a dual potential prebiotic system from Mexican agave "Metlin® and Metlos®", incorporated to an infant formula for term newborn babies: a randomized controlled trial

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ABSTRACT

Rationale. Infant formulae are being supplemented with probiotics, prebiotics, or symbiotic despite uncertainties regarding their efficacy. Mexican agave is an interesting source of fructans with particular features and with potential prebiotic effects. **Material and methods.** RCT in 600 healthy term babies (20 ± 7 days), allocated to receive standard infant formula (control) or infant formula added with a dual prebiotic system "Metlin® and Metlos®", from Mexican agave. Primary outcomes include stools frequency, stools consistency, gastrointestinal intolerance (frequency of abdominal distension, flatulency, regurgitations, vomiting). Secondary outcomes include changes on weight and height along the study and frequency of dermatologic problems (eczema). **Results.** In 66,120 days of total follow-up, there were no differences on the frequency of stools passage (Human Milk: 3.8 ± 2.4 evacuations per day; Pro + Metlin + Metlos 3.6 ± 2.0; Pro + Metlin 3.6 ± 2; only Pro 3.4 ± 2.3¸ only formula 3.4 ± 2.0; p NS). Consistency of stools was similar between human milk and prebiotics supplemented groups. Also the frequency of gastrointestinal symptoms was significantly low between these groups. **Conclusions.** Fructans derivate from agave and added to infant formula are safe and well tolerated by Mexican healthy term babies.

Seguridad de un sistema de fibra derivada del agave con potencial acción prebiótica incorporado a una fórmula infantil en recién nacidos de término

RESUMEN

Justificación. *Las fórmulas infantiles se han suplementado con probióticos, prebióticos o simbióticos, a pesar de que continúan existiendo dudas acerca de la seguridad y eficacia de los mismos. El agave mexicano es una fuente de fructanos con características particulares y con potencial efecto prebiótico.* ***Material y métodos.*** *Ensayo clínico controlado (RCT) realizado en 600 recién nacidos de término sanos (20 ± 7 días), aleatorizados a recibir una fórmula infantil estándar (control) o una fórmula adicionada con mezcla variable de prebióticos derivados del agave mexicano. Los desenlaces primarios fueron la frecuencia y consistencia de las evacuaciones, los datos de tolerancia gastrointestinal (distensión abdominal, flatulencia, regurgitaciones y vómito). Los desenlaces secundarios fueron la ganancia pondo-estatural y la frecuencia de eczema.* ***Resultados.*** *En el análisis de 66,120 días de seguimiento no se observaron diferencias en la frecuencia diaria de las evacuaciones (leche materna 3.8 ± 2.4 evacuaciones/día; Pro + Metlin + Metlos 3.6 ± 2.0; Pro + Metlin 3.6 ± 2; fórmula sólo con probióticos 3.4 ± 2.3 y sólo fórmula 3.4 ± 2.0; p NS). La consistencia de las evacuaciones fue similar entre los grupos de leche materna y quienes recibieron la mezcla de prebióticos. La frecuencia de manifestaciones gastrointestinales fueron significativamente menores en estos mismos grupos.* ***Conclusiones.*** *La adición de fructanos derivados del agave mexicano en las fórmulas infantiles*

es segura y bien tolerada por los recién nacidos sanos mexicanos.

Key words. Metlin and Metlos. Agave fructans safety. Children.

Palabras clave. *Metlin y Metlos. Seguridad de los fructanos de agave. Niños.*

BACKGROUND

Infant formulae are increasingly being supplemented with probiotics, prebiotics, or symbiotic despite uncertainties regarding their efficacy.[1-4] In 2004 a position papers published by the Committee on Nutrition of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition, commented on the addition of prebiotic oligosaccharides to infant and follow-on formulae.[5] On the basis of evidence obtained in a search up to January 2004, the Committee concluded that only limited studies have evaluated the effects of the addition of prebiotic substances to dietetic products for infants. The Committee stated that although the administration of prebiotic oligosaccharides has the potential to increase the total number of bifidobacteria in feces and may also soften stools; there was until that moment no published evidence of any clinical benefits of adding prebiotic oligosaccharides to dietetic products for infants. Of note, according to the Commission Directive 2006/141/ EC of 22 December 2006 on infant formulae and follow-on formulae, fructooligosaccharides (FOS) and galactooligosaccharides (GOS) may be voluntarily added to infant formulae if their content does not exceed 0.8 g/100 mL. In 2010 the same Committee carried out a systematic review of randomized controlled trials (RCT) according to the guidelines from the Cochrane Collaboration. Only those studies that compared the use of infant formula or follow-on formula supplemented with prebiotics during the manufacturing process were included.[6] Twenty-three publications were identified for analysis.[7-29] All of the studies were carried out in healthy term infants. The studies varied in the types of prebiotics used. The most commonly studied prebiotic was a 9:1 mixture of short-chain galactooligosaccharides (scGOS) and long-chain fructooligosaccharides (lcFOS). Other prebiotics studied were GOS, acidic oligosaccharides (AOS), GOS/FOS/AOS, oligofructose plus inulin, and polydextrose (PDX) plus GOS (with or without lactulose). The doses of prebiotics ranged from 0.15-to 0.8-g/100 mL, and the duration of the intervention ranged from 2 weeks to 6 months. All but 2 RCT (which used follow-on formula) reported the prebiotic supplementation of a standard infant formula. The evidence-based findings, reported in this paper were that prebiotic supplementation of infant formula:

- Has no adverse effects on growth or adverse effects in healthy term infants.
- Has the potential to reduce fecal pH.
- Has the potential to increase stool frequency and soften stools, and
- Increase the stool colony counts of bifidobacteria or lactobacilli.

The Mexican Agave plant (Tequilana Weber Blue Variety) is an interesting source of fructans (non inulin-like), which are formed by a complex mix of FOS with a DP between 3 to 29, containing principally β (2 $\rightarrow$ 1) linkages, but also β (2 $\rightarrow$ 6) and branch moieties.[30,31] Indeed, it had been demonstrated the bifidogenic and physiologic effects of these fructans *in vitro* and in animal models.[31,32] Considering this evidence, we conducted an RCT in order evaluate the safety of "Metlin® and Metlos®", a dual fructan system from Mexican agave, incorporated to an infant formula for term Mexican newborn babies.

MATERIAL AND METHODS

Randomized double blind clinical controlled trial (RDBCT), approved by Research Ethical Committee of the Instituto Nacional de Pediatría (INP), México, conducted in 600 term babies, 20 $\pm$ 7 days old at study admission, any gender, weight of 2,490 g or more at born in apparently good health, without medical history of maternal pathologies during pregnancy. The sample size was calculated, considering the primary outcomes, an α error of 0.05, a β error of 0.10 and a 20% of attrition. In order to be enrolled in the study, Mexican women at $\approx$ 30 weeks of gestation were invited to assist to INP after their children were born. Those women were regular patients of medical centers at the south part of Mexico City. Infants were eligible for the study if they met the following criteria:

- The infant was clinically healthy.
- The infant was term-born.
- Age $\leq$ 27 days.
- Weight $\geq$ 2,490 g.

- No allergic response to cows milk, and
- Signed written consent.

The major exclusion criteria were:

- Evidence of heart, respiratory, gastrointestinal, hematological or metabolic diseases.
- Mother had a medical history of diabetes (gestational diabetes was accepted if the infant weight at born was ≤ to that of percentile 95).
- Infant was product of a multiple delivery (twins, triplets, etc.).

Sealed envelopes were prepared containing the sequence of treatment assignation, which were obtained using random allocation software version 1.0.1, through a balanced blocking process. Products under research were coded with seven characters printed in the outside of the box. Neither the researcher, co-investigators, Nekutli personnel, nor parents, were informed about the identity of research products. The study personnel did not make any analysis to identify the study products. The principal researcher was assured that, if necessary, the blinded would be broken. Infant formula does not contain prebiotics and probiotics, but fulfills the nutrient levels for infants according to the regulations described by Codex Alimentarius Comission on ALINORM 06/29/26, and is regularly commercialized in our country. Metlin®, Metlos® and probiotic were added in powder to the infant formula (Table 1) using a V-Mixer in batches of 200 kg. Mixer speed was constant and time of mix was of 40 min (determined by segregation tests). All steps were made on sterile conditions and cans were sealed following the same methods used by the industries of infant formulas. The dual potential prebiotic system are fructans obtained from *Agave tequilana* var Weber with β (2 → 1) and β (2 → 6) linked fructofuranosyl units, resulting in branched molecules of high solubility. Metlos contains more monosaccharides and disaccharides than Metlin. Metlos has an average DP of 15, 55% > DP10 > 45% and polydispersion index of 3.3, whereas Metlin has an average DP of 27, 84.5% > DP10 > 15.5% and polydispersion index of 2.3. Compared with other fructans previously studied (e.g. those obtained from chicory), Metlin and Metlos show branched molecules instead of linear ones of inulin and inulin-like molecules, given them higher solubility and different specificity for enzymatic hydrolysis.

Parents were instructed to administrate the formula *ad libitum* as the only nutritional source, until visit 4. After visit 4, there was no restriction about the complementary food the participant could receive. In case of acute diarrhea or dehydration, the participants could use rehydration therapy without prebiotics. It was recommended not to use any other drug that could modify the outcomes. To collect the data of the participants, a Case Report Form (CRF) was given and explained to each mother since the first clinical visit. Babies were evaluated monthly, until they reach six months old. Primary outcomes include stools frequency, stools consistency, gastrointestinal intolerance (frequency of abdominal distension, flatulency, regurgitations, vomiting). Secondary outcomes include the changes on weight and height along the study and the

Table 1. Infant formula® composition.

Ingredient content in 100 mL	Probiotics + Metlin + Metlos	Probiotics + Metlin	Probiotics + Metlos	Only probiotics	Only formula
Energy (Kcal)	53	53	53	53	53
Protein (g) (75% whey)	1.4	1.4	1.4	1.4	1.4
Fat (g) (42% sunflower; 30% coconut; 28% soy oil)	3	3	3	3	3
Carbohydrates	6	6	6	6	6
Lactose (g)	0.2	0.5	0	0	0
Metlin® (g)	0.3	0	0.5	0	0
Metlos® (g)	7	7	7	7	7
Nucleotides (mg)					
Lactobacillus GG (CUF) 107	0.3	0.3	0.3	0.3	0
DHA (g)*	0.006	0.006	0.006	0.006	0.006
AA (g)**	0.03	0.03	0.03	0.03	0.03

* DHA: docosahexaenoic acid. ** AA: araquidonic acid.

frequency of dermatologic problems (eczema). Measurements (arm circumference, thickness of cutaneous fold, etc.) were done by personnel previously standardized, using the methods of Lothman[33] and Habicht.[34] All available data from participants were included in an intention to treat analysis (ITT) using STATA for Mac, version 11. Variables at admission were contrasted by Chi squared, ANOVA or Kruskall Wallis Test. Primary and Secondary outcomes were also compared with Chi square for categorical variables or by ANOVA or Kruskall Wallis for numeric ones. If necessary a covariant adjustment with ANCOVA or trough a logistic regression for categorical outcomes was made. A $p \leq 0.05$ value was considered as significant.

RESULTS

From a total of 937 potentially eligible babies, 187 (20%) had at least one exclusion criterion. In 150, their parents did not accept to participate in the study. Thus, the final sample size for the study was conformed by 600 babies. Allocation process divided the sample in six groups (group 1: formula added with probiotics + Metlin + Metlos. Group 2: formula added with probiotics + Metlin. Group 3: formula added with probiotics + Metlos. Group 4: formula added only with probiotics. Group 5: Standard Formula without probiotics or prebiotics. Group 6: human Milk). There were not significant differences between groups according to the age at the moment of entry the study, the distribution by gender, gestational age, APGAR history, weight or height at birth (Table 2). No significant differences between groups were observed about weight, height, medium-arm circumference (MAC) and skinfold thickness (Table 3). Human milk group was fed 9.7 ± 2.7 times in 24 h. There were not differences between the infant formula groups according to the amount of ingested infant formula (Table 4). In relation to gastrointestinal intolerance, we are reporting a total of 66,120 days of monitoring, considering the 600 babies, the days they were in the study and the frequency of stools and gastrointestinal events. There were no differences on the frequency of stools passage between groups (Human Milk: 3.8 ± 2.4 evacuations per day; Pro + Metlin + Metlos 3.6 ± 2.0; Pro+Metlin 3.6 ± 2; only Pro 3.4 ± 2.3, only formula 3.4 ± 2.0; p NS). Consistency of stools was similar between Human milk and Metlin + Metlos groups (Table 5). There were significant differences related to gastrointestinal symptoms with less frequency in human milk and Metlin + Metlos group (Figure 1). The weight and height gain were similar between groups (Figures 2 and 3). There were no differences among the groups related to eczema, considering the 66,200 days of follow-up (Breast Milk: 9.9%; Pro + Metlin + Metlos 7.9%; Pro + Metlin 6.6%; Pro + Metlos 7.2%; only Pro 10.9%; only Formula 8.7%, p NS).

DISCUSSION

Inulin (long chain oligosaccharides or IcFOS), short chain oligosaccharides (scFOS), transgalactic-oligosaccharides (also called galacto-oligosac-

Table 2. Basal characteristics of children.

Variable	Probiotics + Metlin + Metlos (n = 93)	Probiotics + Metlin (n = 93)	Probiotics + Metlos (n = 89)	Only probiotics (n = 89)	Only formula (n = 89)	Human Milk (n = 147)
Age [days] (x ± ds)	14.2 ± 6.3	14.0 ± 4.2	14.6 ± 6.4	14.8 ± 6.5	14.0 ± 5.8	14.8 ± 5.2
Males (%)	45	46	57	59	56	54
Gestational age (x ± ds)	38.6 ± 1.5	38.1 ± 5.4	39.0 ± 1.3	37.2 ± 7.5	38.9 ± 1.4	38.4 ± 4.3
APGAR (x ± ds)	8.9 ± 0.29	9.0 ± 0.20	8.9 ± 0.33	8.8 ± 0.44	8.9 ± 0.36	8.9 ± 0.36
Birth weight [g] (x ± ds)	3,102 ± 341	3,041 ± 407	3,087 ± 352	3,015 ± 405	2,997 ± 378	3,038 ± 409
Birth length [cm] (x ± ds)	49.4 ± 7.0	48.9 ± 6.9	47.9 ± 9.8	49.1 ± 2.3	49.4 ± 2.1	48.3 ± 7.7

Table 3. Basal anthropometric variables.

Variable	Probiotics + Metlin + Metlos (n = 93)	Probiotics + Metlin (n = 93)	Probiotics + Metlos (n = 89)	Only probiotics (n = 89)	Only formula (n = 89)	Human Milk (n = 147)
Weight [g] (x ± ds)	3,070 ± 650	3,320 ± 440	3,370 ± 460	3,330 ± 510	3,280 ± 640	3,250 ± 460
Length [cm] (x ± ds)	49.7 ± 2.27	50.7 ± 2.19	50.8 ± 2.05	50.7 ± 2.22	50.0 ± 2.76	50.3 ± 2.06
MAC ϕ [cm] (x ± ds)	9.8 ± 0.95	10.1 ± 0.94	9.9 ± 0.91	10.3 ± 0.94	10.0 ± 1.67	9.9 ± 1.07
Tricipital [mm] (x ± ds)	4.1 ± 0.86	4.4 ± 1.16	4.4 ± 0.61	4.3 ± 1.11	4.4 ± 1.02	4.3 ± 1.23
Bicipital [mm] (x ± ds)	5.5 ± 1.13	5.8 ± 1.03	5.4 ± 0.83	5.8 ± 1.22	5.7 ± 1.21	5.6 ± 1.07
Subscapular [mm] (x ± ds)	5.8 ± 1.38	6.3 ± 1.36	5.8 ± 1.34	6.1 ± 1.67	6.2 ± 1.67	5.7 ± 1.54
Suprailiac [mm] (x ± ds)	4.0 ± 1.11	4.2 ± 1.02	4.1 ± 0.83	4.3 ± 1.04	4.1 ± 0.90	4.1 ± 0.97

ϕ : medium arm circunference.

Table 4. Volume of ingested formula per day.

Month of follow-up (x ± ds)	Probiotics + Metlin + Metlos (n = 93)	Probiotics + Metlin (n = 93)	Probiotics + Metlos (n = 89)	Only probiotics (n = 89)	Only formula (n = 89)
1-2	740 ± 67	801 ± 43	711 ± 72	770 ± 36	810 ± 51
3-4	1180 ± 109	1206 ± 139	1170 ± 89	1240 ± 112	1165 ± 89
5-6	1510 ± 185	1423 ± 119	1470 ± 108	1456 ± 187	1498 ± 196

Table 5. Stool consistency (analysis of 187,070 evacuations during 66,200 monitoring days).

Group	Stools (n)	Liquid or semiliquid (%)	Pasty or semipasty (%)	Coprolitic or hard (%)
Breast Milk	65,112	34.7	65.1	0.2
Pro+Metlin+Metlos	30,334	38.0	61.9	0.1
Pro+Metlin	28,190	38.7	59.0	2.3
Pro+Metlos	27,489	31.9	64.9	3.2
Probiotics only	19,214	23.5*	76.4	0.1
Formula only	16,731	22.4*	76.9	0.7

charides or GOS) and the lactulose, have been added to several foods.[35,36] The inulin-like fructans as IcFOS/scGOS, have been widely used in infants and, in general terms, most of the evidence leads to the fact that they show an acceptable range of safety, and considerable bifidogenic effect.[37] Related to the safety aspects, although there is some concern by the existence of the possibility of adverse events related with the regular and periodic consumption of prebiotics, the exhaustive analysis of the evidence does not give a consistent evidence about some kind of adverse events associated to the use of prebiotics in humans, independently of age of the participants. Ziegler, *et al.*, reported in 2007 an increase of frequency in diarrhea events in the infant group that received a supplemented formula with 4 g/L of a mixture with polidextrose and GOS (50:50) or 8 g/L of a polidextrose, GOS and lactulose mixture (50:30:17),

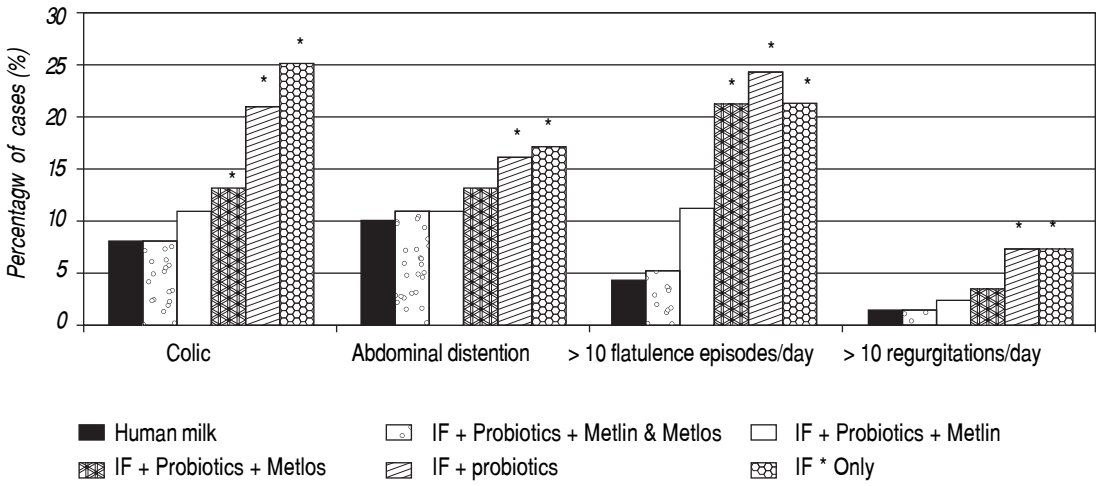

*Figure 1. Gastrointestinal adverse events. IF: infant formula. *p < 0.05.*

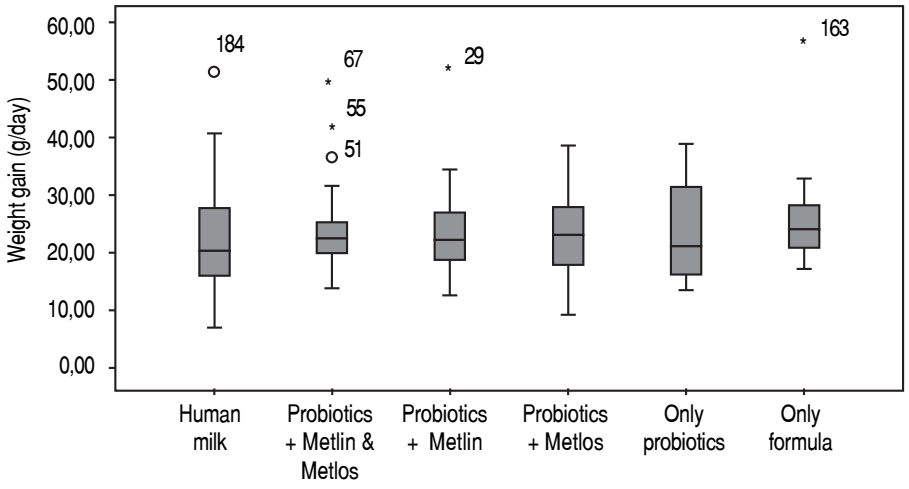

Figure 2. Dairy weight gain during the study.

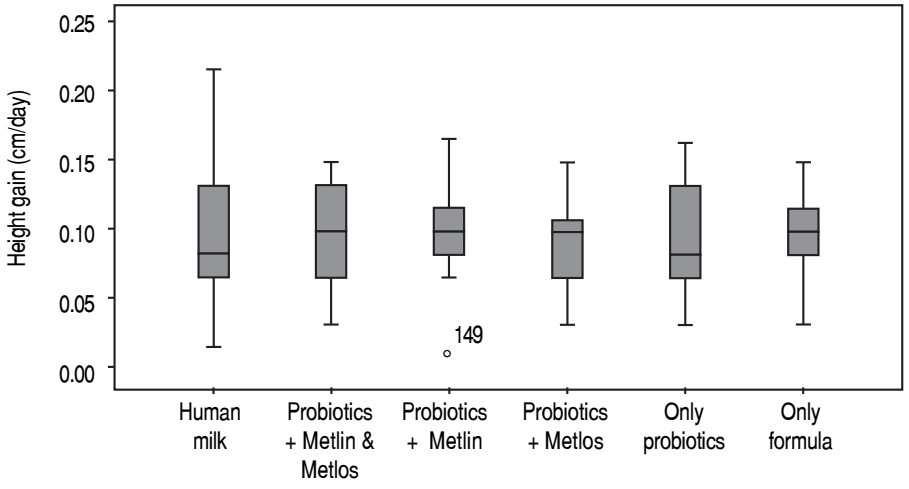

Figure 3. Dairy height gain during the study.

compared with the group without prebiotics (18 *vs.* 4%), the rest of the recently published studies have not identified yet this potential association.[38] Nonetheless, it is important to test the fibers added to foods in order to guaranty the safety to use them in population, especially in children that is a susceptible group. In our study, according to the identified results in the infant cohort, we observed that the frequency of bowel movements in the total monitored days, as well as the consistency of the feces were similar among the groups fed with a formula enriched with fructans (independently of the mixture). Moreover, there were no differences in relation with the group exclusively fed with breast milk. Such results allow us to conclude, in relation with these aspects, that the addition of this kind of fructans derived from the Agave in the infant formula, is a safe strategy of effective supplementation in terms of gastrointestinal health. The aforementioned is supported with the analyses of other gastrointestinal symptoms (vomit, regurgitations, abdominal distention or flatulence), which were similar among the groups. In the same study of Ziegler, the authors reported a higher incidence of eczema in the group supplemented with 4 g/L of prebiotics in relation with the control group (18 *vs.* 7%, p 0.046). In our study the proportion of dermatological problems of this type were similar among all the analyzed groups (including the group of breast feeding). The results in this study allow us to support the use of the fructans Metlin and Metlos as safety soluble fiber with potential as functional bioactive ingredients and with a demonstrated safety similar to that showed by others with GOS and SCFOS (Chouraqui, *et al.*, 2008).[39] Indeed, diminution of colic in those groups fed with infant formula added with agave fructans supports the idea for a prebiotic effect; increasing the beneficial bacteria and with replacement of pathogenic bacteria, which improves the intestinal movements and has direct effect on the visceral nerve fibers related with pain (Savino, *et al.,* 2006).[40] The importance to our country on using fructans derived from agave as dietetic fibers in the foods, is highlighted because the crops of *Agave tequilana* Weber var. *azul* are very extended in several regions of Mexican fields; moreover, those fructans show high solubility and very low glycemic index, conditions that are very convenient to add them to almost any food. One of the most important parts of a clinical trial (and the first) is to demonstrate the safety of the products on study. That was why the principal aim of this work was to test the safety of these fructans in children. However, there are some studies, which stand the efficacy of these fructans in animals and *in vitro* models (Rivera-Huerta, *et al.,* unpublished data; Koenen and Venema, unpublished data). Shortly, we will reporting the efficacy of this dual system of fructans derived from Mexican agave, it will be after a complex but concise analysis of the biological samples related to the before and after the use of supplemented formulas on these Mexican infants.

REFERENCES

1. Norwegian Scientific Committee for Food Safety. Panel on Biological Hazards & Panel on Nutrition, Dietetic Products, Novel Food and Allergy. Risk assessment on use of Lactobacillus rhamnosus (LGG) as an ingredient in infant formula and baby foods (II). Available from: www.vkm.no/dav/63bb45d3eb.pdf [Accessed November 16, 2010].
2. Norwegian Scientific Committee for Food Safety. Panel on Nutrition, Dietetic Products, Novel Food and Allergy. Risk assessment on use of Lactobacillus rhamnosus (LGG) as an ingredient in infant formula and baby foods (opinion). Available from: www.vkm.no [Accessed November 16, 2010].
3. European Food Safety Authority. Scientific opinion on the substantiation of a health claim related Immunofortis and strengthening of the baby's immune system pursuant to Article 14 of Regulation (EC) No 1924/2006. *EFSA J* 2010; 8: 1430. Available from: http://www.efsa.europa.eu/en/scdocs/doc/1430.pdf [Accessed November 16, 2010].
4. Koletzko B, Bohles H, Fusch C, et al. Recommendations on pre- and probiotics in infant formula [in German]. *Monatsschr Kinderheilkd* 2009; 157: 267-70.
5. ESPGHAN Committee on Nutrition. Prebiotic oligosaccharides in dietetic products for infants: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2004; 39: 465-73.
6. Braegger C, Chmielewska A, Decsi T, Kolacek S, Mihatsch W, Moreno L, van Goudoever JB. Supplementation of infant formula with probiotics and/or prebiotics: A systematic review and comment by the ESPGHAN Committee on Nutrition. Journal of Pediatric Gastroenterology and Nutrition 2011; 52: 238-50.
7. Bakker-Zierikzee AM, Alles MS, Knol J, et al. Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable Bifidobacterium animalis on the intestinal microflora during the first 4 months of life. *Br J Nutr* 2005; 94: 783-90.
8. Bakker-Zierikzee AM, Tol EA, Kroes H, et al. Faecal SIgA secretion in infants fed on pre- or probiotic infant formula. *Pediatr Allergy Immunol* 2006; 17: 134-40.
9. Brunser O, Figueroa G, Gotteland M, et al. Effects of probiotic or prebiotic supplemented milk formulas on fecal microbiota composition of infants. *Asia Pac J Clin Nutr* 2006; 15: 368-76.
10. Alliet P, Scholtens P, Raes M, et al. Effect of prebiotic galactooligosaccharide, long-chain fructo-oligosaccharide infant formula on serum cholesterol and triacylglycerol levels. *Nutrition* 2007; 23: 719-23.
11. Arslanoglu S, Moro GE, Boehm G. Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first 6 months of life. *J Nutr* 2007; 137: 2420-4.
12. Arslanoglu S, Moro GE, Schmitt J, et al. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces

the incidence of allergic manifestations and infections during the first two years of life. *J Nutr* 2008; 138: 1091-5.
13. Ben XM, Zhou XY, Zhao WH, et al. Supplementation of milk formula with galacto-oligosaccharides improves intestinal micro-flora and fermentation in term infants. *Chin Med J (Engl)* 2004; 117: 927-31.
14. Ben XM, Li J, Feng ZT, et al. Low level of galacto-oligosaccharide in infant formula stimulates growth of intestinal Bifidobacteria and Lactobacilli. *World J Gastroenterol* 2008; 14: 6564-8.
15. Bettler J, Euler AR. An evaluation of the growth of term infants fed formula supplemented with fructo-oligosaccharide. *Int J Probiotics Prebiotics* 2006; 1: 19-26.
16. Brunser O, Gotteland M, Cruchet S, et al. Effect of a milk formula with prebiotics on the intestinal microbiota of infants after an antibiotic treatment. *Pediatr Res* 2006; 59: 451-6.
17. Costalos C, Kapiki A, Apostolou M, et al. The effect of a prebiotic supplemented formula on growth and stool microbiology of term infants. *Early Hum Dev* 2008; 84: 45-9.
18. Decsi T, Arato' A, Balogh M, et al. Randomised placebo controlled double blind study on the effect of prebiotic oligosaccharides on intestinal flora in healthy infants [in Hungarian]. *Orv Hetil* 2005; 146: 2445-50.
19. Fanaro S, Jelinek J, Stahl B, et al. Acidic oligosaccharides from pectin hydrolysate as new component for infant formulae: effect on intestinal flora, stool characteristics, and pH. *J Pediatr Gastroenterol Nutr* 2005; 41: 186-90.
20. Haarman M, Knol J. Quantitative real-time PCR assays to identify and quantify fecal Bifidobacterium species in infants receiving a prebiotic infant formula. *Appl Environ Microbiol* 2005; 71: 2318-24.
21. Knol J, Scholtens P, Kafka C, et al. Colon microflora in infants fed formula with galacto- and fructo-oligosaccharides: more like breast-fed infants. *J Pediatr Gastroenterol Nutr* 2005; 40: 36-42.
22. Magne F, Hachelaf W, Suau A, et al. Effects on faecal microbiota of dietary and acidic oligosaccharides in children during partial formula feeding. *J Pediatr Gastroenterol Nutr* 2008; 46: 580-8.
23. Moro G, Minoli I, Mosca M, et al. Dosage-related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. *J Pediatr Gastroenterol Nutr* 2002; 34: 291-5.
24. Moro GE, Mosca F, Miniello V, et al. Effects of a new mixture of prebiotics on faecal flora and stools in term infants. *Acta Paediatr Suppl* 2003; 91: 77-9.
25. Moro G, Arslanoglu S, Stahl B, et al. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Child* 2006; 91: 814-9.
26. Nakamura N, Gaskins HR, Collier CT, et al. Molecular ecological analysis of fecal bacterial populations from term infants fed formula supplemented with selected blends of prebiotics. *Appl Environ Microbiol* 2009; 75: 1121-8.
27. Scholtens PA, Alliet P, Raes M, et al. Fecal secretory immunoglobulin A is increased in healthy infants who receive a formula with short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides. *J Nutr* 2008; 138: 1141-7.
28. van Hoffen E, Ruiter B, Faber J, et al. A specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides induces a beneficial immunoglobulin profile in infants at high risk for allergy. *Allergy* 2009; 64: 484-7.
29. Ziegler E, Vanderhoof JA, Petschow B, et al. Term infants fed formula supplemented with selected blends of prebiotics grow normally and have soft stools similar to those reported for breast-fed infants. *J Pediatr Gastroenterol Nutr* 2007; 44: 359-64.
30. Lopez MG, Mancilla-Margalli NA, Mendoza-Diaz G. Molecular structures of fructans from Agave tequilana Weber var. azul. *J Agric Food Chem* 2003; 51(27): 7835-40.
31. Gomez E, Tuohy KM, Gibson GR, Klinder A, Costabile A. In vitro evaluation of the fermentation properties and potential prebiotic activity of Agave fructans. *J Appl Microbiol* 2010; 108(6): 2114-21.
32. Urías-Silvas JE, Cani PD, Delmée E, Neyrinck A, López MG, Delzenne NM. Physiological effects of dietary fructans extracted from Agave tequilana Gto. and Dasylirion spp. *Br J Nutr* 2008; 99(2): 254-61.
33. Lohman T, Roche A, Martorell R (eds). Anthropometric standardization reference manual. Illinois, EUA: Human Kinetics Books; 1991.
34. Habicht JP. Estandarización de métodos epidemiológicos cuantitativos sobre el terreno. *Bol Oficina Sanit Panam* 1974; 375.
35. Gibson GR, McCartney AL, Rastall RA. Prebiotics and resistance to gastrointestinal infections. *Br J Nutr* 2005; 93(Suppl. 1): S31-S34. International Scientific Association for Probiotics and Prebiotics (ISAPP) 6th Annual Meeting. November 9-11, 2008; London, Ontario Canada.
36. de Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. *Adv Biochem Eng Biotechnol* 2008; 111: 11-66.
37. Jenkins DJ, Kendall CW, Vuksan V. Inulin, oligofructose and intestinal function. *J Nutrition* 1999; 129(Suppl. 7): 1431-3.
38. Ziegler E, Vanderhoof JA, Petschow B, Mitmesser SH, Stolz SI, Harris CL, et al. Term infants fed formula supplemented with blends of prebiotics grow normally and have soft stools similar to those reported for breast-fed infants. *J Pediatr Gastroenterol Nutr* 2007; 44: 359-64.
39. Chouraqui JP, Grathwohl D, Labaune JM, Hascoet JM, de Montgolfier I, Leclaire M, Giarre M, et al. Assessment of the safety, tolerance, and protective effect against diarrhea of infant formulas containing mixtures of probiotics or probiotics and prebiotics in a randomized controlled trial. *Am J Clin Nutr* 2008; 87(5): 1365-73.
40. Savino F, Palumeri E, Castagno E, Cresi F, Dalmasso P, Cavallo F, Oggero R. Reduction of crying episodes owing to infantile colic: a randomized controlled study on the efficacy of a new infant formula. *European Journal of Clinical Nutrition* 2006; 60: 1304-10.

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