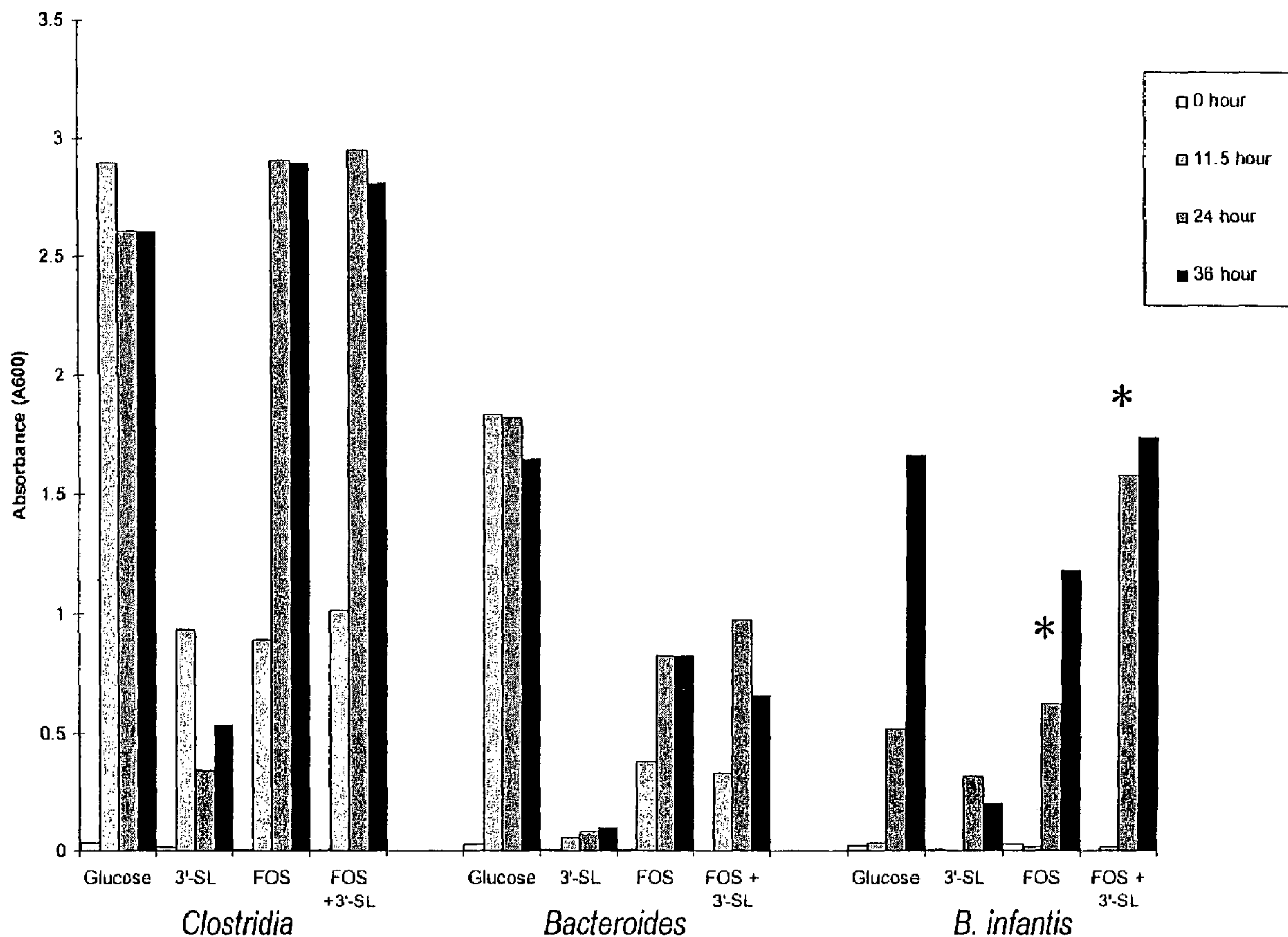




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 (54) Title: NUTRITIONAL FORMULATION CONTAINING PREBIOTIC SUBSTANCES



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 A nutritional composition is provided which comprises oligofructose and sialyllactose.

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(54) Title: NUTRITIONAL FORMULATION CONTAINING PREBIOTIC SUBSTANCES

(57) Abstract: A nutritional composition is provided which comprises oligofructose and sialyllactose.



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5           Moreover, it is known that a metabolic by-product of Bifidobacteria is short chain fatty acids, resulting in a reduction of the pH in the digestive tract. This pH effect has been observed clinically and documented in Mitsuoka et al, "Effect of Fructo-oligosaccharides on Intestinal Microflora", Die Nahrung, 3,5-6: 427 – 438 (1987).

10

          Sialyllactoses are oligosaccharides which occur naturally in human milk as well as in milk of other mammals. However, sialyllactoses are present at noticeably higher concentrations in human milk compared to other mammalian species.

15

          The two primary species of sialyllactose are 3'-sialyllactose and 6'-sialyllactose. These species occur naturally in human milk at a relative ratio of 1:3 (3':6'). Sialyllactose is known to have anti-adhesive properties for specific pathogenic bacteria. For example, it has been suggested that sialyllactose acts to inhibit cholera toxin (see, Idota et al, "Inhibition of Cholera Toxin by Human Milk Fractions and Sialyllactose," Biosci. Biotech. Biochem. 59:417-419) and Helicobacter pylori (see, Simon et al, "Inhibition of Helicobacter pylori Binding to Gastrointestinal Epithelial Cells by Sialic Acid-Containing Oligosaccharides," Infection and Immunity, 750-757, (1997)). In light of its antiadhesive properties, sialyllactose has been used to treat a number of medical conditions. For example, U.S. Patent Nos. 5,514,660 and 20 5,753,630, describe the use of sialyllactose in the treatment and inhibition of duodenal ulcers. U.S. Patent No. 5,883,079 describes the use of sialyllactose to inhibit H. pylori infection in mammalian tissue.

25

          However, the use of two prebiotic substances, specifically, oligofructose and 30 sialyllactose, in combination has heretofore not been described. Accordingly, it can be seen that there is a need for such a combination.

## SUMMARY OF THE INVENTION

35

          The present invention is related to a nutritional composition, which is effective in increasing beneficial Bifidobacteria and inhibiting the binding of pathogenic microorganisms to human tissue. More specifically, the present invention is directed to nutritional compositions comprising oligofructose and sialyllactose. The present

- 3 -

5 invention is further directed to a method of increasing the concentration of Bifidobacteria and inhibiting the binding of pathogenic bacteria in a human subject comprising enterally administering to said subject a composition comprising oligofructose and sialyllactose.

## 10 BRIEF DESCRIPTION OF THE DRAWING

Fig 1 shows the growth of Bifidobacteria, Clostridia and Bacteroides as set forth in Example 1.

15 Fig 2 shows the growth of B. lactis as set forth in Example 2.

Fig 3 shows the growth of Clostridia, Bacteroides and B. lactis as shown in Example 3.

## 20 DETAILED DESCRIPTION OF THE INVENTION

The sialyllactose useful in the present compositions comprises a mixture of 3'-sialyllactose and 6'-sialyllactose. Preferably, the sialyllactose used herein is 3'-sialyllactose. The sialyllactose useful in the present compositions may be prepared  
25 according to any of the methods described, e.g., in U.S. Patent No. 5,575,916; 5,714,075; 5,278,299; 5,374,541; and 5,876,980. However, it will be recognized by those skilled in the art that any other known method of synthesizing and purifying sialyllactose may be used to prepare the sialyllactose, useful in the present compositions.

30

The oligofructose component of the present composition may be prepared from a naturally occurring polyfructose (inulin) which may be found in many plants, including onions, leeks, wheat, chicory and artichoke. Chicory is most commonly used. Oligofructose can be recovered in sufficient quantities, from these plants, by  
35 methods known in the art. The naturally occurring inulin comprises oligofructose and higher polymers of fructose. The inulin can be separated as it is soluble in hot water. If desired, the naturally occurring oligofructose can be separated as it is additionally soluble in cold water. The inulin, optionally after removal of the naturally occurring

- 4 -

5 oligofructose, may be converted into oligofructose by hydrolysis. The inulin may be broken down into oligomeric chain lengths by using an inulase enzyme. Inulin can also be degraded into oligomeric chain lengths by chemical hydrolysis. The oligofructose component of the present composition may also be prepared by synthesis rather than by extraction procedures. Oligofructose may be synthesized  
10 from sucrose by transfructosylation, which is accomplished by means of an enzyme,  $\beta$ -fructofuranosidase, that links additional fructose monomers to the sucrose molecule. Oligofructose formed in this manner contains fructose units linked to a terminal glucose unit. Oligofructose derived from inulin from plants such as chicory contains both fructose chains and fructose chains with a terminal glucose unit. Oligofructose  
15 prepared by methods such as these is commercially available. A preferred form of oligofructose for purposes of this invention is Raftilose® available from Orafiti S.A., Tienen, Belgium. Again, it is understood that any known method of synthesizing and/or isolating oligofructose may be suitable for the present invention.

20 The nutritional composition of the present invention may comprise 0.1g/L to 10g/L of oligofructose and 6 mg/L to 10 g/L of sialyllactose. Preferably, the present composition contain 0.3 g/L to 6 g/L of oligofructose and 60 mg/L to 1 g /L of sialyllactose, more preferably 1 g/L to 3 g/L of oligofructose and 100 mg/L to 600  
25 ml/L of sialyllactose and even more preferably about 3 g/L of oligofructose and about 100 mg/L of sialyllactose.

The present inventors have found that the combination of oligofructose and sialyllactose in the present nutritional formulations produces a synergistic prebiotic effect, i.e., a prebiotic effect greater than the additive effect of the substances alone.  
30 The present use of oligofructose and sialyllactose specifically increases the concentration of beneficial bacteria (Bifidobacteria) in the gut while having no effect on pathogenic bacteria (e.g., Clostridia, Bacteroides, E. coli, etc). In addition, although the administration of oligofructose is known to lower the pH of its environment, the combination of oligofructose with sialyllactose has been found to  
35 lower the pH to an even greater extent. This reduction in gut pH results in an environment which is less conducive to the growth of certain organisms, specifically, the less beneficial or more pathogenic bacteria such E. coli or Bacteroides.

- 5 -

5           The nutritional compositions of the present invention can be utilized in  
 conjunction with various nutritional products, such as infant formula, follow-on  
 formula, toddler's beverage, milk, yogurt, fruit-based products for older children  
 (such as fruit juices) candies, chewing gum, lozenges, powders, tablets, etc.  
 Preferably the present compositions are added to infant formula. The infant formula  
 10 can be in the form of a ready to feed liquid or a powder, which may be mixed with  
 water and fed to the infant. It is most preferred that the present formulation be added  
 to infant formula in liquid form.

          Infant formula suitable for use with the present invention should contain all  
 15 vitamins and minerals considered to be essential in the daily diet. These vitamins and  
 minerals should be present in nutritionally significant amounts. Examples of  
 vitamins, minerals and other nutrients which may be included in infant formulas in  
 which the present formulations are to be added include vitamin A, vitamin B complex,  
 vitamin C, vitamin D, vitamin E, vitamin K, calcium, magnesium sodium, potassium,  
 20 phosphorous, copper, zinc, chloride, iodine, selenium, iron, niacin, folic acid,  
 pantothenic acid, biotin, chlorine, Inositol and manganese.

          The infant formula may contain one or more lipid sources as will be  
 recognized by those skilled in the art. The infant formula may further contain other  
 25 substances than to have a beneficial effect, such as, nucleotides, immunoglobulins,  
 polyunsaturated fatty acids, etc.

A preferred infant formula according to the present invention is as follows:

<u>Ingredient</u>	<u>Units</u>	<u>Per Liter</u>
Energy	Kcal	672
Protein	g	15
Whey: Casein ratio		60:40
Fat	g	36
Carbohydrate	g	72
Oligofructose	g	3.0
Sialyllactose	mg	100
Vitamin A	RE	750
Mixed natural carotids	IU	400

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<u>Ingredient</u>	<u>Units</u>	<u>Per Liter</u>
Vitamin D	mcg	10.6
Vitamin F	mg	7.4
Vitamin K	mcg	67.0
Vitamin B <sub>1</sub> (thiamin)	mcg	1000
Vitamin B <sub>2</sub> (riboflavin)	mcg	1500
Vitamin B <sub>6</sub> (pyridoxine)	mcg	600
Vitamin B <sub>12</sub> (cyanocobalmine)	mcg	2.0
Niacin	mcg	9.0
Folic Acid	mcg	80
Pantothenic Acid	mcg	3000
Biotin	mcg	90
Vitamin C (ascorbic acid)	mg	90
Choline	mg	100
Inositol	mg	33
Calcium	Mg	460
Phosphorous	Mg	333
Magnesium	Mg	64
Iron	Mg	8.0
Zinc	Mg	6.0
Manganese	mcg	50
Copper	mcg	560
Iodine	mcg	100
Sodium	mg	160
Potassium	mg	650
Chloride	mg	433
Selenium	mcg	14

5

The present invention is further described with reference to the following examples:

#### Example 1

Identical inoculua ( $A_{600} = 1.0$ ) of overnight cultures (PYG broth, 37° C, anaerobic) of each organism (Clostridia, Bacteroides, or B. lactis) were grown in  
 10 batch culture (37° C, anaerobic) with constant stirring. Carbohydrate-deficient batch

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5 culture media (PYG) was supplemented with either glucose (3.0 g/L), oligofructose (FOS at 3.0 g/L), sialyllactose (3'- or 6'-sialyllactose at 100 mg/L), or oligofructose plus sialyllactose (3.0 g/L and 100 mg/L, respectively). Growth was monitored at various time points (see Figure 1) by determining the titers of the bacteria on selective  
10 agar (BIM-25 agar for Bifidobacteria and Wilkens-Chalgren agar for Clostridia and Bacteroides). Results of the growth analysis are shown in Figure 1.

As shown in Figure 1, substituting 3'-sialyllactose for glucose in the growth media resulted in essentially no growth of Clostridia or Bacteroides in comparison to glucose. However, the growth of Clostridia and *B. infantis*, but not Bacteroides, in  
15 the presence of oligofructose was similar to that seen in the presence of glucose. The combination of oligofructose and sialyllactose had no effect on the growth of Clostridia or Bacteroides. However, there was a noticeable effect on the growth of *B. infantis*, especially at the twenty four hour time point. It can be seen that the combination of oligofructose and sialyllactose had synergistic effect on the growth of  
20 *B. infantis*.

### Example 2

Identical inocula ( $A_{600} = 1.0$ ) of overnight cultures (PYG broth, 37° C. anaerobic) of *B. Lactis* were grown in batch culture (37° C anaerobic) with constant  
25 stirring. Carbohydrate-deficient batch culture media (PYG) was supplemented with either glucose (3.0 g/L), oligofructose (3.0 g/L), or oligofructose (3.0g/L) plus sialyllactose (3'- or 6'-sialyllactose at 100 mg/L). Growth was monitored at various time points (see Figure 2) by determining the titers of the *B. lactis* bacteria on selective agar (BIM-25 agar). Results of the growth analysis are shown in Figure 2.

30

Figure 2 demonstrates that *B. lactis* grew significantly better in the presence of oligofructose than glucose. However, the growth of Bifidobacteria lactis was significantly enhanced in the presence of the present combination of oligofructose and either 3'- or 6'- sialyllactose at 46 hours.

35

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5 **Example 3**

A metabolic by-product of Bifidobacteria is short-chain fatty acids, the presence of which lowers the intestinal pH.

10 Figure 3 shows an analysis at different times post-inoculation of batch cultures of Clostridia, Bacteroides, and B. lactis. Identical inocula ( $A_{600} = 1.0$ ) of overnight cultures (PYG broth, 37°C, anaerobic) of each organism was grown in batch culture (37°C, anaerobic) with constant stirring. Carbohydrate-deficient batch culture media (PYG) was supplemented as above. The media pH were monitored at various time  
15 points. Results of the pH analysis are shown in Figure 3.

As can be seen in Figure 3, in the presence of glucose, all three colonic bacteria lowered the pH. In the presence of sialyllactose alone, the pH increased in all cases. This is consistent with the poor microbial growth observed in the presence of  
20 sialyllactose alone. In the presence of oligofructose, the pH lowered, but to a lesser extent than glucose in batch cultures of Clostridia and B. infantis, but was similar to the pH change observed in the Bacteroides culture with glucose. In the presence of the combination of oligofructose and sialyllactose of the present invention, the pH lowered to a greater extent than with glucose.

25 However, this reduction in pH was less than that seen with glucose (Clostridia) or similar to the glucose pH change (Bacteroides and B. lactis). Of particular interest is the observation that while oligofructose alone shows some pH reduction over time in the bifidobacteria culture, the presence of sialyllactose  
30 dramatically reduced the pH compared to the oligofructose-only B. infantis culture. Thus, the synergistic effects of oligofructose and sialyllactose are present.

The present invention may be embodied in their specific forms without departure from the spirit or essential attributes thereof and, accordingly, reference  
35 should be made to the appended claims, rather than to the foregoing specification as indicating the scope of the invention.

**CLAIMS**

1. A nutritional composition comprising 0.1 g/L to 10 g/L of oligofructose and 6 mg/L to 10 g/L of sialyllactose.  
5
2. A composition as in Claim 1, wherein the sialyllactose is a mixture of 3'-sialyllactose and 6'-sialyllactose.
3. A composition as in Claim 1, wherein the sialyllactose is 3'-sialyllactose.  
10
4. A composition as in Claim 1, wherein the sialyllactose is 6'-sialyllactose.
- 15 5. A nutritional composition as in Claim 1, comprising 0.3 g/L to 6 g/L of oligofructose and 60 mg/L to 1 g/L of sialyllactose.
6. A nutritional composition as in Claim 1, comprising 1 g/L to 3 g/L of oligofructose and 100 mg/L to 600 mg/L of sialyllactose.  
20
7. A nutritional composition comprising 1.5 g/L of oligofructose and 100 mg/L of sialyllactose.
8. A nutritional composition as in Claim 1, wherein said composition is in liquid form.  
25
9. A nutritional composition as in Claim 1, wherein said composition is in powder form.
- 30 10. A nutritional composition as in Claim 1, further comprising a least one of Vitamin A, Vitamin D, Vitamin E, and Vitamin B complex.
11. A nutritional composition as in Claim 1, further comprising at least one of

calcium, magnesium, sodium, potassium, phosphorous, copper, zinc, iodine, selenium, and iron.

5 12. A nutritional composition as in Claim 1, which is in the form of an infant formula, follow-on formula, toddler's beverage, milk yogurt, fruit juice, fruit-based drink, candy, chewing gum, or a lozenge.

10 13. A method of increasing the amount of Bifidobacteria and inhibiting the binding of pathogenic bacteria in a human, comprising administering to said human nutritional composition comprising oligofructose and sialyllactose.

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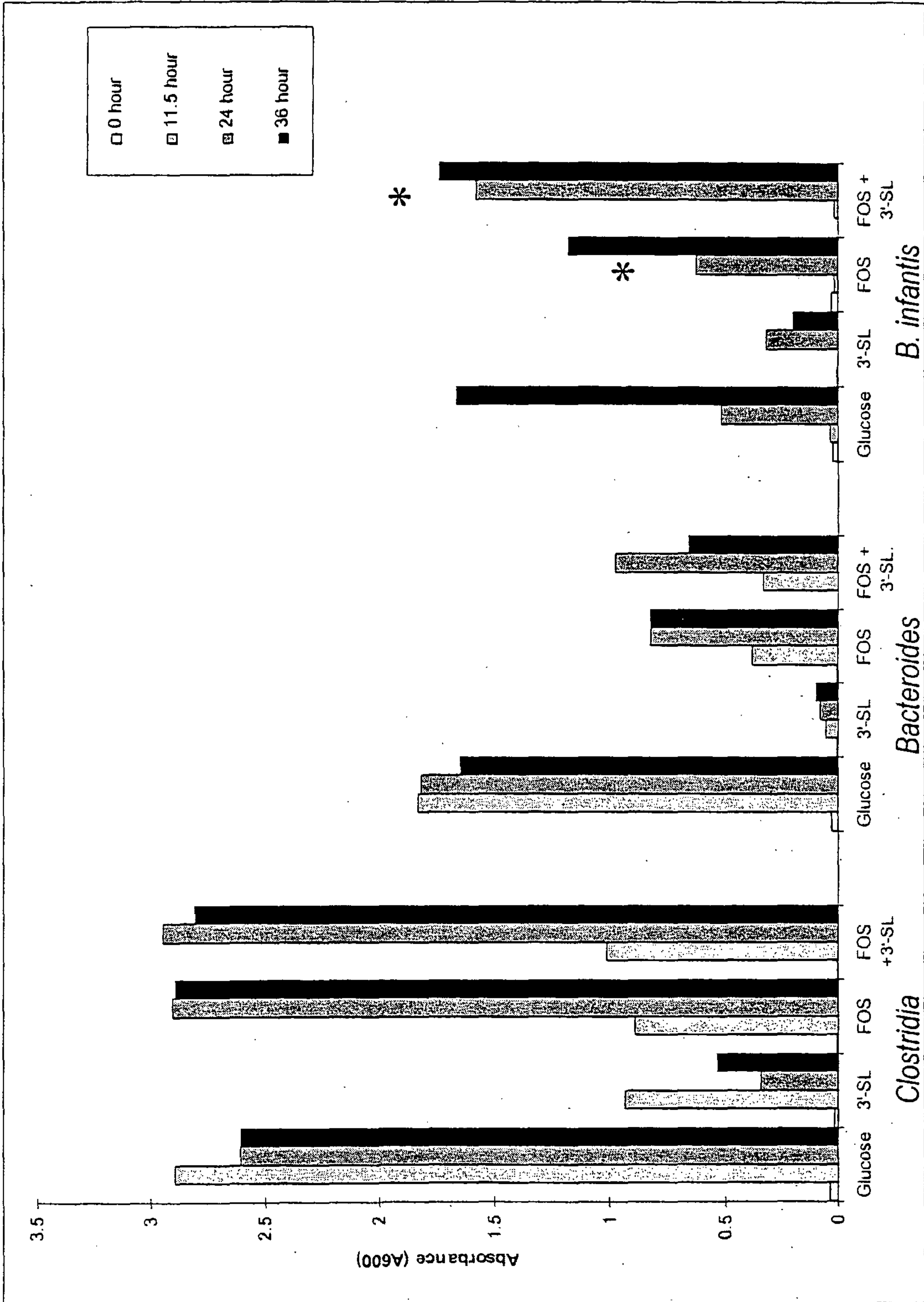


FIG. 1

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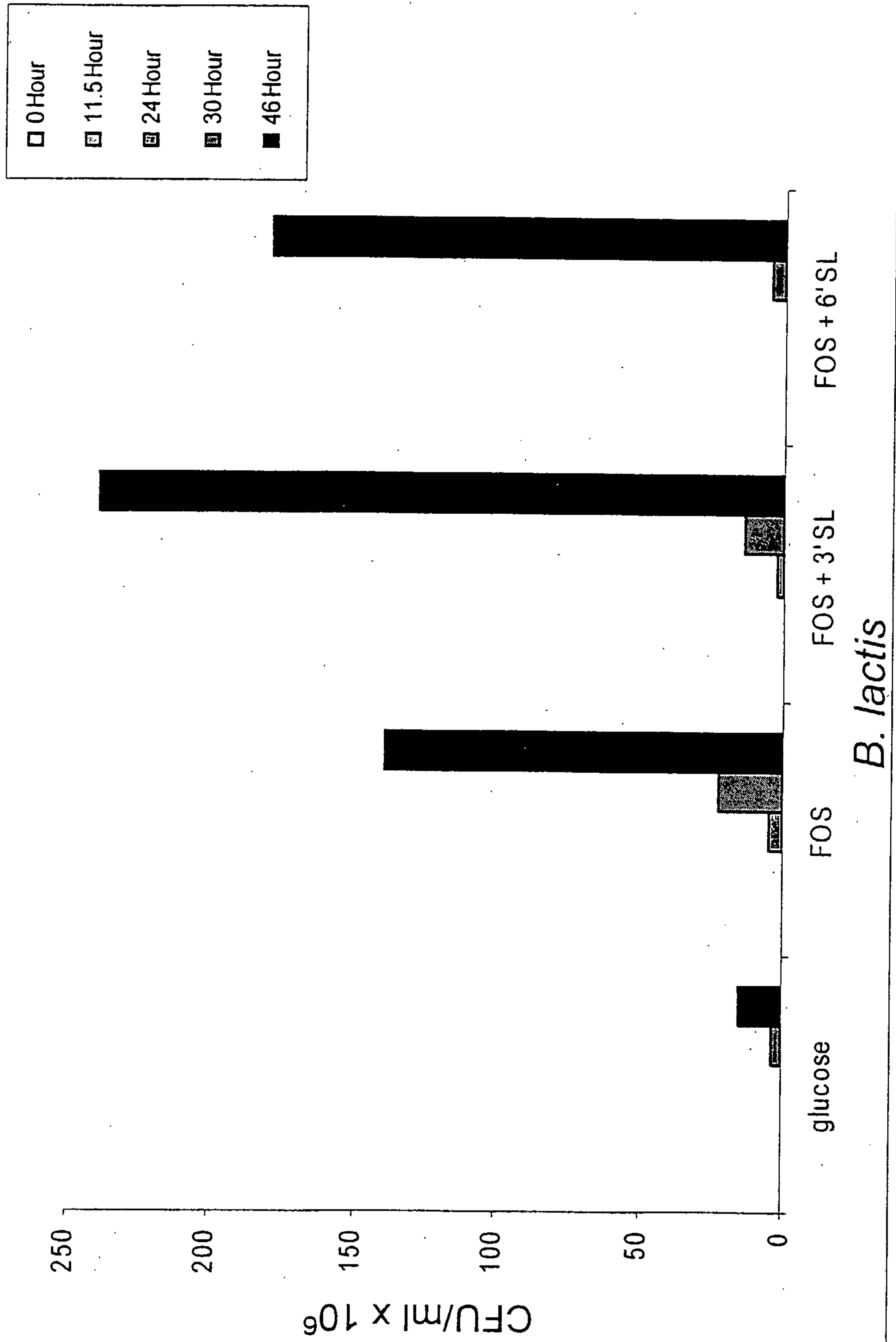


FIG. 2

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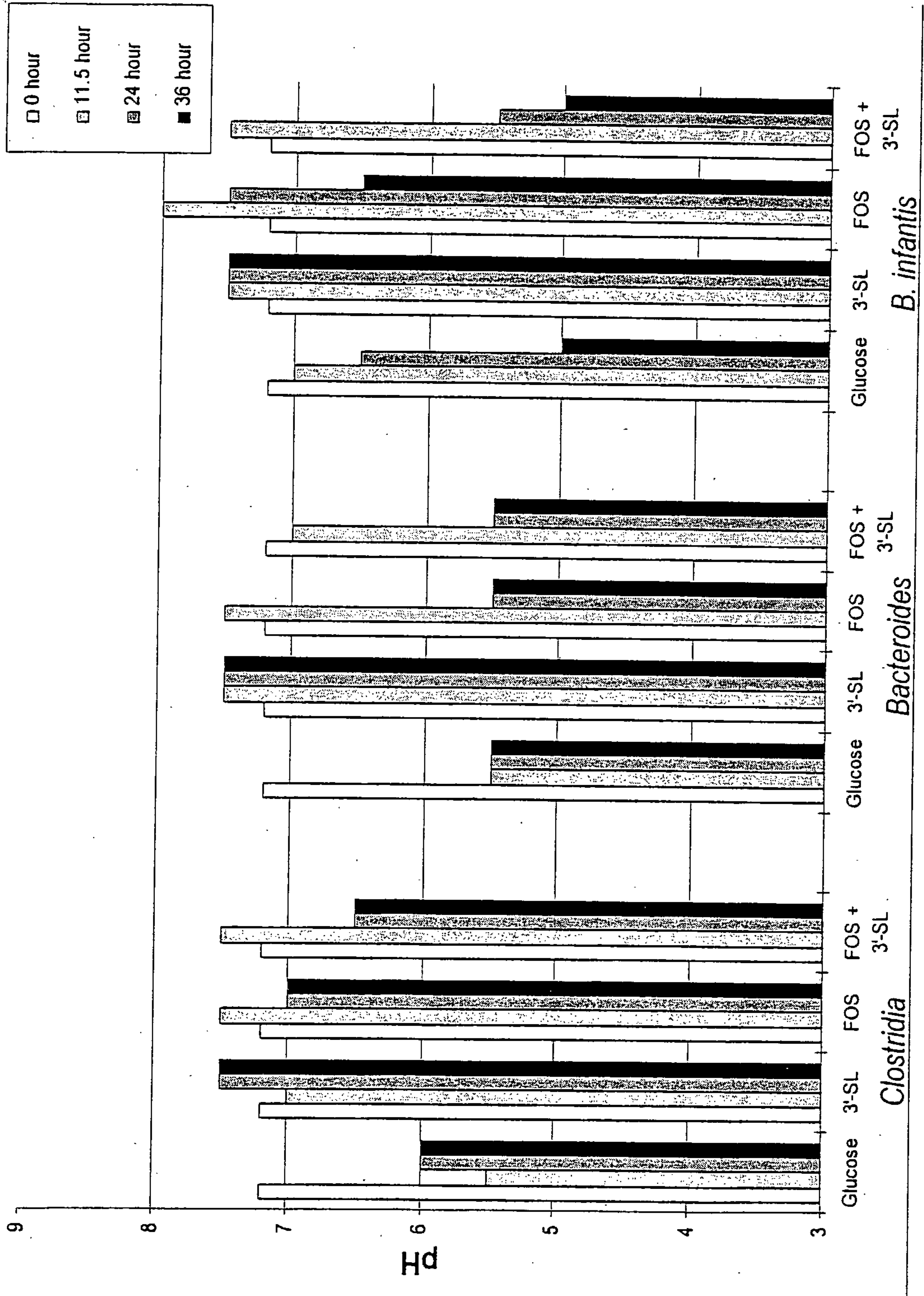


FIG. 3

