

2018/11/01

来自妈妈的“第一口糖”

母乳低聚糖最新研究进展及其与微生态
健康的关系

中国营养保健食品协会

杜邦营养与健康大中华区产品经理

李灵



母乳喂养是金标准

> 母乳喂养促进了婴儿的健康成长

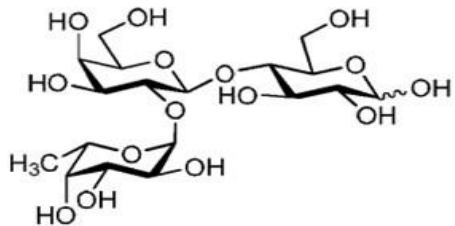
> 婴儿配方粉无法完全模仿母乳的成分

> 母乳低聚糖可以模仿一部分母乳促进健康的效用



母乳低聚糖

- **母乳低聚糖 (HMO)** 是一组独特的来源于母乳的低聚糖
- HMO是母乳中仅次于乳糖和脂肪之外的第三大可溶物
- 在所有从母乳中被发现的130种不同的低聚糖中，2'-岩藻糖基乳糖 (2'-FL) 是含量最丰富的~ 2.4 g/L
- 母乳低聚糖的种类和水平在不同女性中，不同的地域中以及泌乳的不同阶段都差异巨大



中国营养保健食品协会



HMO帮助构建健康的肠道菌群

HMO是重要的生物活性物质
能从生命早期开始帮助人体促进和维持健康

- HMO有效促进双歧杆菌增长，并且能够帮助形成以双歧杆菌占主导的肠道菌群环境
- HMO增强了肠屏障功能并且扮演了病原微生物诱饵的角色

阻止病原微生物（弯曲杆菌，大肠杆菌）
在肠上皮细胞定植

认知健康

免疫调节作用

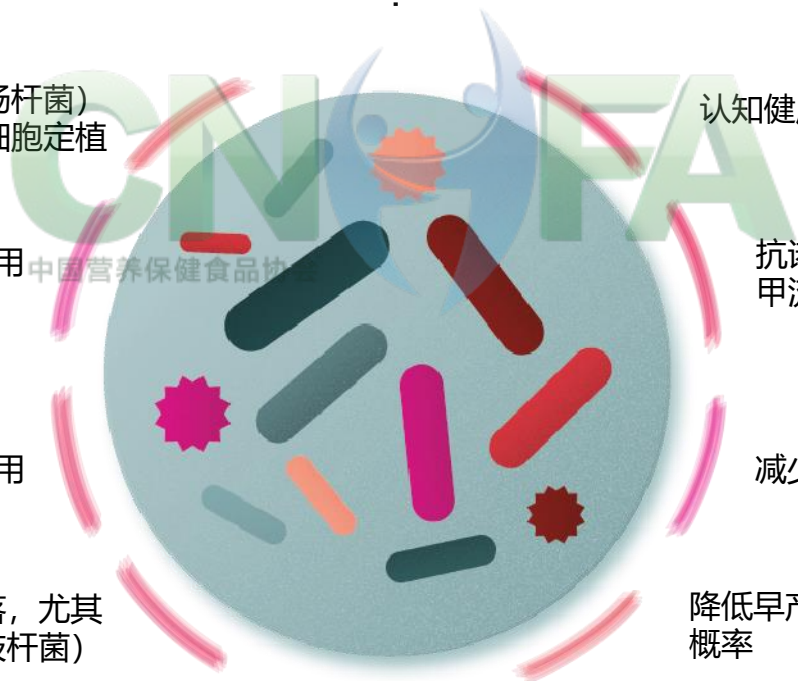
抗诺如病毒定植（病毒性胃肠炎），
甲流病毒

呼吸道健康作用

减少肠痉挛（肠绞痛）

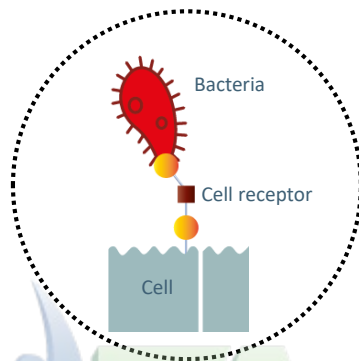
益生元效用（影响人体微生物群落，尤其
双歧杆菌）

降低早产儿发生新生儿小肠坏死综合征的
概率

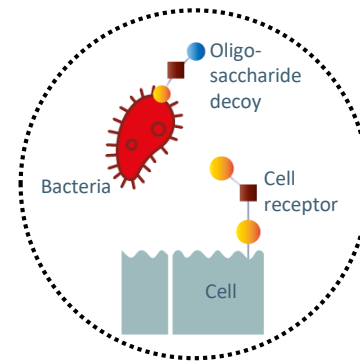


HMO充当病原微生物的诱饵

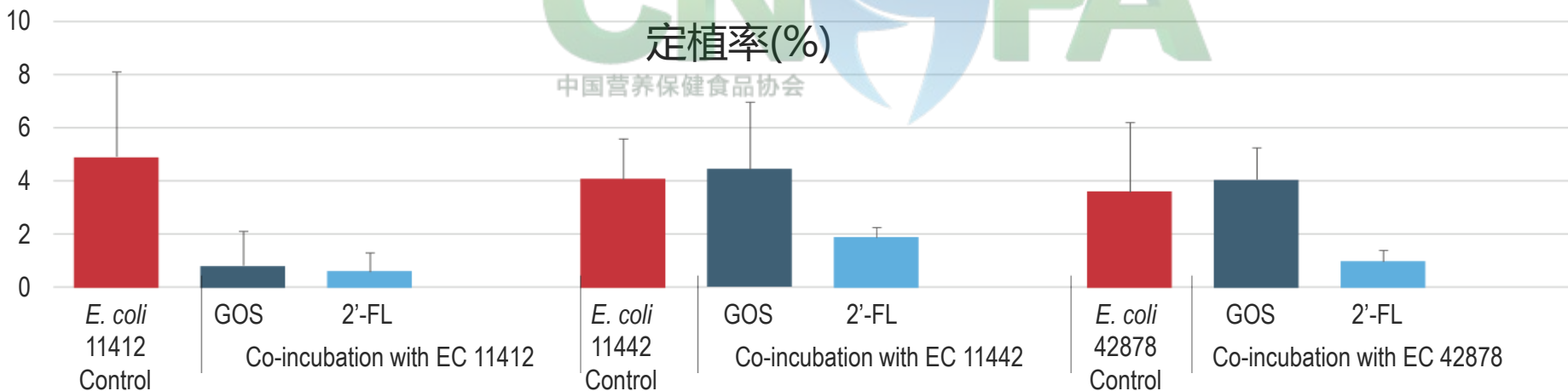
- 研究发现HMO充当了诱饵受体，阻止病原微生物定植在上皮细胞，因此HMO可能抑制了感染性疾病的发生
- 我们的研究显示2'-FL减少了导致婴儿腹泻的3株大肠杆菌在Caco-2肠上皮细胞的定植



无低聚糖



诱饵低聚糖与病原体结合



2'-岩藻糖基乳糖和低聚半乳糖对于大肠杆菌11412, 11442和42878在Caco-2细胞上定植的影响, 使用了SYTO24细菌标记和荧光检测

HMO有效促进双歧杆菌生长

- 体外婴儿肠道模拟器模型是研究HMO的益生元特性的十分有用的工具，它获得的是与人类相关的数据，而不是动物数据
- 当使用具有相对高双歧杆菌含量的婴儿粪便作为接种物时，2'-FL增加了模拟器中双歧杆菌的总量，但是在其他没有检测到双歧杆菌的粪便样品的模拟器中双歧杆菌数量没有增加。



In vitro infant colon simulator
in Kantvik Finland



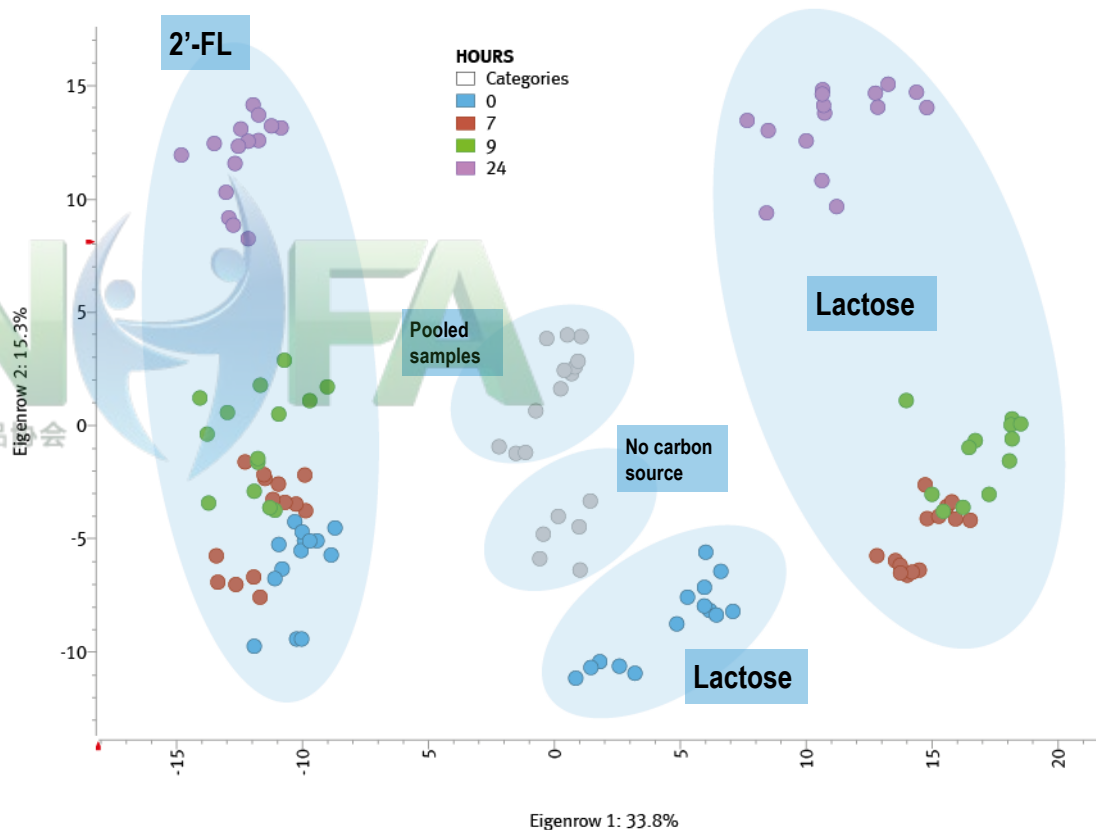
BIOSCREEN

Bacterium	Strain	Carbon source			
		GLU	LACT	GOS	2'-FL
<i>Clostridium perfringens</i>	ATCC 3626	+	+	+	-
<i>Clostridium perfringens</i>	ATCC 13124	+	+	+	-
<i>Escherichia coli</i>	ATCC 11775	+	+	+	-
<i>Salmonella typhimurium</i>	EELA	+	+	-	-
<i>Escherichia coli</i> (O111:K(58):H-)	CCUG 11412	+	+	+	-
<i>Escherichia coli</i> (O142:K86(B):H4)	CCUG 11442	+	+	+	-
<i>Escherichia coli</i> (O111:K58(B4):H2)	CCUG 42878	+	+	+	-
<i>Lactobacillus acidophilus</i>	NCFM	+	+	+	-
<i>Lactobacillus paracasei</i>	Lpc-37	+	+	+	-
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	Bi-07	+	+	+	-
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	Bi-04	+	+	+	-
<i>Bifidobacterium longum</i> subsp. <i>longum</i>	BI-05	+	+	+	-
<i>Lactobacillus rhamnosus</i>	HN001	+	+	+	-
<i>Bifidobacterium lactis</i>	HN019	+	+	+	-
<i>Bifidobacterium longum</i> subsp. <i>infantis</i>	Bi-26	+	+	+	+
<i>Bifidobacterium longum</i> subsp. <i>infantis</i>	ATCC 15697	+	+	+	+
<i>Bifidobacterium breve</i>	Bb-03	+	+	+	-
<i>Bifidobacterium bifidum</i>	Bb-06	+	+	+	+

母乳低聚糖区别于乳糖的不同代谢产物

PRINCIPAL COMPONENT ANALYSIS OF GC-TOF-MS PROFILING

- > 当婴儿双歧杆菌Bi-26以2'-FL作为碳源时的代谢产物与以乳糖作为碳源时的代谢产物差异很大
- > 与以乳酸相比，当以2'-FL为碳源时，Bi-26产生更多的1,2-丙二醇，醋酸酯，甲酸盐和丙酮酸等
- > 除了已知的代谢产物，以2'-FL作为碳源时，与以乳糖作为碳源相比，Bi-26产生了一系列更高含量的未知分子
- > Bi-26含有转运和降解母乳低聚糖的基因簇



近期临床研究

临床研究显示使用HMO的配方是安全并且有良好的耐受性的

- Correlation between less diarrhea incidents in infants and higher 2' FL concentration in human milk (identified via epidemiological studies)
- *Nestlé intervention study, 2016*: Infant formula supplemented with HMOs (2'Fucosyllactose and Lacto-N-neotetraose) shifts stool microbiota and metabolic signatures closer to that of breastfed Infants
- *Simeoni et al, 2016*: B.lactis + bovine oligosacch → demonstrated the role of nutritional intervention in modulating the diversity and composition of the gut microbiota so that it becomes closer to those of breastfed infants
- *Goehring et al. 2016*: Infants fed formula supplemented with 2' FL exhibit lower plasma and ex vivo inflammatory cytokine profiles, similar to those of a breastfed reference group.
- *Abbott, 2016*: Growth and 2' FL uptake were similar between infants fed formula and human milk fed with the same caloric density
- *Elison et al. 2016*: Supplementing the diet with 2'FL and LNnT may be a valuable tool to restore homeostasis in adults having an imbalanced microbiota
- *Puccio et al. 2017*: Infant formula with 2' FL and LNnT is safe, well-tolerated, and supports age-appropriate growth. Infants receiving HMOs-supplemented formula had significantly fewer parental reports of bronchitis and respiratory tract infections, and medication use (antipyretics and antibiotics)

HMO应用广泛



INFANT NUTRITION

- 标准婴儿配方粉（1-2段）
- 3-4段婴儿配方粉
- 成长奶粉
- 特殊用途婴儿配方粉



DIETARY SUPPLEMENTS

- 肠易激综合征
- 旅行者腹泻
- 流感和诺如病毒感染的预防
- 呼吸道疾病预防
- 孕期营养



FOOD APPLICATION

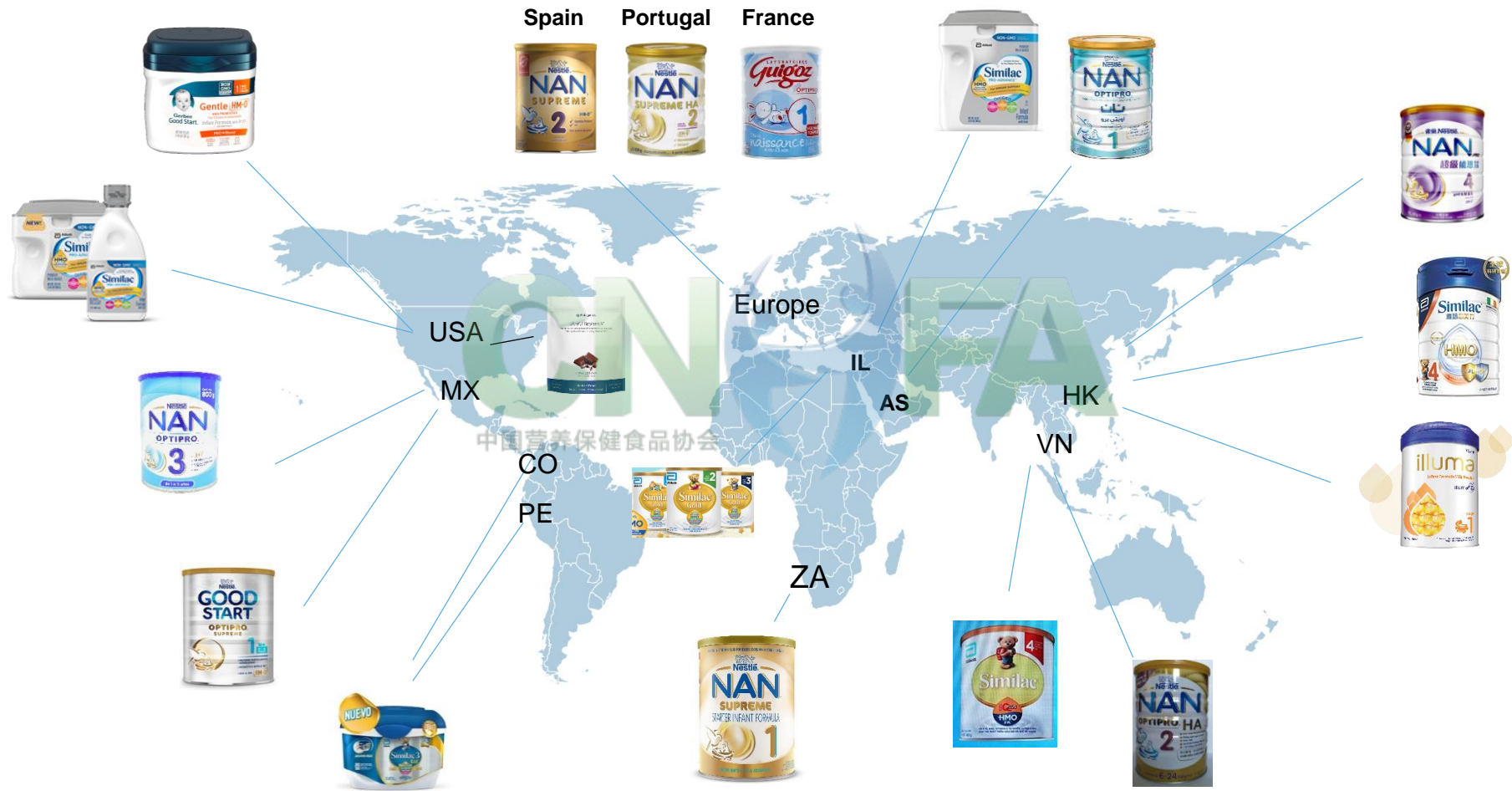
- 婴儿食品
- 婴幼儿酸奶
- 儿童营养
- 老年营养（双歧杆菌不足引起的问题）



PETFOOD

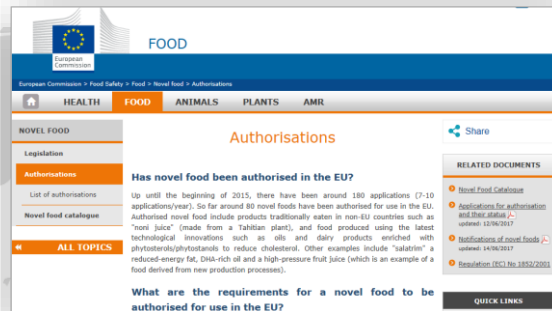
- 腹泻预防-牛奶替代品
- 动物免疫系统激发
- 家禽-预防弯曲杆菌

全球已上市的HMO产品



欧盟和美国法规现状

- > 2017年12月杜邦2' -FL获得欧盟新资源食品批准
- > 2018年4月杜邦2' -FL获得欧盟在婴儿奶粉中的使用批准
- > 从2017年7月开始，杜邦2' -FL就获得了美国GRAS通知
- > 在2018年4月，杜邦2' -FL得到了在婴幼儿奶粉中使用的FDA无异议通知，最大添加量为2.4g/L



GRN No. 546	
Substance:	2'-O-fucosylactose
Intended Use:	Intended for use in term infant formula at a maximum level of 2,400 milligrams (mg)/Liter. Also intended for use in baked goods and baking mixes, beverages and beverage bases, coffee and tea, dairy product analogs, infant and toddler foods, grain products and pastas, milk (whole and skim), processed fruits and juices, processed vegetables and juices, and sugar substitutes at maximum levels ranging from 0.084 to 2.4 grams/serving.
Basis:	Scientific Procedures
Notifier:	Glycom AG Djølmejs 3720K-2800 Kgs, Lingsby, Denmark
Date of filing:	Oct 16, 2014
GRAS Notice (releasable information):	GRN 546 (in PDF) (1.1 MB)
Date of closure:	Sep 16, 2015
FDA's Letter:	FDA has no questions
Notes:	Correction letter issued September 24, 2014.

GRN No. 735	
Substance:	2'-Fucosylactose
Intended Use:	For use as an ingredient in beverages and beverage bases; breakfast cereals; dairy product analogs; frozen dairy desserts and mixes; gelatins, puddings, and fillings; grain products and pastas; jams and jellies; milk, whole and skim; milk products; processed fruits and fruit juices; sweet sauces, toppings, and syrups; non- exempt infant and follow-on formula; baby foods; and medical foods at levels ranging from 0.24 to 4 grams/serving.
Basis:	Scientific procedures
Notifier:	Glycosyn, LLC and Friesland Campina Dome B.V. 890 Winter Street Suite 200 Waltham, MA 02451
FDA's Letter:	Pending

中国法规现状

中国政府机构刚经历了重组，包括

- 新的农业农村部(过去的农业部)
- 新的国家卫健委(过去的国家卫计委)
- 新的市场监督管理总局(过去的国家食品药品监督管理总局+国家质量监督检验检疫总局+国家工商总局)
- 政府对监管批准和监管的责任需要澄清
- HMO的供应商和应用企业正在通力合作，积极推动法规批准的进程，但是目前还没有时间表



杜邦人体微生物组平台

益生菌
领先地位



杜邦益生菌
杜邦营养与健康

母乳低聚糖
2018进入市场



中国营养保健食品协会

杜邦在2016年宣布与Inbiose合作

下一代微生物组解决方案



2017 杜邦在2017年宣布成立微生物组
创投部门

CARE4U™ 2'-FL



高纯度，婴儿级2'FL，粉体质量优异：

- >98% 2'-FL
- 密度 > 0.6 kg/L (稀疏孔洞)
 - 最高的纯度和密度 → 成品中产品使用量少
 - 给益生菌更多的空间
- 高度可溶，几乎清澈的浊度，优异的即时溶解性
- 知识产权保护程序（已在世界各地申请多项专利）

DU PONT CERTIFICATE OF ANALYSIS BR0001 221

Part#: 14181 05/01/2018

Table 1. 2'-FL, pure dry and carbohydrate content

mg	sample	Substrate (g)	Area %	Manitol (g)	Area %	Fructose (g)	Area %	2'-FL (g)	Area %	2'-FL (g)	Area %	2'-FL (g)	Area %
1	2'-FL Lactosa batch 100017	nd	nd	nd	0.1	nd	0.1	99.8	99.9	100.0	100.0	100.0	100.0
2	2'-FL Lactosa batch 100017	nd	nd	nd	0.1	nd	0.1	99.8	99.9	100.0	100.0	100.0	100.0
3	2'-FL Lactosa batch 100017	nd	nd	nd	0.1	nd	0.1	99.8	99.9	100.0	100.0	100.0	100.0

1. Lact - 100% lactose - 100%
nd=not detected

Remarks: 1. Added reference to control better

CULTURE DIVISION
www.danisco.com

Page 1 of 2
Valid from: January 24, 2018

TEMPORARY PRODUCT DESCRIPTION - TPD 274362-0.1EN
Material no. 61011643

CARE4U™ 2'FL 15 kg

Description
CARE4U™ 2'FL, 15 kg in high purity infant grade 2'-Fucosylactose (2'FL) powder. 2'FL is a monosaccharide that occurs naturally in human milk and is comprised of glucose, D-galactose and D-glucose. It is a white to very colored powder that is soluble in water.

Usage levels
Product: 1.2 - 2.7 g / (depending on infant formula)
Toddler formula: 1.2 - 2.7 g / (depending on total lactation)

Microbiological specifications
Aerobic contaminant (25 °C) = 1000
Aerobic contaminant (35 °C) = 5000 CFU/g
Yeast and Moulds = 100 CFU/g
Enterobacteriaceae = 10³ CFU/g
Listeria monocytogenes = 10² CFU/g
Clostridium perfringens (5 x 1 g) = 10² CFU/g
Clostridium perfringens (5 x 1 g) = 10² CFU/g
Bacillus spores (5 x 1 g) = 50 CFU/g
Enterococcus = 100 CFU/g
Clostridia spores (5 x 1 g) = 10 CFU/g

Composition
2'-Fucosylactose (Disaccharide (DFL))
Lactose

Physical/chemical specifications
2'-Fucosylactose = 94 Area %
Appearance (Color) = White to cream colored
Appearance in solution = 300 ICUMSA units color
Water content = 5.0 %
Protein content = 100 mg/g
Total Ash = 0.5 %
Densification = 300 EU/g
*Absorbing 20 g in 100 ml water at 40 °C

Storage
24 months from date of manufacture, when stored in original and unopened packaging under cool dry conditions (-20 °C, max 60 % relative humidity).

Packaging
Sealed aluminum bag of 15 kg with PE inner bag, packed in a cardboard box.

Purity and legal status
CARE4U™ 2'FL, 15 kg complies with applicable EU legislation.
Applicable local regulations should always be reviewed and checked by the user of this product, as regulations and legislation may vary between countries.

供应链-三步走策略



首批产品

- 1到2个第三方工厂来满足市场引进和中短期需求

初步上市阶段

中国营养保健食品协会 Grindsted, 丹麦

- 灵活的工厂生产线满足规模扩张和市场开发的需求

大规模生产阶段

- 时间，地点和规模将取决于市场渗透程度
- 由于规模效应，大批量生产将带来更大的价格优势
- 多种生产设施保证供应

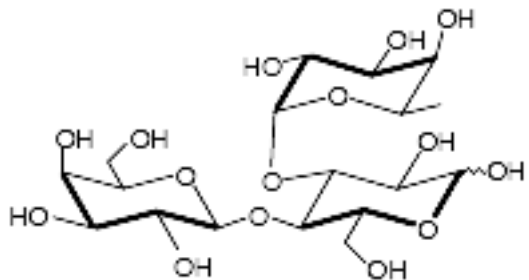
3-FL

- 3-FL 存在于所有母亲的母乳中，在哺乳期浓度或许会增加
- 与2'FL（以及唾液酸化HMOs）相比，3-FL已被证明在减少结肠运动收缩方面更有效
- 3-FL与Caco-2细胞中的2'FL相比表现出更强的细菌粘附抑制作用



- 安全实验和临床研究将于2018年底完成
- 2019年底之前获得监管批准，2020年初商业化

中国营养保健食品协会



inbiose Pre-clinical differentiator for 3-FL

Fucosylated but Not Sialylated Milk Oligosaccharides Diminish Colon Motor Contractions

John Blumenthal^{1,2}, Michael H. Buck¹, Hwey-Lin Lin¹, Paul Forsythe¹, Andrew M. Sklarer, Wolfgang A. Kump^{1,3}

Abstract
Human milk oligosaccharides (HMOs) are being studied by different groups exploring a broad range of potential beneficial effects in the mammalian infant. Many of these effects have been attributed to a growth promotion effect on certain gut organisms such as bifidobacteria. Additionally, evidence indicates that HMOs are able to directly promote positive changes in gut epithelium and immune responses under certain conditions. This study utilizes a standardized ex vivo mouse colonic preparation to examine the effects of sialylated, fucosylated and other HMOs on gut motor contractions. Only the fucosylated molecules 2'FL and 3'FL increased contractility in a concentration dependent manner. In the case of 2'FL, independent 2'FL was greater than 2'FL plus sialylated 2'FL. The HMO 2'FL and 3'FL, both in monomer (2'FL) and galactooligosaccharide (GOS) (2'FL/3'FL) forms, were used as a regional control. For validation, we used the known functional regulator of gut contractility by digoxigenin and L-Hecosyl, while it was also capable of reducing contractility, was substantially less effective than 2'FL and 3'FL. These results suggest that specific HMOs are unlikely to be having these effects via bifidobacteria, but through direct action on neurally dependent gut migrating motor complexes in ileum and colon. It is important in providing this information to understand conditions where gut motility and contractility are affected. Furthermore, this research has the potential that for optimal design and future might be useful as therapeutic or preventative adjuncts in disorders of gut motility, and possibly also have beneficial central nervous system effects.

3-FL effect on ex vivo mouse colon model

3'FL decreased gut motor contractions 2 times better than 2'FL (DL Unit and GOS: no effects)

inbiose Also antiviral action indications

Structural Basis for Norovirus Inhibition by Human Milk Oligosaccharides

Shan Wu^{1,2}, Joon Kwon^{1,2}, Daniel K. Singh^{1,2}, Leticia Hernandez^{1,2}, Stefan Jermolov^{1,2}, Peter Sauer^{1,2}

• Currently, there is no treatment or vaccine available for human norovirus infections, which cause a massive burden of disease

inbiose Similar effects known earlier with probiotics: "Reducing colic – fussiness"

Neurophysiology & Motility

Spatiotemporal maps reveal regional differences in the effects on gut motility for *Lactobacillus reuteri* and *rhizosinus* strains

Abstract
Beneficial Gut-derived bacteria such as probiotics that are restructive acutely affect the amplitude of neuronal respiratory motor complexes (RMCs). What is lacking for an improved understanding of these complex effects are region-specific measurements of reactivity and frequency. We have combined intraluminal pressure recordings with spatiotemporal maps to describe novel neurophysiological effects of different strains of beneficial bacteria on motility. **Methods:** Distal ileum and proximal colon were used and colon motility made of mouse ex vivo neuronal and colon segments before and after intraluminal application of *Lactobacillus reuteri* DSM 17938 or *Lactobacillus reuteri* DSM 17938. Respiratory motor complex frequency and velocity were calculated. Key findings: (1) distal ileum motility: by 30% and 35% in colon. Animal subjects received 25%, but decreased 20% in colon. Animal 20% decreased by 10% and in colon by 20%. DSM 17938 increased animal respiratory 60% in colon. DSM 17938 increased animal respiratory 17%, but increased in colon 60%. Animal 20% was reduced 60% and 20% in colon. DSM 17938 decreased frequency by 75% and increased

Lactobacillus reuteri (DSM 17938)

JB-1 and DSM 17938 affect jejunal and colon motility

Probiotics have already shown impacts on motor function ...

结论

HMO促进健康

- > 母乳低聚糖有效促进双歧杆菌生长，并显示了与一些特定双歧杆菌菌株的共生特性
- > 母乳低聚糖主动预防病原微生物和病毒的侵害，维护肠道微生态平衡



HMO应用广泛

- > 各欧美国家和地区陆续上市含HMO的婴儿配方粉，并获得市场认可
- > 除了婴儿配方粉，HMO作为优质功能性低聚糖，还可以广泛应用于其他领域



杜邦为您提供

- > 杜邦CARE4U系列HMO产品纯度高，粉体质量优异
- > 杜邦益生菌平台搭配HMO的应用，将成为市场热点
- > 杜邦3FL将于2020年初推出商业化产品

IT'S WHAT'S INSIDE.

DuPont Nutrition & Health, a DowDuPont Specialty Products Division business, combines in-depth knowledge of food and nutrition with current research and expert science to deliver unmatched value to the food, beverage, pharmaceutical and dietary supplement industries. We are innovative solvers, drawing on deep consumer insights and a broad product portfolio to help our customers turn challenges into high-value business opportunities. More information is available at www.food.dupont.com.

