Impact of Farm Environment on Key Bioactive Components in Breast Milk

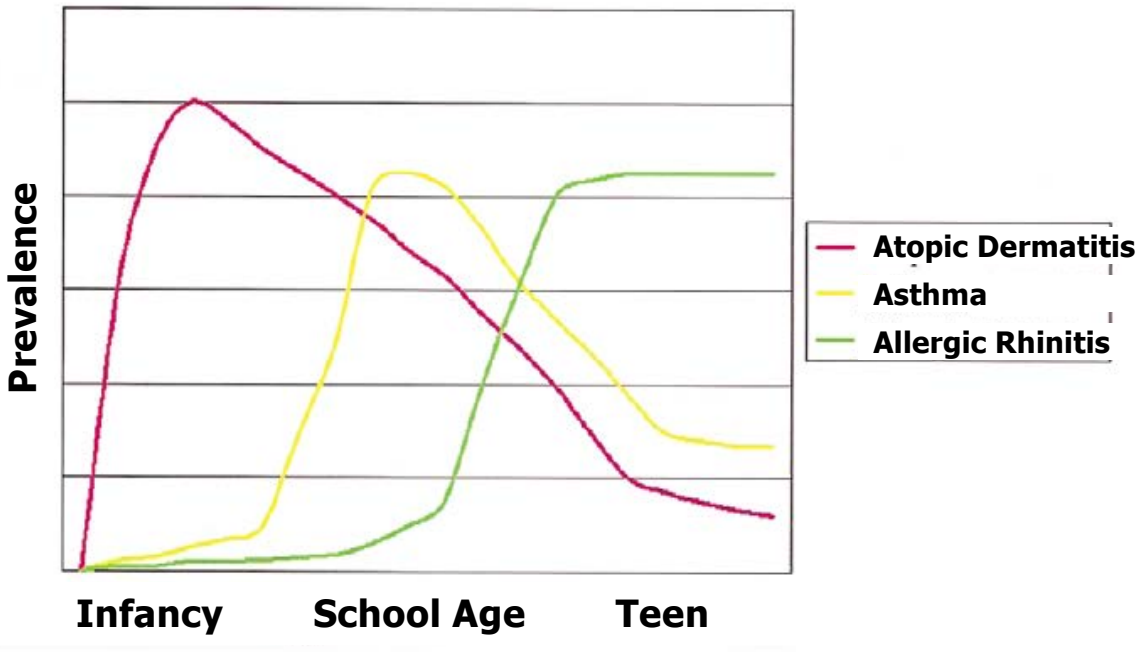
Anastasia Tackett, Anne-Marie Singh, Christine Seroogy, John Lucey

Immune health issues in children

- Food Allergy
  - 8% of children have a food allergy.
- Asthma
- Atopic Dermatitis (Eczema)
  - AD globally affects 15%–20% of children

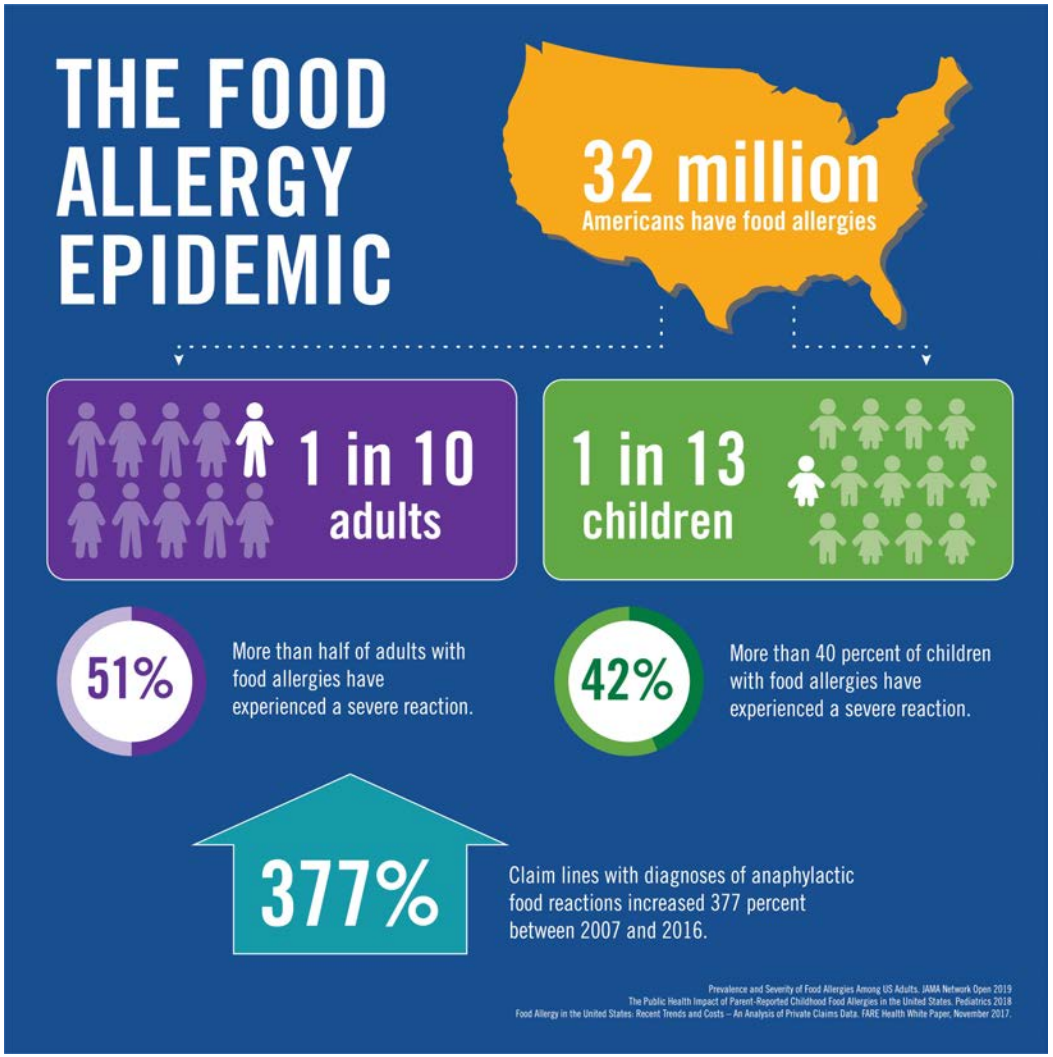
- All increasing in incidence in recent years. Why?
- What can we do?

Atopic Dermatitis is an Early Expression of the Atopic March



Spergel and Paller, JACI 2003

- There is over a 50% increase in incidence of food allergy since 1997!

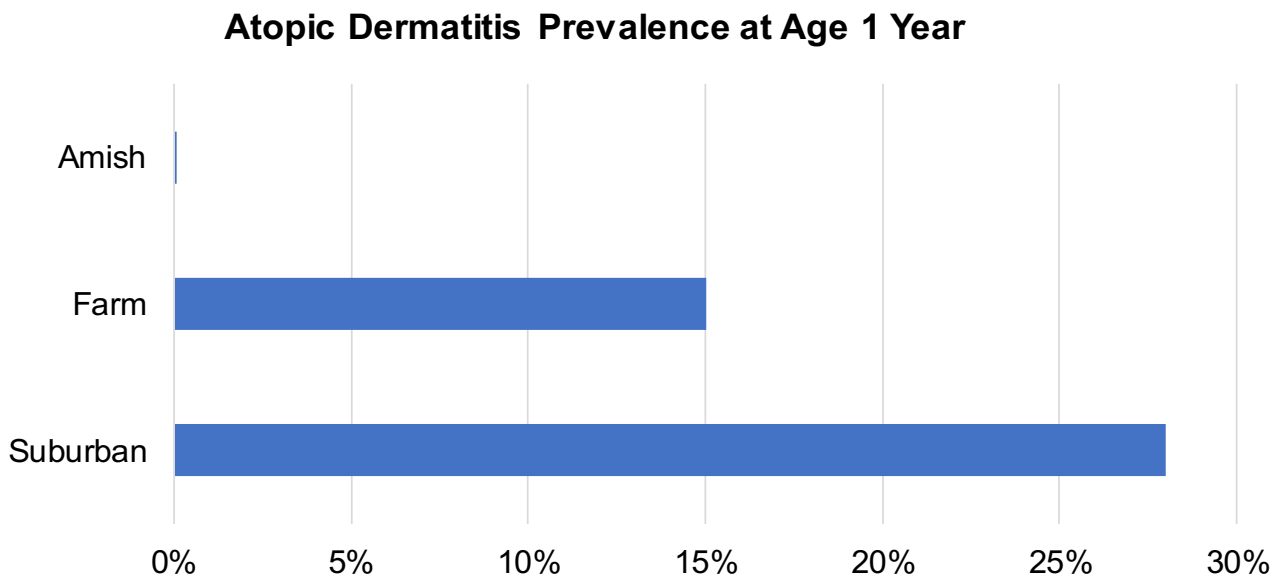


Farm Exposure

https://i.pinimg.com/originals/90/d6/cc/90d6cc9b136cfce844daf4b4437a4d9c.jpg

- Evidence of reduction in allergies with farm exposure.
- Wisconsin Amish reported significantly decreased allergic disease prevalence, including food allergy (Tantoco et. al., 2018).
- Exact mechanism is unknown
- Is it farm milk? Dust? Microbial exposure?

Amish



- WISC cohort demonstrates the rate of atopic dermatitis is approx. 15% in infants born to farming families, <1% in Amish families, vs. 28% in suburban families from Wisconsin at 1 year of age.

European Studies

- PASTURE cohort study suggests consumption of unprocessed cow's milk protects infants from common respiratory infections (Loss et al., 2015).
- Various microorganisms have been identified from the farming environment that have been reported to have an allergy-protective effect (Debarry et al., 2007).

Farm environment and breast milk composition

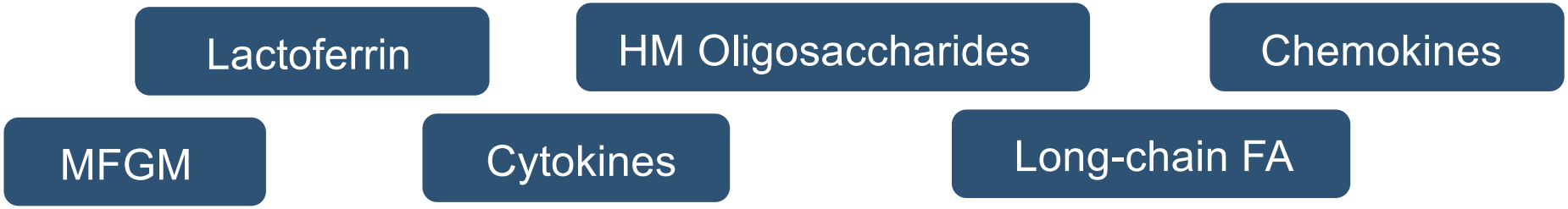
- Early exposure to allergens may benefit health of kids
  - Early dietary exposure to peanuts in high-risk infants prevented development of peanut allergy (Du Toit et al., 2016).

- Maybe human breast milk composition changes based on mother's diet and environment?

Hypothesis

- We hypothesize that maternal diet and farm exposure alter breast milk composition to prevent food allergy and atopic dermatitis.

What could be in breast milk to help prevent allergy?



The Team

- Dr. John Lucey
- Dr. Anne Marie Singh, MD
  - Associate Professor in Pediatric Allergy and Immunology, Director of the Food Allergy Research and Education Center of Excellence
- Dr. Christine Seroogy, MD
  - Professor in Pediatric Allergy and Immunology

Project

- Wisconsin Infant Study Cohort (WISC)
- 270 participants
  1) Farming families
  2) Non-farming Rural families
  3) Amish ~30 maternal/infant pairs
- Breast milk samples collected at 2 months and 9-12 months
- Cow milk samples taken as well from households who consume farm milk
- Compare allergy incidence with Suburban birth cohort, 500
  – 125 will be high risk

Project Objectives

1. Determine the immunologic factors, including total and specific antibody levels, cytokine expression and chemokine levels in human breast milk from mothers with different levels of farm exposure.

2. Elucidate the biochemical composition of breast milk and farm milk, including fat globule size, types of protein and fats, and amount and types of human milk oligosaccharides from mothers living in suburban, rural and Amish communities.

3. Determine food allergy and atopic dermatitis disease outcomes and associations with breast milk and farm milk biomolecules.

Target Bioactive Components

1) Lactoferrin
2) Human milk oligosaccharides (HMOs)
3) Milk fat globule membrane

Bioactive Component #1

- Human Milk Lactoferrin
  - Major protein in mature breast milk at about 1–4 g/L
  - Antimicrobial properties
  - Anti-inflammatory, by turning off the production of numerous pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and IL-8
  - Released in secondary granules by Neutrophils, a type of innate immune cell.
  - Reduction of inflammation and infection in an infant helps save energy for growing.

  (Hanson et al., 2003).

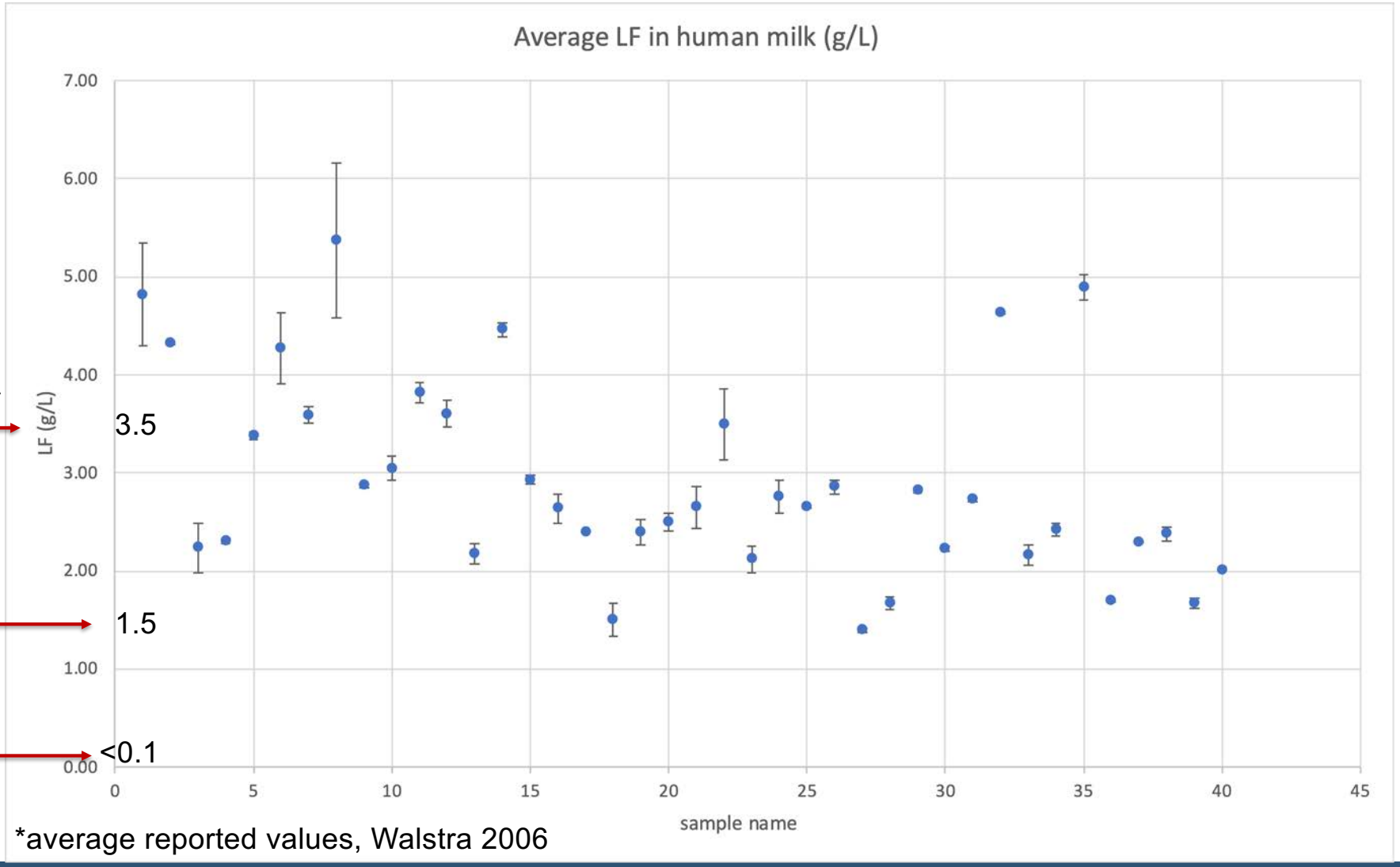
Lactoferrin sample plate - ELISA

Expected Values

Human Colostrum* → 3.5

Human milk* → 1.5

Cow milk* → <0.1

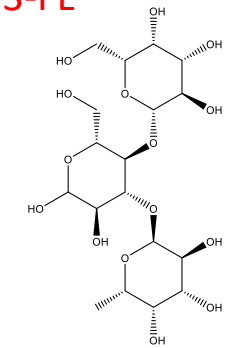


*average reported values, Walstra 2006

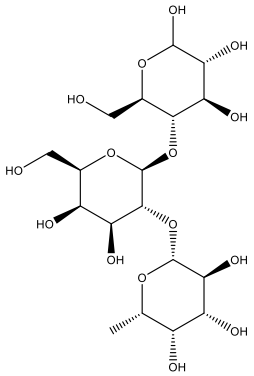
Bioactive Component #2

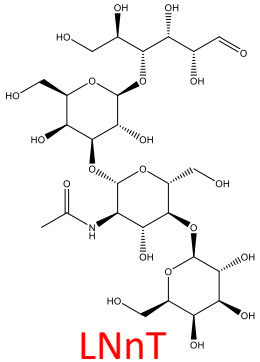
- Human Milk Oligosaccharides (HMOs)
  - 3rd largest fraction in breast milk after lactose and lipids, but largely undigestible by the infant.
  - Typically, 10-15 g/L total in mature milk and 20-25 g/L in colostrum
  - Potential applications for health and wellness
    - Prebiotic, stimulating beneficial colonization and reducing colonization with pathogens; reduced inflammation

3-FL



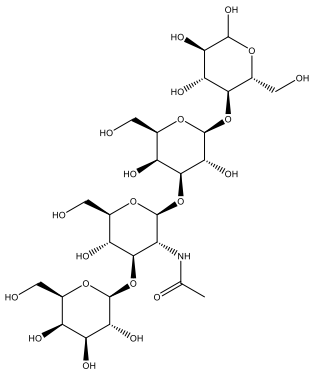
2'-FL

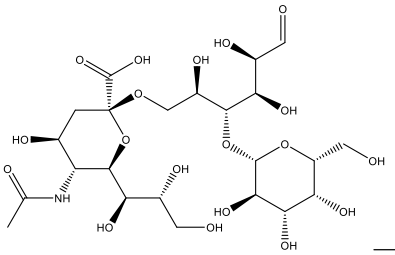




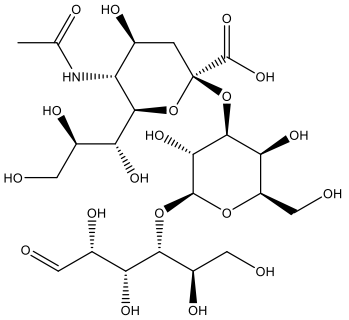
LNnT

LNT





6'-SL



3'-SL

GRAS Notices

FDA Home › Generally Recognized as Safe › Food Ingredient & Packaging Inventories › GRAS Notices

The inventory of GRAS notices provides information about GRAS notices filed since 1998, when FDA received its first GRAS notice. As of October 17, 2016, the GRAS final rule (81 FR 54960; August 17, 2016) requires a specific format for a GRAS notice. Prior to that date, FDA processed GRAS notices under the framework of the GRAS proposed rule (62 FR 18938; April 17, 1997). Notices received prior to the effective date of the GRAS final rule provide examples for potential notifiers for the types of information that may support a GRAS conclusion. In the inventory, notices follow the requirements for the format and content of a GRAS notice as of the effective date of the GRAS final rule.

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- To search for a specific substance/term, enter the term in the Search box and select Show Items to display only those records that contain the selected term. (The search results also includes terms not shown on this page, but included in the full record on the detail page.)
- The search results will return hits of records containing words that include the search term. For example, a search for the color ***red*** will return results that include terms such as *red*uce, ing*red*ient, and cultu*red*. To limit results to only the searched term, place a space before and after the word in the basic search or in the advanced search "this exact phrase" field.

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Basic Search | Advanced Search | Field Search

Search: Show Items Clear

Records Found: 1017 Show All Page 1 of 21

GRN No. (sorted Z-A)	Substance	Date of closure	FDA's Letter
1017	Lacto-*N*-tetraose		Pending
1016	6'-sialyllactose sodium salt		Pending
1015	3'-sialyllactose sodium salt		Pending
1014	2'-fucosyllactose		Pending
1013	*Lactobacillus rhamnosus* DSM 33156		Pending
1012	Powdered sap from *Angelica keiskei* (ashitaba) containing 8% chalcones (ashitaba sap)		Pending

Yu et al. Microb Cell Fact (2018) 17:101
https://doi.org/10.1186/s12934-018-0947-2

Microbial Cell Factories

RESEARCH Open Access

Production of a human milk oligosaccharide 2′-fucosyllactose by metabolically engineered *Saccharomyces cerevisiae*

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Abstract

Background: 2′-Fucosyllactose (2-FL), one of the most abundant oligosaccharides in human milk, has potential applications in foods due to its health benefits such as the selective promotion of bifidobacterial growth and the inhibition of pathogenic microbial binding to the human gut. Owing to the limited amounts of 2-FL in human milk, alternative microbial production of 2-FL is considered promising. To date, microbial production of 2-FL has been studied mostly in *Escherichia coli*. In this study, 2-FL was produced alternatively by using a yeast *Saccharomyces cerevisiae*, which may have advantages over *E. coli*.

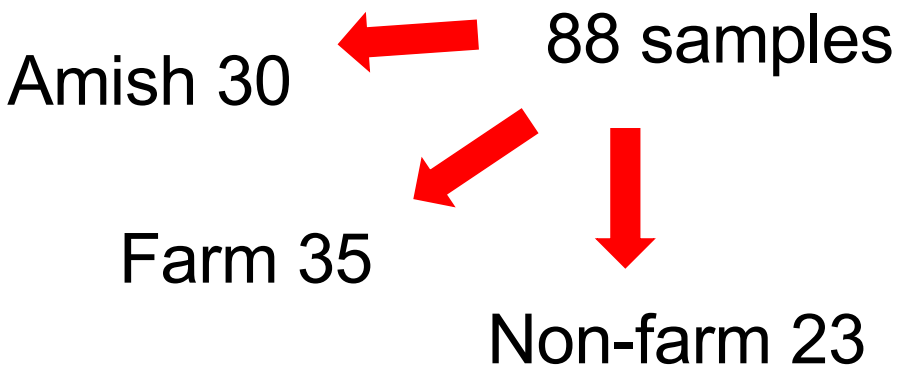
Results: Fucose and lactose were used as the substrates for the *salvage* pathway which was constructed with *fkp* coding for a bifunctional enzyme exhibiting L-fucokinase and guanosine 5′-diphosphate-L-fucose phosphorylase activities, *fucT2* coding for α-1,2-fucosyltransferase, and *LAC12* coding for lactose permease. Production of 2-FL by the resulting engineered yeast was verified by mass spectrometry. 2-FL titers of 92 and 503 mg/L were achieved from 48-h batch fermentation and 120-h fed-batch fermentation fed with ethanol as a carbon source, respectively.

Conclusions: This is the first report on 2-FL production by using yeast *S. cerevisiae*. These results suggest that *S. cerevisiae* can be considered as a host engineered for producing 2-FL via the *salvage* pathway.

Keywords: 2′-Fucosyllactose, Human milk oligosaccharide, *Salvage* pathway, α-1,2-Fucosyltransferase, Lactose permease, *Saccharomyces cerevisiae*

HMO Method

- Human Oligosaccharides (HMOs) – HPIC
  - amounts and 6 types
    - 3-fucosyllactose (3-FL)
    - 2′-fucosyllactose (2’-FL)
    - Lacto-N-neotetraose (LNnT)
    - Lacto-N-tetraose (LNT)
    - 6'-Sialyllactose (6’-SL)
    - 3′-Sialyllactose (3’-SL)



  - Tan, J., F. Chai, & C. Zhang (2015). Determination of Human Milk Oligosaccharides in Human Breast Milk by HPAE-PAD with On-Line Sample Cleanup. Thermo Fisher Scientific, Singapore, CMD Application and Training Center

HMOs

- Mothers vary in the specific structures of HMOs present in their breast milk
- May be influenced in part by gene expression and environment.
- Some mothers don’t make 2’FL, some no 3-FL
  - FUT2 (secretor gene) and FUT3 (Lewis gene)

HPIC Calibration

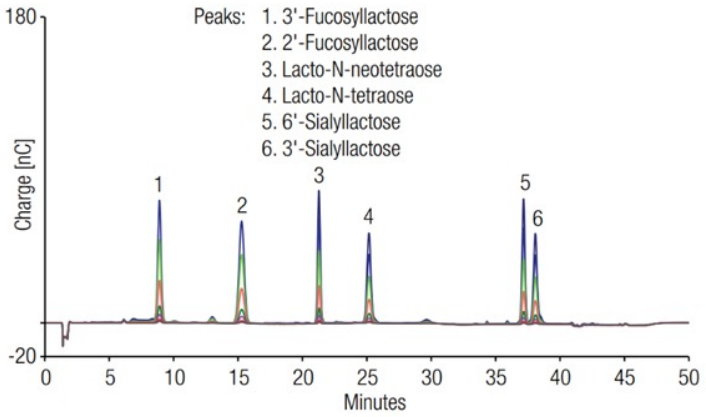
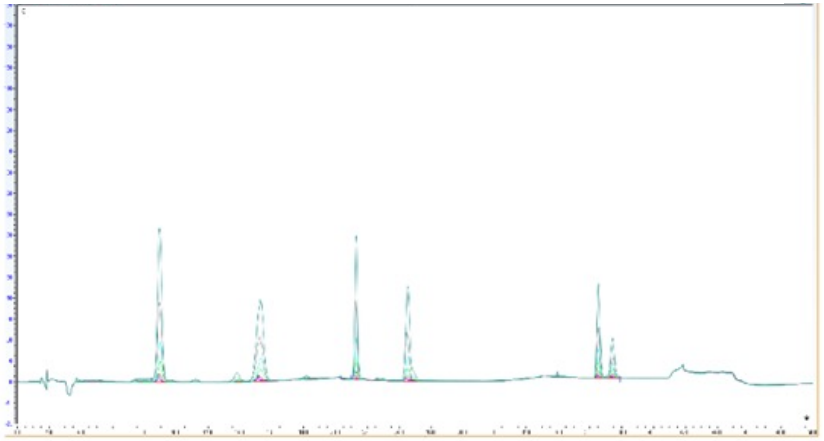


Figure 2. Overlay of chromatograms of standards.



First Human Milks

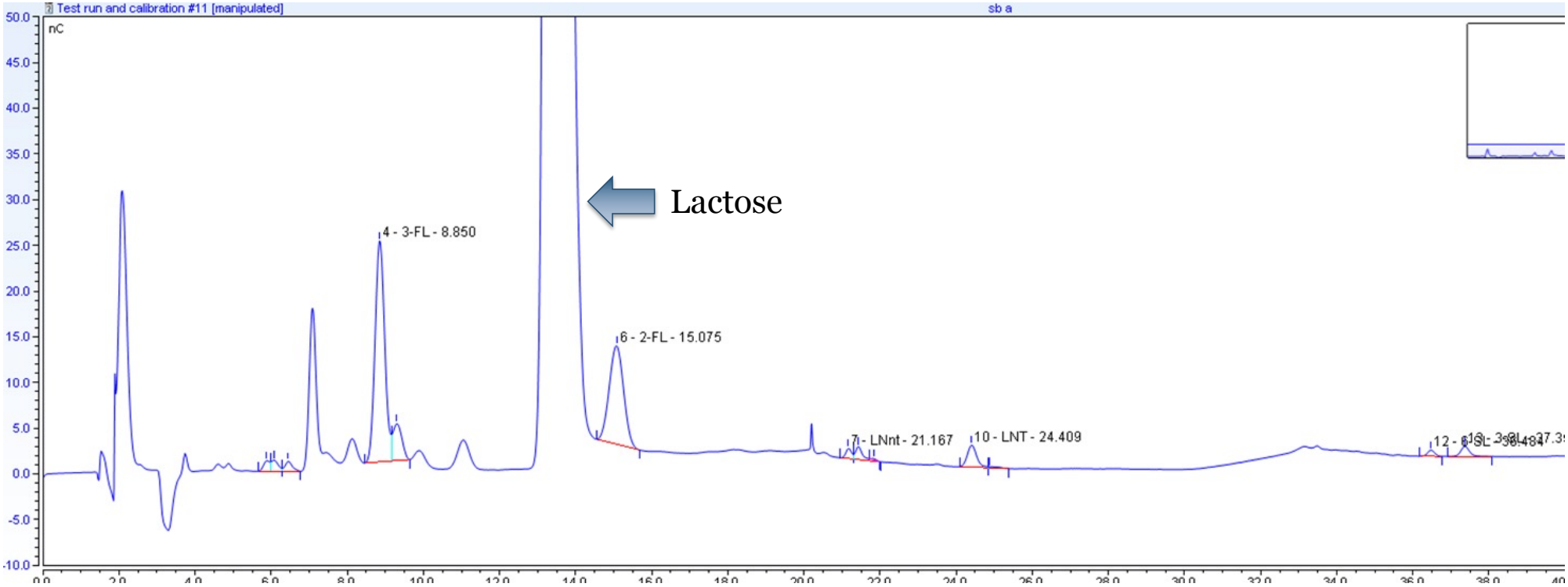
HMOs in NICU and SB milks						
Sample	Average ug/ml in milk					
	3-FL	2-FL	LNnT	LNT	6-SL	3-SL
1	217	2528	498	845	494	100
2	258	2379	442	1021	711	118
3	235	2347	489	995	652	107
4	233	n.d.	60	4456	544	170
SB	1249	1054	46	174	39	123

Samples 1-4 are from the NICU and so are from early lactation.

SB human milk is from 4 months.

Std Dev < aprox. +/- 10 ug/ml

SB Breast Milk Injection Chromatogram

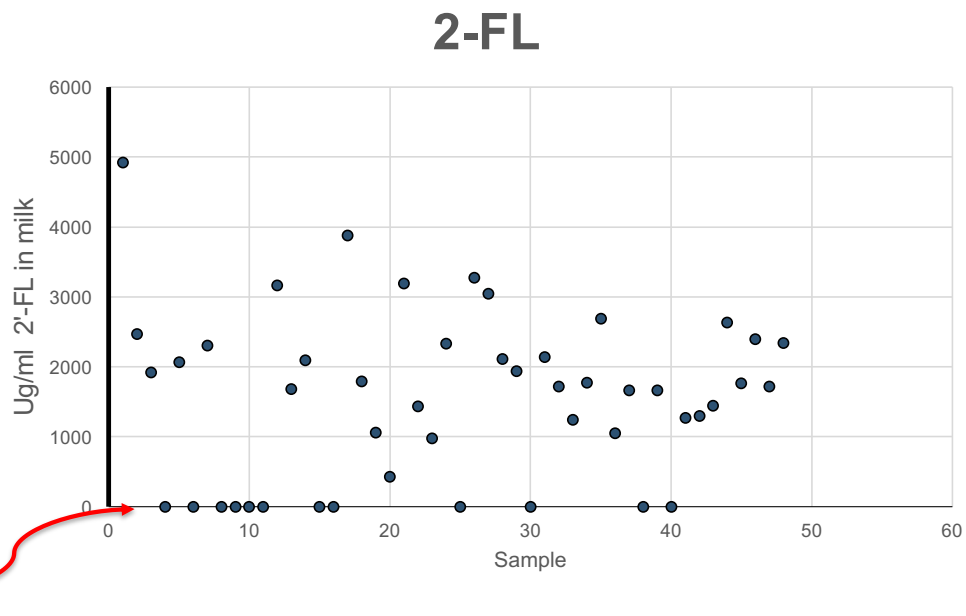


Sample Results

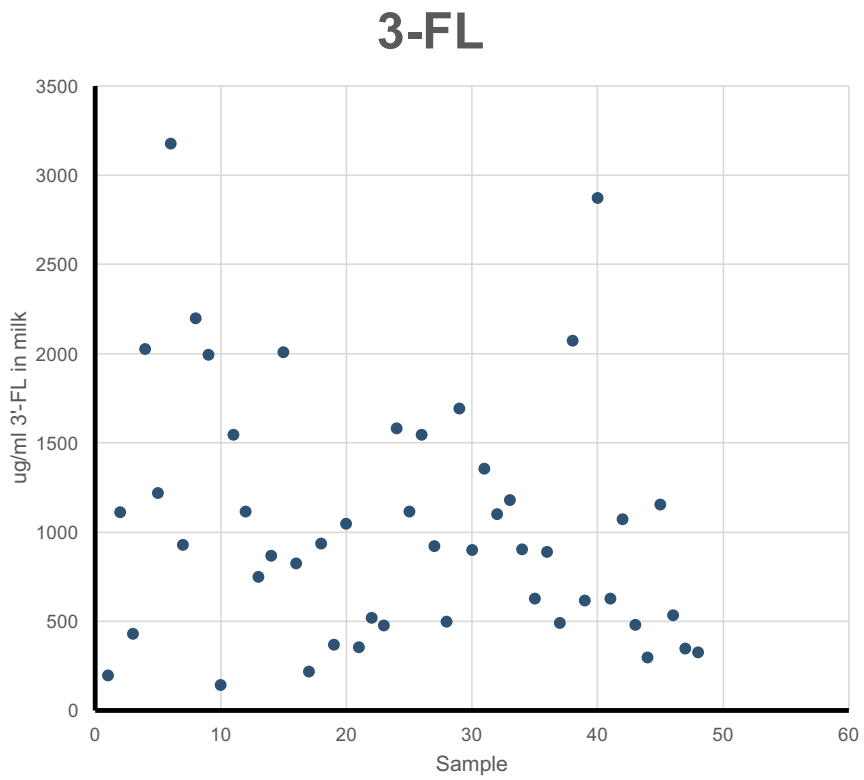
Non-Secretors for 2’-FL

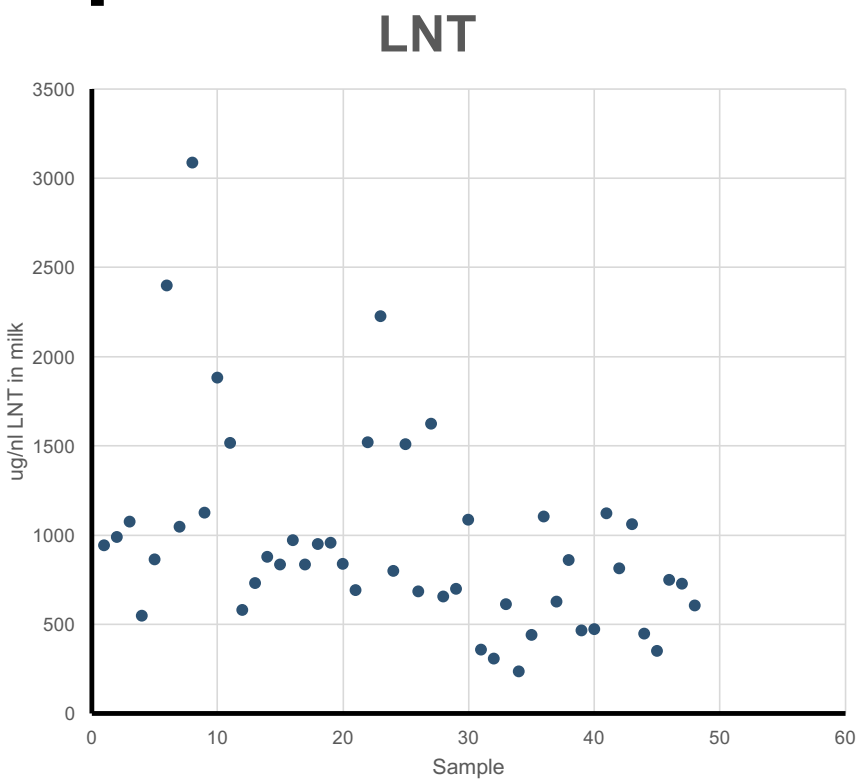
- 15 milks so far fit the non-secretor category based on their lack of 2’-FL.
- 45 milks so far fit the secretor category.

- Above 0.1 g/L or 100 ug/ml of 2’-FL has been used as a cutoff of secretors vs. non-secretors. (Durham et al., 2021)

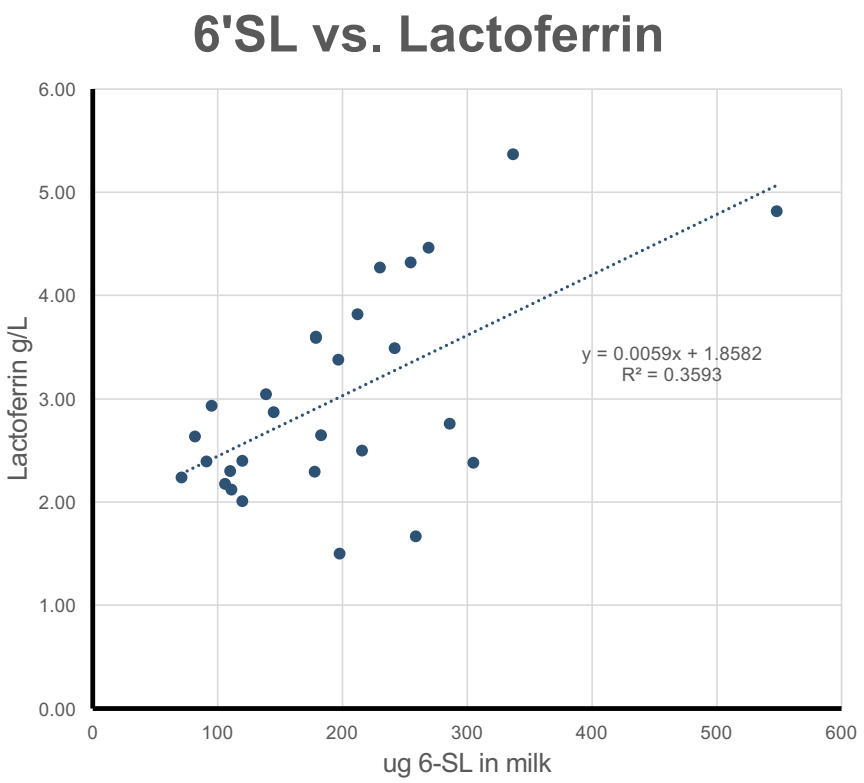


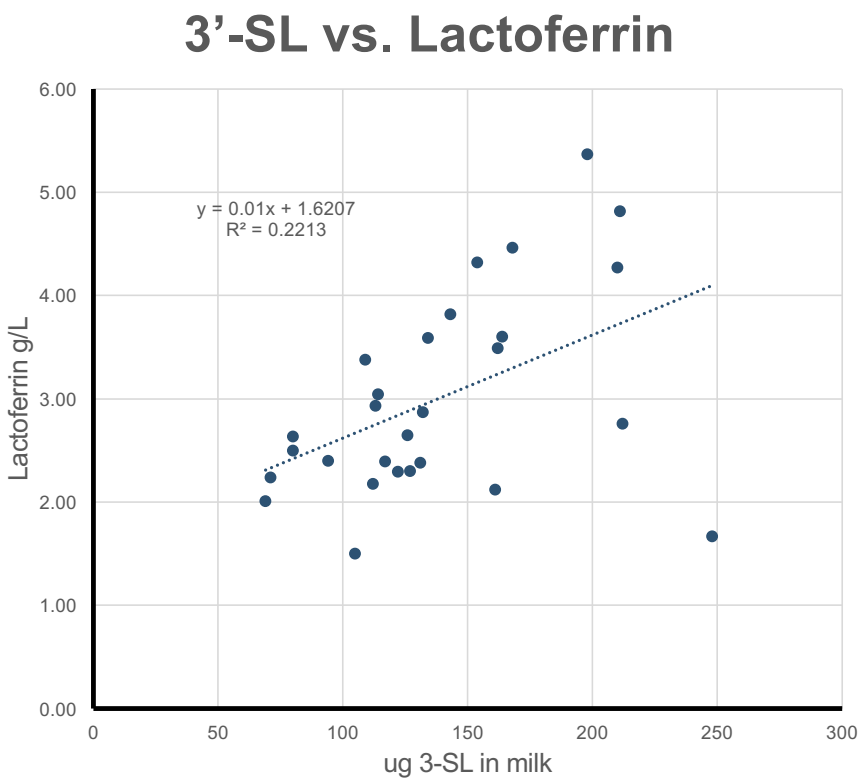
Variability in HMO levels from Breast Milk Samples





HMO vs. Lactoferrin in Breast Milks



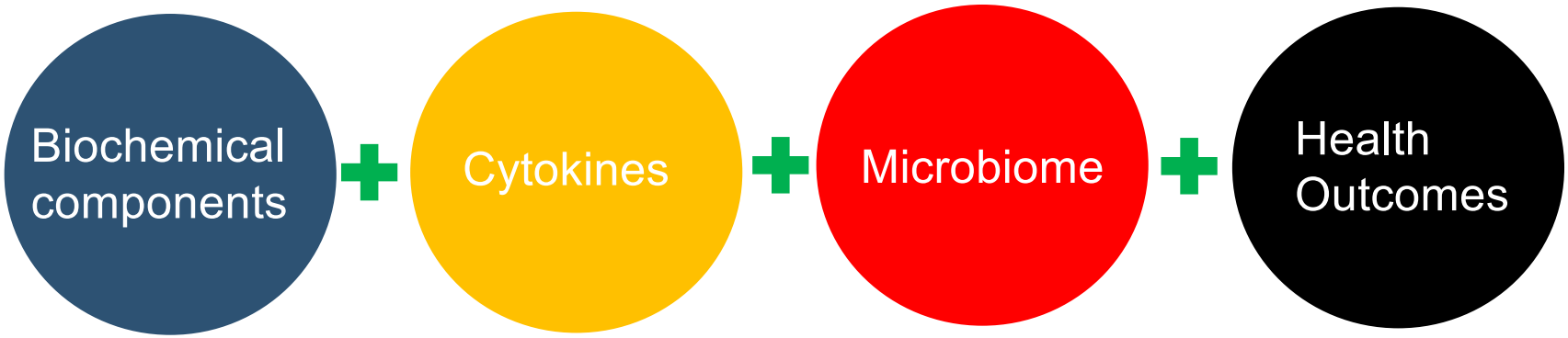


Bioactive Component #3

- Fat globules
  - Farm milk has naturally occurring membrane around fat globules, while in homogenized milk, these membranes are replaced by caseins.
  - Milk fat globule membrane material may be a bioactive component, with benefit for infant and children neurodevelopment and defense against infections.

Future Work

- HMO samples continue
  - Try to identify peaks we don't know.
- Helping finish Lactoferrin tests
- See Amish vs. farming vs. non-farming groups and compare with results



Closing

- The goal is to determine what factors in breast milk may be modulated by a farm environment and possibly are linked to less allergy.
- These components may be used to the benefit of infants who are at risk.

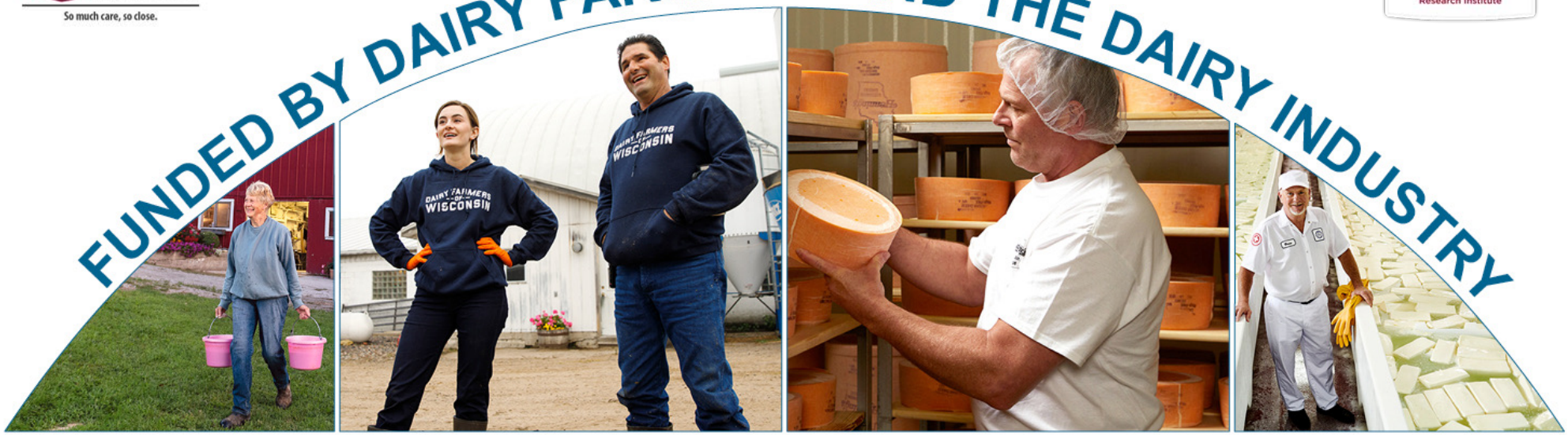
Thank You to Our Supporters

Wisconsin and US Dairy Farm Families | Dairy Farmers of Wisconsin

National Dairy Council | CDR Industry Team | WCMA

USDA
Hatch Act

The Wisconsin Farm Study



Teddy Bear Tackett ☺

Questions??

Samples 1.1

Data shown in ug/ml of milk

Sample ID	3-FL	2-FL	LNnT	LNT	6-SL	3-SL
3251	195	4928	109	944	548	211
2641	1112	2477	120	988	255	154
7121	431	1921	258	1076	71	71
7011	2027	n.d.	n.d.	547	110	127
2171	1220	2071	367	863	197	109
2231	3178	n.d.	131	2399	230	210
2481	929	2310	< 68*	1048	179	134
7161	2198	n.d.	n.d.	3089	337	198
2121	1995	n.d.	< 68*	1127	145	132
2901	144	n.d.	149	1884	139	114
2221	1544	n.d.	n.d.	1518	212	143
2241	1116	3165	127	582	179	164

n.d. < limit of detection

* below limit of quantification < 68 ug/ml

Samples 1.2

Data shown in ug/ml of milk

Sample ID	3-FL	2-FL	LNnT	LNT	6-SL	3-SL
2821	749	1685	< 68*	730	106	112
2401	868	2094	225	877	269	168
2131	2008	n.d.	n.d.	836	95	113
7131	823	n.d.	110	970	82	80
1321	217	3881**	702	834	120	94
2391	937	1798	< 68*	949	198	105
7031	368	1062	82	956	91	117
7231	1047	433	74	838	216	80
1861	354	3195	269	692	183	126
1791	519	1438	73	1522	242	162
7211	475	981	196	2229**	111	161
2991	1581	2337	156	800	286	212

n.d. < limit of detection

* below limit of quantification < 68 ug/ml

** over max area

Samples 2.2

Data shown in ug/ml of milk

Sample ID	3-FL	2-FL	LNnT	LNT	6-SL	3-SL
1241	1116	n.d.	n.d.	1508	178	122
2971	1545	3274	172	685	305	131
2031	921	3046	152	1625	259	248
7281	499	2112	186	656	120	69
2811	1693	1939	79	698	194	169
7001	900	n.d.	< 46*	1088	102	50
3331	1354	2146	< 46*	359	189	162
1391	1100	1726	47	308	179	86
1781	1181	1245	178	612	256	109
7251	903	1781	95	235	121	85
7311	627	2690	160	439	248	87
2561	890	1056	n.d.	1105	198	96

n.d. < limit of detection * below limit of quantification < 46 ug/ml ** over max area

Samples 3.1

Data shown in ug/ml of milk

Sample ID	3-FL	2-FL	LNnT	LNT	6-SL	3-SL
1111	492	1671	78	627	n.d.	165
3261	2074	n.d.	< 44*	861	< 68*	89
7221	615	1670	90	467	< 68*	92
7021	2874	n.d.	n.d.	473	125	91
3471	627	1270	122	1123	124	89
3991	1072	1303	76	813	188	99
7181	481	1450	96	1061	140	99
2011	298	2638	177	449	129	79
7081	1156	1772	< 44*	352	138	77
3451	534	2401	147	748	148	103
3491	348	1725	338	727	81	101
7071	327	2343	224	606	225	77

n.d. < limit of detection * below limit of quantification < 44 ug/ml ** over max area