

Green algae as a platform for protein production: Food, Feed, and Nutritional Supplements

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Chlamydomonas reinhardtii is a model organism, but lacked a viable commercial production process and food safety record before Triton

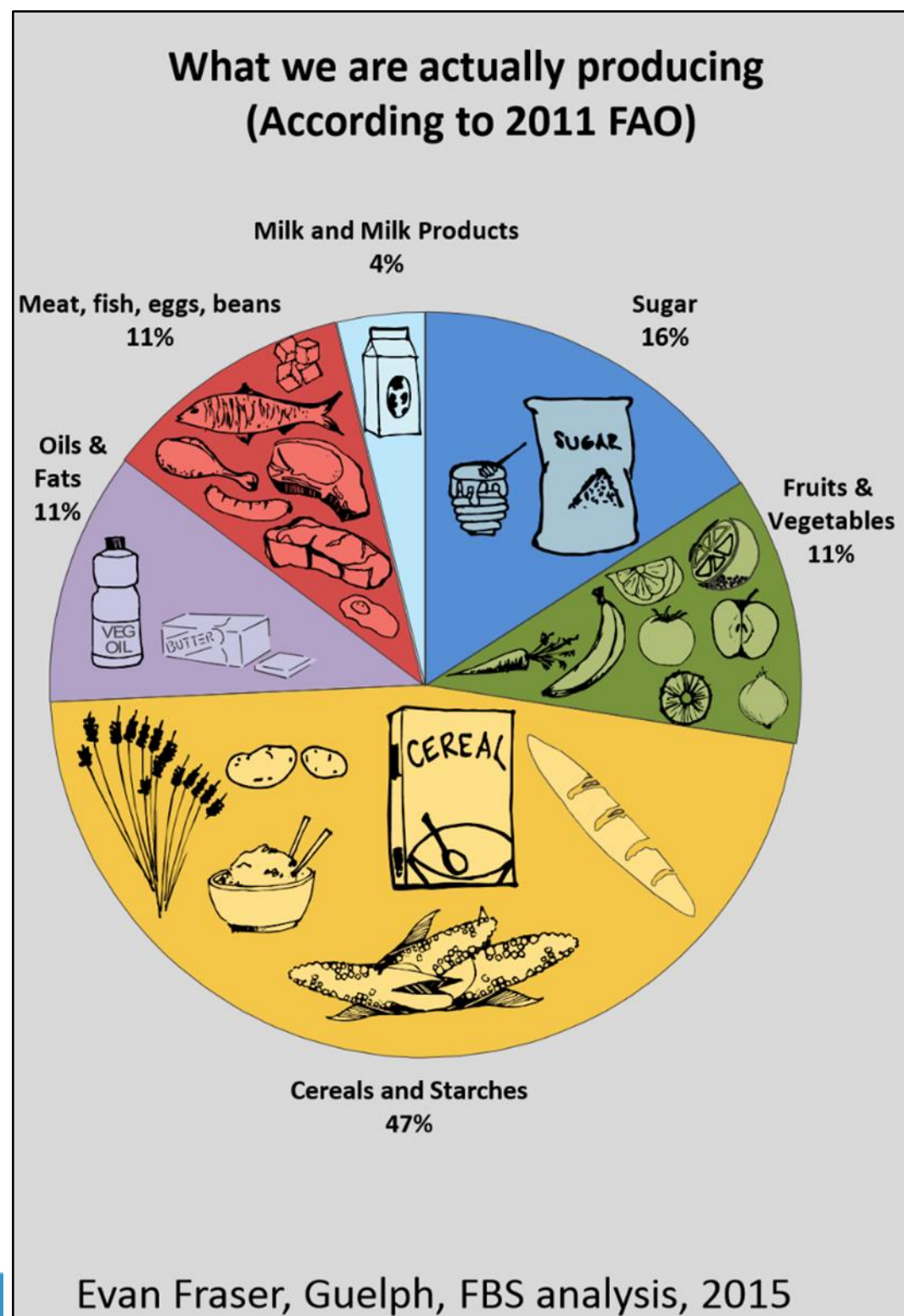
- *Chlamydomonas reinhardtii* is a single-cell green alga.
- *Chlamydomonas* species are **distributed worldwide**.
- *Chlamydomonas reinhardtii* is a model organism, mostly due to its lab scale **synthetic biology**: an ideal host to produce mammalian/plant proteins, antibodies, vaccines, and hormones.
- But lack of an economic large scale production process.



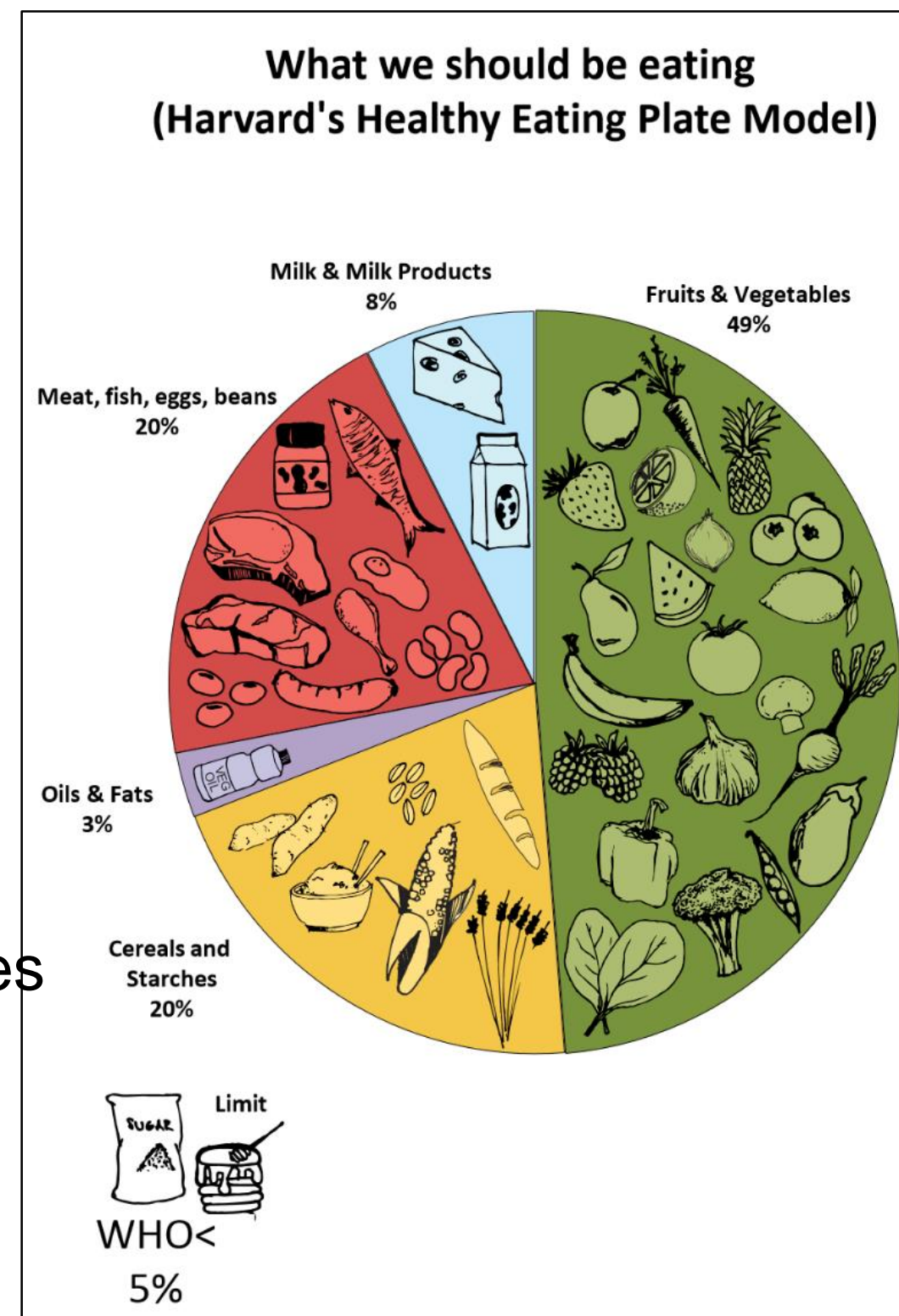
Triton, founded in 2013, is a San Diego start-up company

- Triton has developed a cost effective, scalable, heterotrophic production process for *Chlamydomonas reinhardtii*.
- *Chlamydomonas reinhardtii* is rich in proteins, omega oil, vitamin A, and minerals, with taste like wheatgrass shake.
- Triton has completed toxicity studies on *Chlamydomonas reinhardtii*, expects GRAS status in Q3 2017.
- Triton has proprietary protein expression and production technology with 17 colostrum proteins for synthetic colostrum.
- Lead protein, osteopontin, technology is ready for regulatory and commercial development.

Commercial production of food grade *Chlamydomonas reinhardtii*, or other nutritious green algae, has the potential to address global nutrition shortages



- More proteins →
- More fish →
- More vegetables →
- Less fats →
- Less carbohydrates →



For the first time, Triton has developed a viable commercial viable production process for *Chlamydomonas reinhardtii* in fermenters

- **Productivity:** Increased from < 0.4 to > 20 grams/liter/day,
- **Titers:** Increased from < 2 grams/liter to > 80 grams/liter.
- **Validation:** Process validation by two independent contract manufactures in 200 liter tanks, and one in 3,000 liter tanks.
- **Costs:** would be between \$2 to \$10 per kg (dried powder) when scale up.




Food grade algae produced in a 3,000 liter fermenter by a CMO in a cGMP facility



CERTIFICATE OF ANALYSIS

PRODUCT NAME: Triton Algae-
Chlamydomonas reinhardtii
LOT NUMBER: 170310
WEIGHT SHIPPED: 20.05 KG
MANUFACTURE DATE: March 10, 2017

	STANDARD SPECIFICATIONS	RESULTS	TEST METHOD
ANALYTICAL			
Moisture %	20.0% Maximum	2.0%	AOAC #926.08
Color	Dark Green	Matches Standard	
Appearance	Powder	Matches Standard	
MICROBIOLOGICAL			
Aerobic Plate Count	NMT 10,000 CFU /g	<10 CFU /g	BAM CH. 3
Yeast & Mold	NMT 30 CFU /g	30 CFU /g	BAM CH. 18
Coliform	NMT 10 CFU /g	<10 CFU /g	BAM CH. 4
E.coli	Negative	Negative	BAM CH.4
Staphylococci (coag. Pos.)	Negative	Negative	AOAC #975.55
Salmonella	Negative	Negative	AOAC #999.08

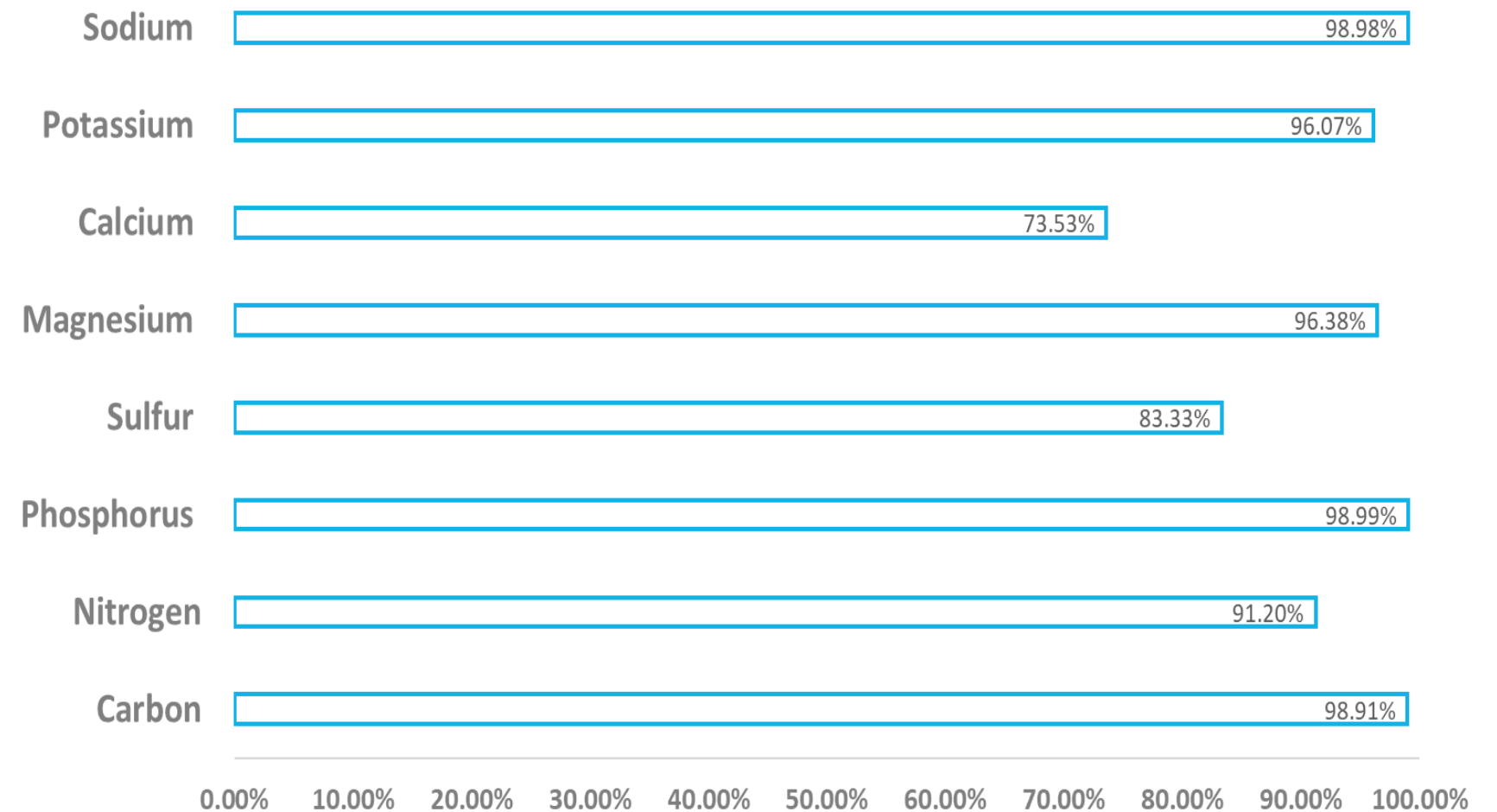

 Reviewed By:

March 27, 2017
 Date:

Triton's media is made of "fertilizers" and acetate. At harvesting, greater than 90% of the Nitrogen, Phosphate, Potassium and Carbon are utilized by the algae

- Petrochemicals can be converted into nutritious green algae powder with nutritional value better than vegetables
- *C. reinhardtii* has a high efficiency of utilizing fertilizers and fresh water
- *C. reinhardtii* does not require crop protection chemicals
- *C. reinhardtii* has a high protein content, unsaturated fatty acids, and minerals
- *C. reinhardtii* is provided in a dried powder like flours, easy for transportation and storage

Utilization at harvesting



Ensuring the safety of this new and healthy food

Generally recognized as safe (GRAS) is a US Food and Drug Administration (FDA) designation that a compound or ingredient added to food is shown to be safe for its intended use

Chlamydomonas reinhardtii safety trials:

- Three in vitro assays (Ames Bacterial Reverse Mutation Assay, Chromosomal Aberration Assay, Mouse Micronucleus Assay) were completed and showed **no adverse or toxic effects**
- 28-day rat feeding trial was completed and showed **no adverse or toxic effects** even at the highest dose levels
- **Self affirmed GRAS status is expected in Q3, 2017**
- The resulting safety data will be published and included in a GRAS notification submitted to the FDA, to achieve **FDA “No Questions” GRAS status in Q2 2018**

Chlamydomonas reinhardtii is a robust host strain for the production of recombinant proteins.

Numerous proteins have been expressed in *Chlamydomonas reinhardtii*. It has the machinery to post-translationally modify complex proteins similar to higher organisms such as plants and mammalian cells.

Antibodies

Industrial enzymes

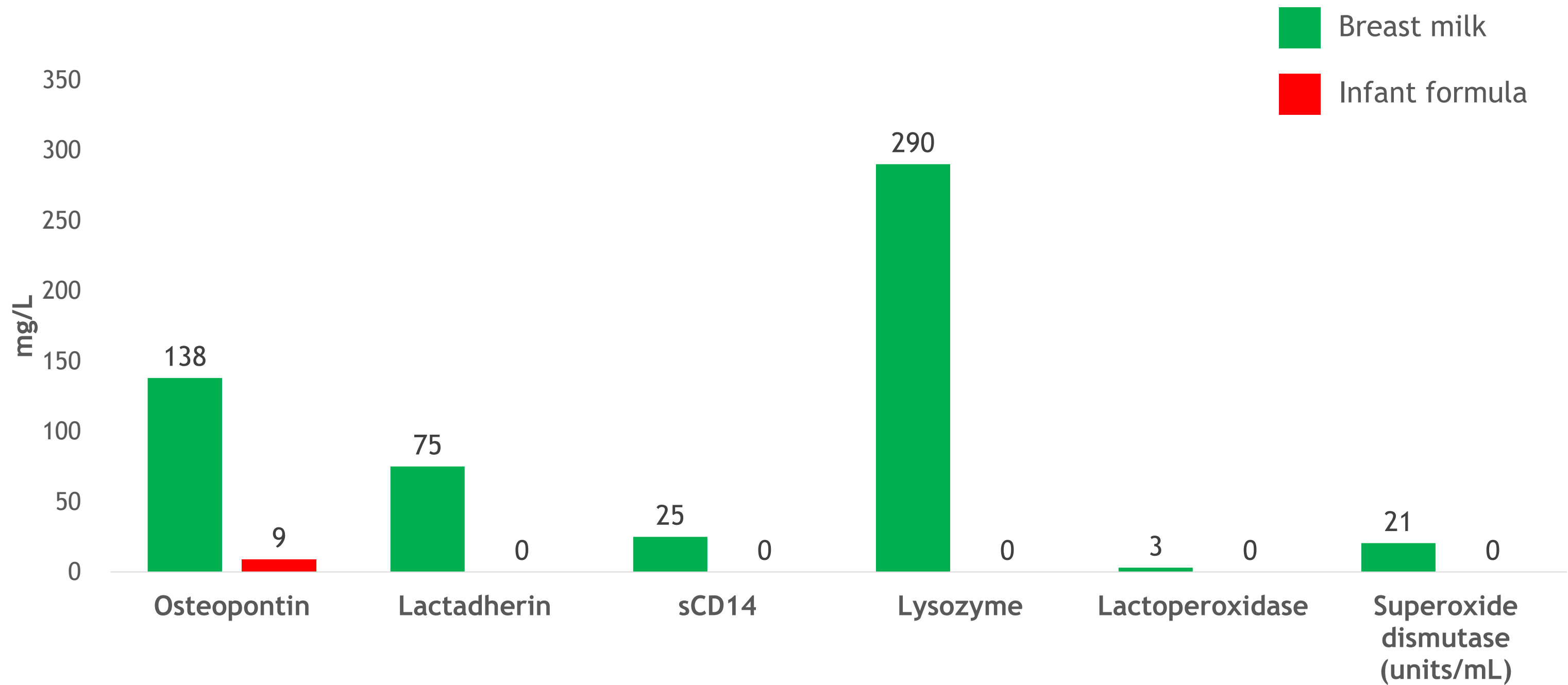
Vaccines

Growth hormones

Colostrum proteins

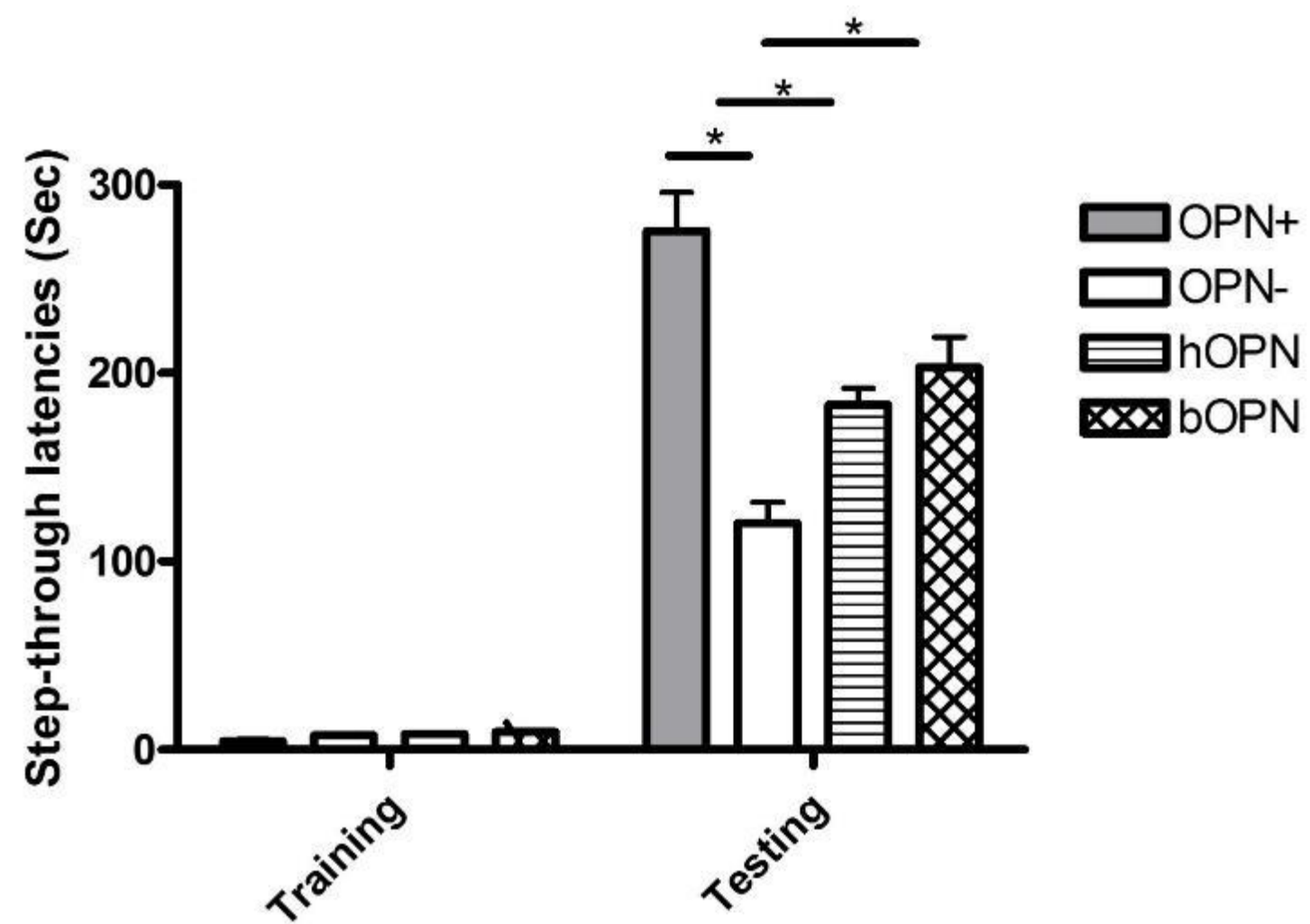
Host	Low cost production	Host protein edible	Complex proteins
E. coli	✓	X	X
Yeast	✓	✓	X
CHO	X	X	✓
Insect	X	X/✓?	✓
Algae	✓	✓	✓

Many components of breast milk are not provided in today's formula, including key functional milk proteins that are important for infant development



Triton's Bovine and Human Osteopontin (OPN) increase the learning ability of mouse pups

Passive avoidance test: Day 30



Conclusions from Prof. Bo Lonnerdal (UC Davis):

- Recombinant hOPN and bOPN **increase the learning ability** of mouse pups
- Recombinant hOPN and bOPN significantly increase the expression of OPN and myelin related proteins in mouse brains
- Milk osteopontin (OPN) and recombinant hOPN and bOPN have no adverse effect on weight gain on baby pups



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