Development of plant-based long-chain omega-3 oils

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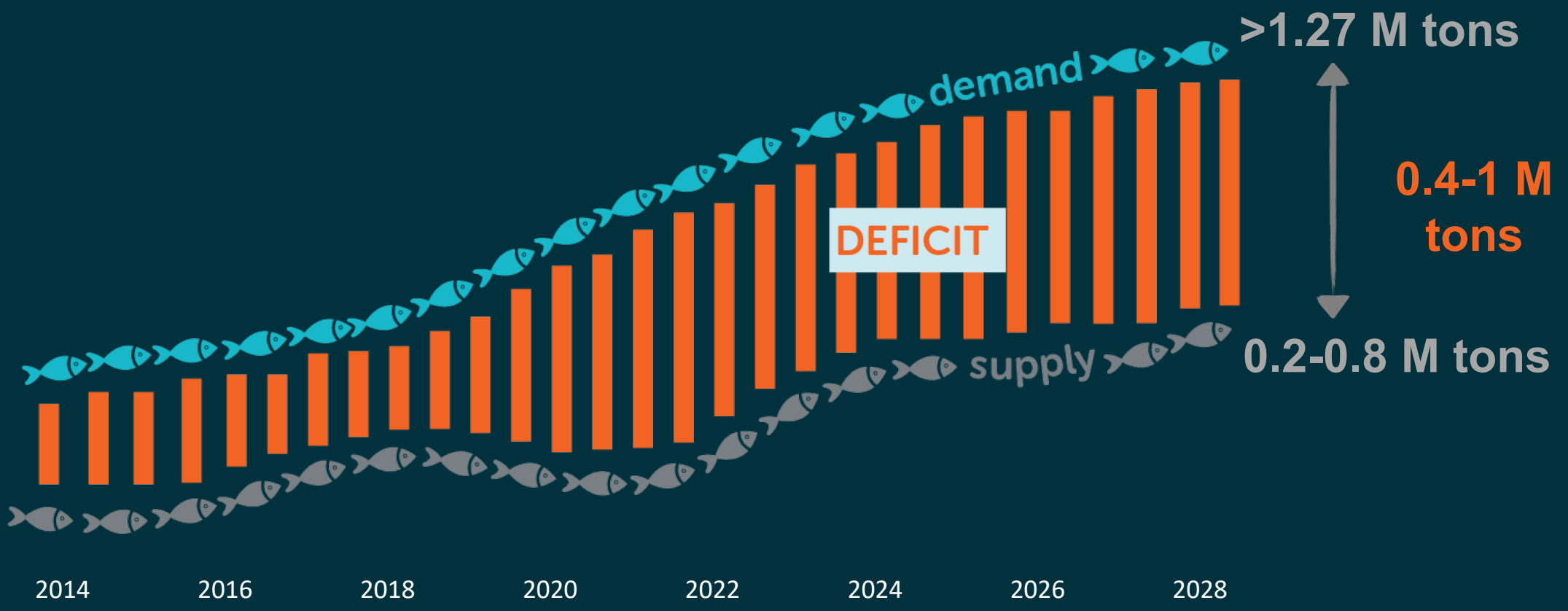
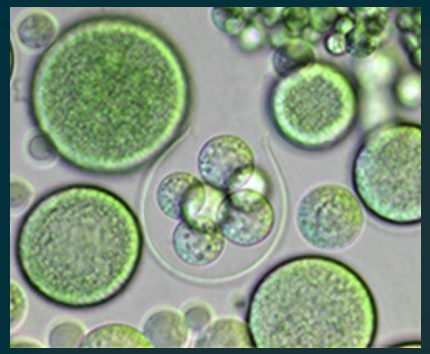
Additional omega-3 sources are required



Tocher et al, 2019

Our strategy



GENES

Microalgae
(EPA and DHA primary producers)

Canola

2005 Proof of concept: EPA/DHA in seed

Seed-specific expression in Arabidopsis

0.9% DHA in seed oil

ALA

18:3

Δ6D

18:4

SDA

Δ6E

C. elegans elongase

20:4

Δ5D

20:5

EPA

Δ5E

Pavlova

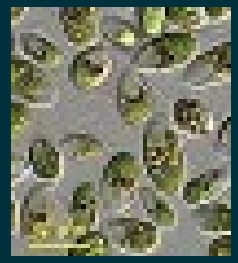
22:5

Δ4D

22:6

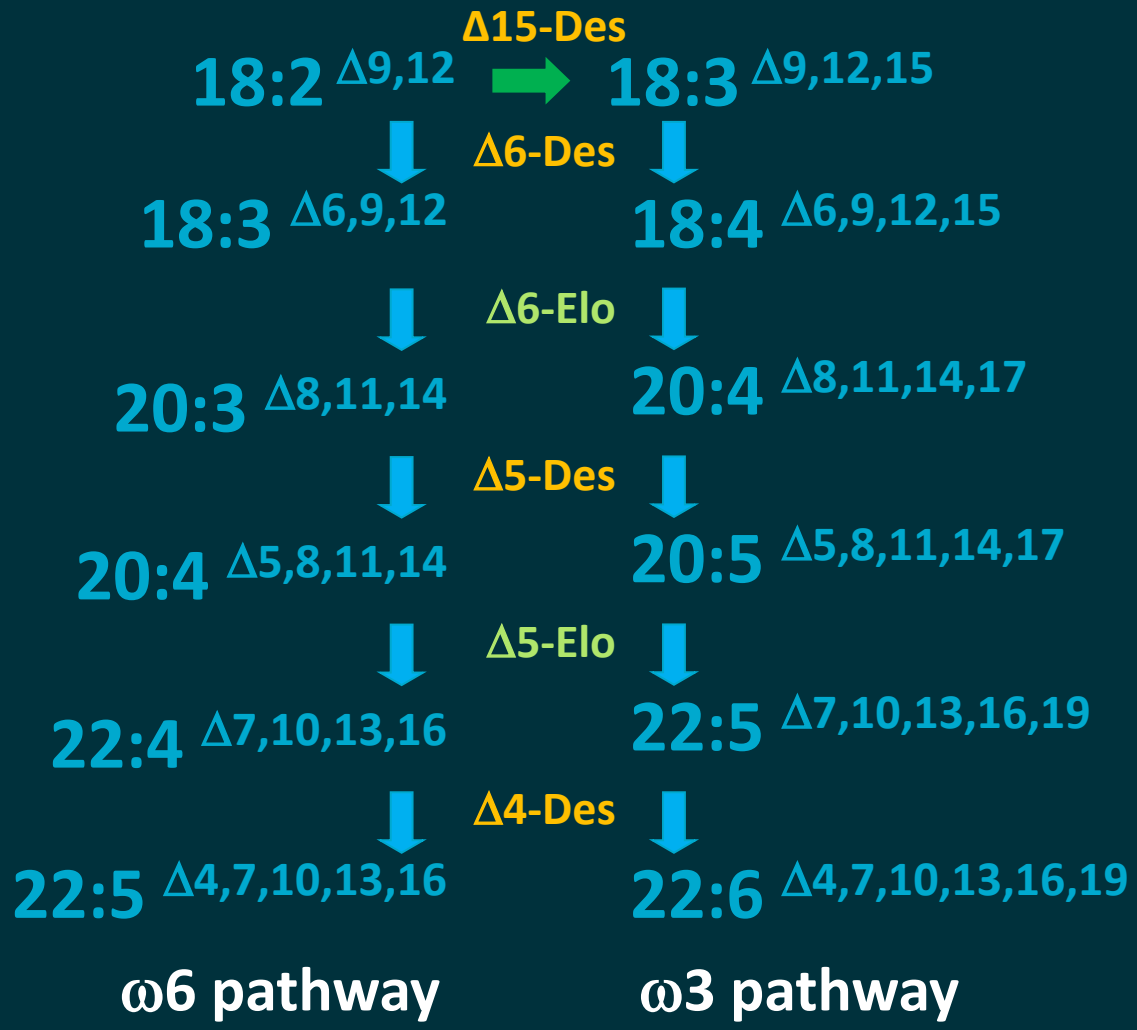
DHA

Zebra fish dual-function desaturase



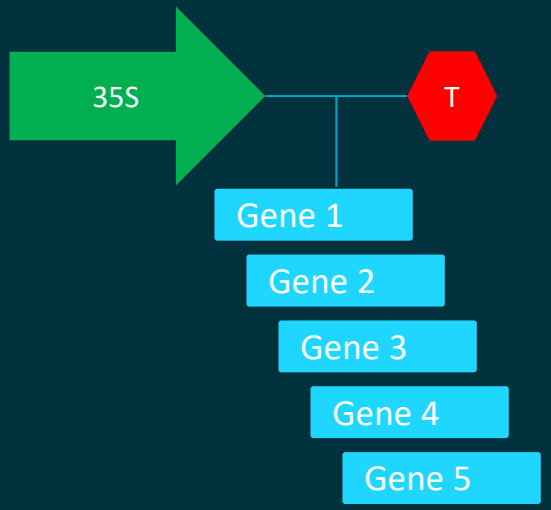
Robert et al, 2005

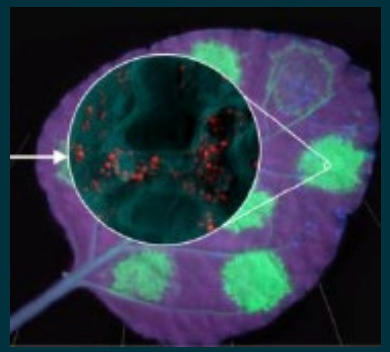
Gene discovery for DHA biosynthesis pathway

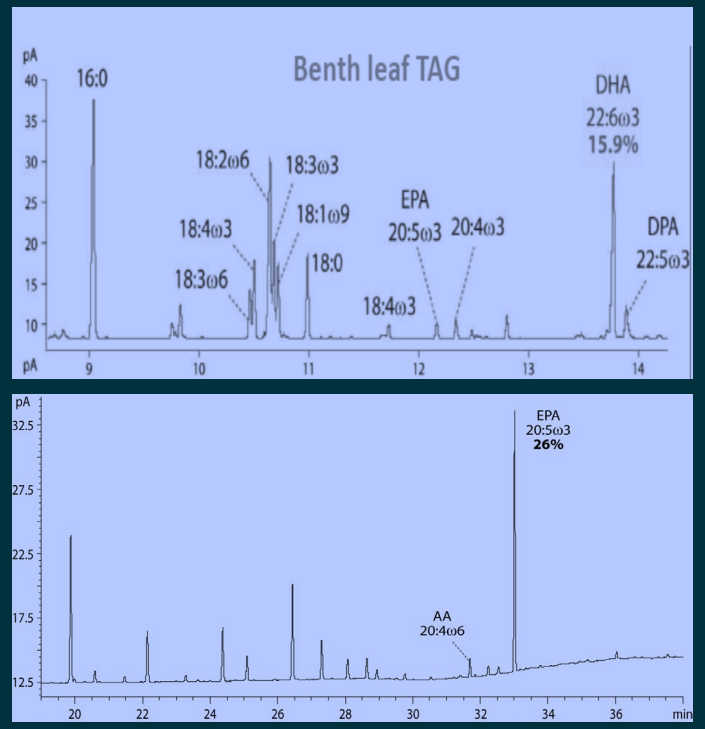


[Australian National Algae Culture Collection – CSIRO https://www.csiro.au/en/about/facilities-collections/Collections/ANACC]

Transient expression system: Leaf







- Efficient pathway to maximise the fatty acid flux to ω3-LCPUFA especially DHA
- High ω3 preference

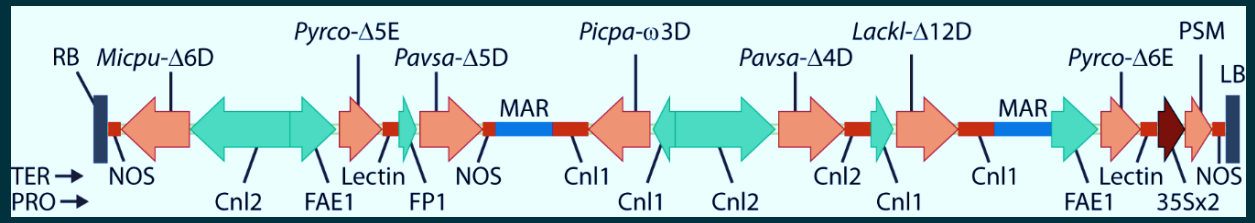
Wood et al, 2009
Petrie et al, 2010
Petrie et al, 2012

Multiple-gene construct for canola

16:0 → 18:0 → 18:1 Δ9 —FAD2→ 18:2 Δ9,12 —FAD3→ 18:3 Δ9,12,15

Δ12-Des *Lachancea kluyveri*

Δ15-Des *Pichia pastoris*



Seed specific expression
8 cassettes, 23-Kb T-DNA

Δ6-Des *Micromonas pusilla*

18:4 Δ6,9,12,15

Δ6-Elo *Pyramimonas cordata*

20:4 Δ8,11,14,17

Δ5-Des *Pavlova salina*

20:5 Δ5,8,11,14,17

Δ5-Elo *Pyramimonas cordata*

22:5 Δ7,10,13,16,19

Δ4-Des *Pavlova salina*

22:6 Δ4,7,10,13,16,19

Zhou et al, 2007
Petrie et al, 2010a
Petrie et al, 2010b

Fish oil-like DHA level in canola

Seed	16:0	18:0	18:1 OA	18:2 ω6 LA	18:3 ω6 GLA	18:3 ω3 ALA	18:4 ω3 SDA	20:3 ω3 ETrA	20:4 ω3 ETA	20:5 ω3 EPA	22:5 ω3 DPA	22:6 ω3 DHA	∑EPA, DPA, DHA	Total ω3	Total ω6	ω3:ω6
WT	4.0	2.2	59.1	19.3	-	9.5	-	-	-	-	-	-	-	9.5	19.3	0.5
DHA canola	4.2	2.7	42.0	7.0	0.4	20.0	**2.2**	**0.6**	**1.3**	**0.5**	**1.0**	**9.7**	**11.1**	**35.3**	7.4	**4.8**

Petrie et al, 2020

ω3-LCPUFA products beyond DHA canola

16:0 → 18:0 → 18:1 Δ9 —FAD2 / Δ12-Des→ 18:2 Δ9,12 —FAD3 / Δ15-Des→ 18:3 Δ9,12,15

↓ Δ6-Des

18:4 Δ6,9,12,15

↓ Δ6-Elo

20:4 Δ8,11,14,17

↓ Δ5-Des

20:5 Δ5,8,11,14,17 (EPA)

>10% EPA

↓ Δ5-Elo

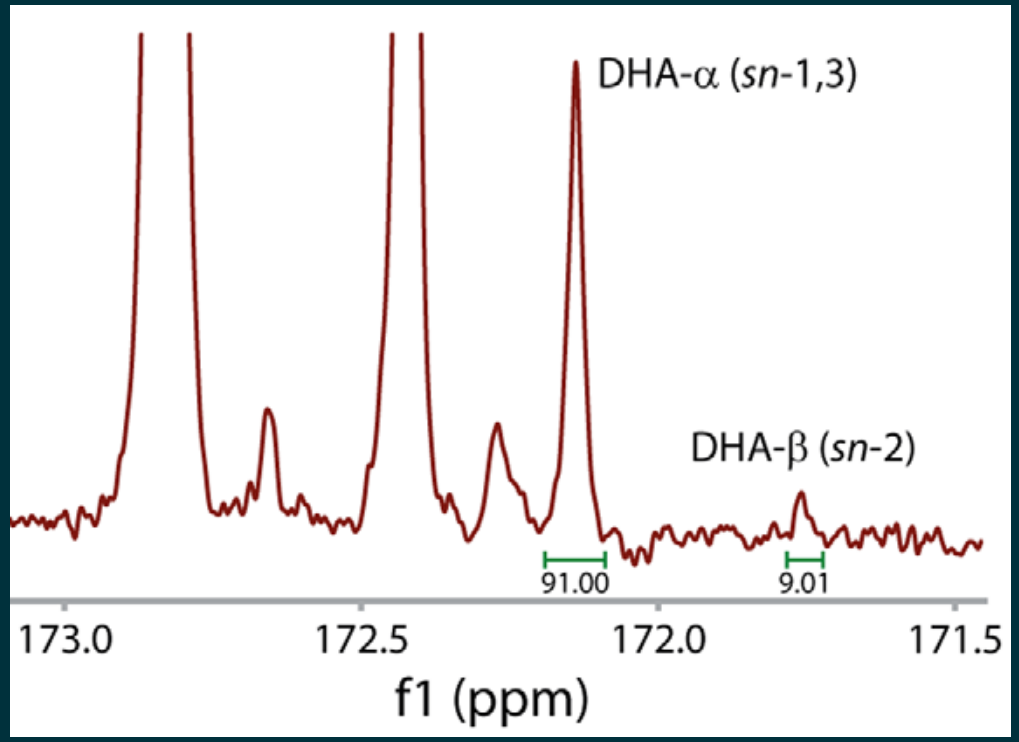
22:5 Δ7,10,13,16,19 (DPA)

>12% DPA

↓ Δ4-Des

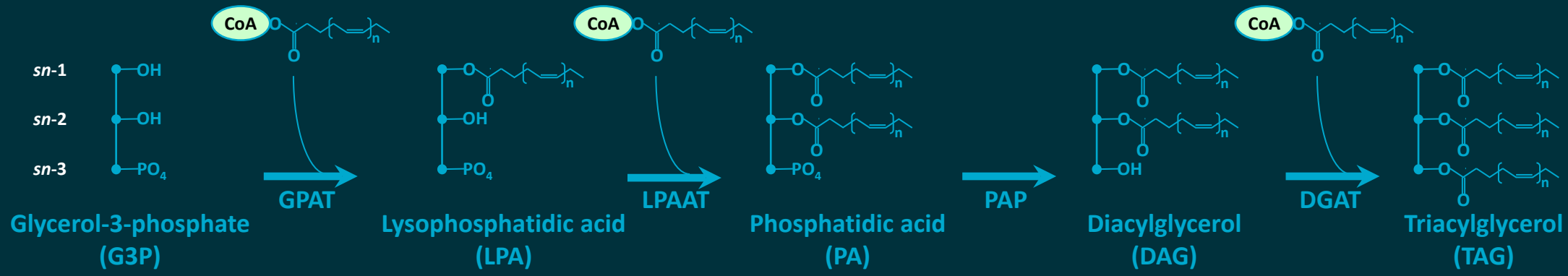
22:6 Δ4,7,10,13,16,19 (DHA)

ω3 LC-PUFAs distribution in TAG



^{13}C-NMR spectrum showed ω3 LC-PUFAs were dominantly distributed at *sn*-1/3 positions of TAG

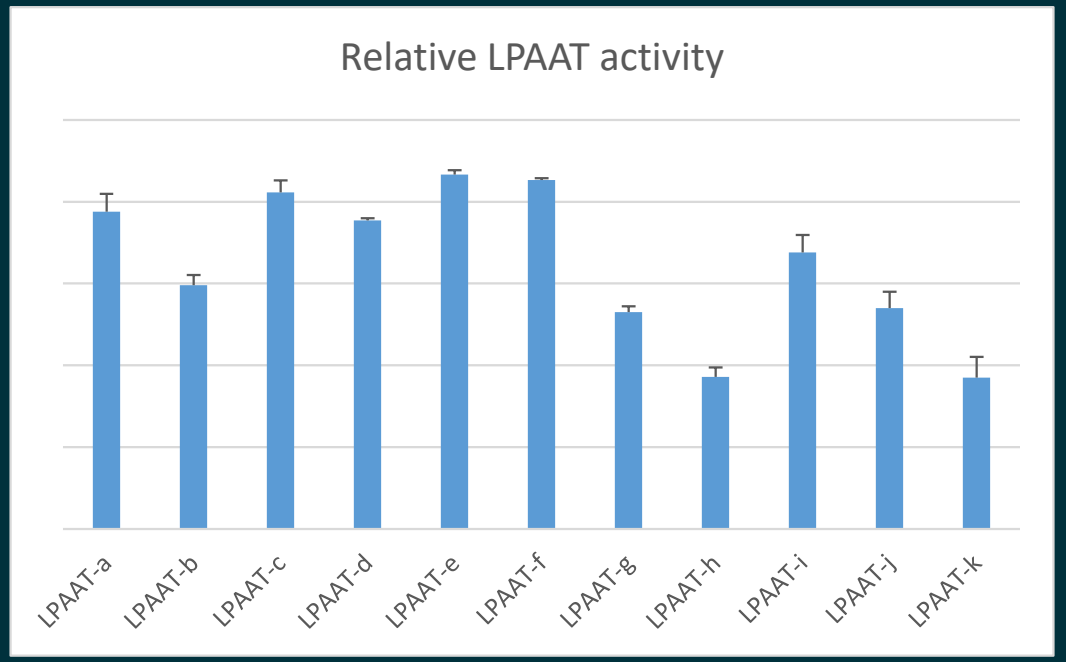
ω3 LC-PUFA preferred TAG assembly enzyme needed



- Lysophosphatidic acid acyltransferase (LPAAT) with ω3 LC-PUFA preference for increasing DHA level at *sn*-2 position
- ω3 LC-PUFA preferable glycerol-3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT) for higher DHA content

Characterisation of other source of GPAT and LPAAT

- Screening of algal and fungal GPAT and LPAAT
- Two GPAT and two LPAAT genes were shortlisted
- Overexpression algal GPAT and fungal LPAAT in DHA canola increased total DHA level as well as *sn*-2 distribution



Line	DHA of total lipid (%)	DHA at *sn*-2 (%)
DHA canola	12.9	7.2
DHA canola + algal GPAT + fungal LPAAT	15.2	11.4

Unpublished

Atlantic salmon fish trials with DHA canola oil

Lipid composition (g) of 100 g fish fillets (NQC)

	Trial 1 (fed on 1.6 kg fish)		Trial 2 (fed on 1.2 kg fish)		Trial 3 (fed on 150 g fish)	
	Control	ω3 Canola	Control	ω3 Canola	Control	ω3 Canola
EPA	0.24	0.23	0.46	0.37*	0.57	0.55
DHA	0.30	0.35*	0.51	0.67*	0.72	0.79*
EPA&DHA	0.55	**0.58**	0.97	**1.04**	1.29	**1.35**
ω3/ω6	0.67	0.71*	0.82	1.00*	0.68	0.75*

2.3 x **4.2 x** **5.4 x**

250 mg recommended daily EPA/DHA intake

Commercial trial in Chile

Control: Standard formulation from feed company

* statistically significant difference $p<0.05$ between groups (Student's t-test)

Silva et al. 2020

DHA canola development timeline

2003 — CSIRO project formally starts

2004 — POC Provision patent

2005 — First Publication

2010 — Nuseed/CSIRO/GRDC partnership

2014 — Elite Event

2014 — Field Trial starts

2017 — Deregulation submission

2018 — OGTR, FSANZ USDA Approval

2020 — CFIA approval

2022 — FDA Approval

2023 — NFSA Approval

Summary

- Ambitious pathway for engineering and GM product commercialisation
- Driven by the need to feed a growing population in the face of environmental challenges
- Deregulated in AU, USA and Canada for cultivation, feed and food
- Approved by Norwegian Food Safety Authority's (NFSA) for use in fish feed
- Commercial sales processed
- Two brands developed by Nuseed:
  - Aquaterra™ for aquaculture
  - Nutriterra™ for human consumption
- 1-2 ha DHA canola = as much DHA as 10,000 kg fish

The team along the journey

CSIRO

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Nuseed

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