

Effect of pH and 22:6 n -3 on the biohydrogenation of 18:2 n -6 by *Butyrivibrio fibrisolvens* and *Propionibacterium acnes*

Lipids in Ruminants 2016 – Lokeren

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22:6 n -3 – docosahexaenoic acid (DHA)

18:2 n -6 – linoleic acid (LA)

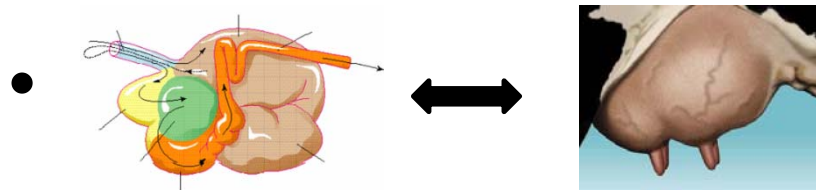
Overview

- Introduction
- Material and methods
- Results and discussion
- Conclusion



Introduction

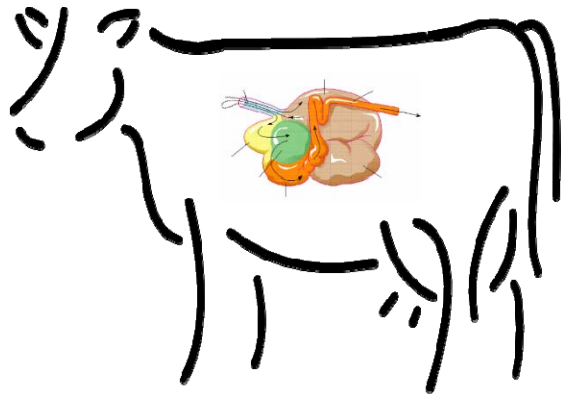
- Highly fermentable carbohydrates
→ milk fat depression (MFD)



- Basis for MFD: biohydrogenation theory
 - shift in biohydrogenation pathway
 - relation with bacterial abundances



Biohydrogenation theory



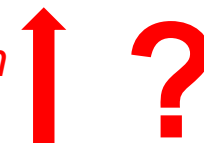
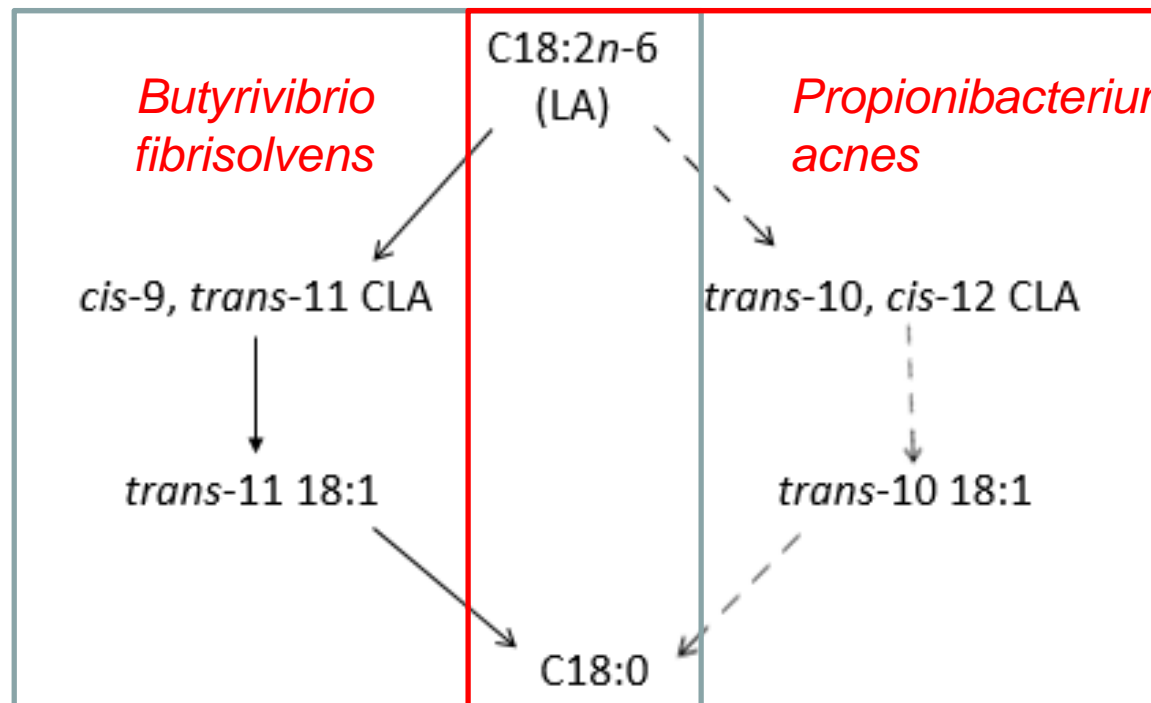
B. fibrisolvens
P. acnes

trans-11 to
trans-10
shift



Dietary conditions:

- Starch
- Low rumen pH
- Marine oils



Aim

- Rate of CLA formation under different rumen conditions *in vitro*
 - *cis-9, trans-11* CLA by *Butyrivibrio fibrisolvens*
 - *trans-10, cis-12* CLA by *Propionibacterium acnes*



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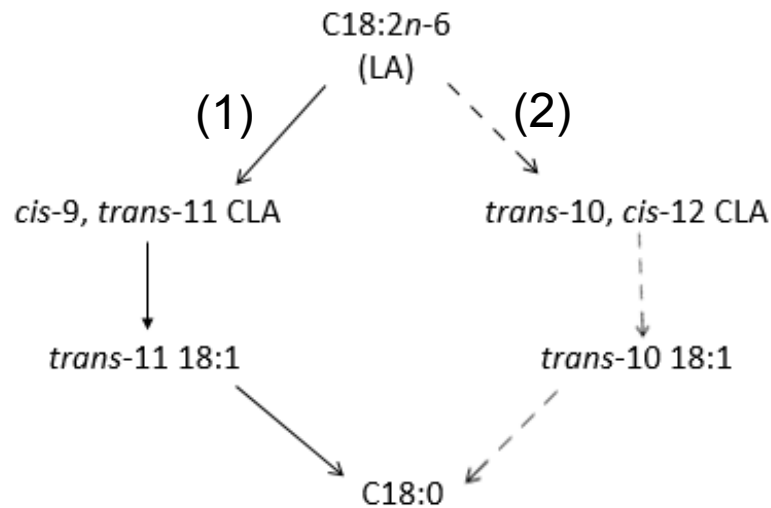


Material and methods

- 3 different growth media (20% (v/v) rumen fluid)
 - (1) Control medium (pH \approx 6.6)
 - (2) Low pH medium (pH \approx 5.5)
 - (3) DHA medium (pH \approx 6.7)
- Hungate-type tubes: 9.5 mL per tube
- Addition of 18:2*n*-6 (40 μ g/mL)
- Autoclave (20 min, 121°C)
- CO₂ flushing



Material and methods (2)



- 2 bacterial species

(1) *Butyrivibrio fibrisolvens* D1:

→ *cis*-9, *trans*-11 CLA

→ inoculum size: 0.5 mL

(2) *Propionibacterium acnes* DSM 1897:

→ *trans*-10, *cis*-12 CLA

→ inoculum size: 0.5 mL or 1.0 mL



Material and methods (3)

- 5 different time points
0 h, 2 h, 4 h, 8 h, 24 h
- Quadruplicates (different days)
bacterium × growth medium × incubation period



Material and methods (4)

- Measurements

Long-chain fatty acids: GC

- Statistical analysis

MIXED procedure of SAS

(fixed factors: bacterium & growth medium;
random factor: day)

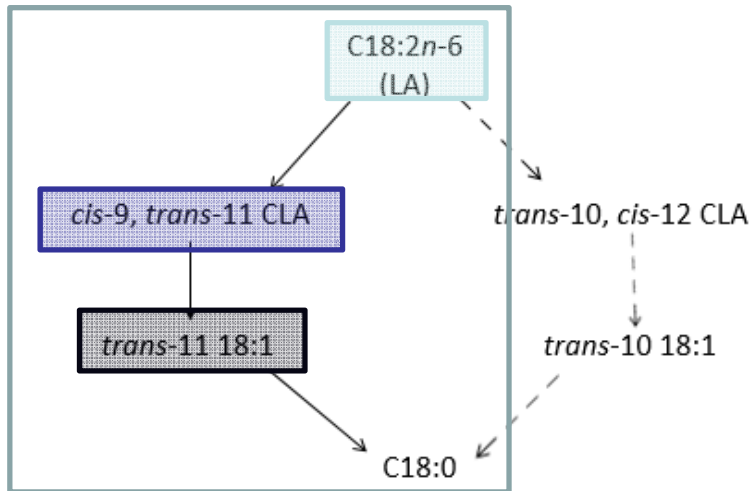


Overview

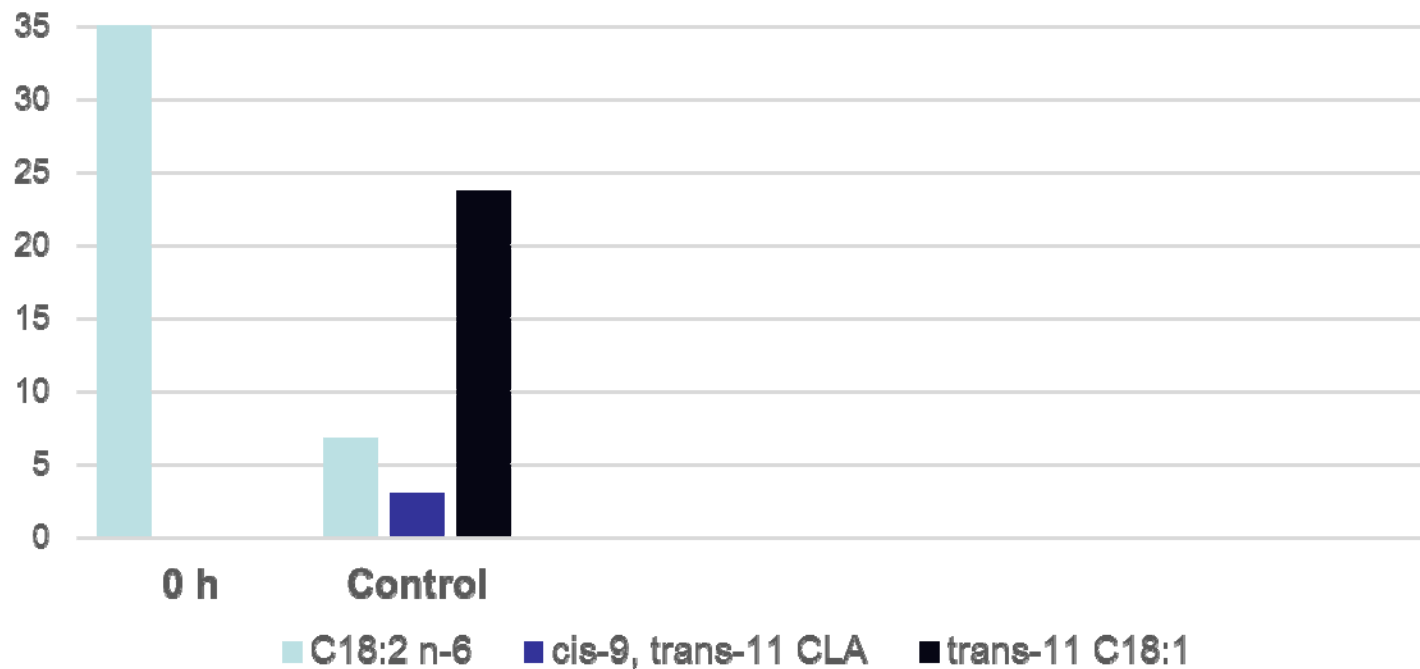
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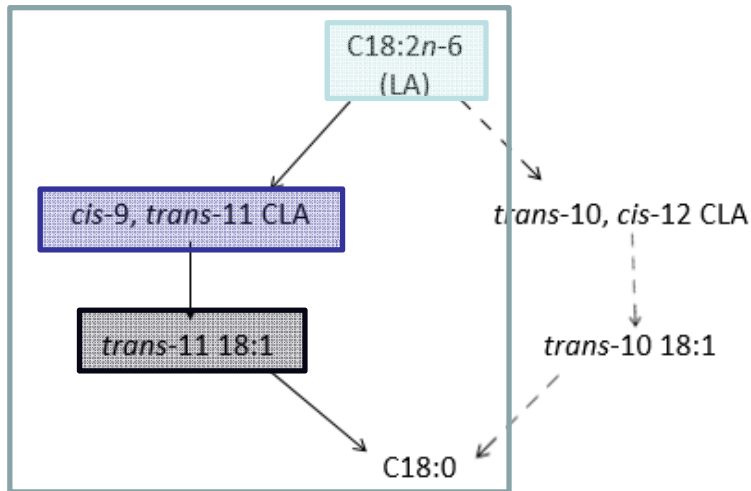
Butyrivibrio fibrisolvens



Mean concentration of C18:2n-6, *cis*-9, *trans*-11 CLA and *trans*-11 C18:1 over 24 h of incubation ($\mu\text{g}/\text{mL}$)

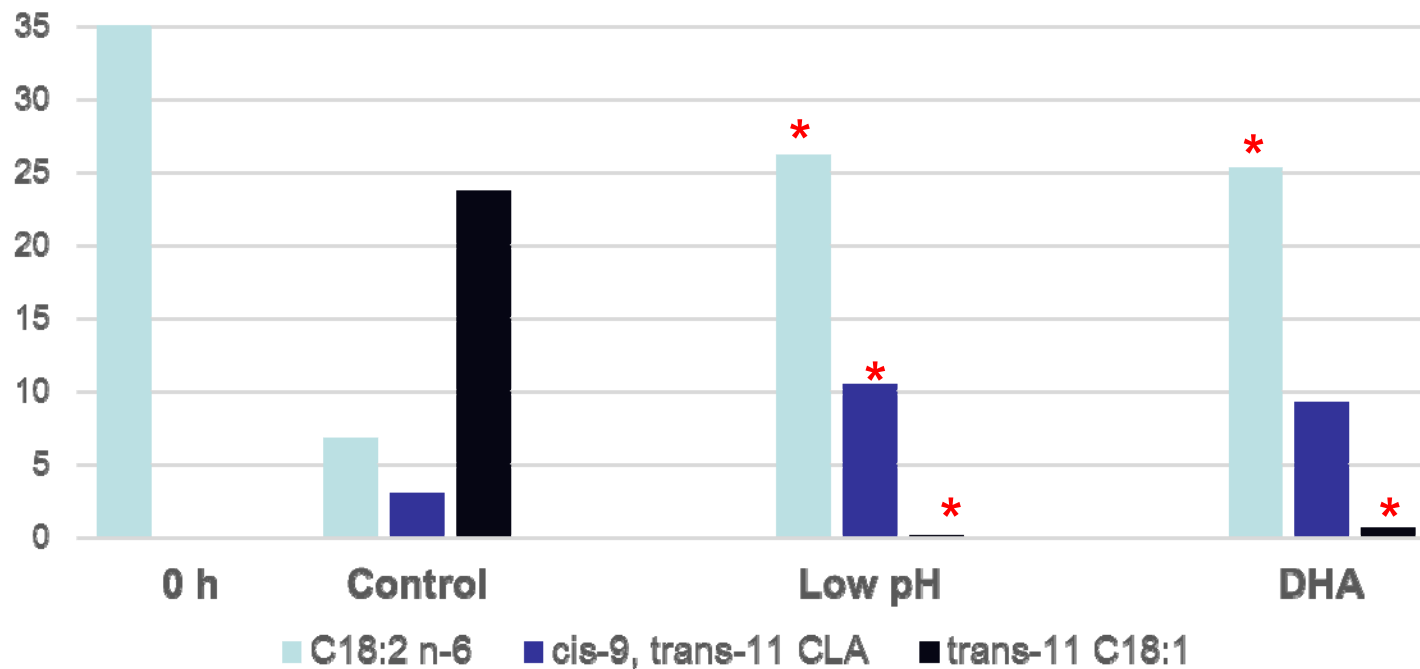


Butyrivibrio fibrisolvens

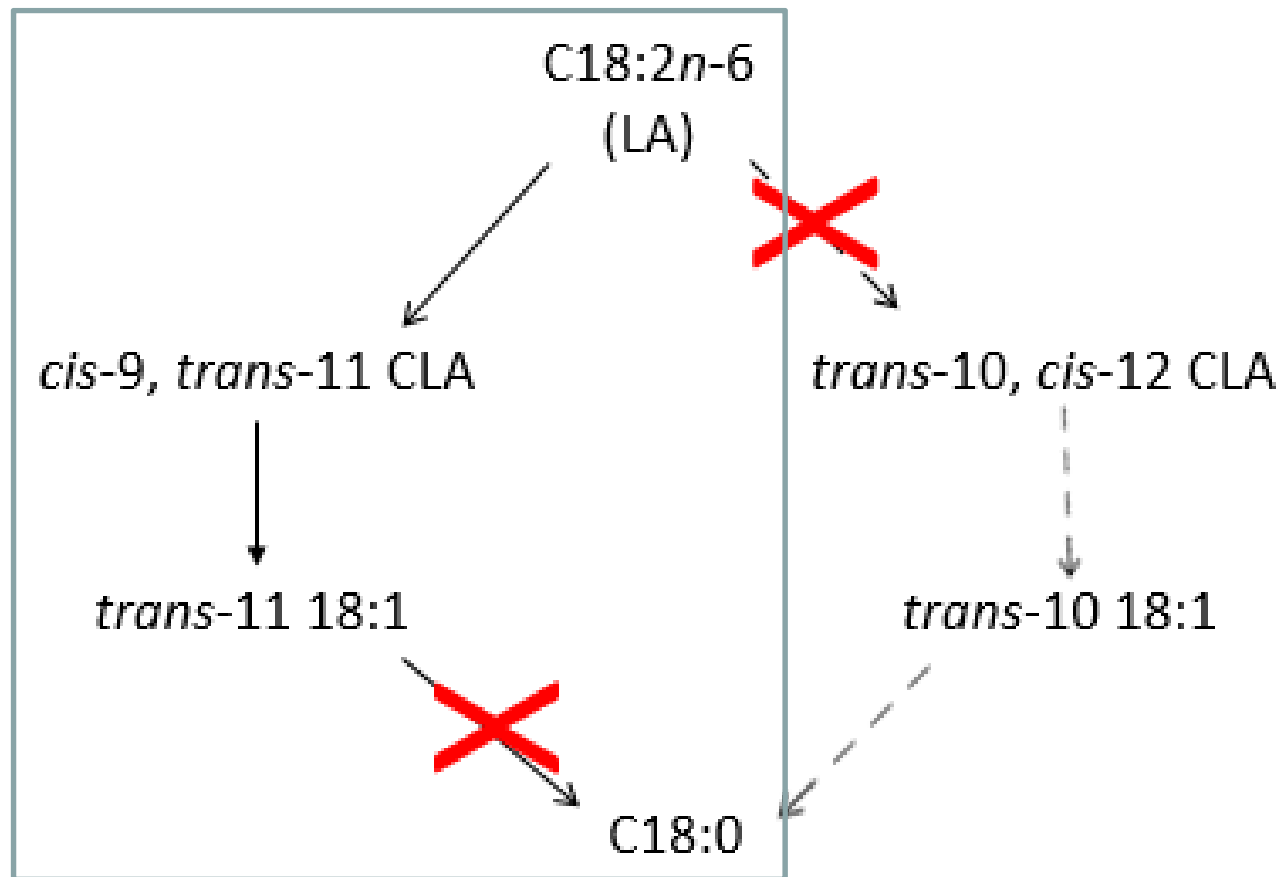


* Different from control (P < 0.05)

Mean concentration of C18:2n-6, *cis*-9, *trans*-11 CLA and *trans*-11 C18:1 over 24 h of incubation (µg/mL)

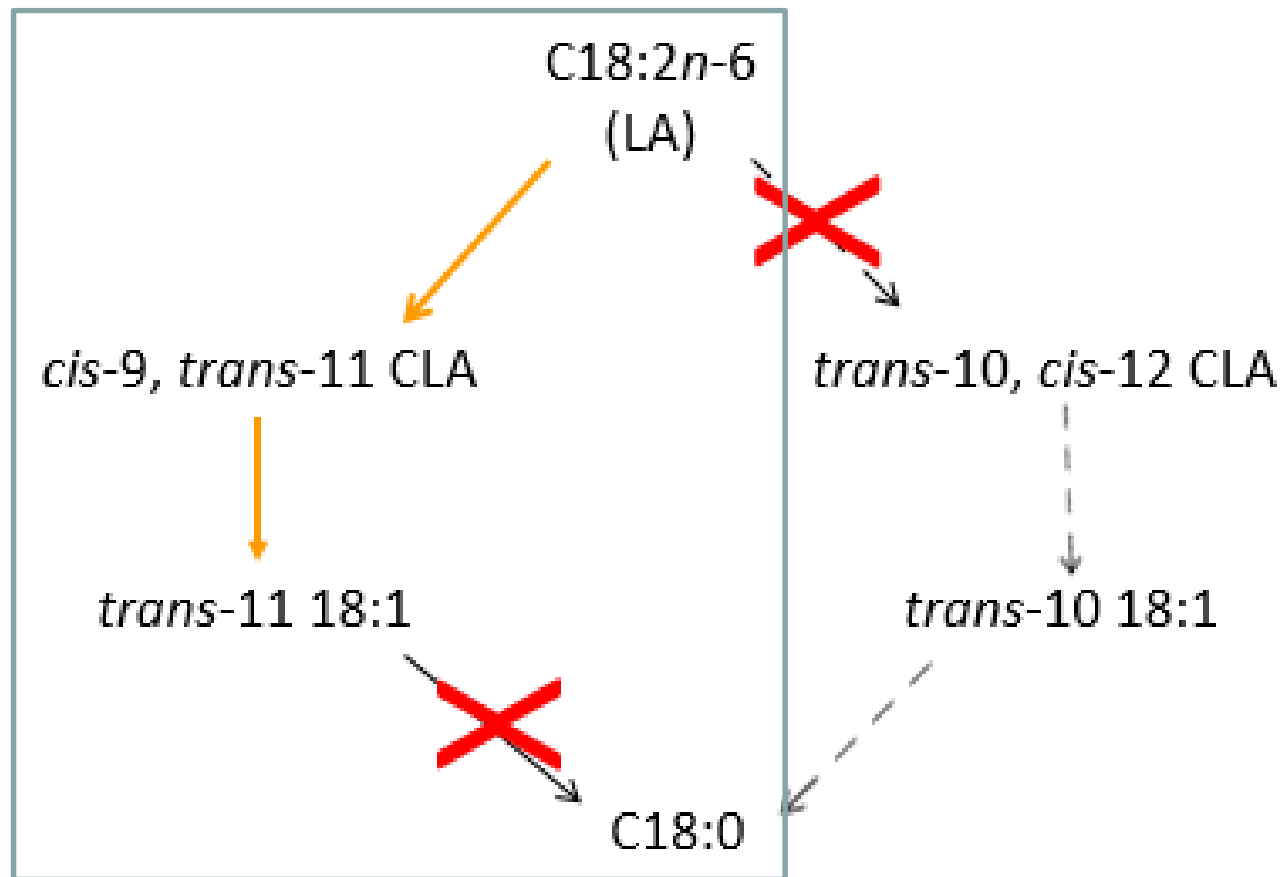


Butyrivibrio fibrisolvens

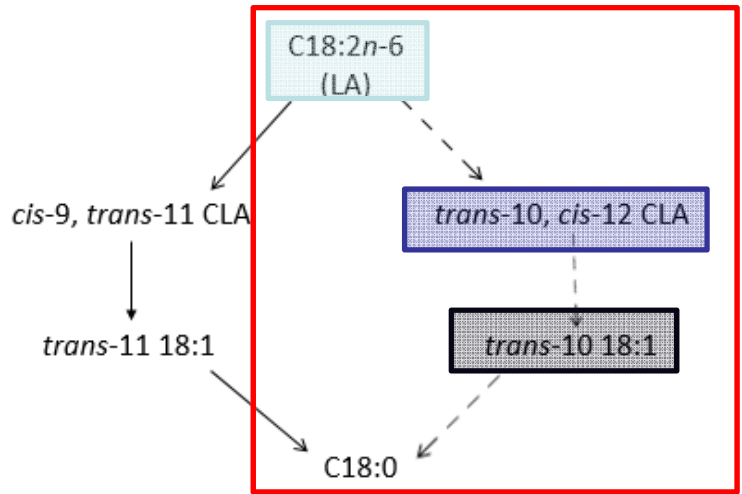


Butyrivibrio fibrisolvens

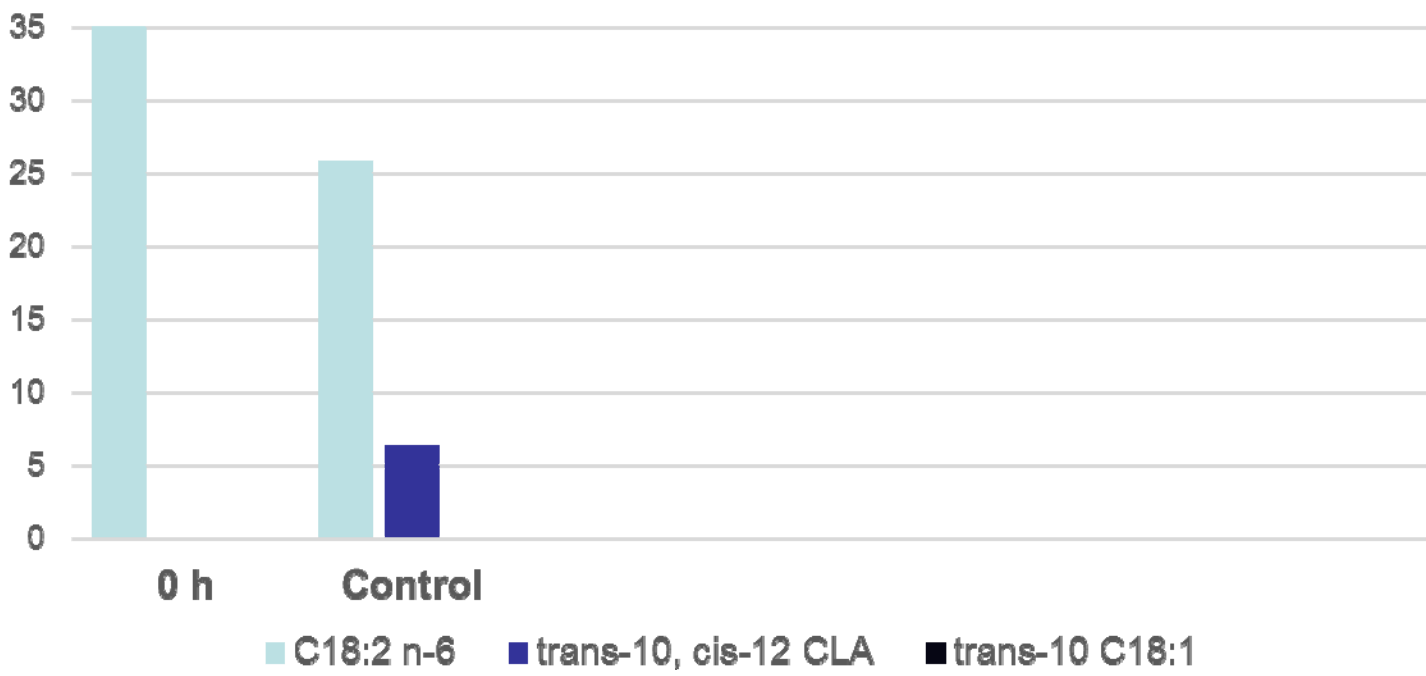
Low pH & DHA



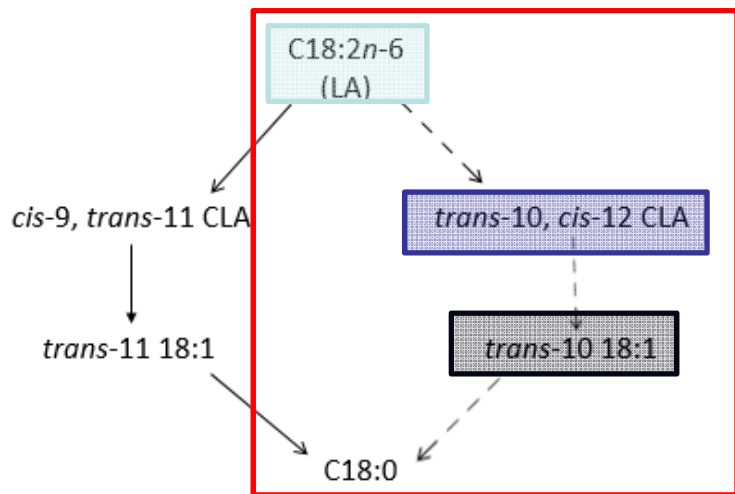
Propionibacterium acnes



Mean concentration of C18:2n-6, trans-10, cis-12 CLA and trans-10 C18:1 over 24 h of incubation (µg/mL)



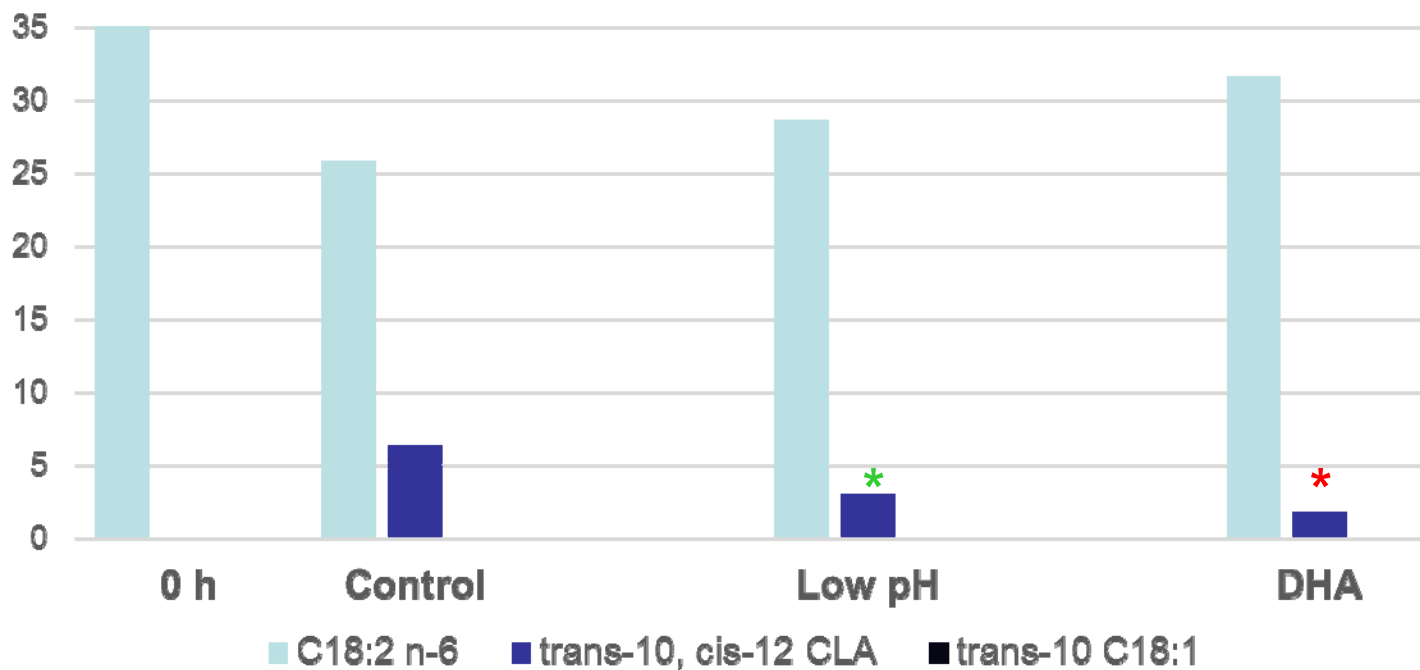
Propionibacterium acnes



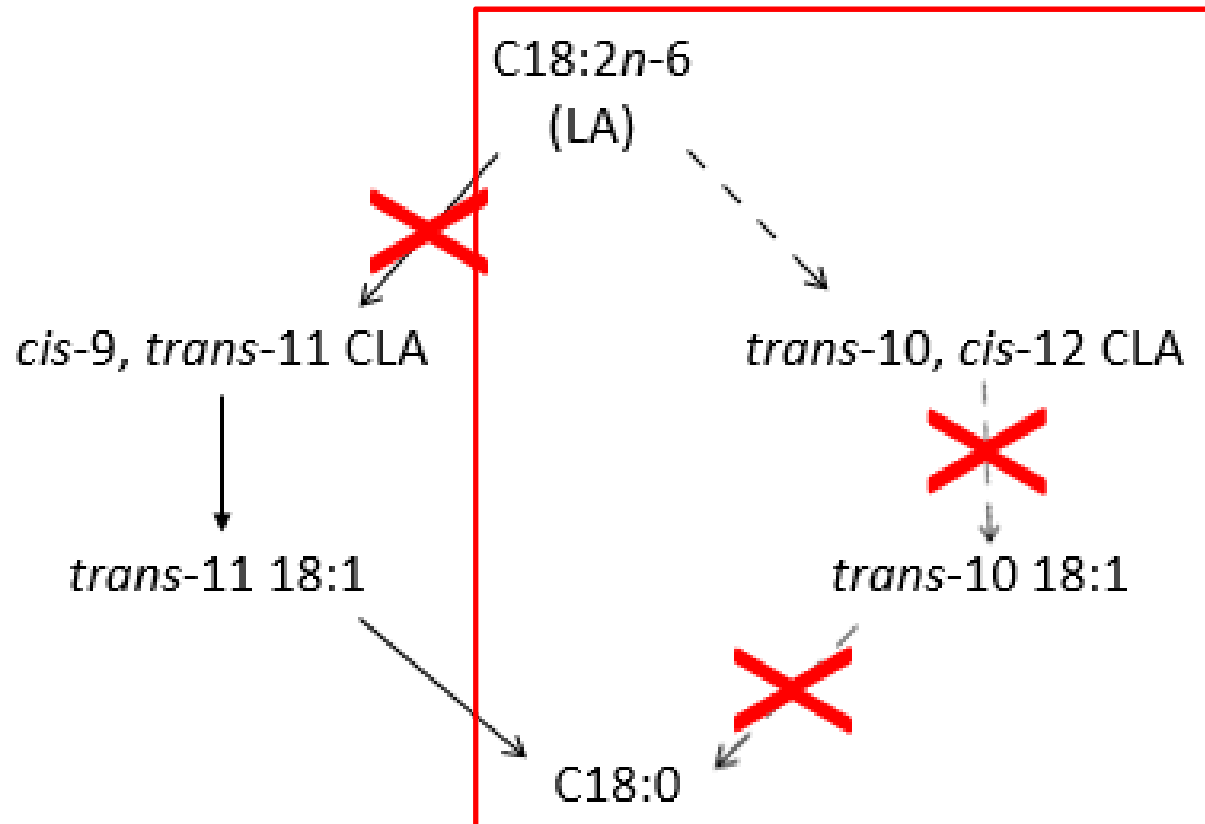
* Different from control (P < 0.05)

** Different from control (P < 0.10)

Mean concentration of C18:2n-6, trans-10, cis-12 CLA and trans-10 C18:1 over 24 h of incubation (µg/mL)

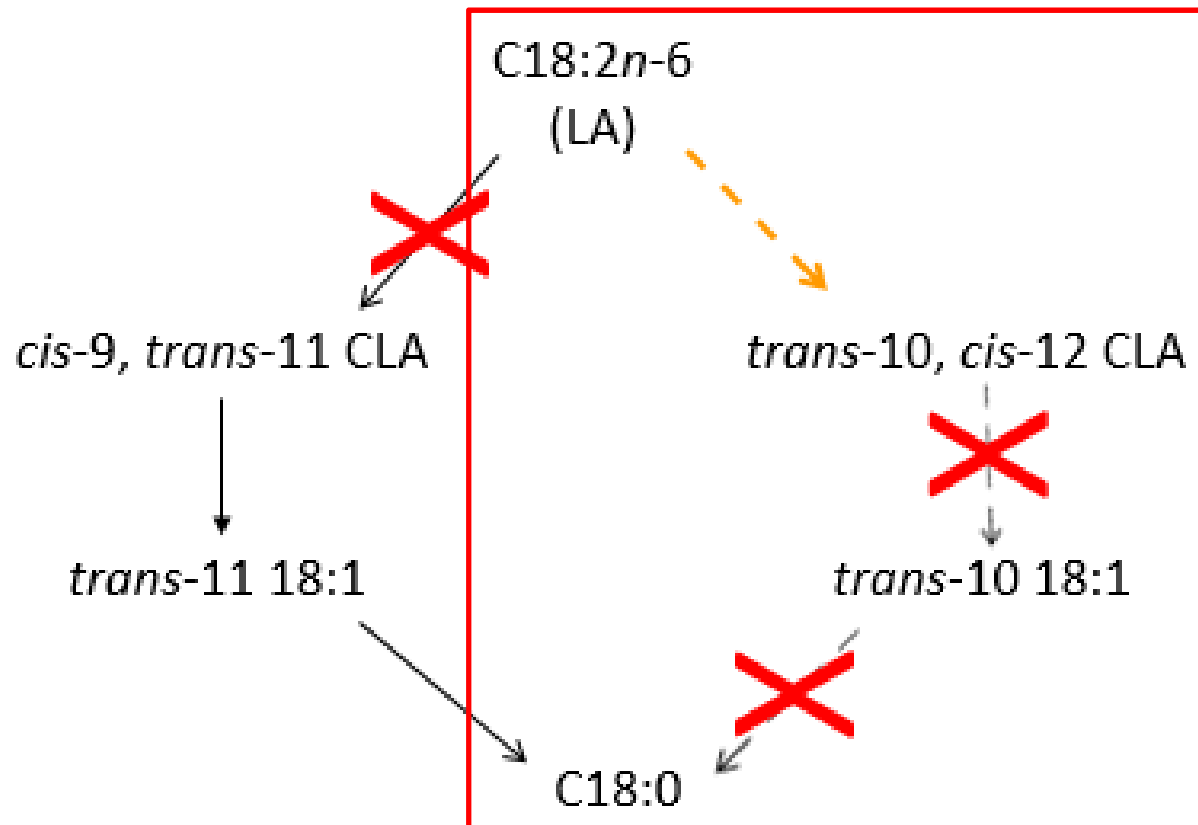


Propionibacterium acnes

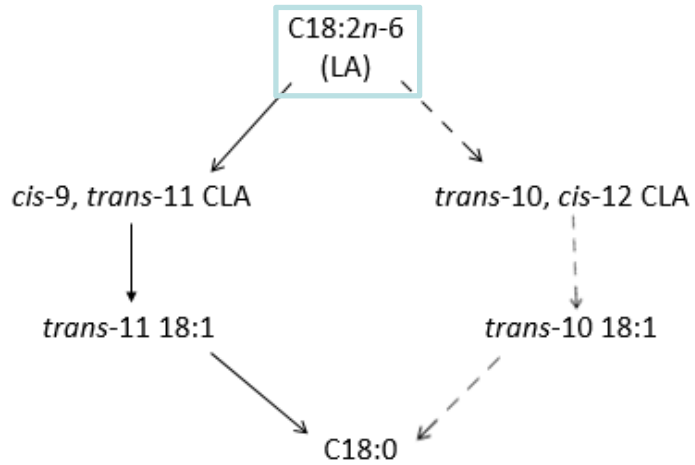


Propionibacterium acnes

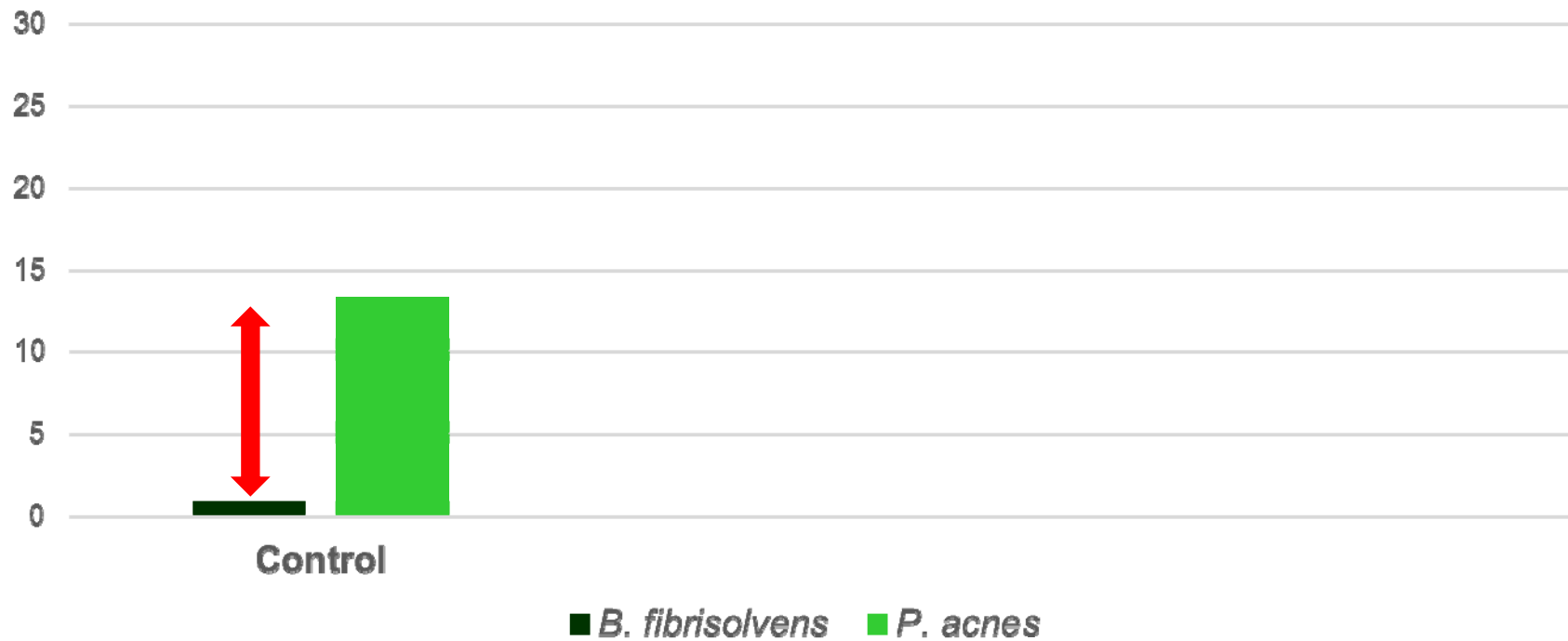
Low pH & DHA



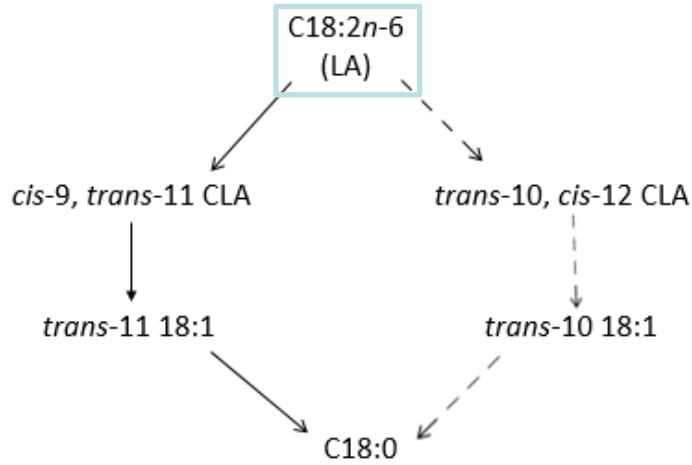
B. fibrisolvens versus *P. acnes*



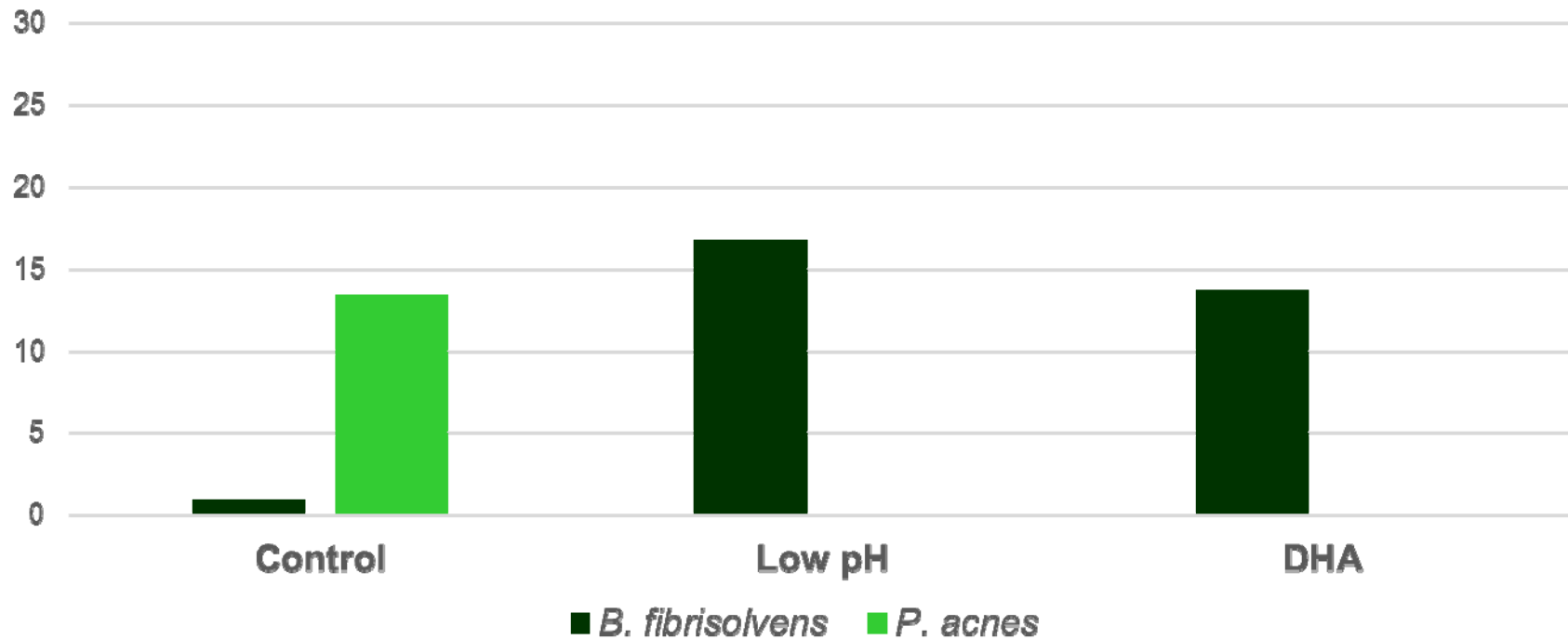
Concentration of C18:2n-6 after 24 h of incubation ($\mu\text{g/mL}$)



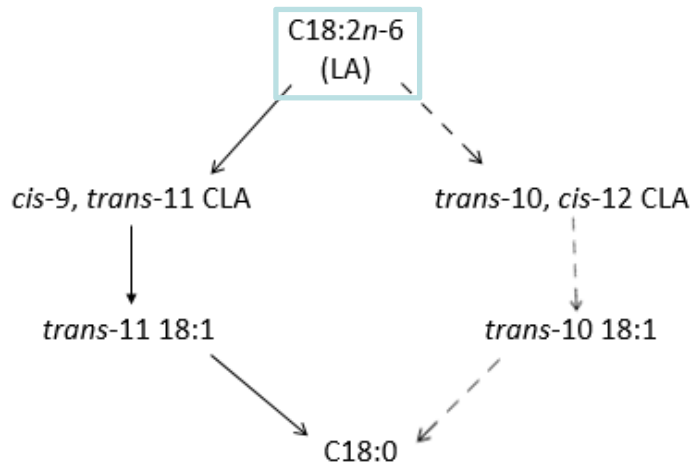
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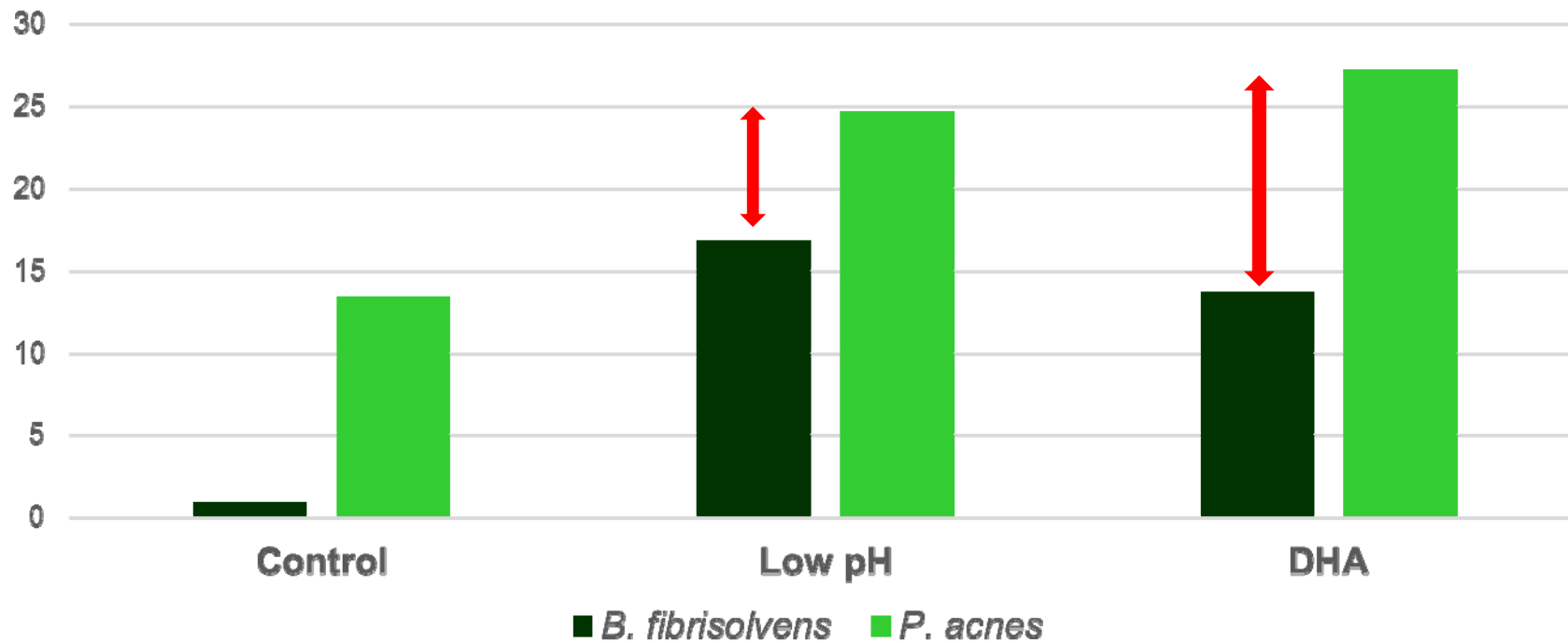
Concentration of C18:2n-6 after 24 h of incubation ($\mu\text{g/mL}$)



B. fibrisolvens versus *P. acnes*



Concentration of C18:2n-6 after 24 h of incubation ($\mu\text{g/mL}$)



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Conclusion

Butyrivibrio fibrisolvens

Low pH & DHA

➔ ↓ *cis*-9, *trans*-11 CLA

➔ ↓ *cis*-9, *trans*-11 CLA to *trans*-11 C18:1

Propionibacterium acnes

Low pH & DHA

➔ ↓ *trans*-10, *cis*-12 CLA



Conclusion

Rate of CLA formation

Butyrivibrio fibrisolvens > *Propionibacterium acnes*

➔ *trans*-11 to *trans*-10 shift due to an increase of ?
trans-10 producers alone

New hypothesis for next experiment

Balance: $\frac{\text{cis-9, trans-11 CLA producers}}{\text{trans-10, cis-12 CLA producers}}$



Thank you for your attention!

Questions?

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Ghent University
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