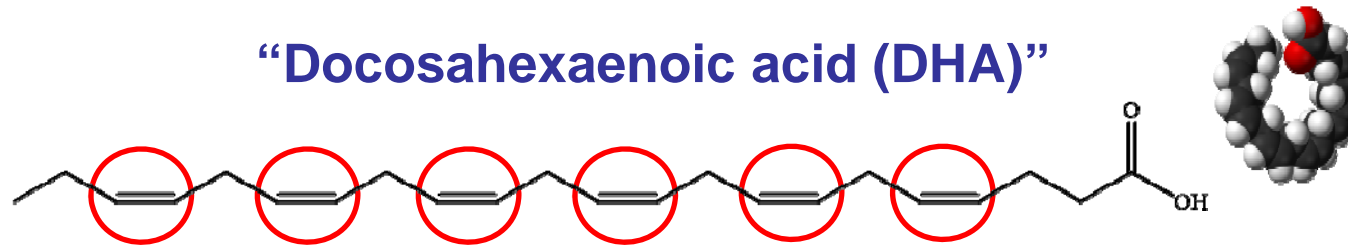


Addition of uncentrifuged-autoclaved rumen fluid allows microbial biohydrogenation of docosahexaenoic acid (DHA) in highly diluted rumen inoculum.

Marlene Escobar
PhD student

Introduction

“Docosahexaenoic acid (DHA)”



Benefits

Humans



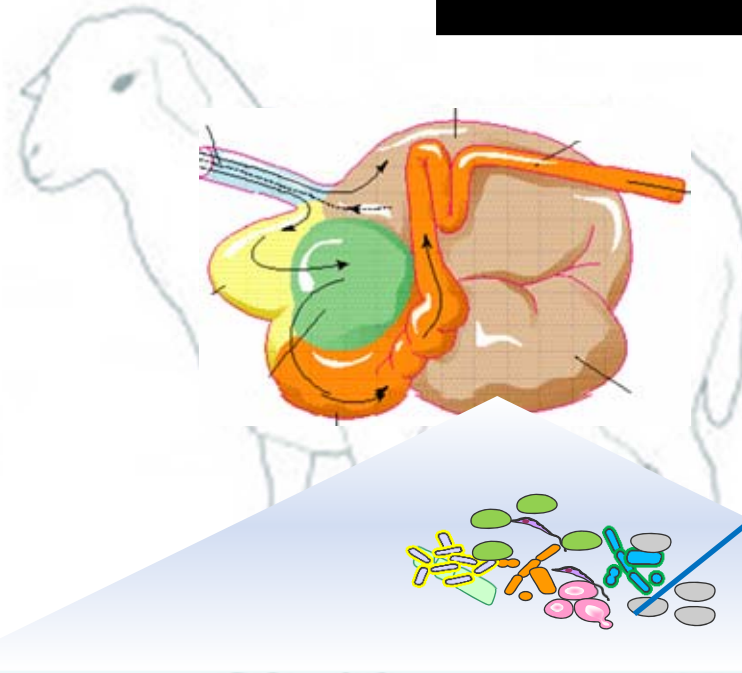
Ruminants



Introduction



DHA rich supplement

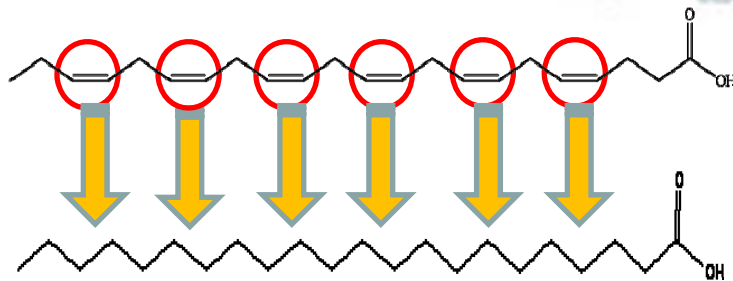


*Butyrivibrio
Proteoclasticus* P-18



(picture: Wallace et al., FEMS Microbiol Lett. 2006)

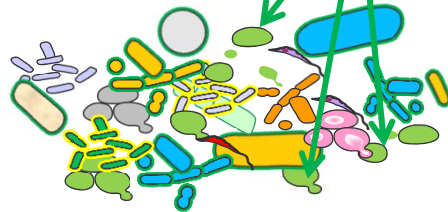
Jeyanathan et al., BMC Microbiol. 2016



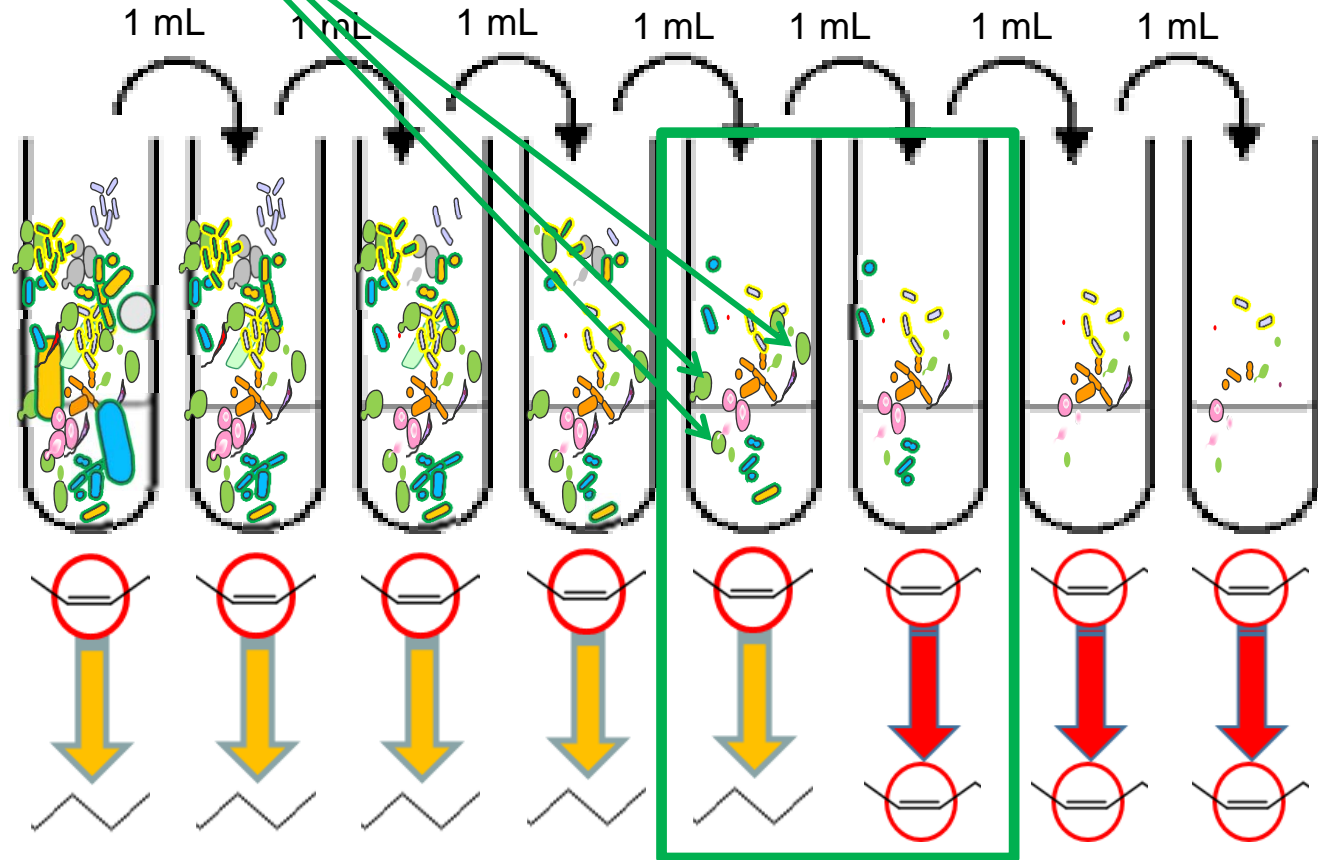
Biohydrogenation

Introduction

Biohydrogenating bacteria
in
rumen mixed cultures



Dilution to extinction
technique:



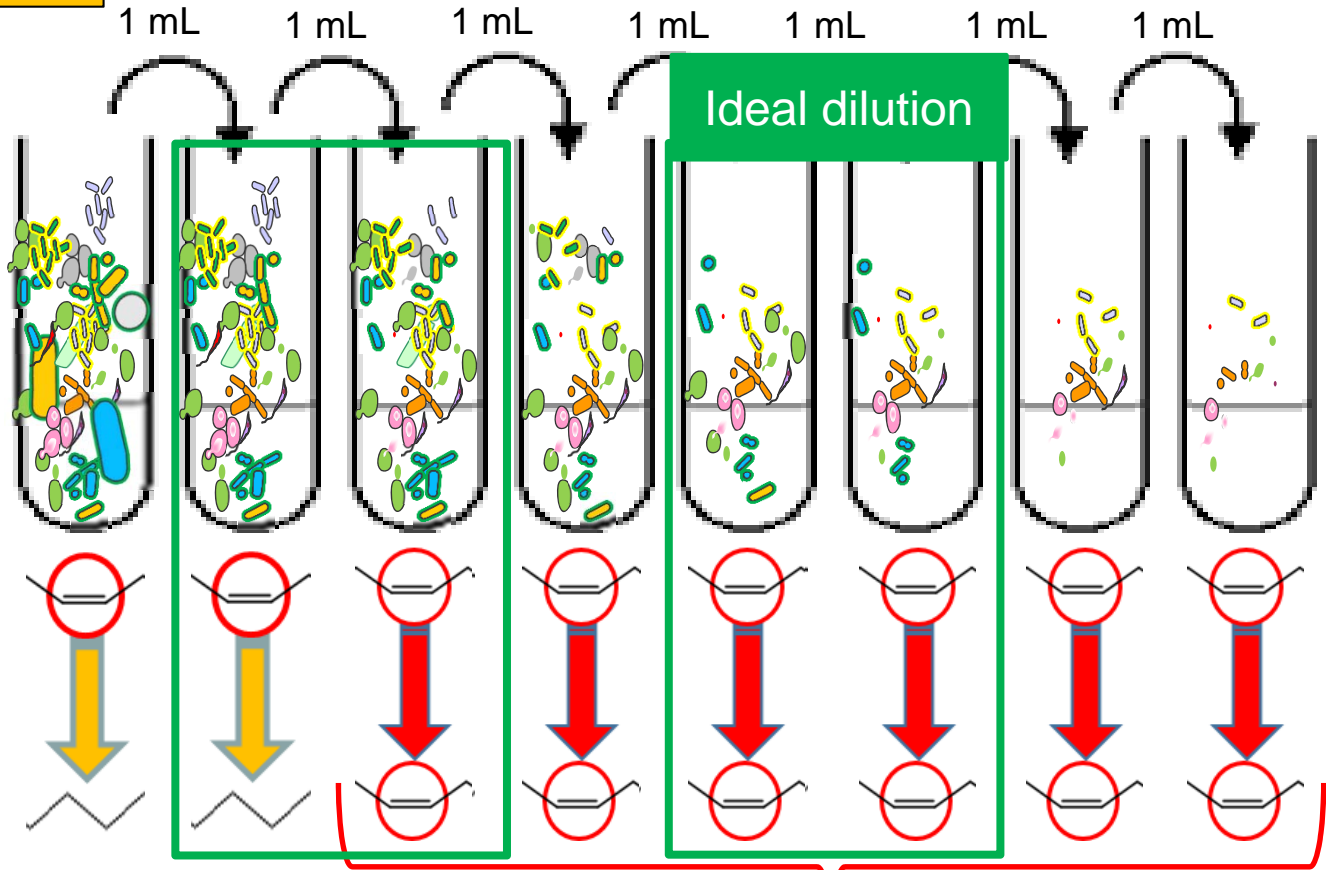
Biohydrogenation
capacity



Previous dilution experiments

Introduction

Biohydrogenation capacity



Toxicity of DHA in *in vitro* conditions



Introduction

Method to reduce the toxicity of DHA



Food particles of the rumen fluid



Stimulation of biohydrogenation by food particles.

Harfoot et al., *Biochem. J.* 132, 829-832 (1973)



Addition of rumen particles
to rumen growth media



uncentrifuged – autoclaved
rumen fluid
(uRF)



Hypothesis

1. Addition of autoclaved-uncentrifuged rumen fluid stimulates biohydrogenation of DHA.

Experiment 1

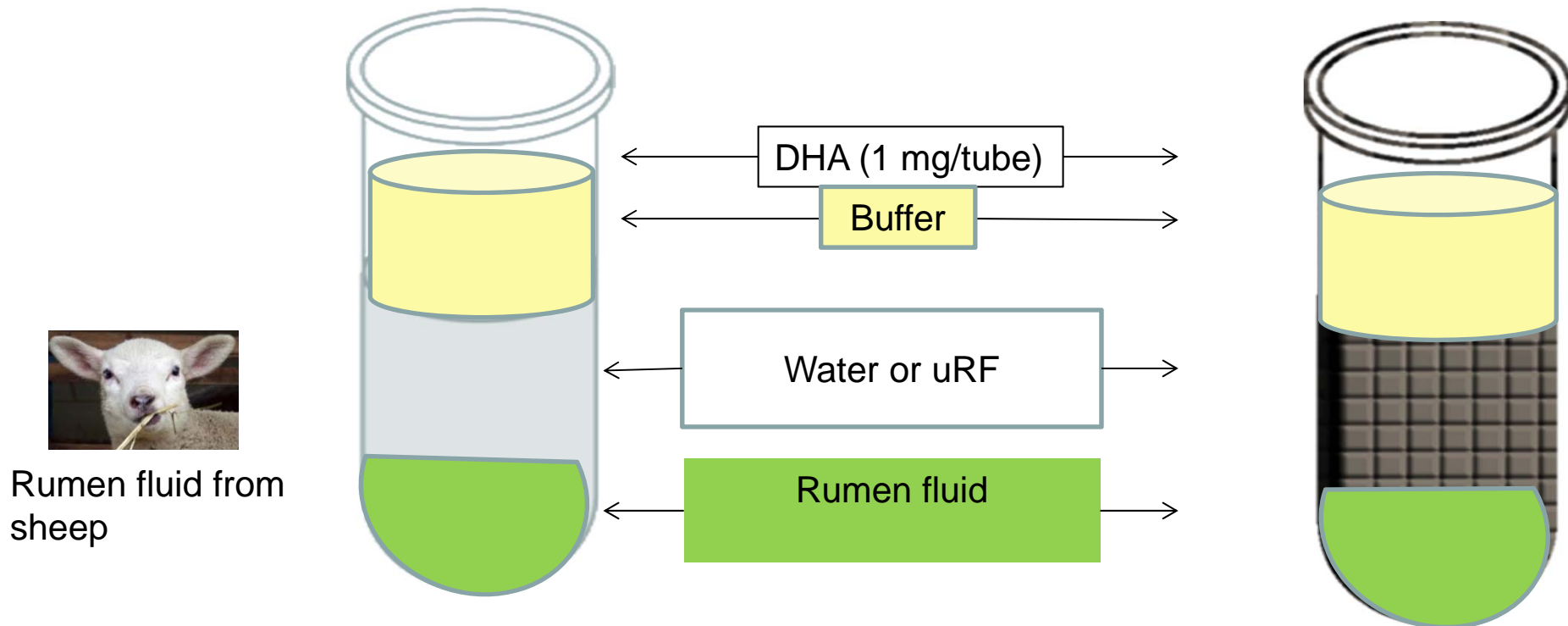
2. Addition of autoclaved-uncentrifuged allow the biohydrogenation of DHA by diluted rumen inoculum

Experiment 2



Materials and methods

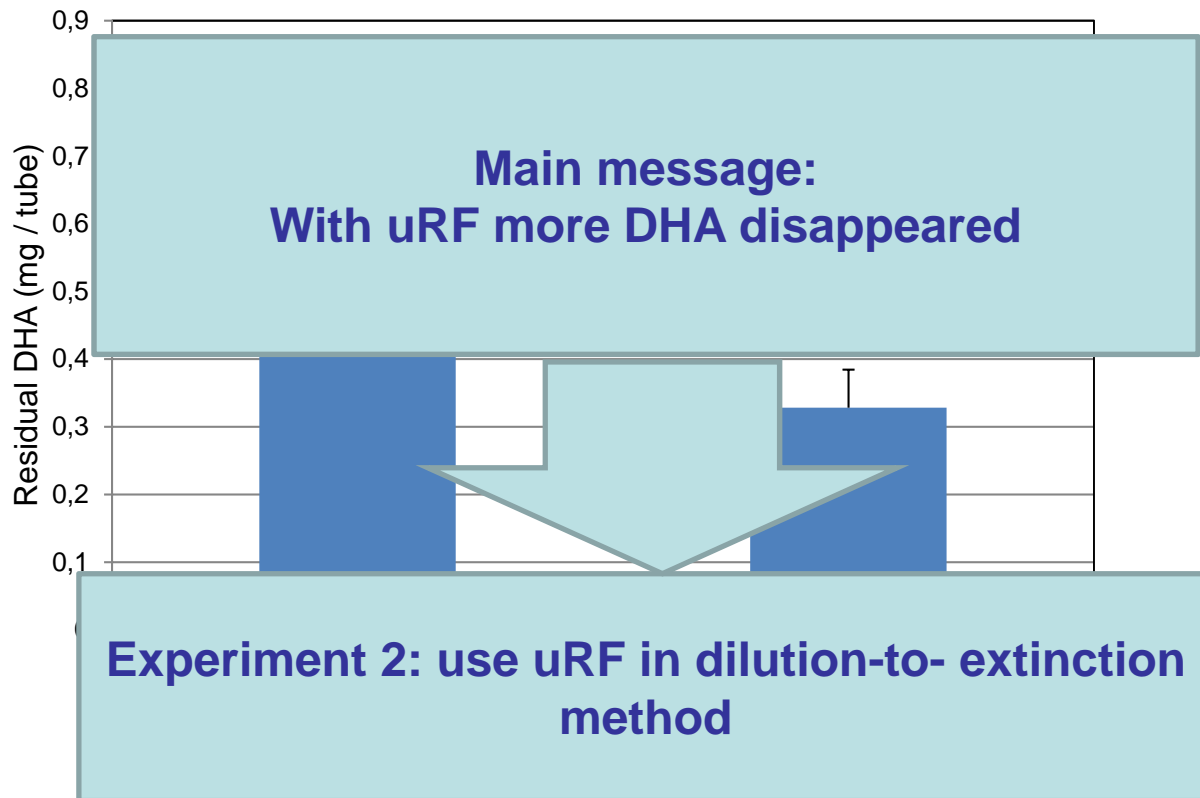
Experiment 1 Addition of uncentrifuged- autoclaved rumen fluid (uRF)



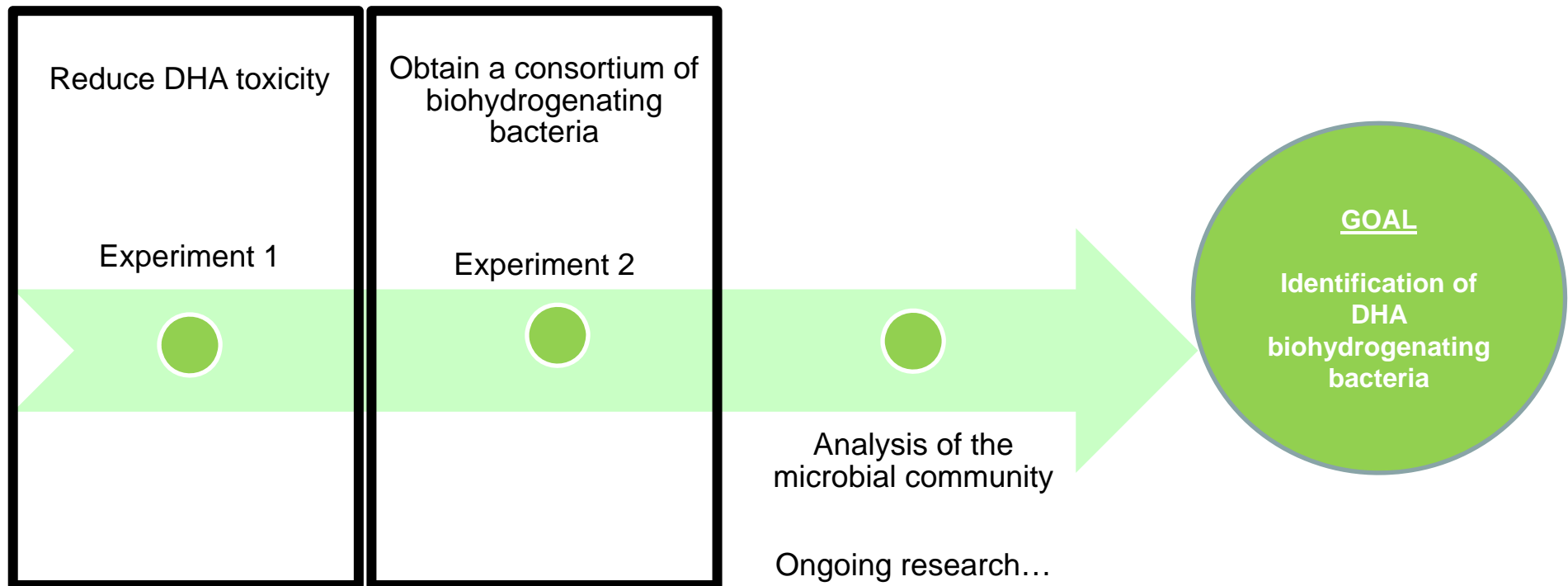
- Incubated 24 h
- Residual amount of DHA mg/tube was measured

Results

Experiment 1
Addition of uncentrifuged-
autoclaved rumen fluid (uRF)



Introduction

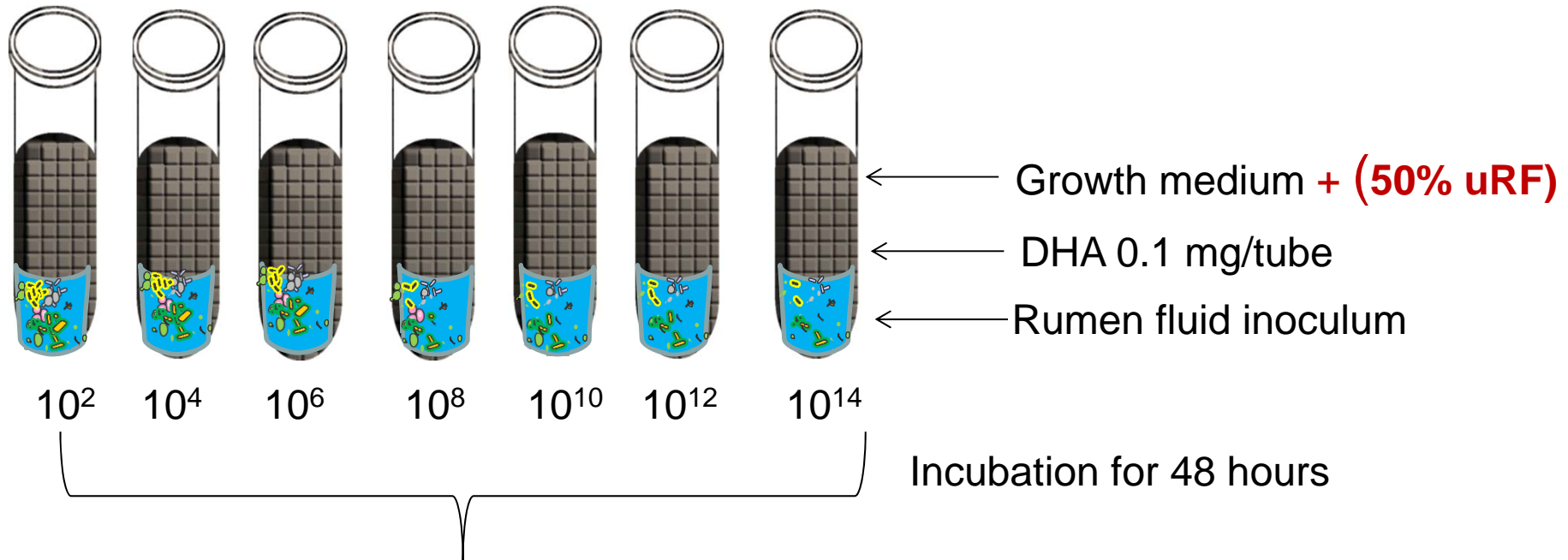


Materials and methods

Experiment 2 Dilution-to-extinction



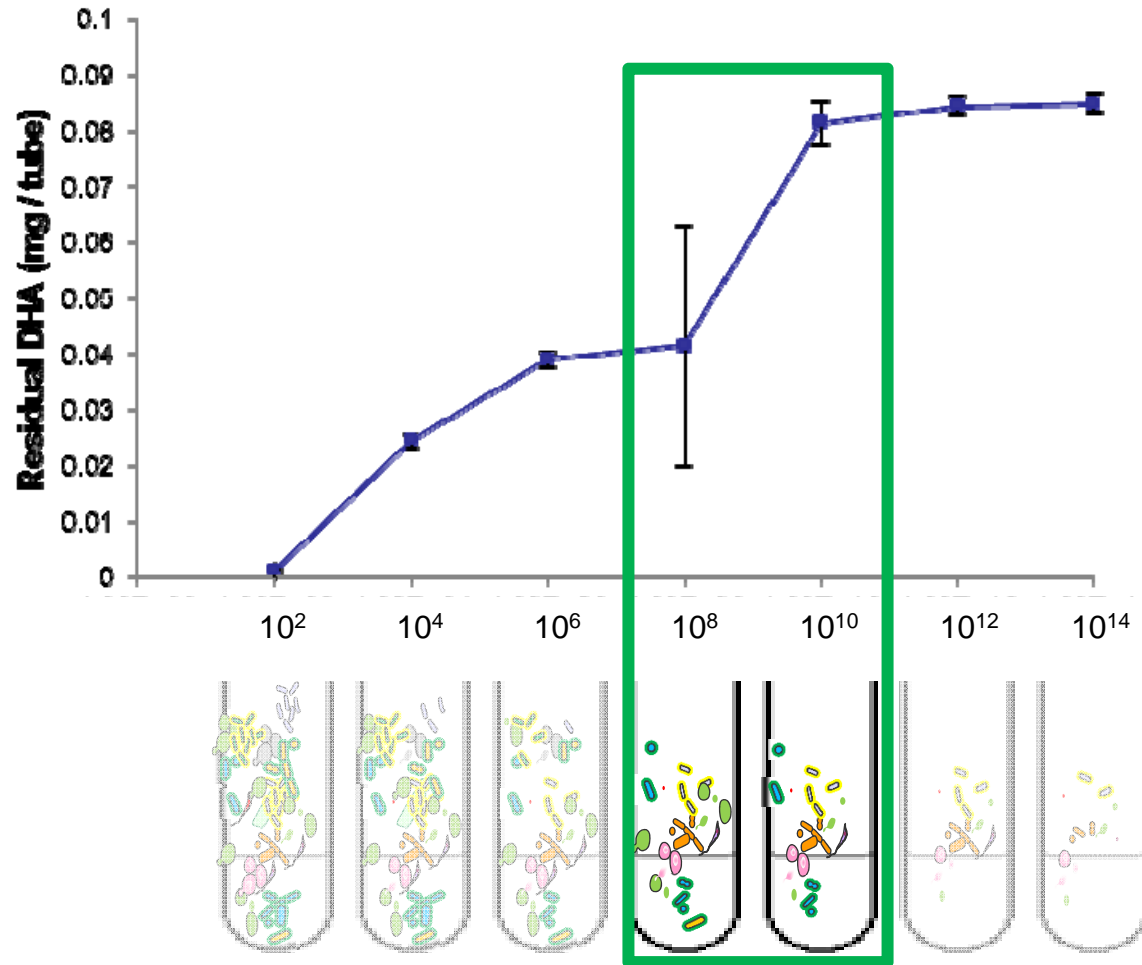
Rumen fluid



Dilution range of inoculum

Experiment 2
Dilution-to-extinction

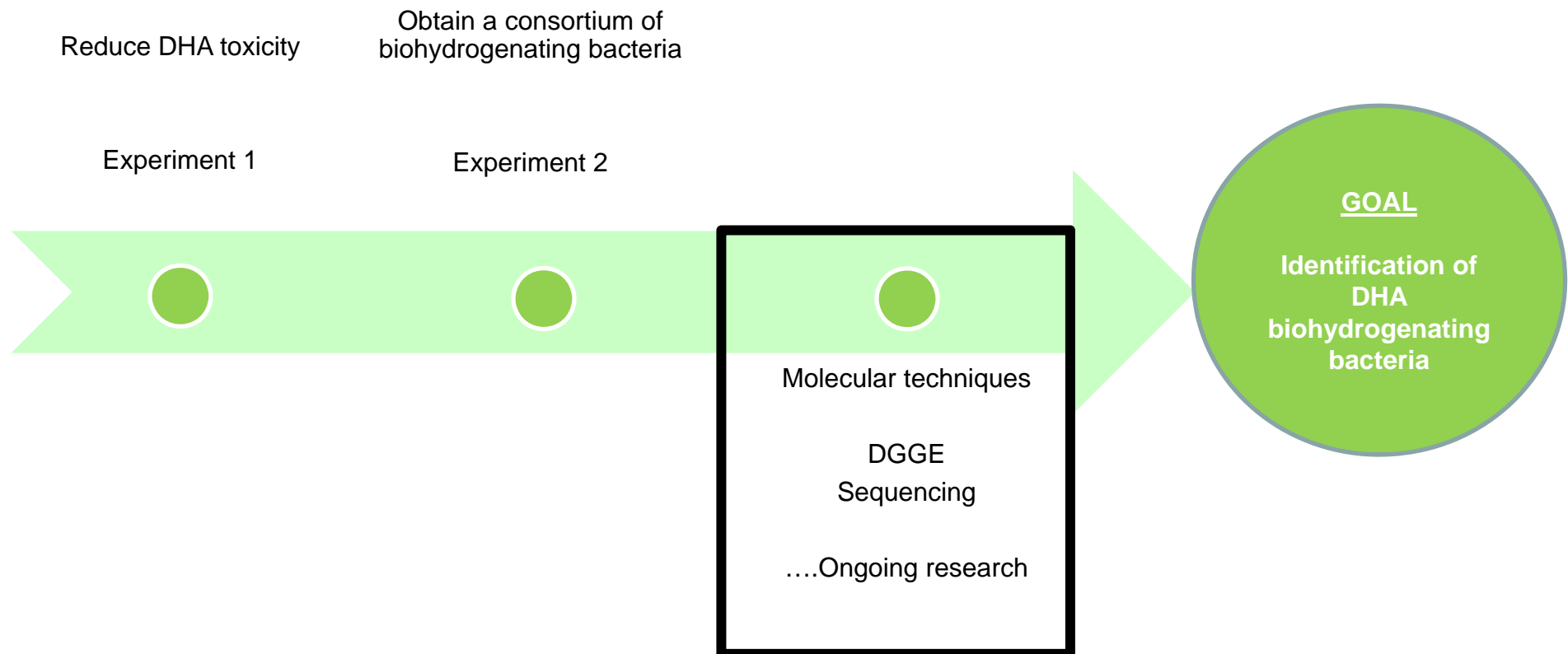
Results



Conclusion

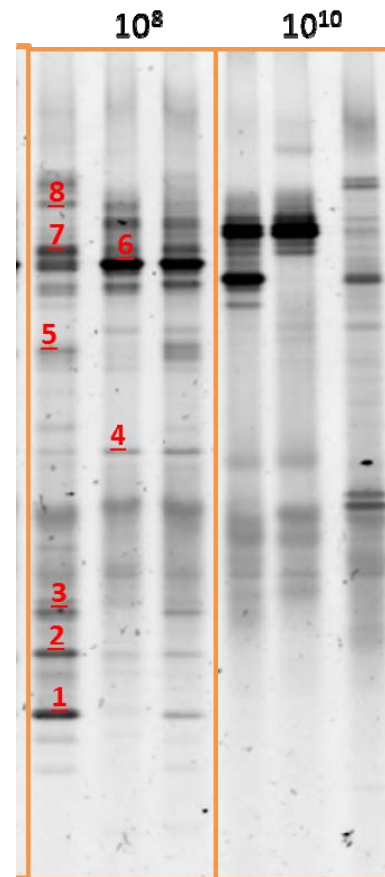
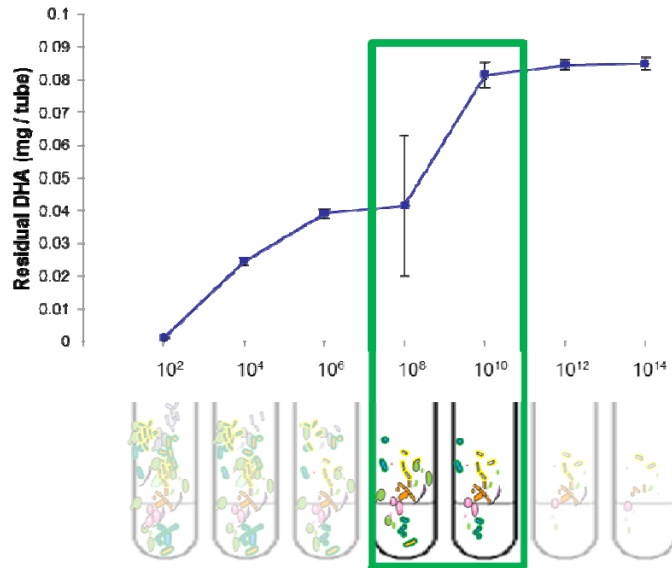
- Addition of uRF to the growth media **stimulated the metabolism of DHA** by ruminal microorganisms in **highly diluted** rumen inoculum.

Introduction



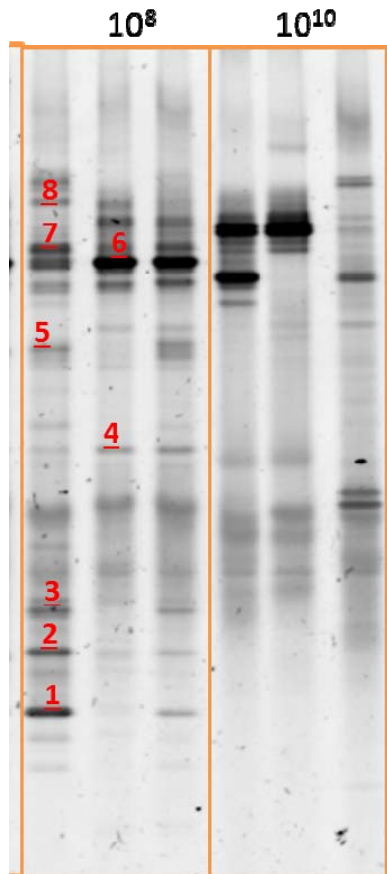
Denaturant gradient gel electrophoresis (DGGE)

Ongoing research



Ongoing research

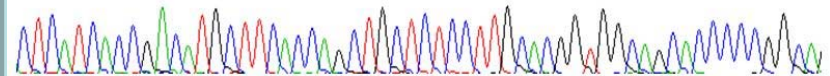
Identification of the bacteria



DNA extraction

DNA sequencing

CTCATCACCGACATGCTTCACCAGCTGCTCTCCTTGCA CGGTGGCAGCACCCCGGCAI



GOAL

Identification of
DHA
biohydrogenating
bacteria

Thank you for your attention!

Acknowledgments

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Ghent University (Belgium)



<http://www.lanupro.ugent.be>



Introduction



Conventional isolation techniques



- Complicated
- Cultivability is limited
- Great sensitivity of bacteria towards DHA

introduction

Toxicity of DHA

Disrupt the cell membrane

