Effects of ten freshwater microalgae on *in vitro* ruminal methane production and digestibility

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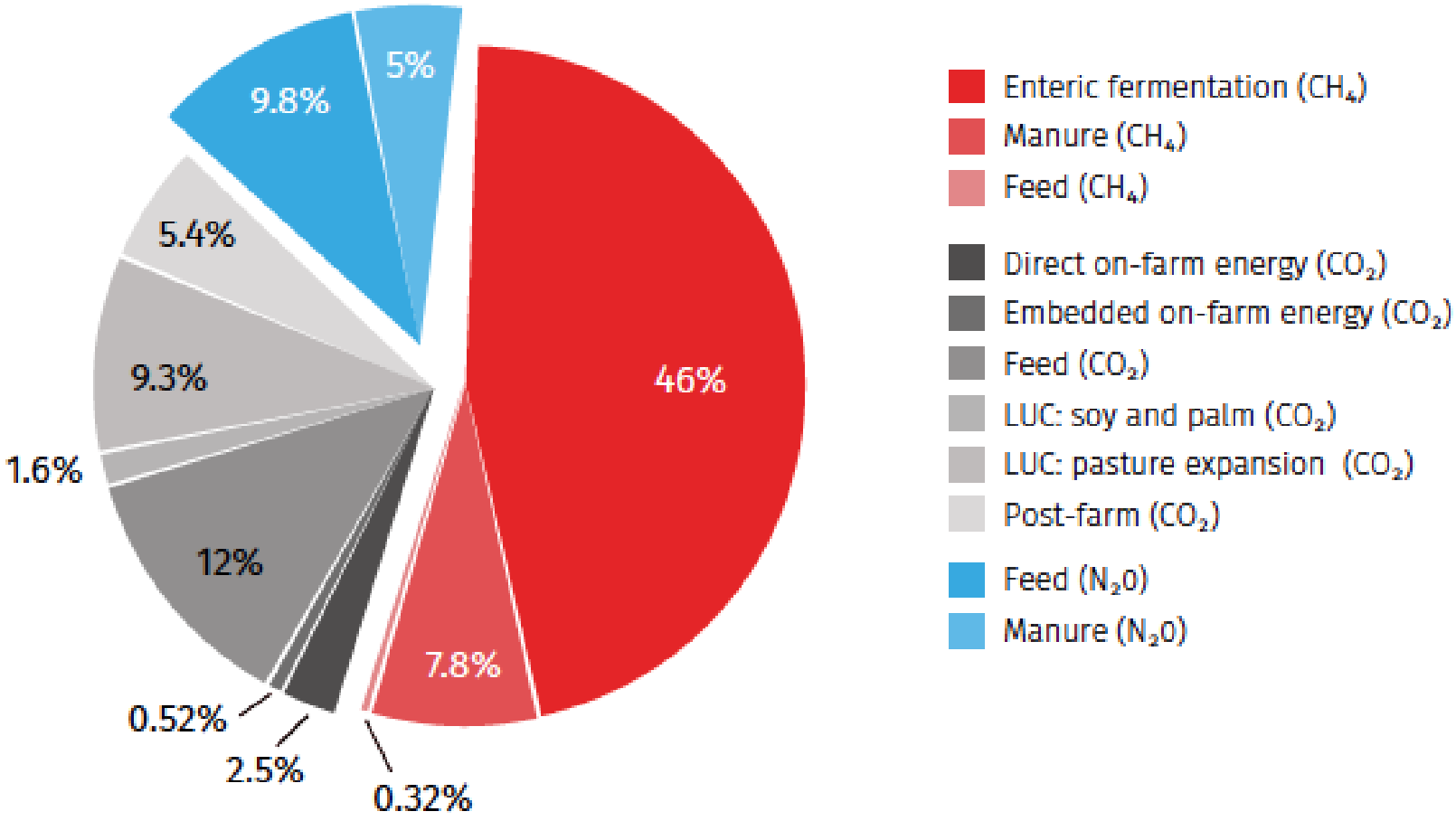
Enteric methane (CH_4) emission from Agrifood systems

- Agrifood systems accounts for 1/3 of total anthropogenic greenhouse gas (GHG) emission
- Almost half of Agrifood system GHG is enteric CH_4
- CH_4 atmospheric half-life: 8.6 years (Muller and Muller, 2017)



FAO, 2023

Can microalgae supplement reduce CH_4 emission?

- *In vitro* CH_4 inhibition (% inhibition, dosage):

Euglena gracillis (9.2~48.5%, 5~100% DM)

Nannochloropsis gaditana (10.2%, 16% DM)

Phaeodactylum tricornutum (16.6%, 16% DM)

Schizochytrium sp. (7.8%, 6.5% DM)

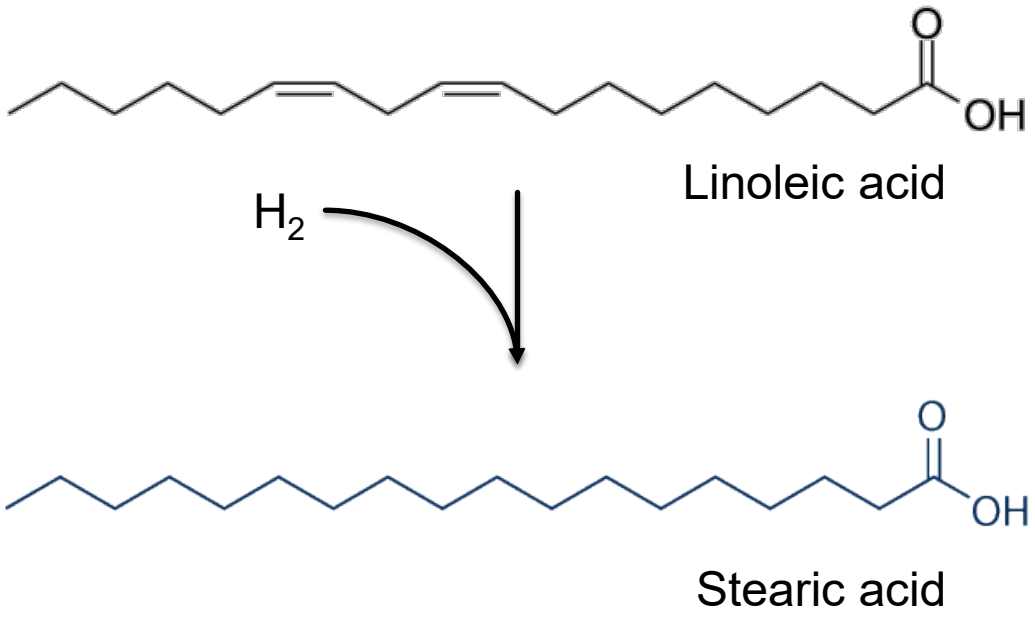
Aquagrow-DHA (*Crypthecodinium cohnii* extract), (23~80.6%, 5~29.3%DM)

Aemiro et al., 2016; Fievez et al. 2007; Kiani et al., 2020

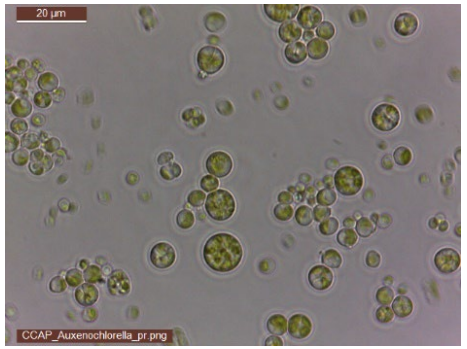
How microalgae may mitigate CH_4 production, and other benefits

- Unsaturated fatty acids (biohydrogenation)
- Rich source of protein
- Does not need arable land or potable water

Biohydrogenation as alternative H_2 sink



Freshwater Microalgae selected for this study

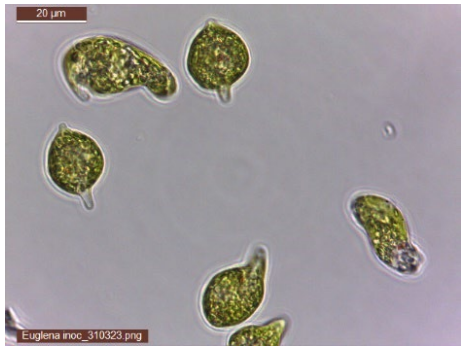


Auxenochlorella protothecoides (Ap)

38-41% lipid,
19.4% PUFA

Chlamydomonas pulvinate (Cp)

C. malina 32.5% lipid,
52.9% PUFA

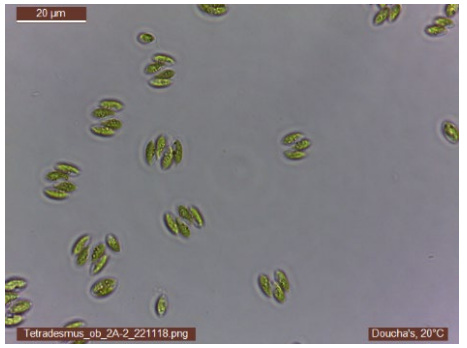


Euglena mutabilis (Em)

E. gracilis 70% lipid,
23.4% PUFA

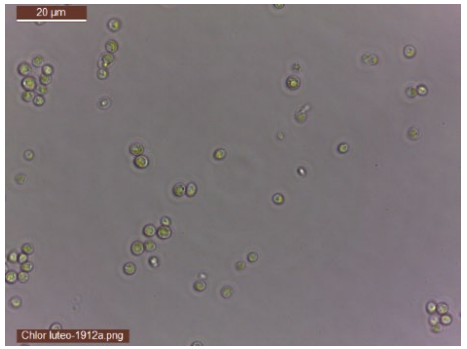
Parachlorella kessleri (Pk)

24.1% lipid,
51.4% PUFA



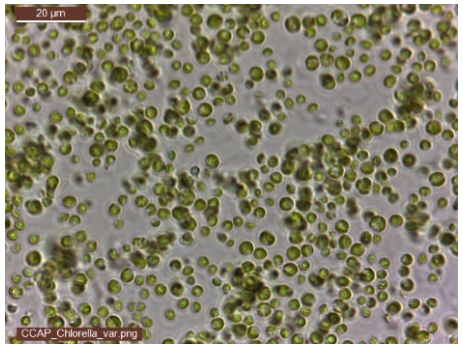
Tetradesmus obliquus (To)

12.6% lipid,
39.4% PUFA



Chlorella luteoviridis (Cl)

9.9% lipid,
40.7% PUFA

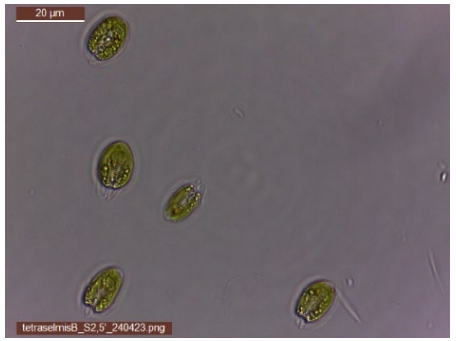


Chlorella variabilis (Cv)

22.6% lipid,
38% PUFA

Stichococcus bacillaris (Sb) 24% lipid,
33% PUFA

Tetradesmus acuminatus (Ta) 42.6% lipid,
26.6% PUFA



Tetraselmis gracilis (Tg)

11.1% lipid,
17.1% PUFA

Campenni et al., 2012; Polat et al., 2023; Kumaran et al., 2023; Morales-Sanchez et al., 2020; Liang et al., 2020; Pohl and Wagner 1971; Mutaf et al., 2019

Cultivation and *in vitro* testing

Culture Microalgae (2% CO_2, light at 115 µmol/m^2.s,16 h:8 h day/night, 110 rpm, optimal temperature, pH, most adapted nutrient media)

↓

Biomass collection (Centrifugation and freeze-drying)

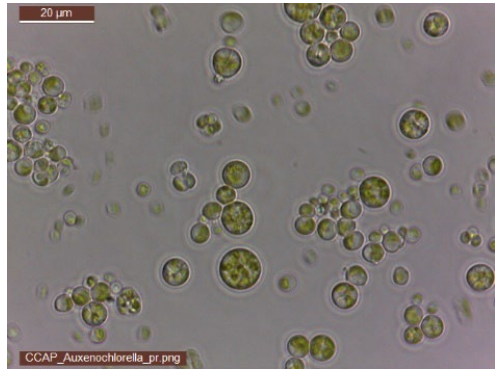
↓

Chemical analyses

↓

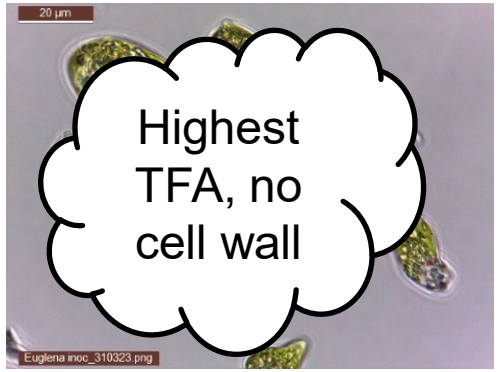
Hohenheim Gas Test (24 h, 15% DM basal diet substitution)

Nutritional properties of cultured microalgae

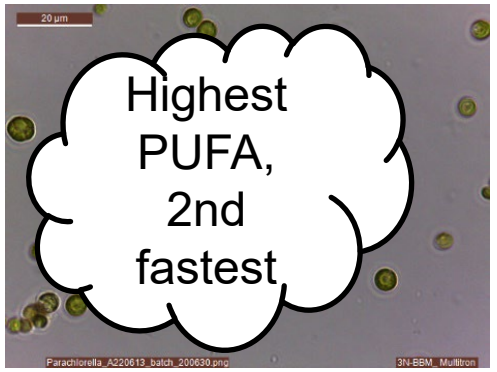


Auxenochlorella protothecoides (Ap)
17.5% CP, 10.3% TFA, 6.08% PUFA

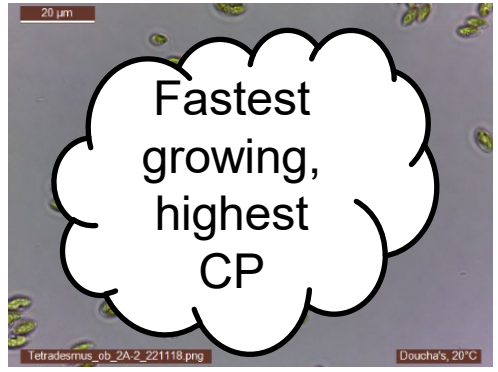
Chlamydomonas pulvinate (Cp)
44.0% CP, 11.8% TFA, 6.08% PUFA



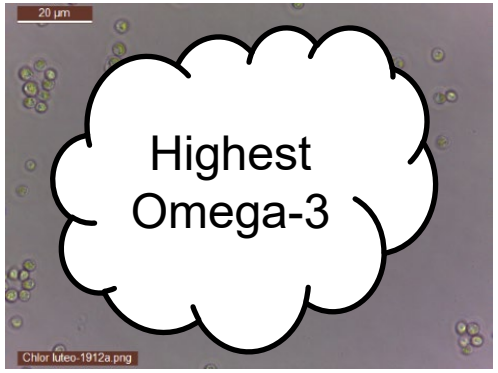
Euglena mutabilis (Em)
44.5% CP, 18.0% TFA, 7.51% PUFA



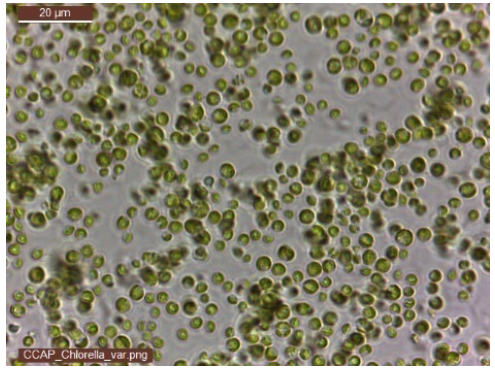
Parachlorella kessleri (Pk)
44.3% CP, 13.5% TFA, 7.55% PUFA



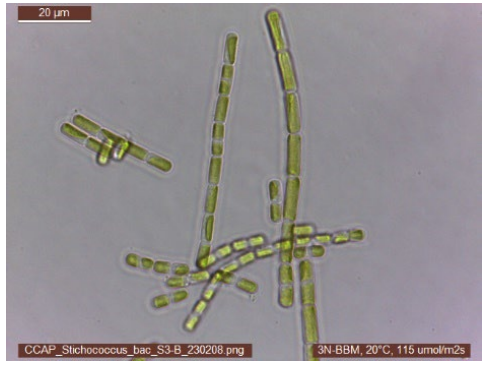
Tetradesmus obliquus (To)
58.7% CP, 13.1% TFA, 4.62% PUFA



Chlorella luteoviridis (Cl)
46.2% CP, 10.3% TFA, 7.12% PUFA

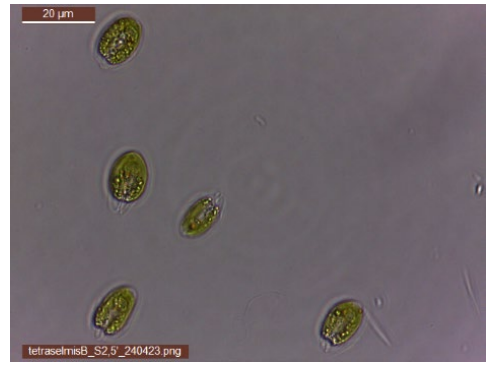


Chlorella variabilis (Cv)
48.5% CP, 10.3% TFA, 3.91% PUFA

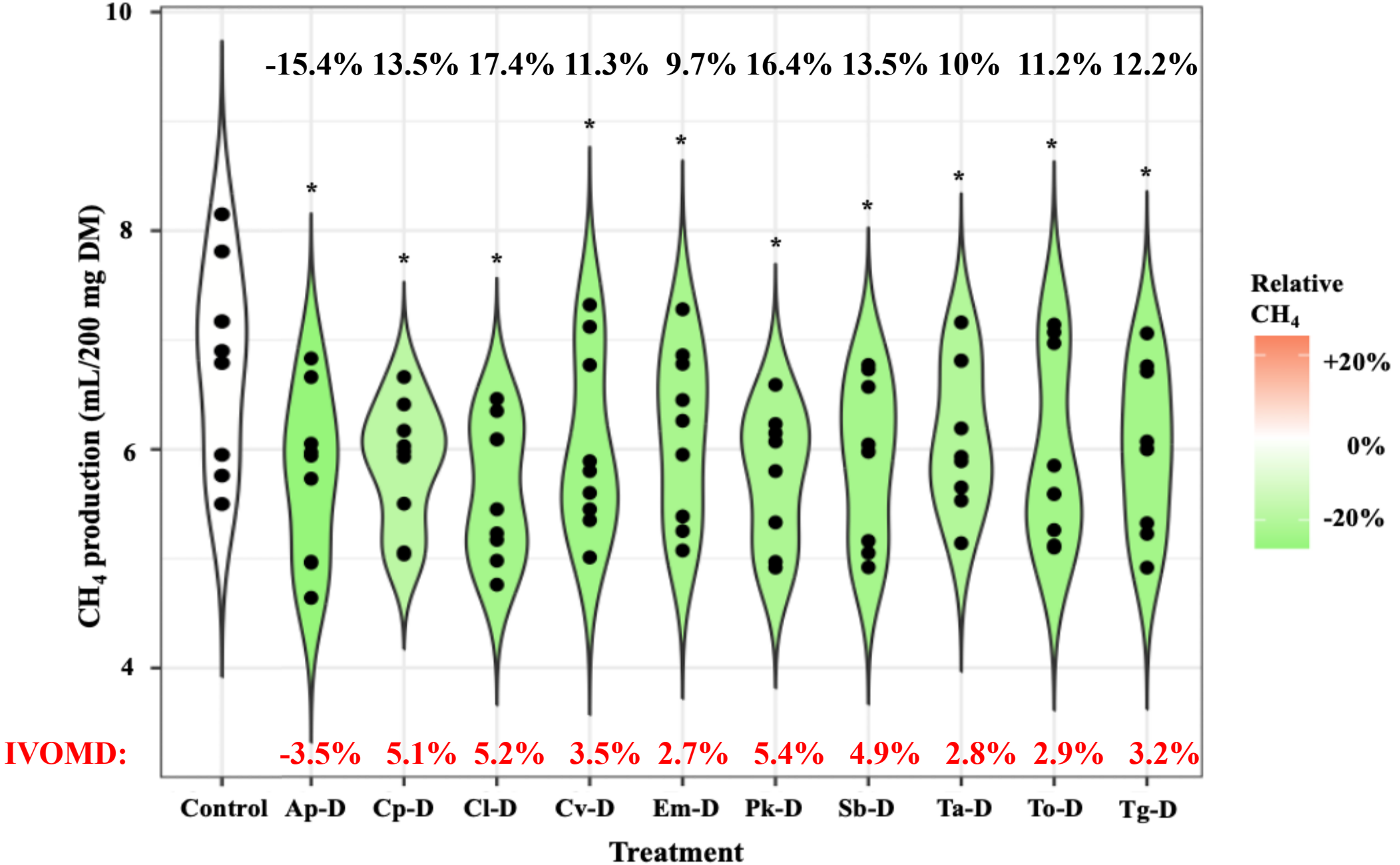


Stichococcus bacillaris (Sb)
38.1% CP, 10.9% TFA, 5.31% PUFA

Tetradesmus acuminatus (Ta)
39.7% CP, 10.5% TFA, 4.29% PUFA



Tetraselmis gracilis (Tg)
30.7% CP, 12.6% TFA, 4.18% PUFA


-15.4% 13.5% 17.4% 11.3% 9.7% 16.4% 13.5% 10% 11.2% 12.2%
IVOMD: -3.5% 5.1% 5.2% 3.5% 2.7% 5.4% 4.9% 2.8% 2.9% 3.2%
CH4 production (mL/200 mg DM)
10
8
6
4
Control Ap-D Cp-D Cl-D Cv-D Em-D Pk-D Sb-D Ta-D To-D Tg-D
Treatment
Relative CH4
+20%
0%
-20%

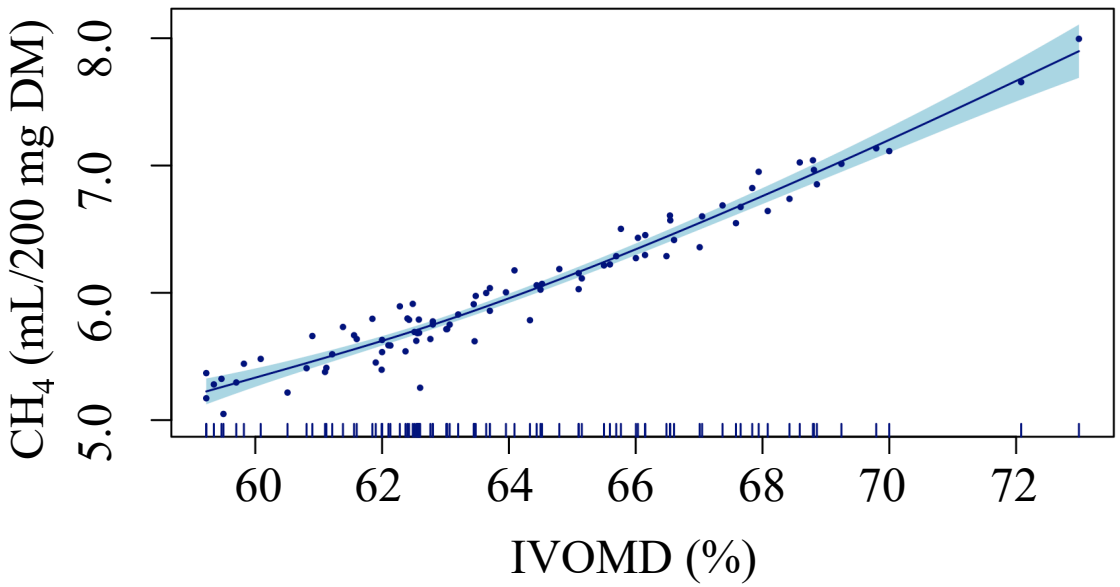
Generalized additive models (GAMs) – a semi-parametric approach

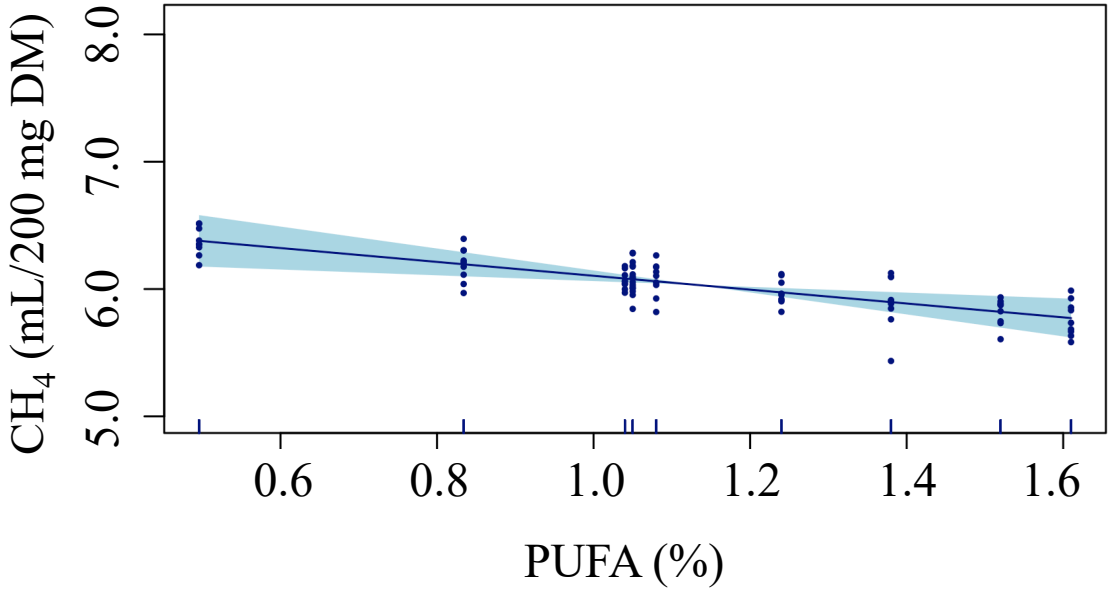
- GAMs are smooth semi-parametric models of the form:

$$g(\mathbb{E}[y|X])=\beta_0+f_1(X_1)+f_2(X_2, X_3)+\ldots+f_M(X_N)$$

- GAMs extend generalized linear models by allowing non-linear functions of features
- Since the model is additive, it is easy to examine the marginal effect of each variable

IVOMD and PUFA content is correlated with CH_4 production

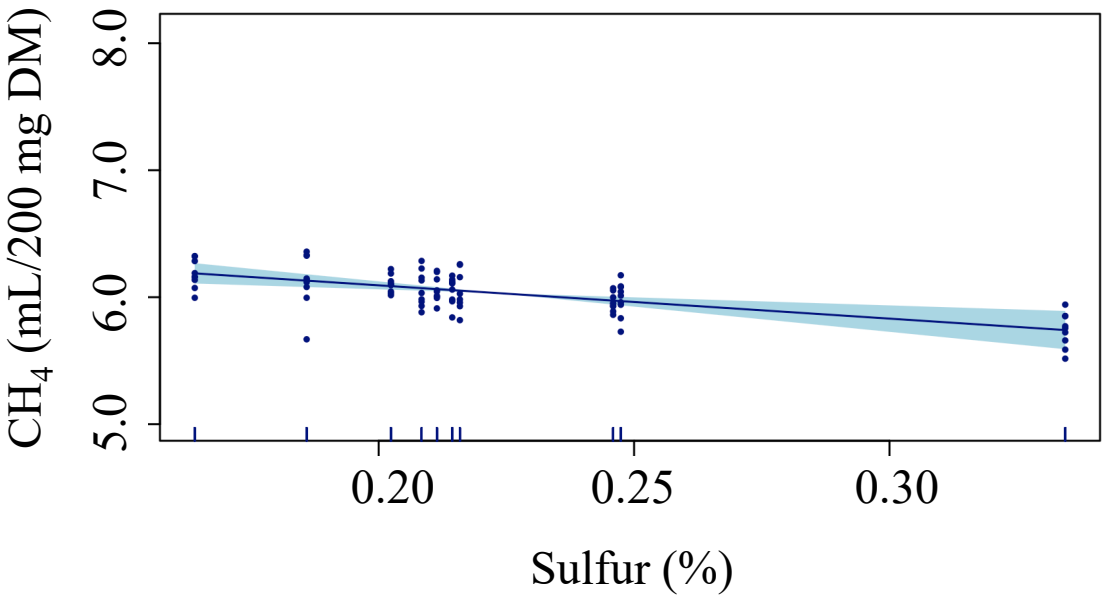


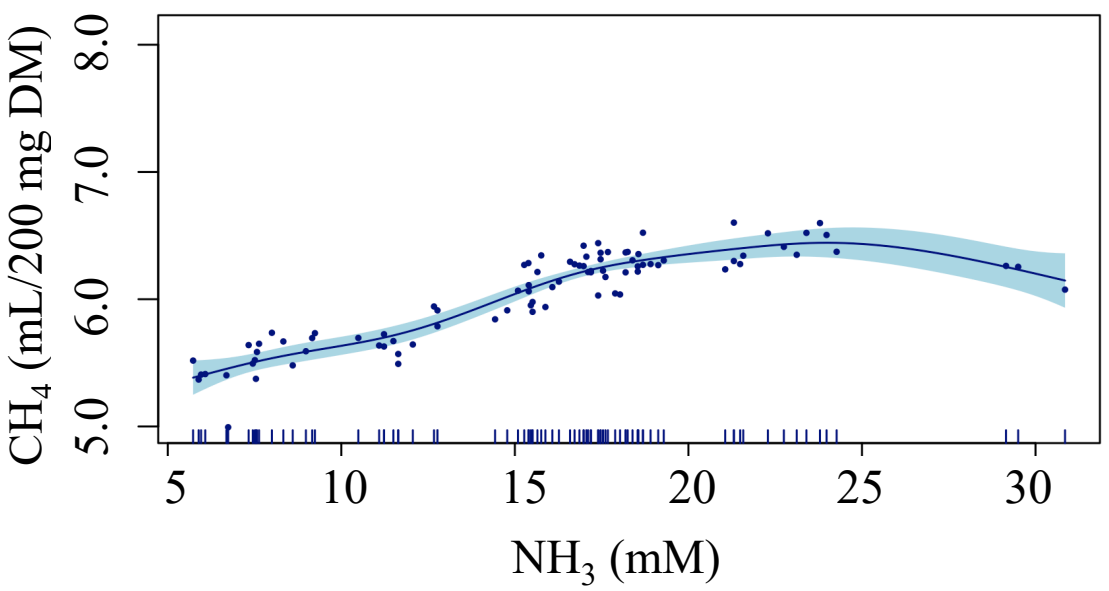


- Positive correlation between CH_4 production and IVOMD
- Negative correlation between CH_4 production and PUFA, but….lipid extracted *Chlorella vulgaris, Micratinium reisseri, Nannochloris bacillaris* and *Tetracystis sp*. reduced CH_4.

Tibbetts et al., 2016

Sulfur and NH_3 content is correlated with CH_4 production





- *Scenedesmus sp. AMDD* increased sulfate-reducing bacteria in anaerobic food sludge
- Increasing NH_3 concentration up to 25 mM were not inhibitory to methanogenesis

Tartakovsky et al., 2013

Conclusions and perspectives

- Freshwater microalgae supplementation could mitigate enteric CH_4 emission, with mild reduction in IVOMD
- IVOMD is positively related to *in vitro* CH_4 production
- PUFA and sulfur content is negatively related to *in vitro* CH_4 production
- *Tetradesmus obliquus* with its high growth speed and CP, moderate PUFA content could be a good supplement for ruminant feed
- Warrants further investigation and *in vivo* trial

Thank you

Agroscope

CoBiEM
Collection of Biotechnologically Exploitable Microalgae

Culture Collection of algae & protozoa

SAG

AgroVet Strickhof

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