

Lessons from SMethane: Assessing the effect of plant extracts on methane production by in vitro screening tests: (dose) responses and sources of variability

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Methodology



In vitro batch incubation system



250 mg substrate



5 ml rumen fluid + 20 ml
phosphate/bicarbonate buffer



24 h at 39 °C



Methane and VFA

Test approach

1/ Screening : limited doses & substrates, 1 pH

2/ Promising compounds :

≠ doses (btw. 3 and 10)

≠ substrates (btw. 1 and 11)

≠ pH levels (3)

3/ Combinations (3 companies; 1 'main' test product)

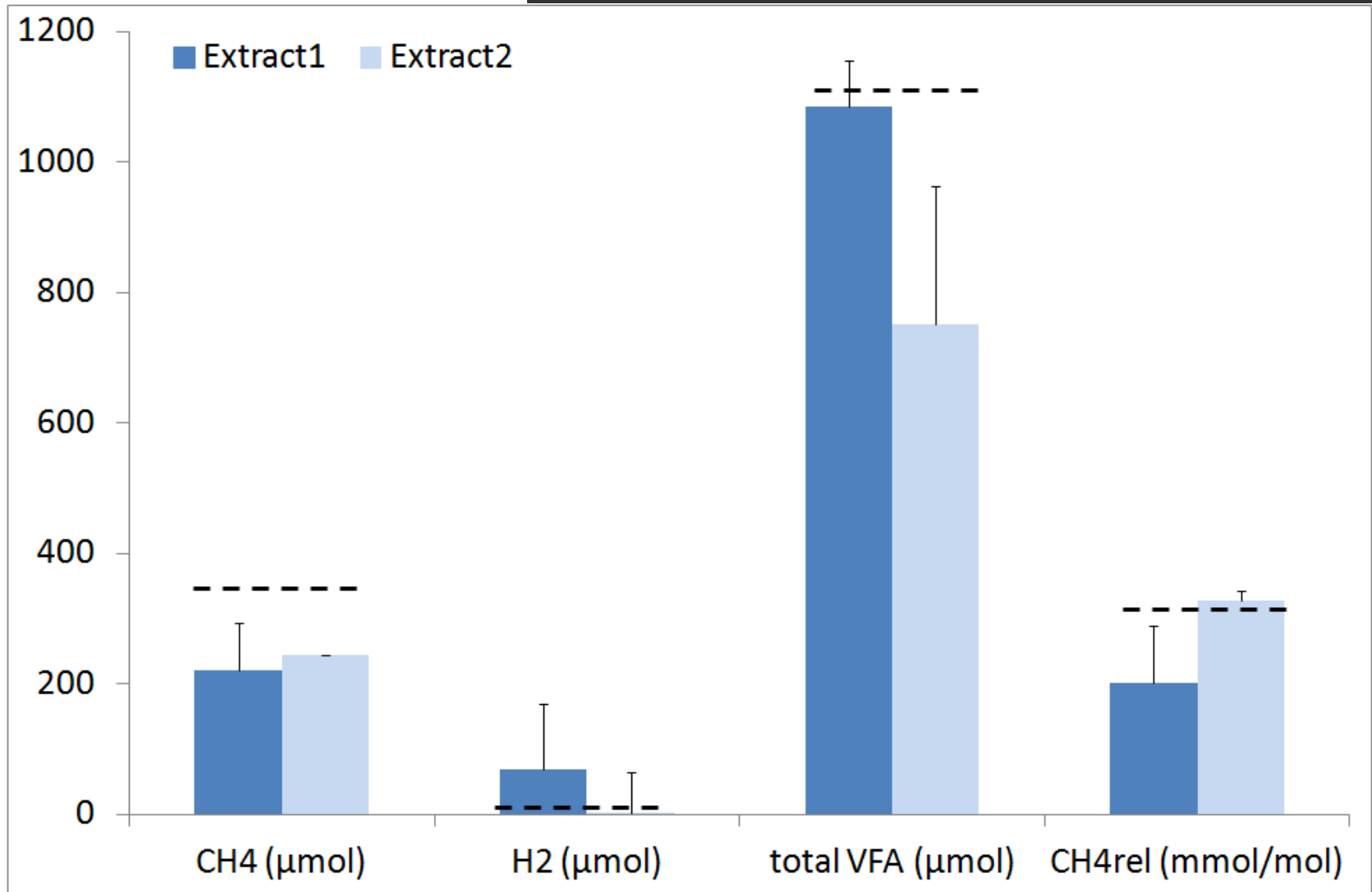
Overview

- Specific vs overall inhibition
- Dose responses
- Substrate dependent inhibition
- pH dependent inhibition

- Other responses

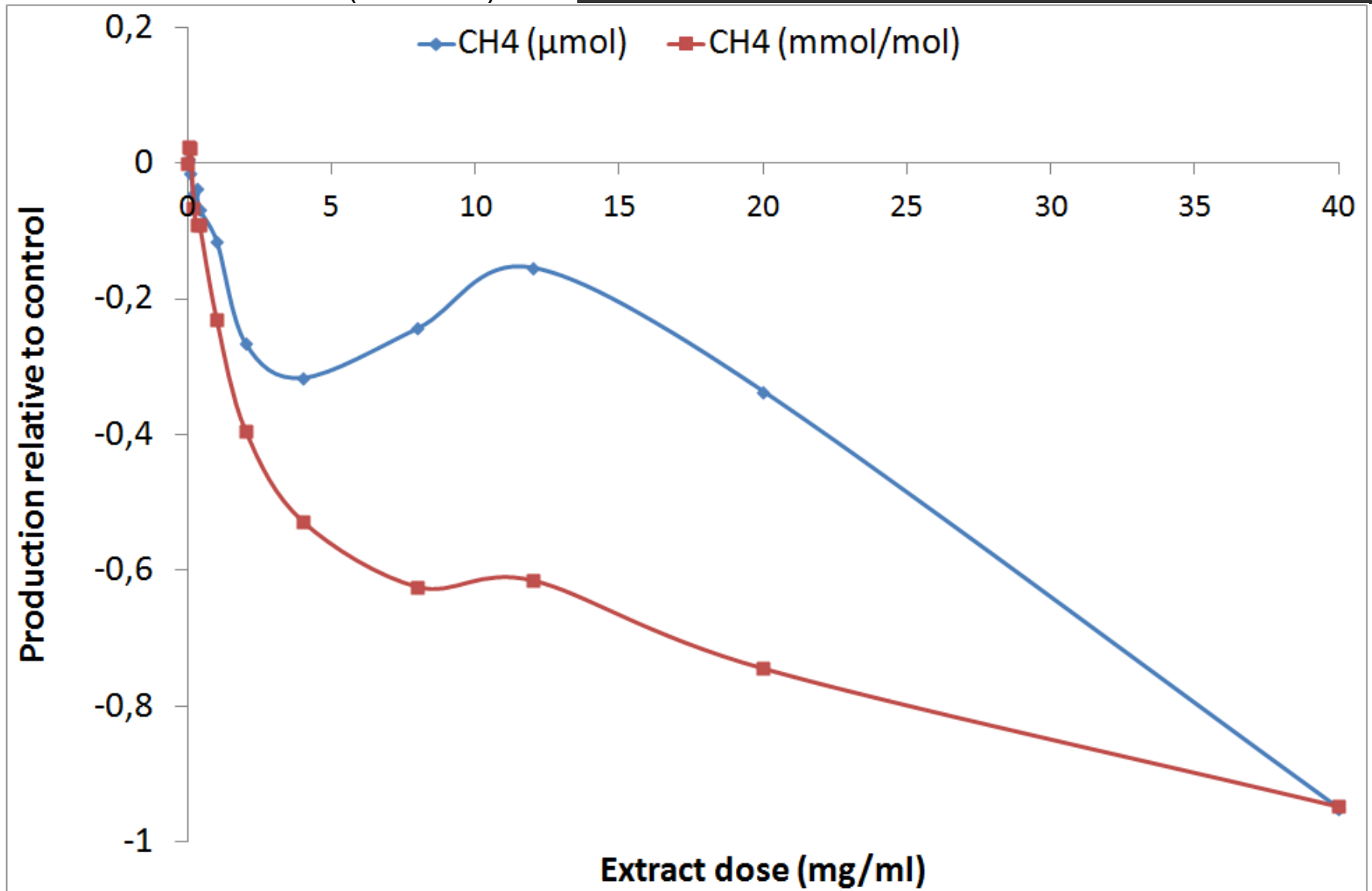
Specific vs overall inhibition

Extract1 – 0.1, 0.2, 0.3 mg/ml
Extract2 – 0.25, 0.50, 0.75 mg/ml
Substrate - grass silage
- - - control



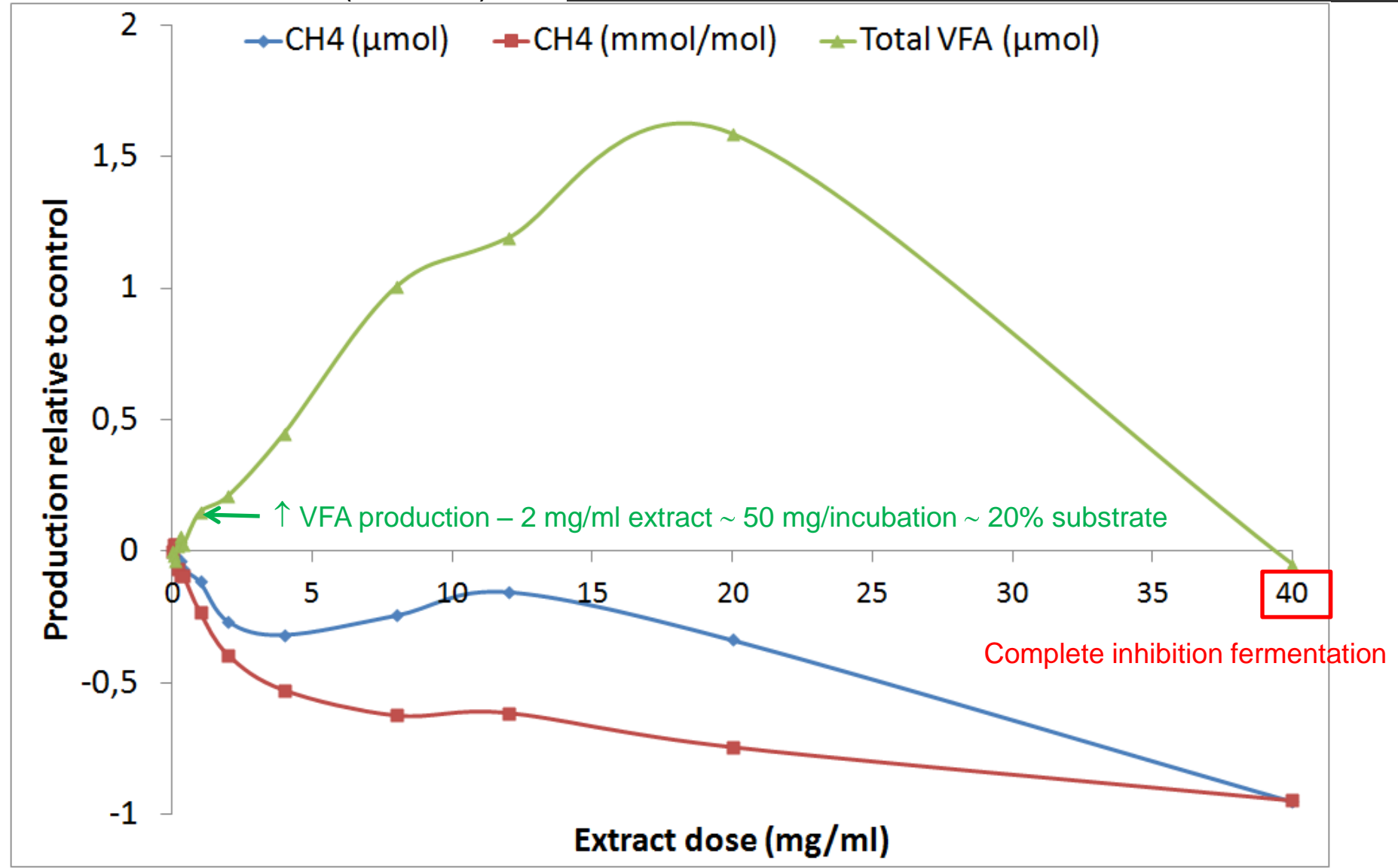
Extract3 – 0, 0.04, 0.1, 0.2, 0.3, 0.4, 1.0,
2.0, 4.0, 20.0, 40.0 mg/ml
Substrate – GS/MS/Conc (35/35/30)

Dose responses



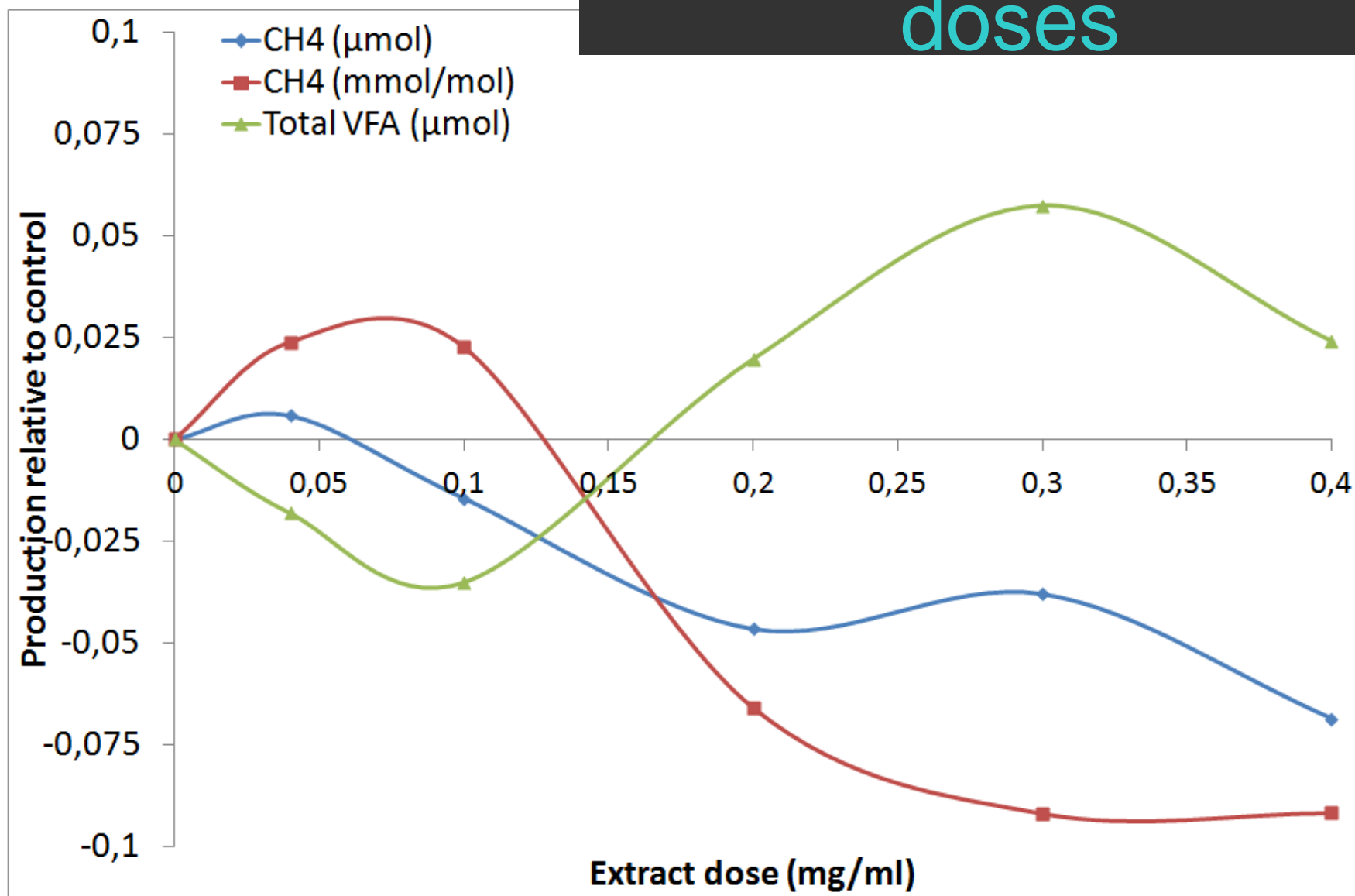
Extract3 – 0, 0.04, 0.1, 0.2, 0.3, 0.4, 1.0,
2.0, 4.0, 20.0, 40.0 mg/ml
Substrate – GS/MS/Conc (35/35/30)

Dose responses



Extract3 – 0, 0.04, 0.1, 0.2, 0.3, 0.4 mg/ml
Substrate – GS/MS/Conc (35/35/30)

Economically feasible doses



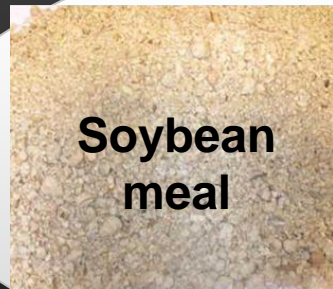
Substrate dependent inhibition

Soybean meal (SBM)

Grass silage (GS)

Maize silage (MS)

MIX (SBM + GS + MS, 25 : 20 : 55 ; 25 : 55: 20)



MIX

Substrate dependent inhibition

Soybean meal (SBM)

Grass silage (GS)

Maize silage (MS)

MIX (SBM + GS + MS, 25 : 20 : 55 ; 25 : 55: 20)

Straw (Straw)

Concentrate (CON)

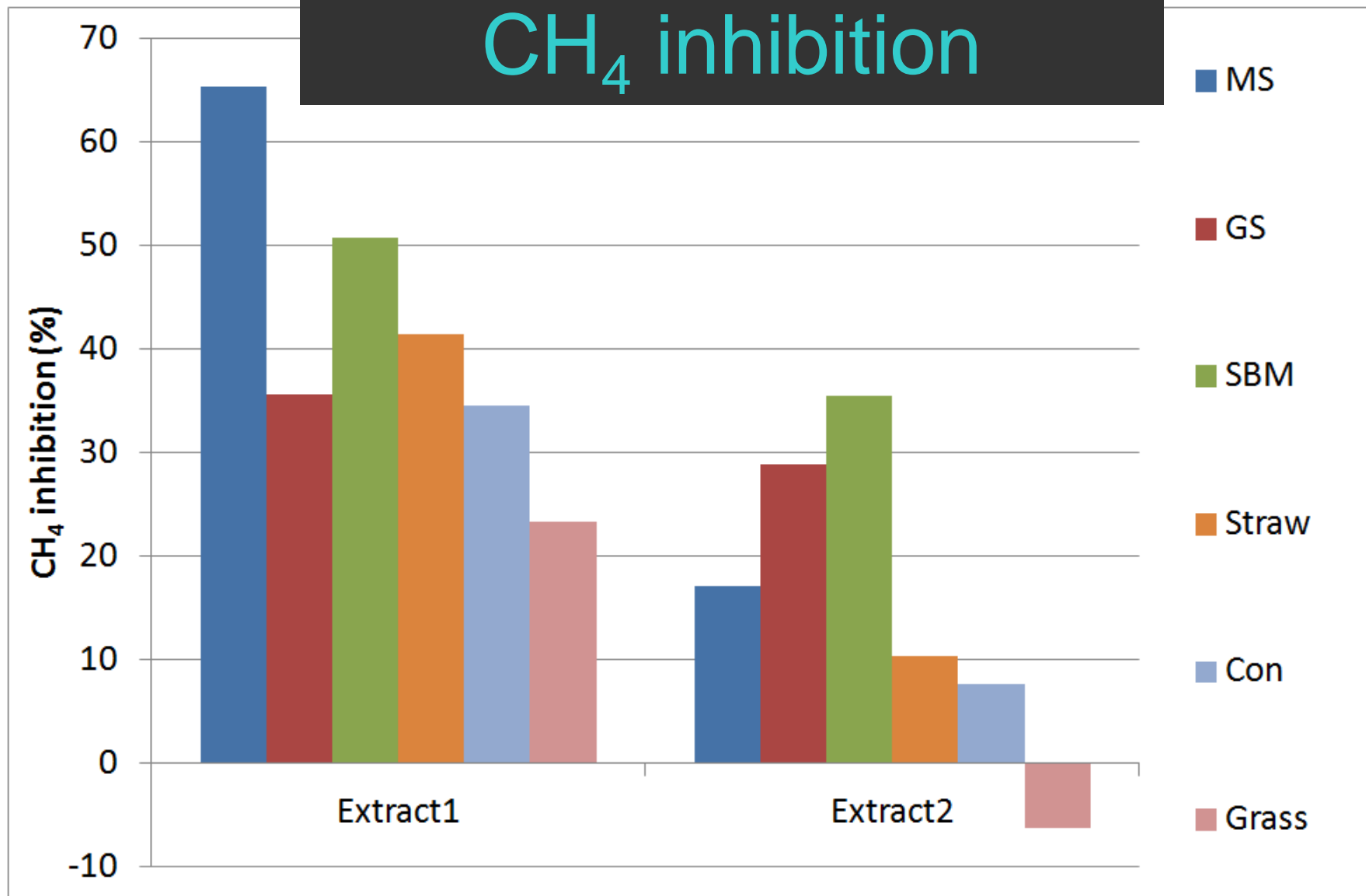
MIX (Straw + CON, 25 : 75)

Lyophilised fresh grass (Grass)

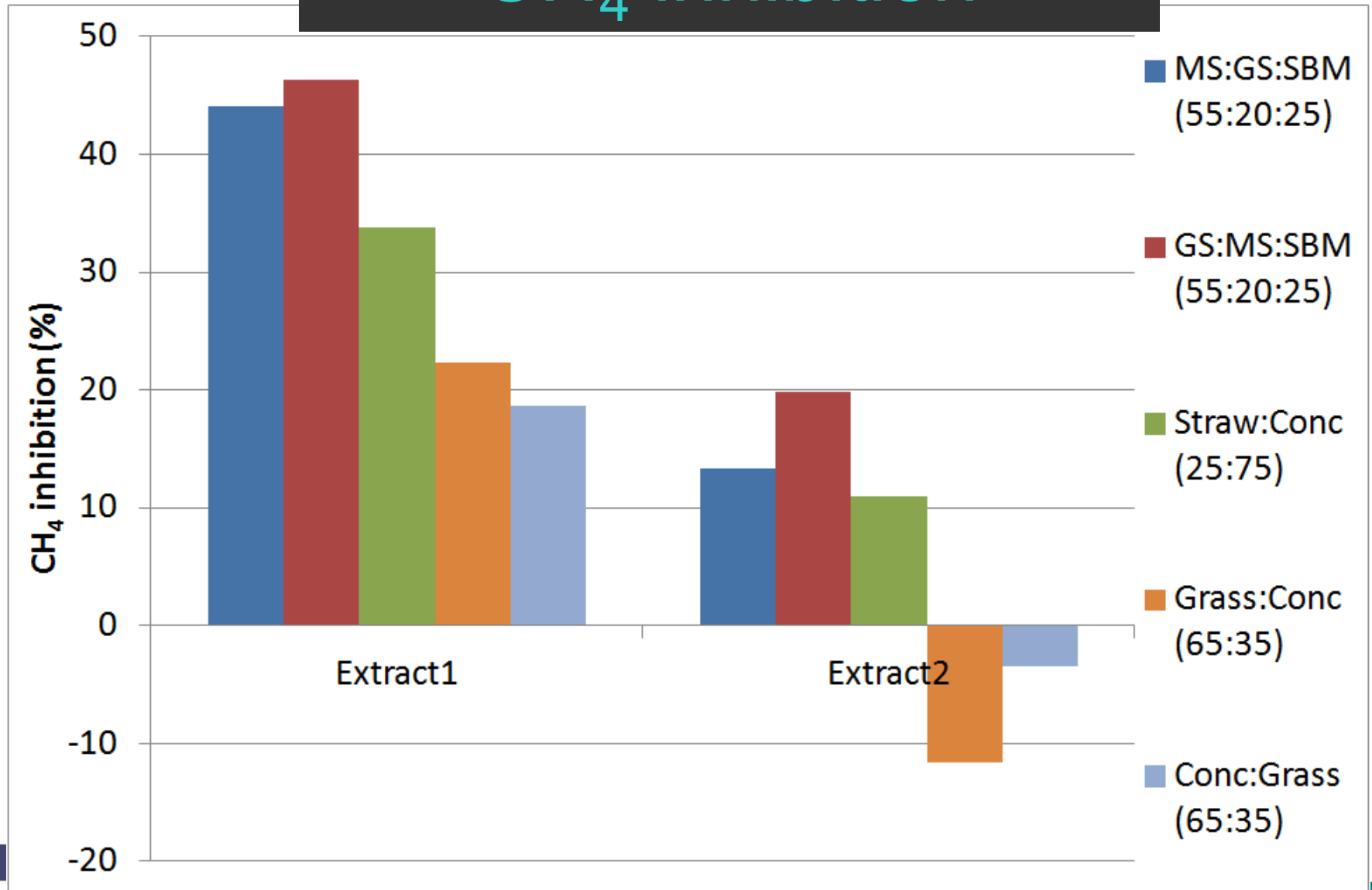
Concentrate (CON)

MIX (Grass + CON, 35 : 65 ; 65 : 35)

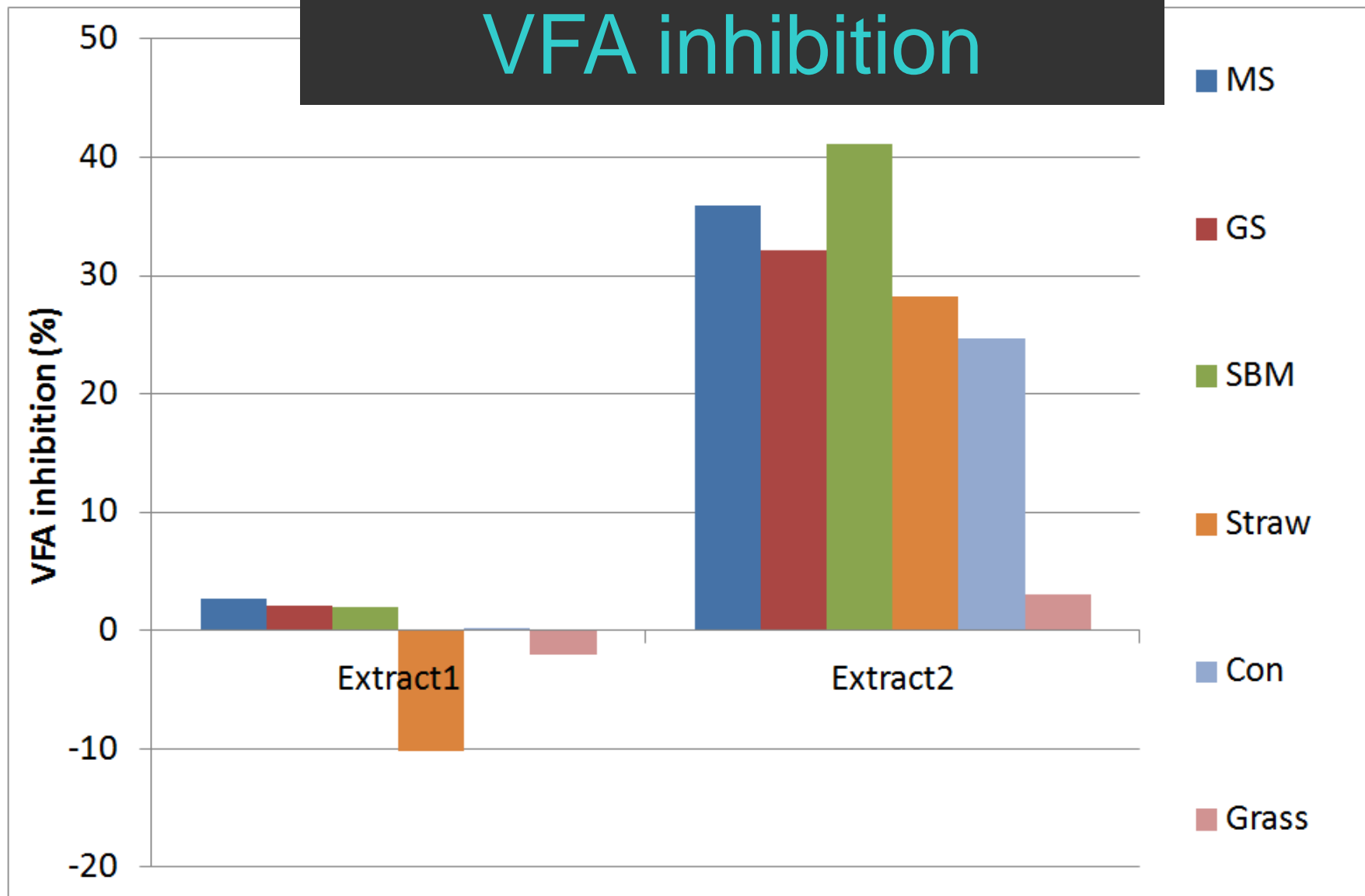
Substrate dependent CH₄ inhibition



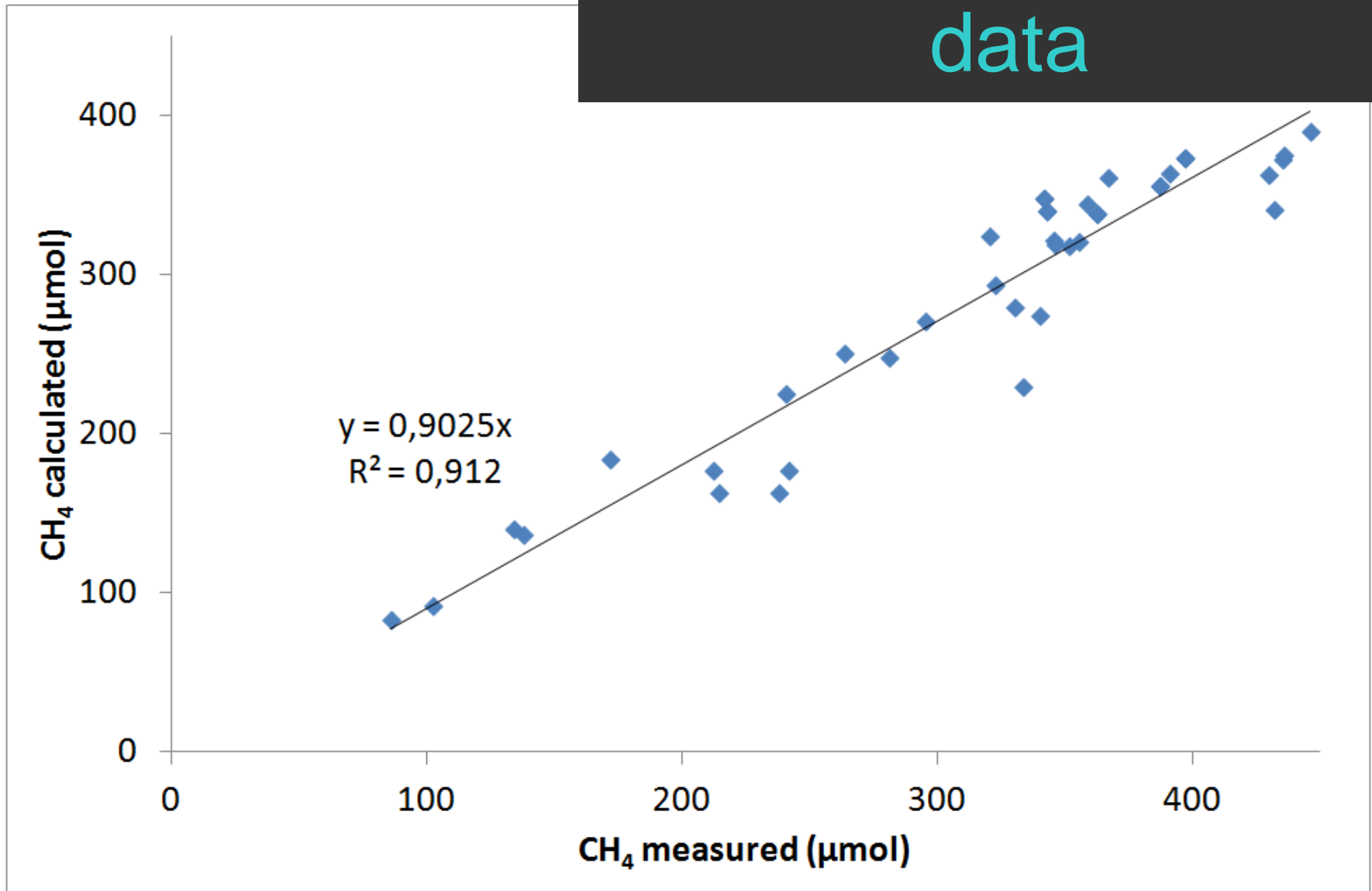
Substrate dependent CH₄ inhibition



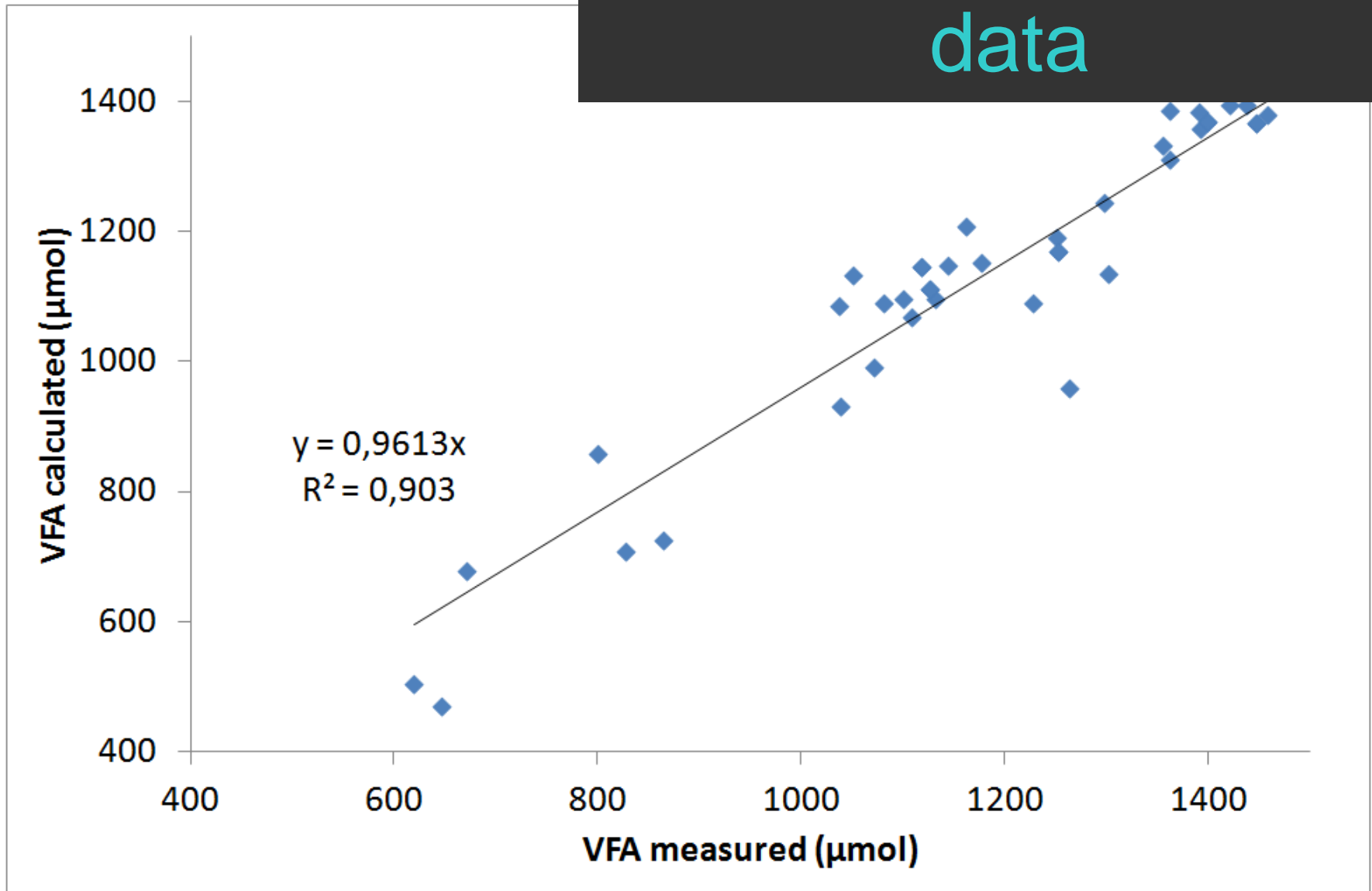
Substrate dependent VFA inhibition



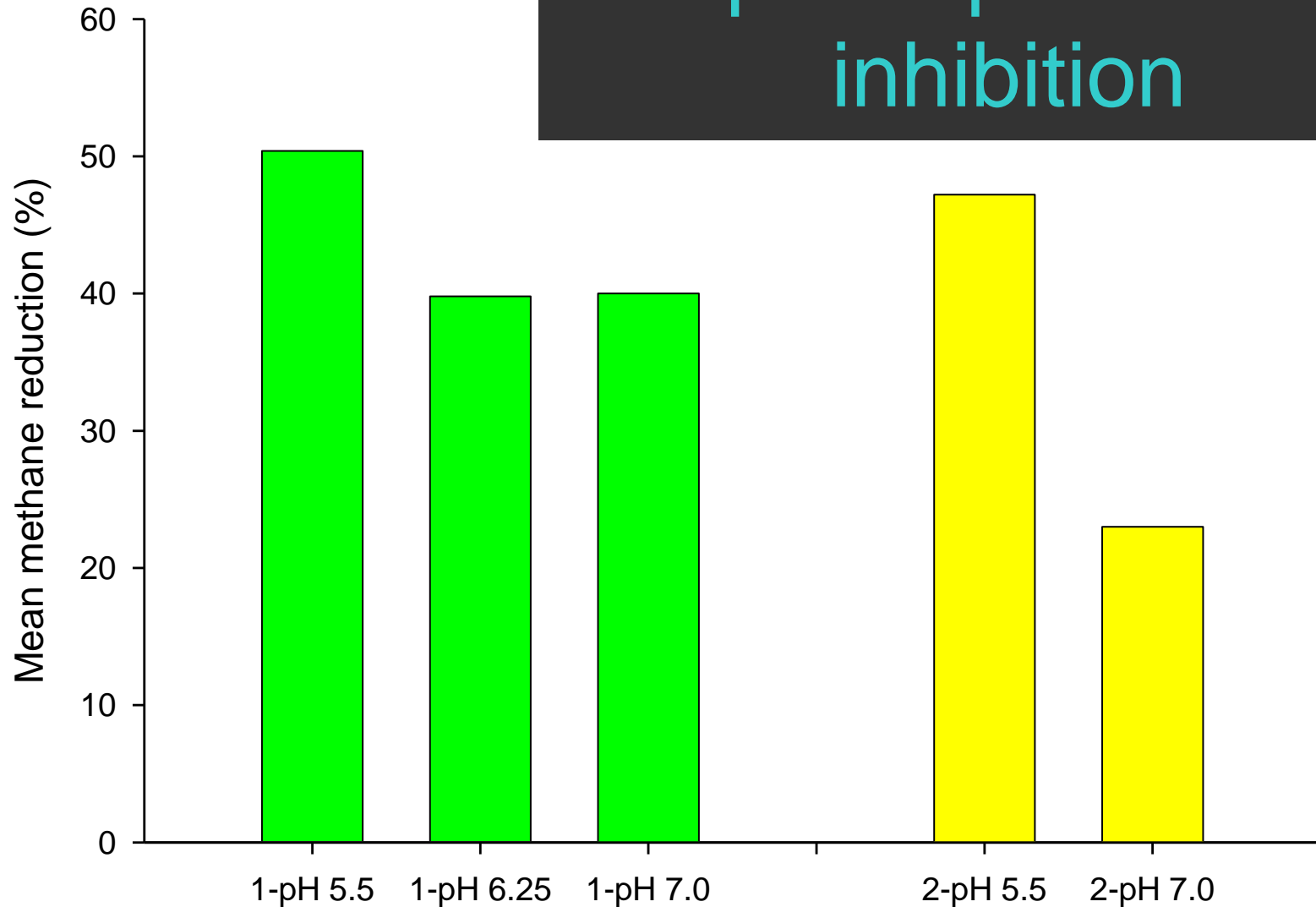
Modelling from in vitro data



Modelling from in vitro data

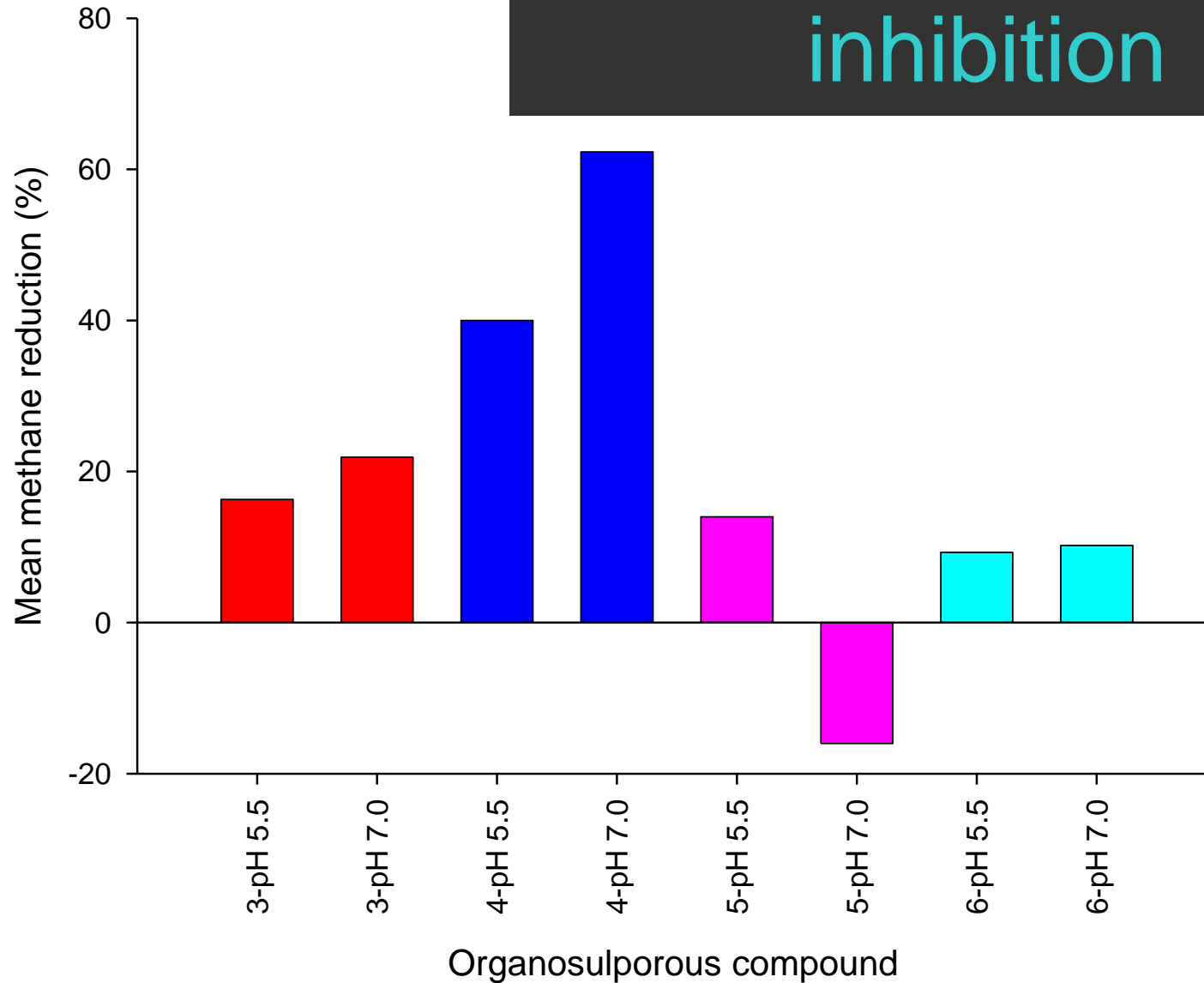


pH dependent inhibition

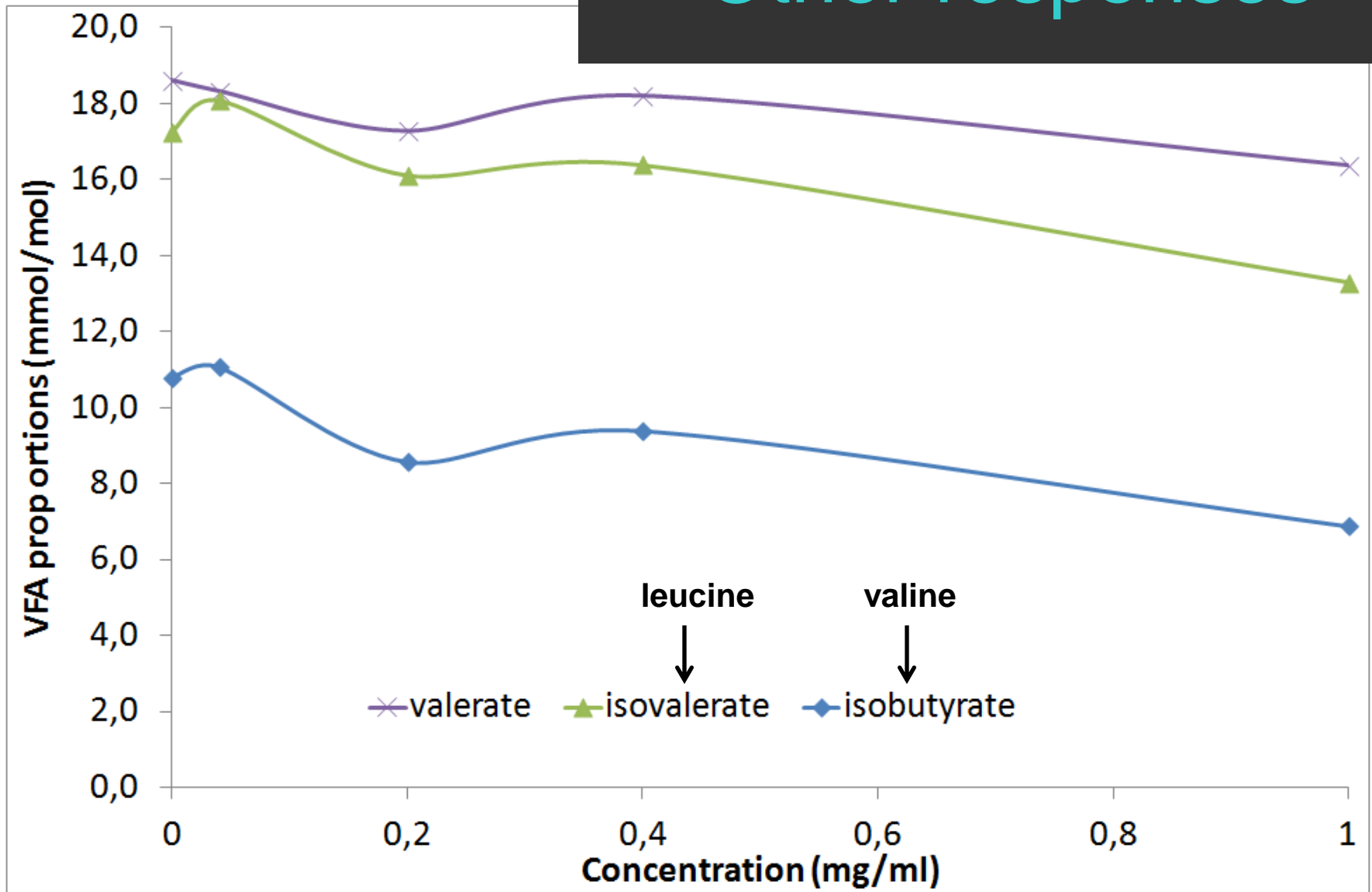


Purified organosulphorous molecule at different pH

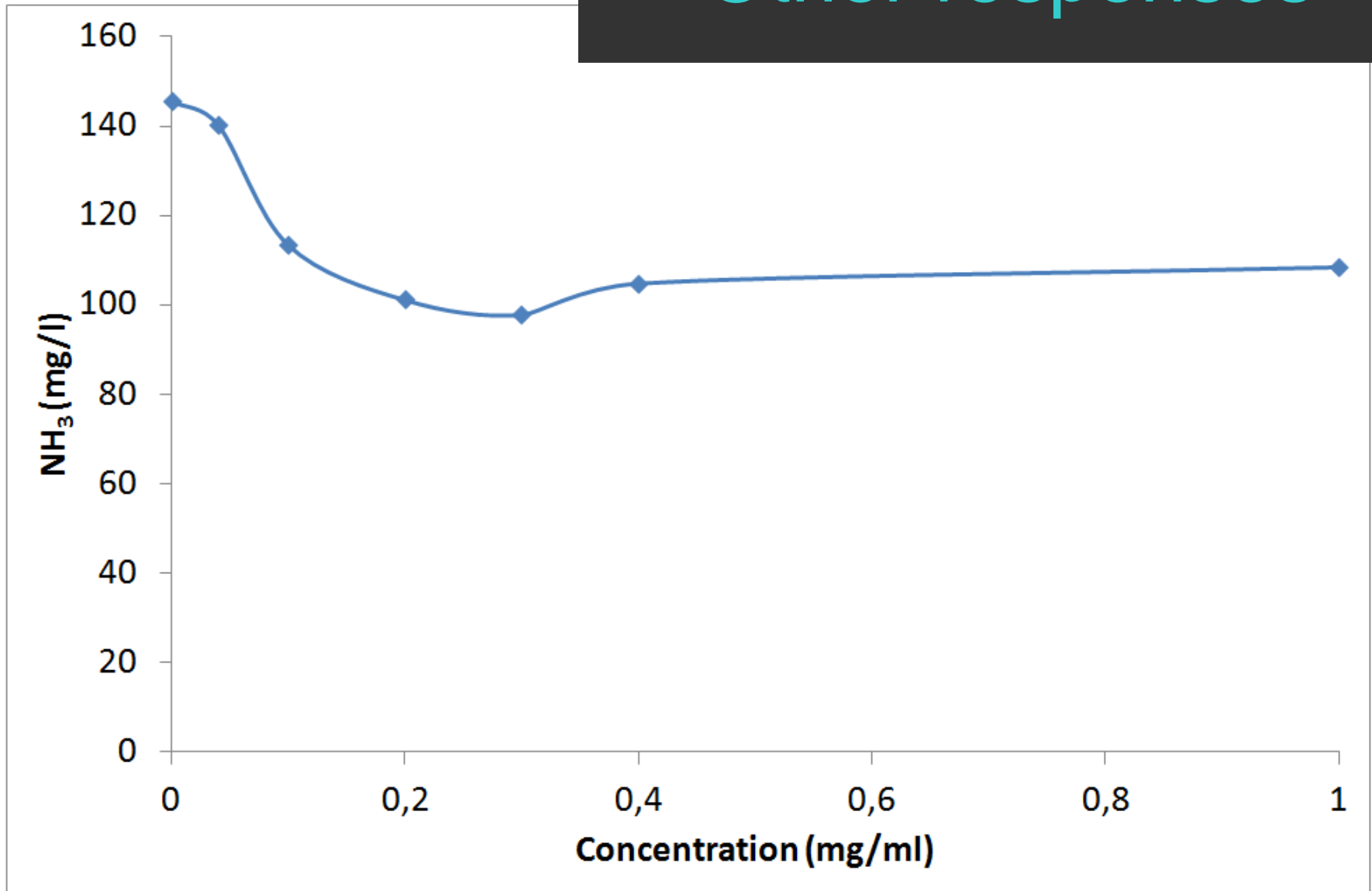
pH dependent inhibition



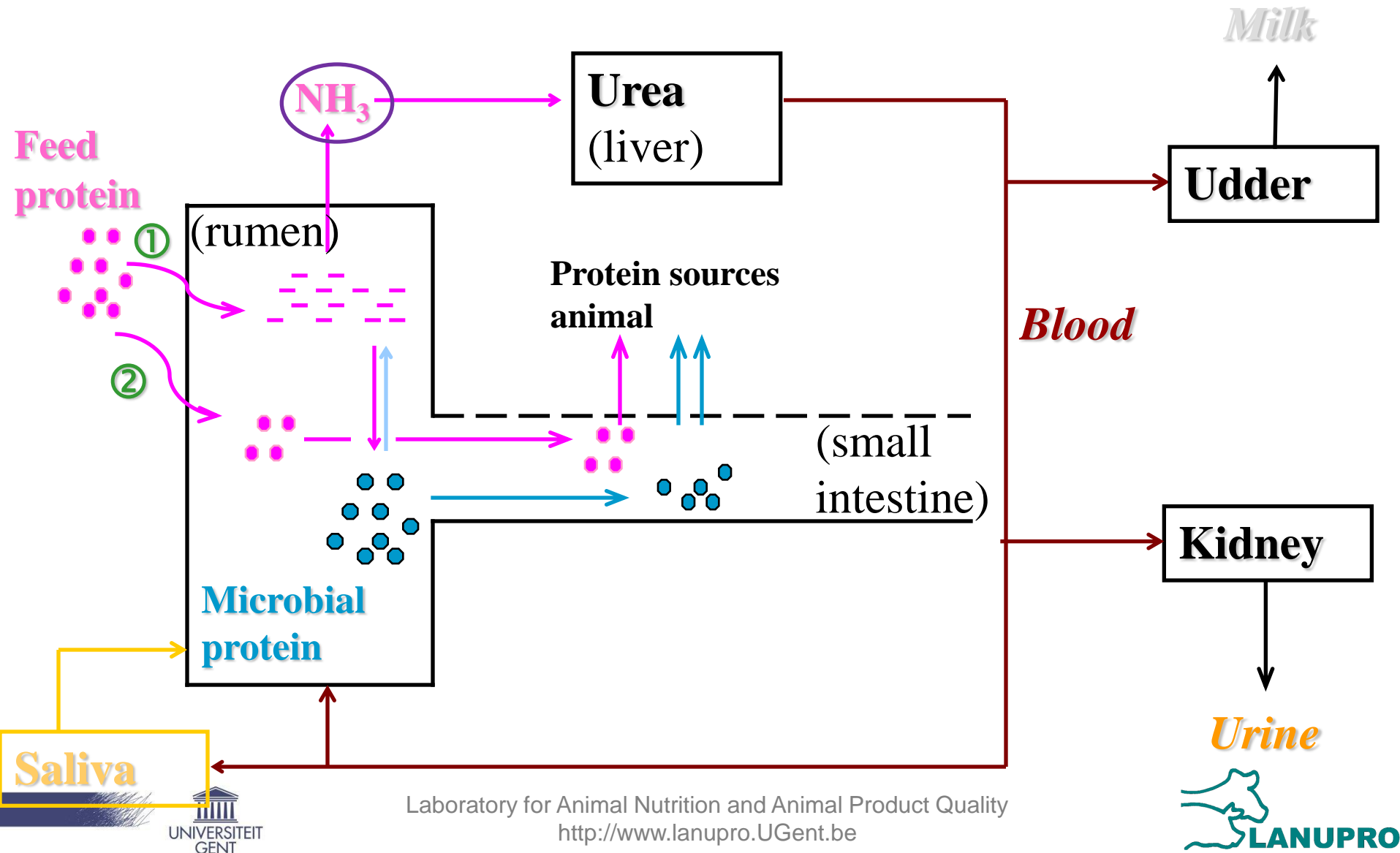
Other responses



Other responses



Other responses



Other responses

Acidi-
fication



Evaporation

Urine
(urea)



uptake



nitrification

leaching

Eutrophication



Greenhouse
effect

Denitrification

Conclusions

- **Specific vs overall inhibition** – some overall
- **Dose responses** – all – economically feasible
- **Substrate dependent inhibition** – e.g. fresh grass, straw
- **pH dependent inhibition** - variable

- **Other responses** – ↓ AA degradation; urinary urea