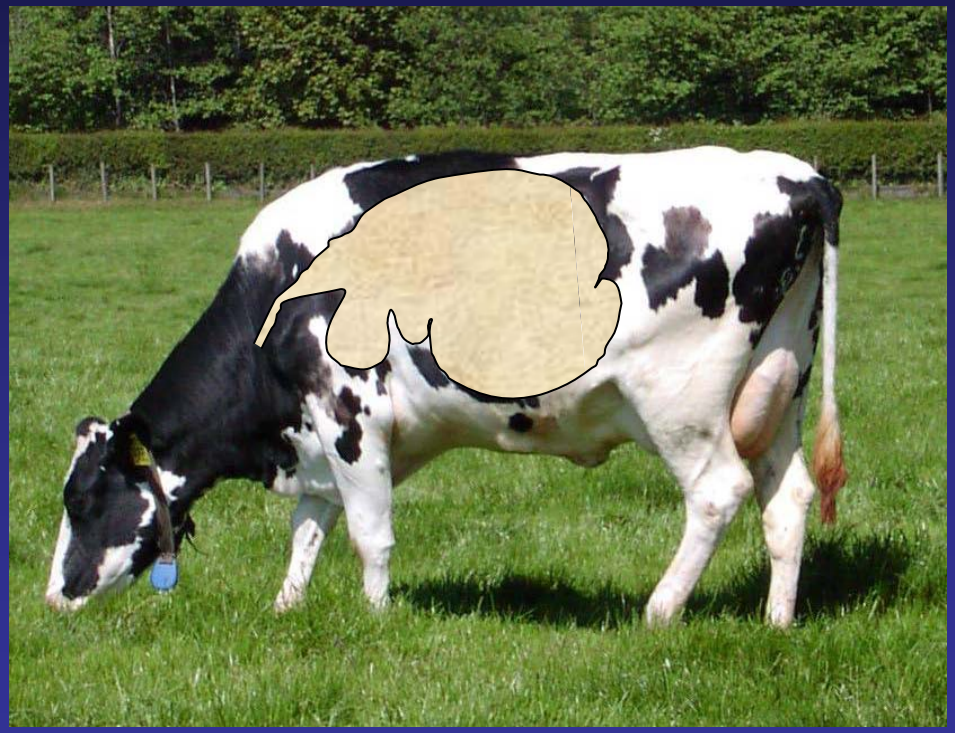
In vitro degradation of fresh forage by ruminal anaerobic fungi

Hugo Jimenez

Supervisors: Joan Edwards, Mike Theodorou, Neil McEwan

The Rumen Microbiota



Rumen Conditions:

- Anaerobiosis
- Temperature (39°C)
- pH (5.5 - 7)
- Fungi (<10^{4-5} ml^{-1}),
  - Biomass (8 - 20%)

Rumen:

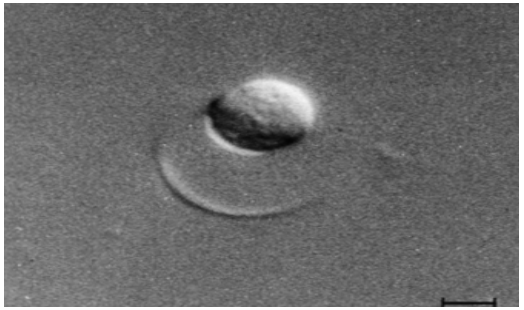
- Bacteria (10^{9-10} ml^{-1})
- Protozoa (10^{5-6} ml^{-1})
- Archaea (10^{5-7} ml^{-1})

What are anaerobic fungi?

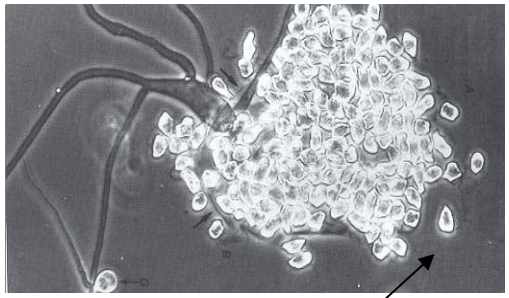
- Important role as fibre degraders in the rumen ecosystem,
  - Fungal rhizoids,
  - Enzymes.
- Unusual group of fungi, strict anaerobes.
  - Phylum: *Neocallimastigomycota*,
  - Six genera currently recognised.

Life Cycle of Anaerobic Fungi

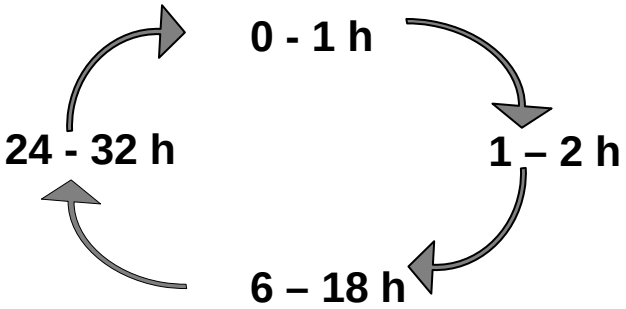
Motile Zoospore →

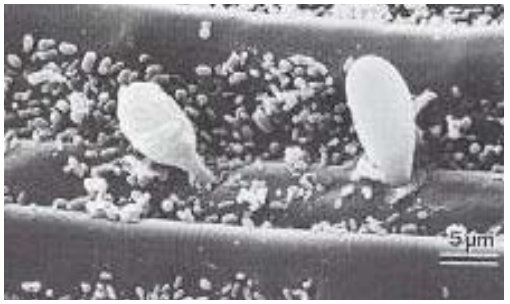


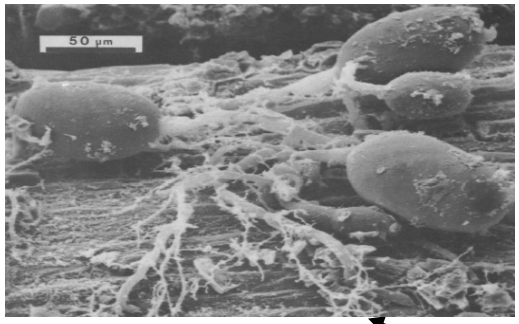
Attachment, Encystment and Germination



Released Zoospores







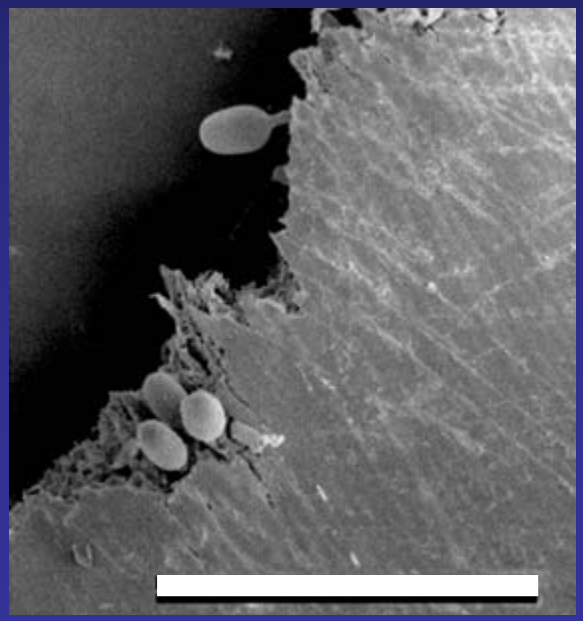
← Zoosporangia

Rhizoid System ←

Images from various sources: Bauchop, 1979; Orpin, 1994

Fungal Attachment Sites

- Attachment to plant material is mainly via 'damaged' areas;
  - Mastication,
  - Mechanical disruption/feed processing.



200 µm

100 µm

Images from Rezaeian *et al* , 2004

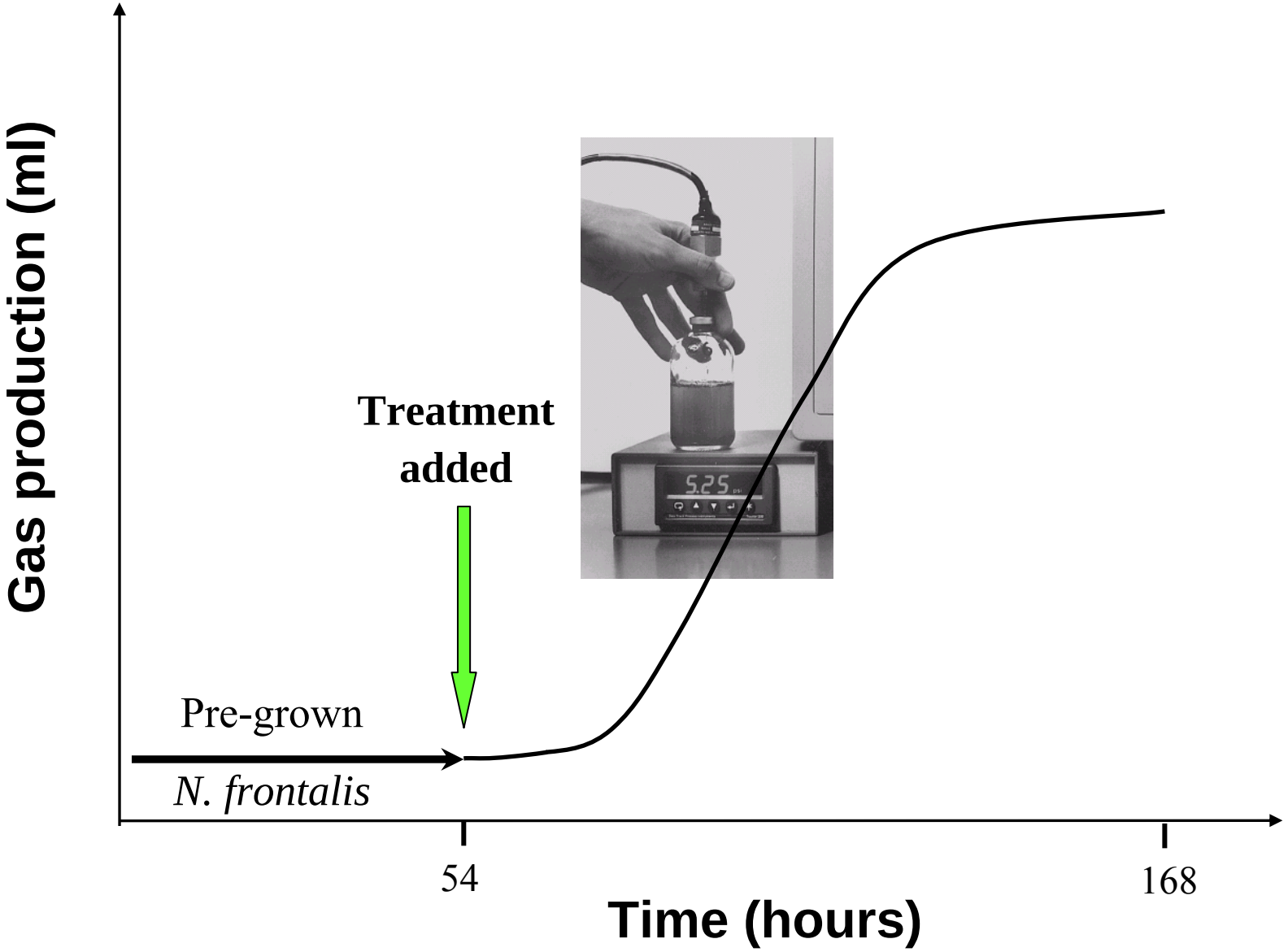
Does fresh forage particle size affect fungal metabolism?

- Decreasing particle size, increases surface area.
- Decreasing particle size has a variable effect on microbial metabolism,
  - Bacteria: increased attachment & enzymatic attack[1] and metabolism[2]. (Walker and Wilson, 1991)[1]
  - Anaerobic fungi: no effect on metabolism. (France *et al*, 1993)[2]
- Why is there no effect on fungi?
  - France *et al*, 1993 used autoclaved particles (<2 cm).
  - Grazing ruminants ingest forage >2 cm (~40 % DM).

Experiment

- *In vitro* experiment needed to enable direct assessment of fungal metabolism.
- Gas production method used to assess the metabolism as:
  - Good indicator of metabolism,
    - End products of gas as well as volatile fatty acids.
  - Real time measurement,
    - Enables assessment of kinetics and rates.
  - Methodology reproducible.
- Pure fungal culture: *Neocallimastix frontalis*

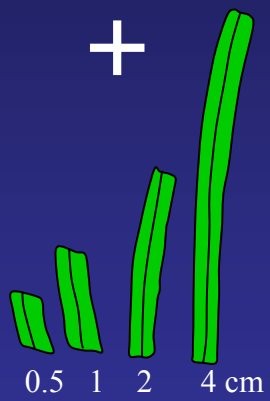
In vitro Gas Production System



Experiment

Pre-grown
N. frontalis culture

\+



& Control

Five treatments (n=4)

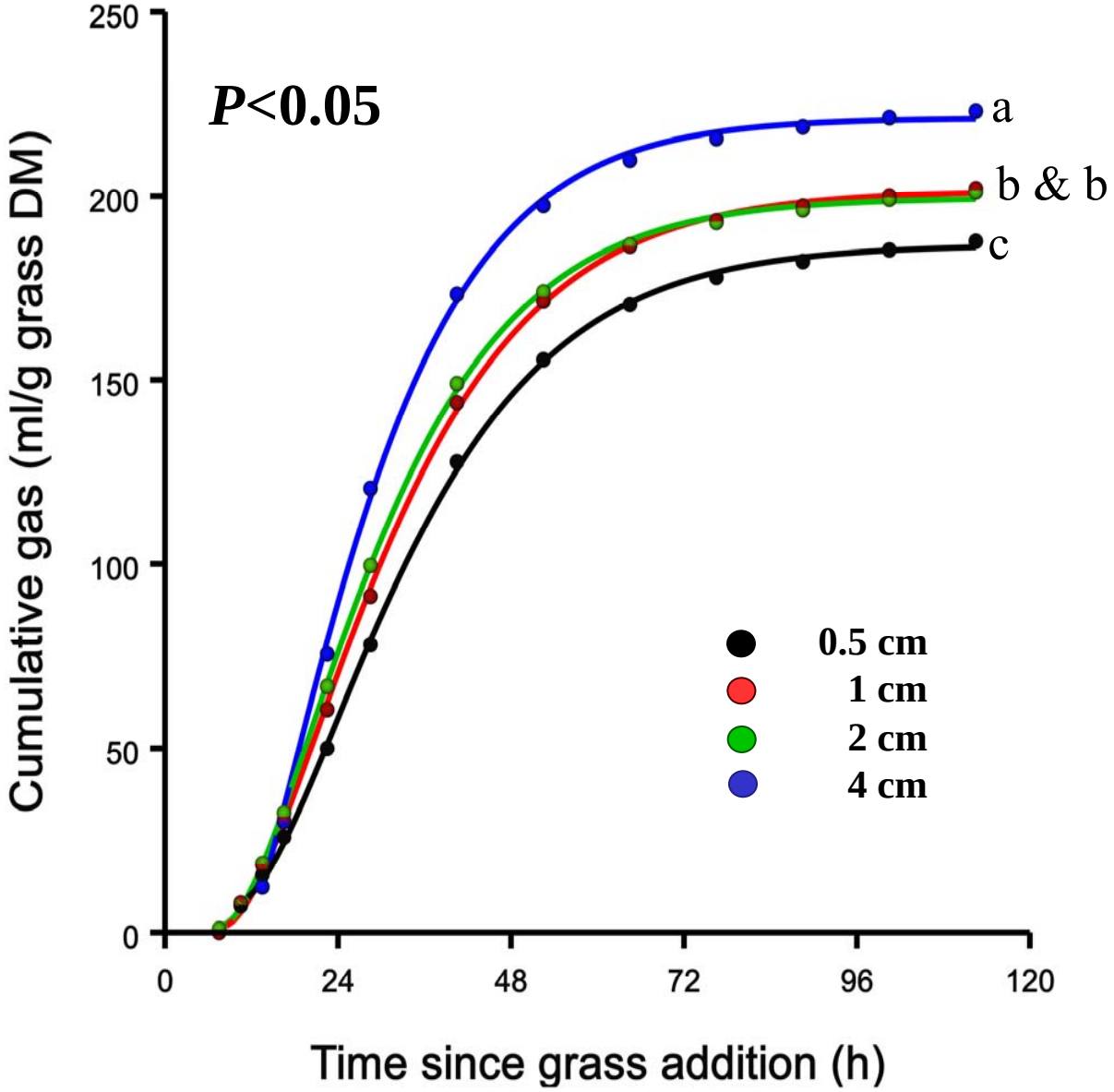
Incubate at 39°C, monitoring gas production.

54 h → 168 h

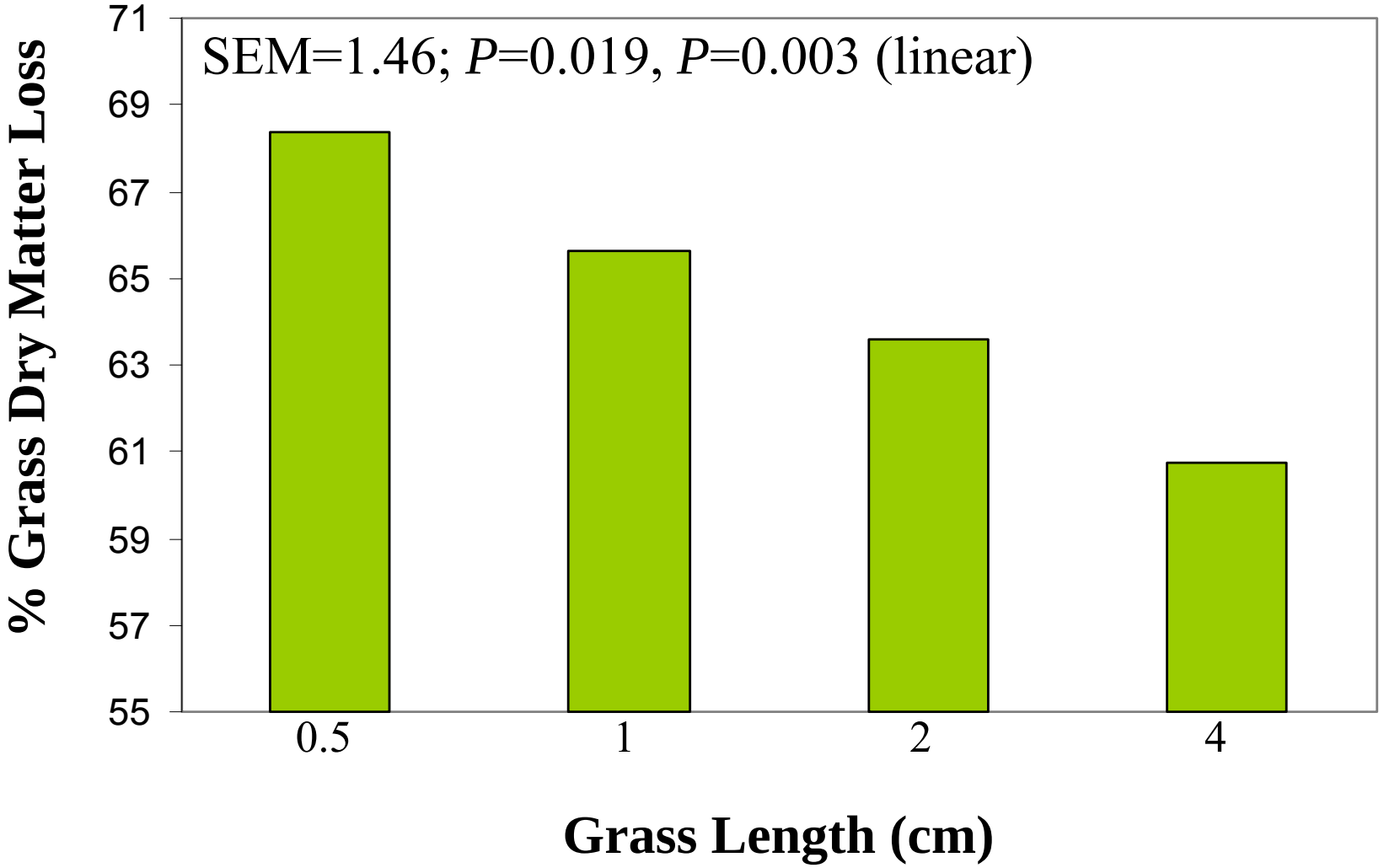
Solid Phase Analysis:
Dry Matter Loss

Liquid Phase Analysis:
-VFA (acetate)
-Lactate
-Formate

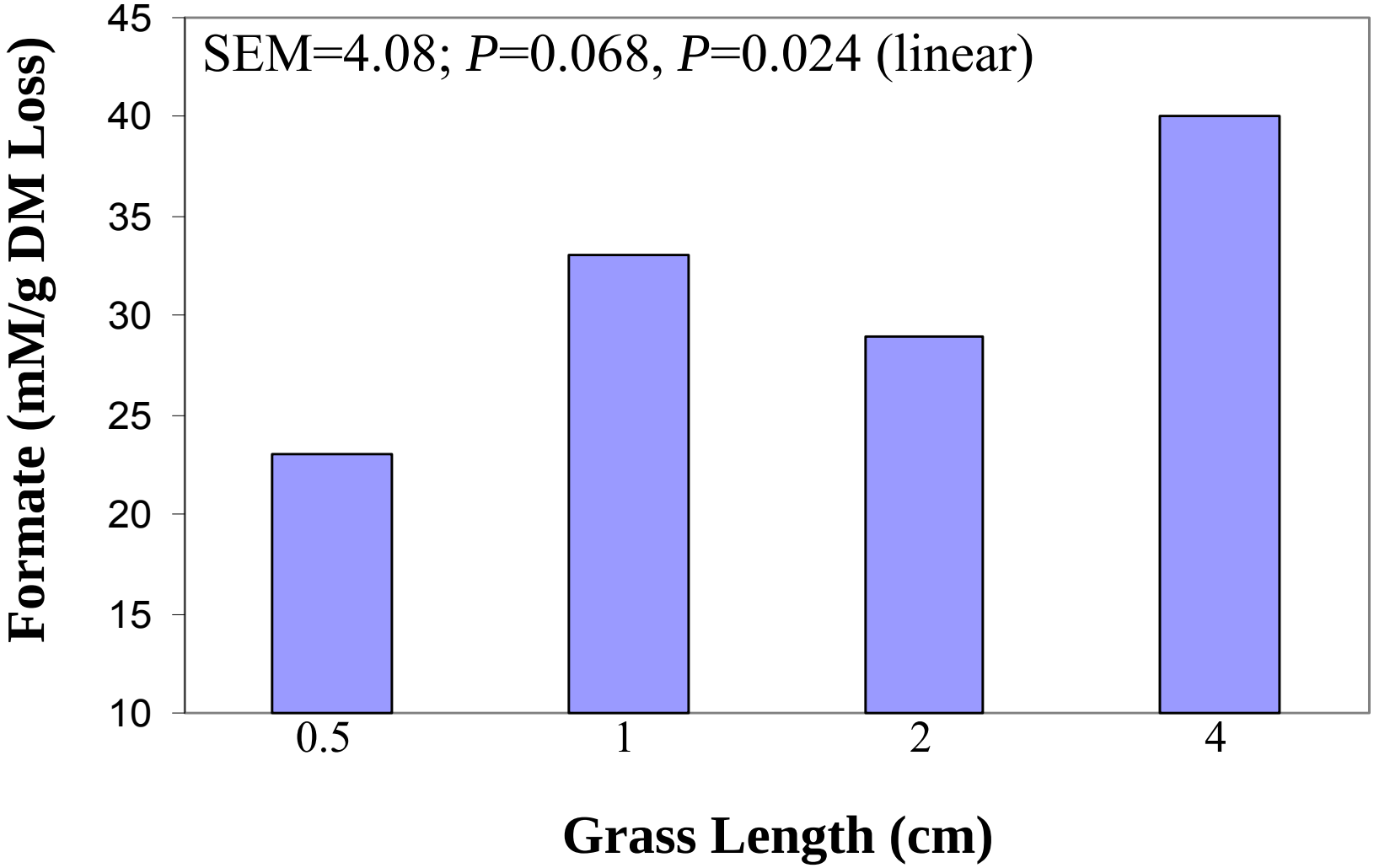
Results


P<0.05
a
b & b
c
0.5 cm
1 cm
2 cm
4 cm
Cumulative gas (ml/g grass DM)
Time since grass addition (h)
0
50
100
150
200
250
0
24
48
72
96
120

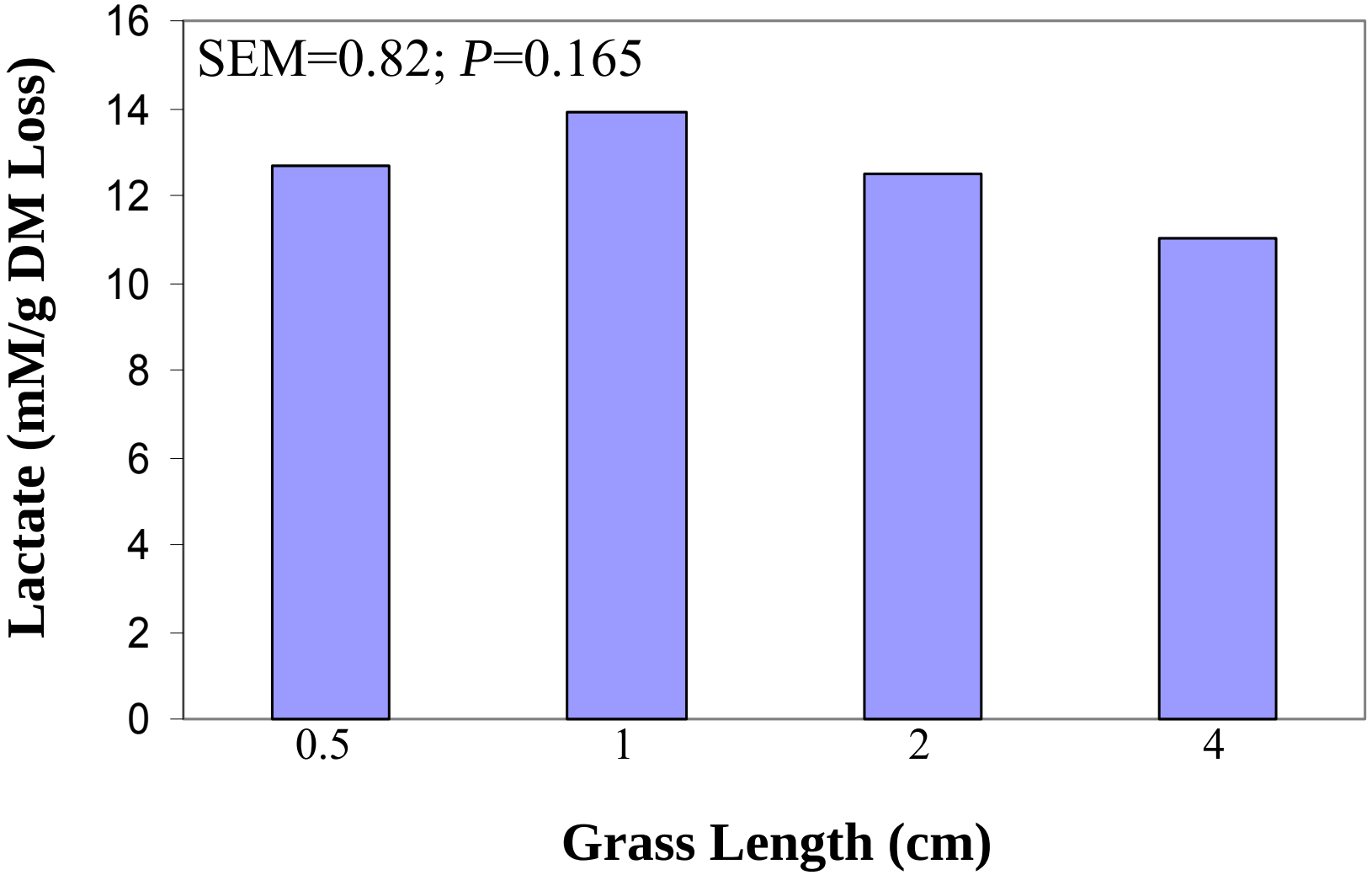
Results


SEM=1.46; P=0.019, P=0.003 (linear)
% Grass Dry Matter Loss
71
69
67
65
63
61
59
57
55
0.5
1
2
4
Grass Length (cm)

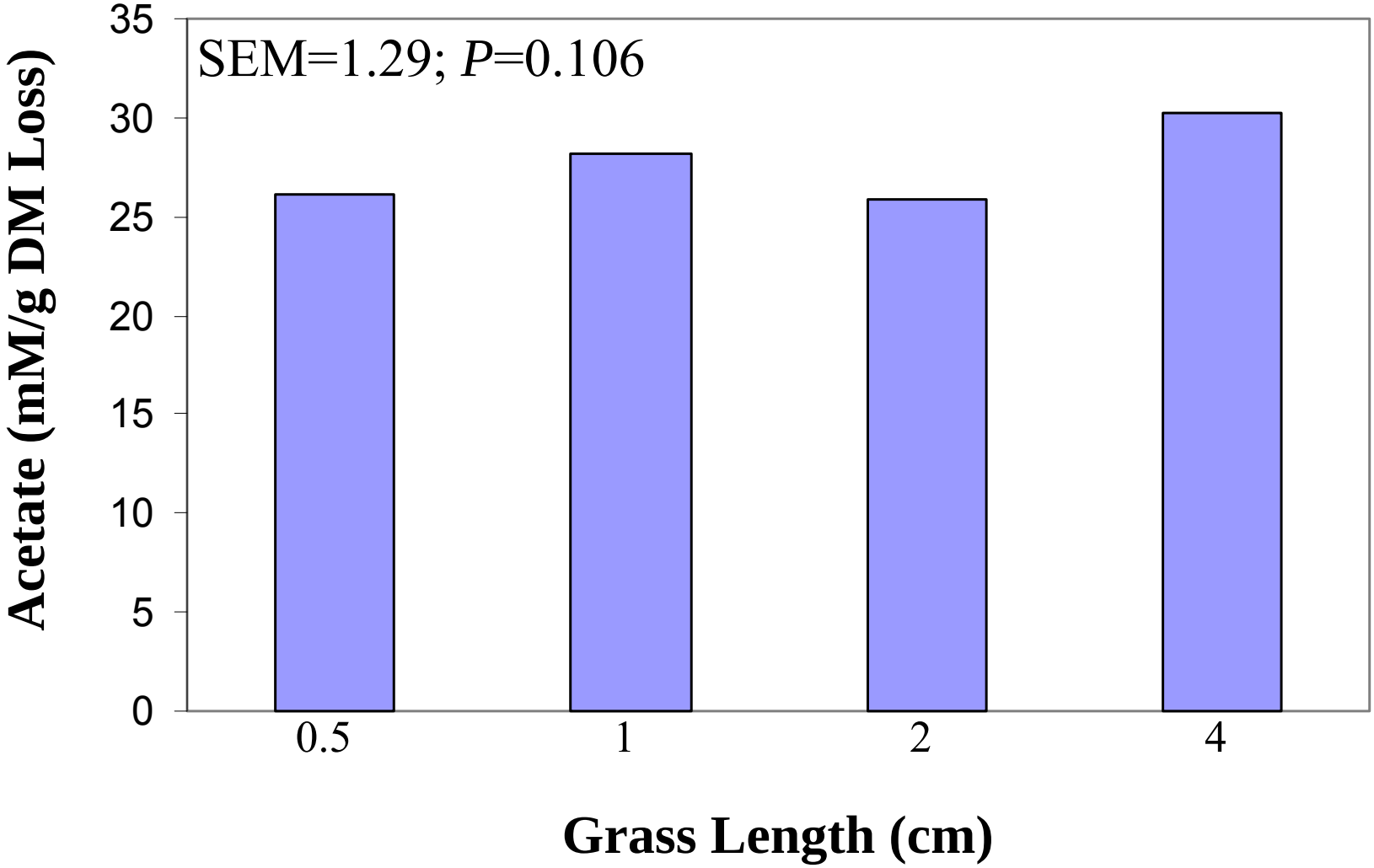
Results


SEM=4.08; P=0.068, P=0.024 (linear)
Formate (mM/g DM Loss)
45
40
35
30
25
20
15
10
0.5
1
2
4
Grass Length (cm)

Results


SEM=0.82; P=0.165
Lactate (mM/g DM Loss)
16
14
12
10
8
6
4
2
0
0.5
1
2
4
Grass Length (cm)

Results


SEM=1.29; P=0.106
Acetate (mM/g DM Loss)
35
30
25
20
15
10
5
0
0.5
1
2
4
Grass Length (cm)

Summary

- Gas production increased with particle length.
- DM loss decreased linearly with particle length.
- Formate increased linearly with particle length.
- Only a tendency for lactate and acetate to change with particle length.

Conclusion

- The length of grass does affect fungal metabolism.
  - Increased particle length, decreased degradation.
- Increased particle length, increased fermentation (gas & formate).
- Fermentation ≠degradation – WHY?
- Role of other factors?
  - Enzymes, hydration, specific gravity, plant-mediated processes…..

Thank you for your attention.

Acknowledgements:

Dr Joan Edwards
Prof. Mike Theodorou
Dr Neil McEwan
Dr Alison Kingston-Smith
Dr Ruth Sanderson

Corpoica

COLCIENCIAS
COLOMBIA

bbsrc
biotechnology and biological sciences
research council

Surface Area - Fungi ?

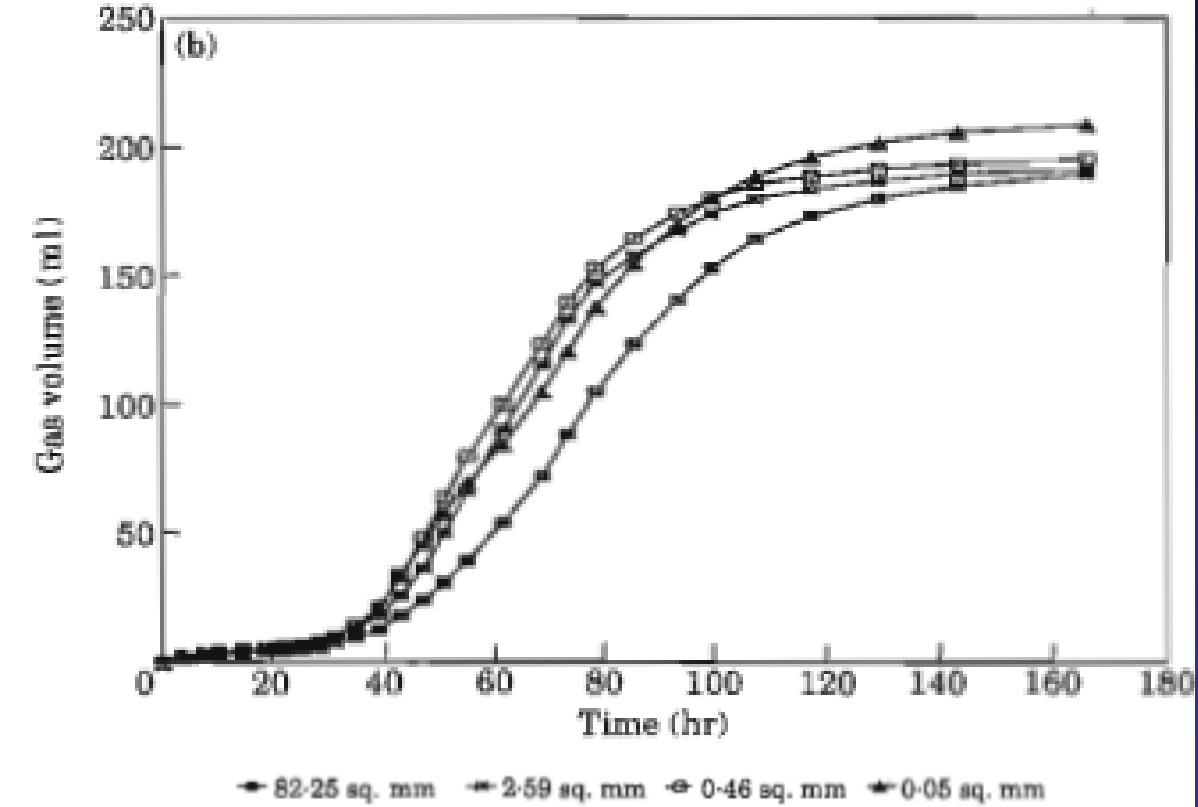
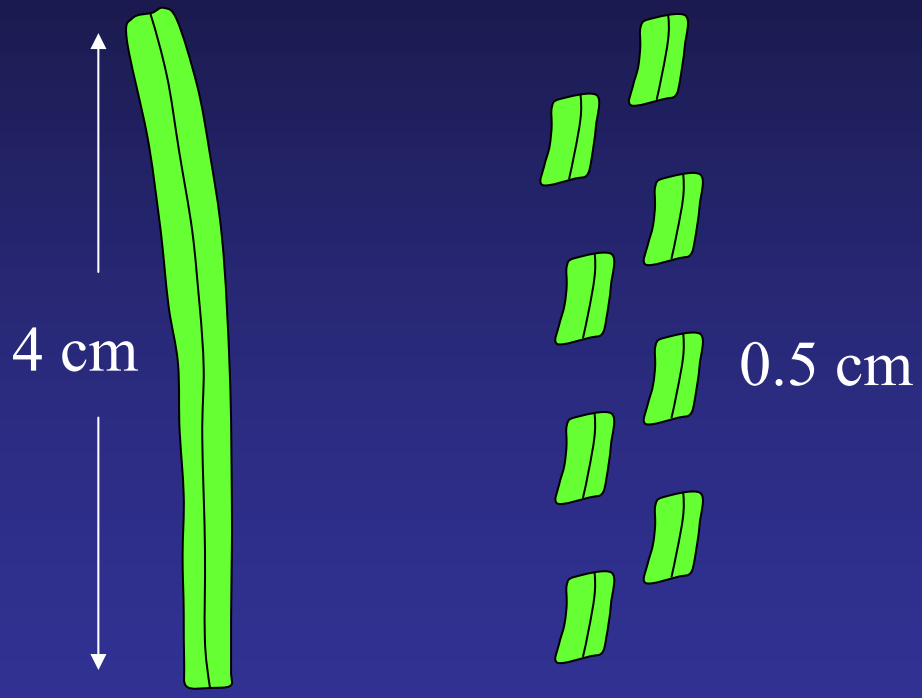


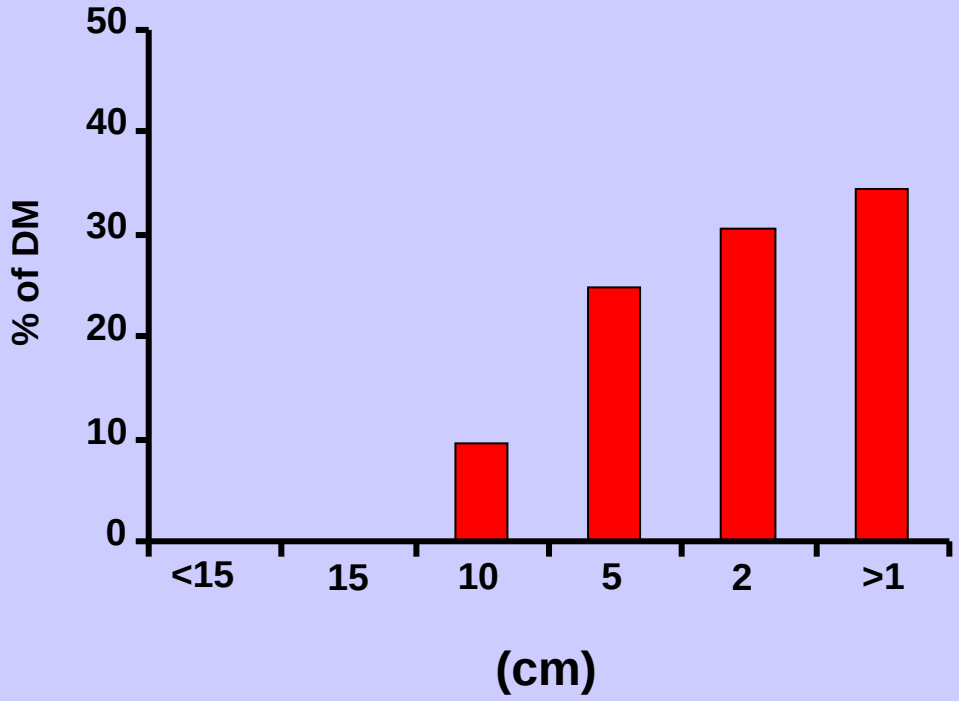
FIG. 3. Gas accumulation profiles obtained experimentally using particles of wheat straw of different sizes and rumen fluid enriched for rumen bacteria (a) and rumen fungi (b). Each profile was procured using 1 g DM of straw, 10 ml of rumen fluid and 90 ml of medium.

Surface Area

Same amount of forage, different surface area



Particle size distribution in the down bolus

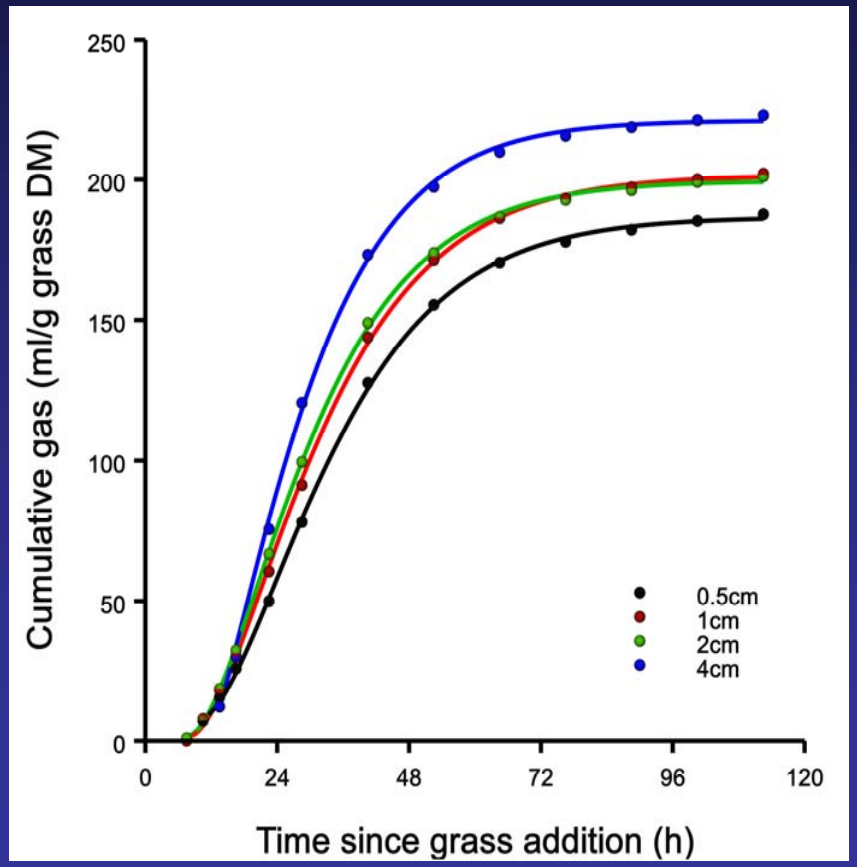


(Kingston-Smith, personal communication)

Model of France *et al* (1993) fitted to mean profiles for each treatment and bootstrapped (n=1000)

$$y = A - BQ^{t}Z^{\sqrt{t}}$$ where y = ml/g grass DM;

t = incubation time (h)



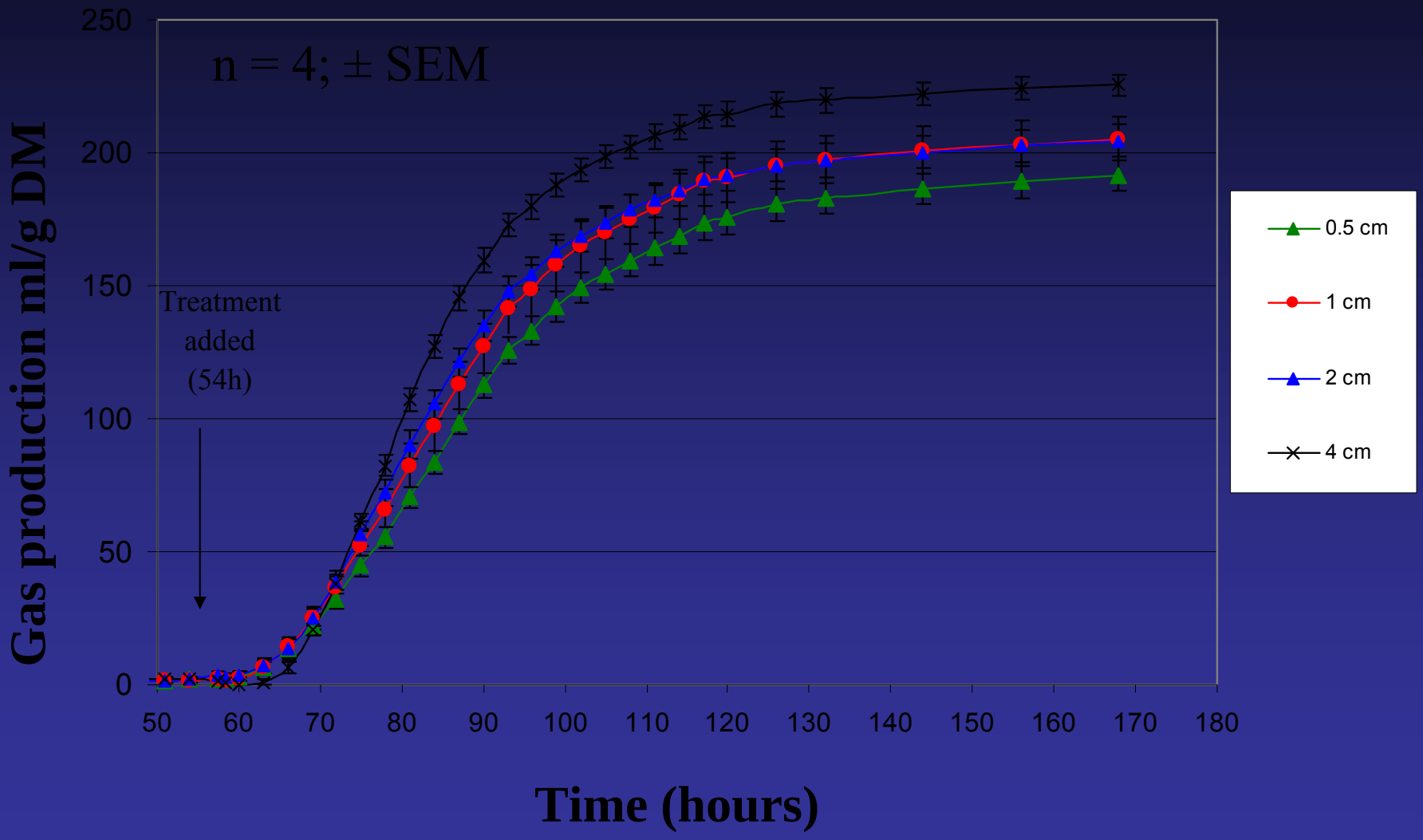
Median estimates

	A (ml/g DM)	t_{50} (h)	μ_{50} (h^{-1})
0.5 cm	186.7[a]	31.7[a]	0.0450[a]
1 cm	201.5[b]	30.1[a]	0.0461[a]
2 cm	199.5[b]	28.6[b]	0.0493[a]
4 cm	221.1[c]	27.2[b]	0.0568[b]

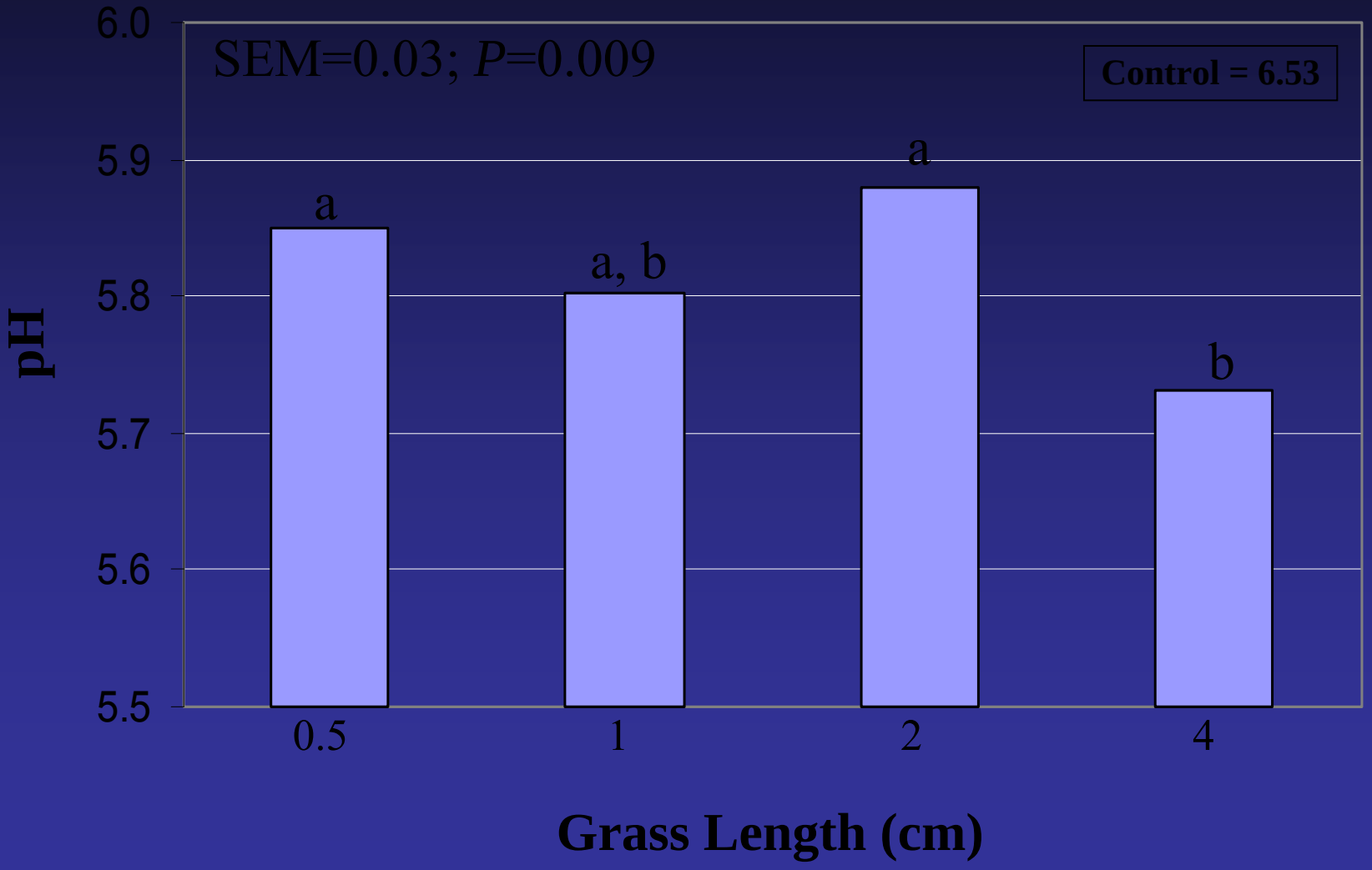
a, b, c ; Values with differing superscripts differ at $P<0.05$ based on distribution percentiles

France *et al* (1993) *J. Theor. Biol.* **163** 99-111.

Results


n = 4; ± SEM
Treatment added (54h)
Gas production ml/g DM
Time (hours)
0
50
100
150
200
250
50
60
70
80
90
100
110
120
130
140
150
160
170
180
0.5 cm
1 cm
2 cm
4 cm

Results


SEM=0.03; P=0.009
Control = 6.53
6.0
5.9
5.8
5.7
5.6
5.5
pH
a
a, b
a
b
0.5
1
2
4
Grass Length (cm)