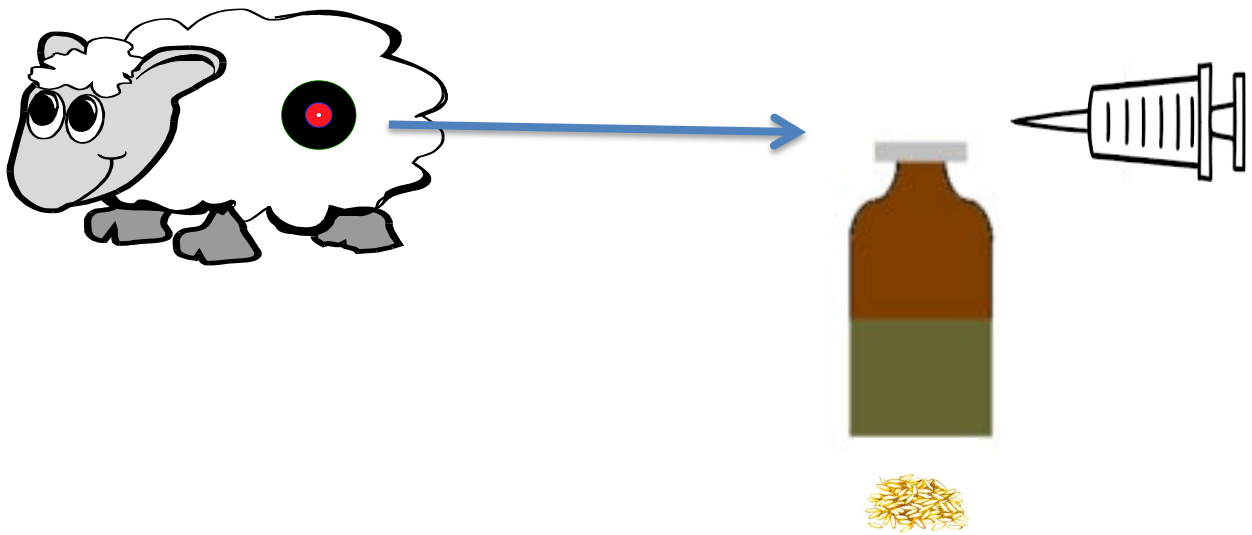
In vitro systems to measure methane production in ruminants



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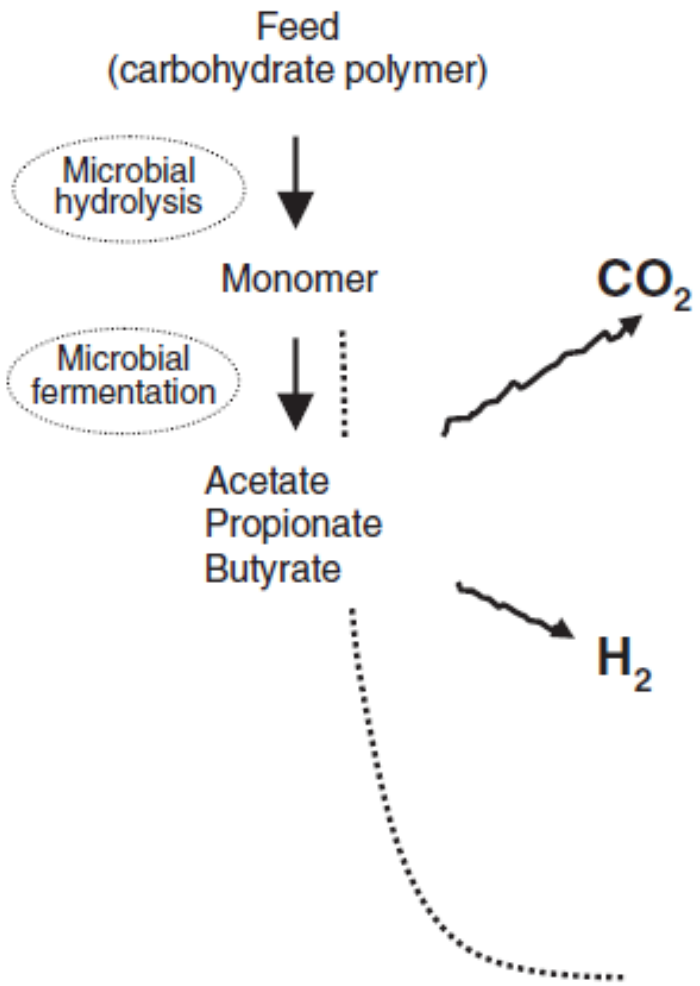
CSIC
Consejo Superior de Investigaciones Científicas

rednueva
network for updating emission values in spanish agriculture

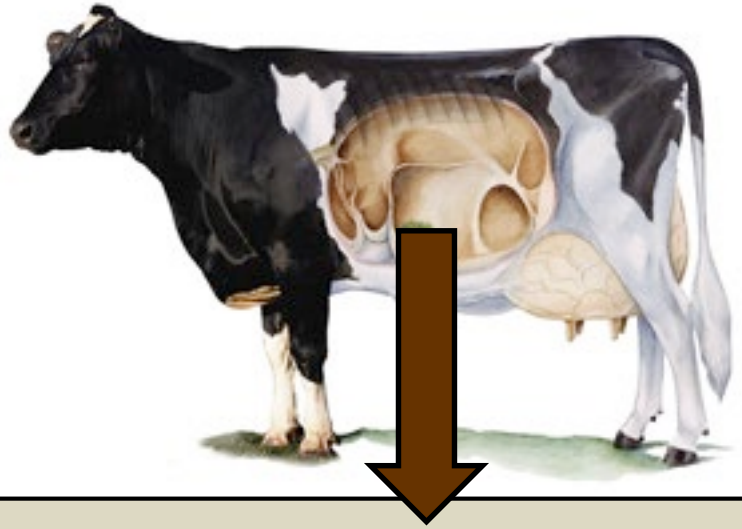
livAGE
Ammonia & Greenhouse Gas Emission from Animal Production Housing

CH4 production in the rumen



Rumen microbiome

Video protozoa



Bacteria

~1000 species
10^{10} to 10^{11}cells/ml

Anaerobic Fungi

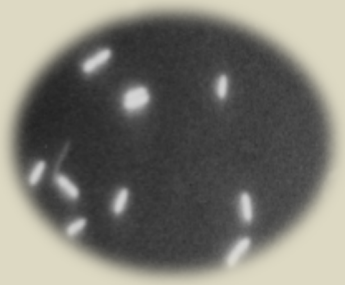
~9 genera
<10^5 cells/ml

Ciliate Protozoa

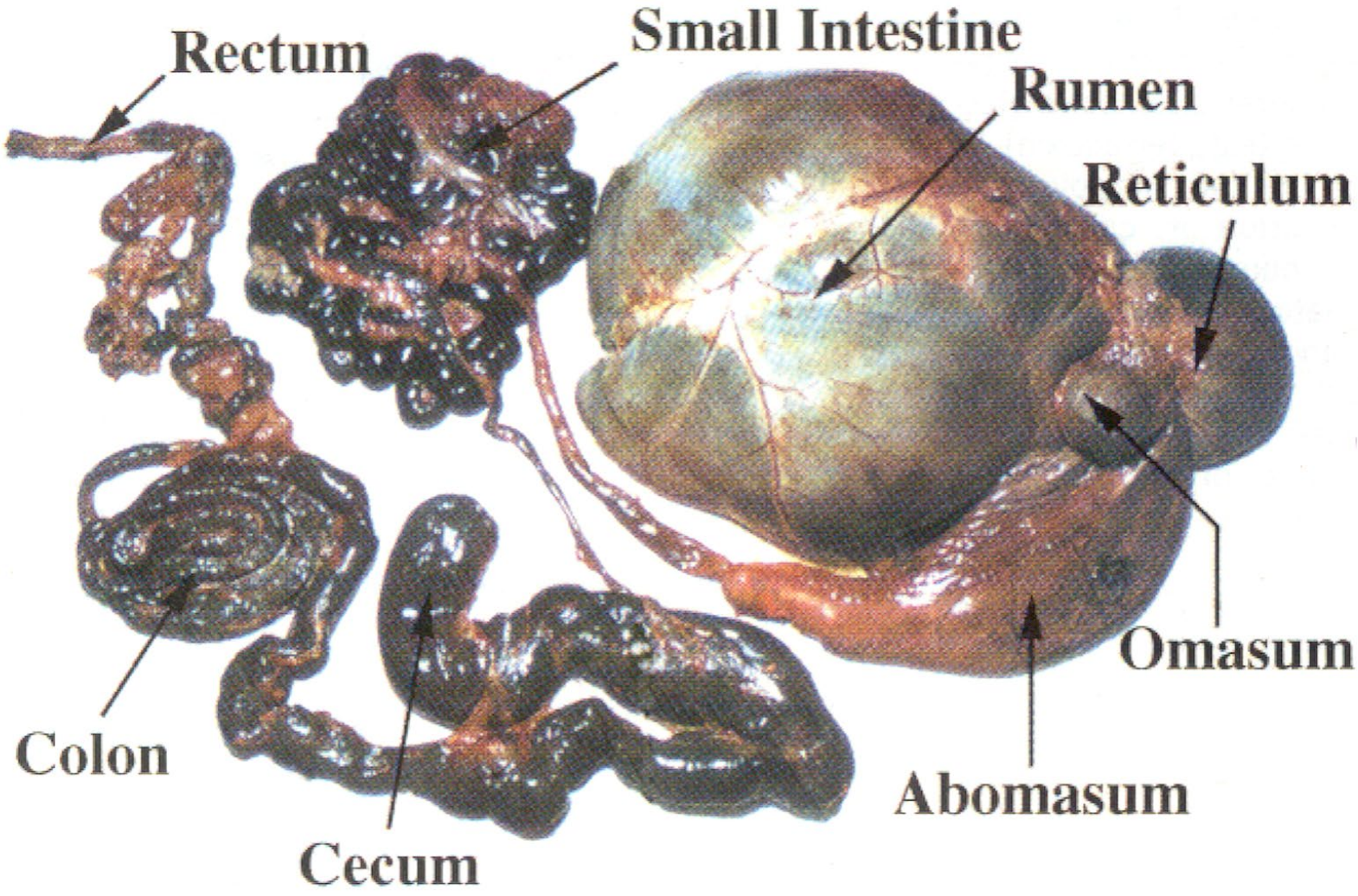
~40 species
<10^5 cells/ml

Methanogenic Archaea

~30 species
10^6 to 10^8 cells/ml

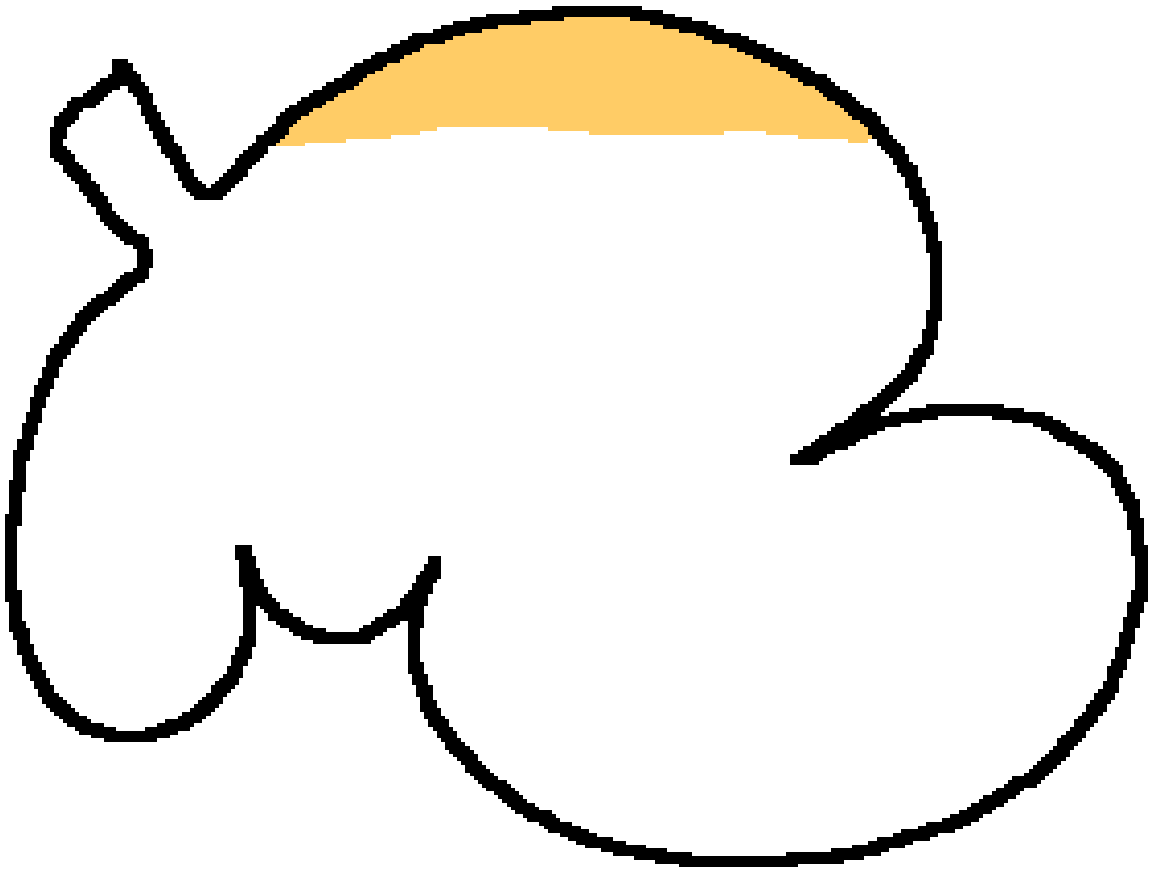


GI tract


Rectum
Small Intestine
Rumen
Reticulum
Omasum
Colon
Cecum
Abomasum

Rumen content layers

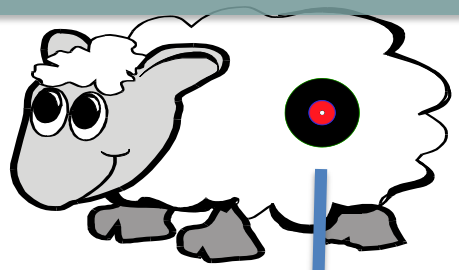
- Sali
- Wa
- Fee



In vitro rumen simulation

(Semi) continuous systems

In vitro rumen simulation



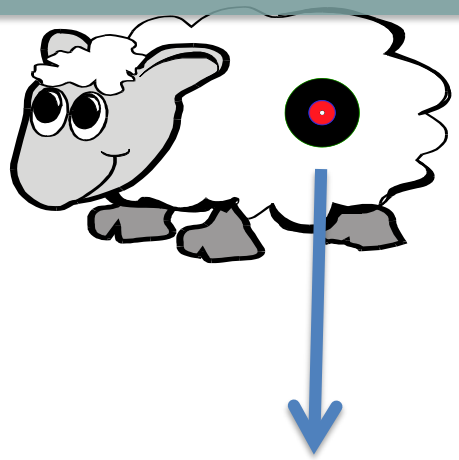
Batch culture

CO_2/N_2 flushing

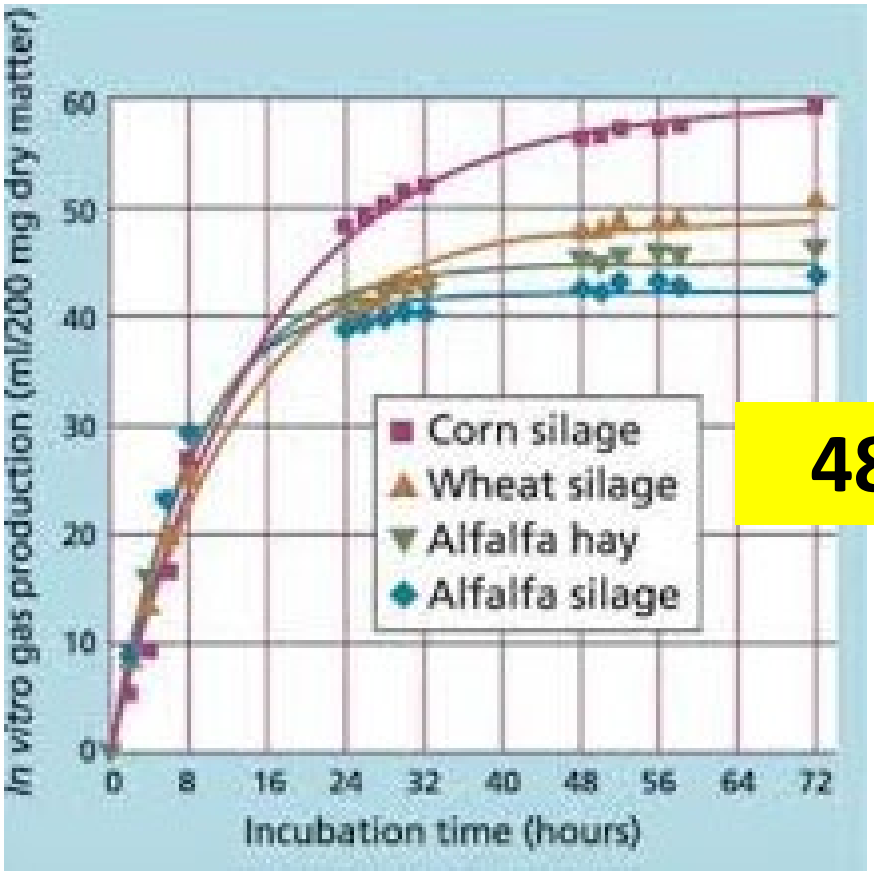
- Filtered rumen fluid -------- 12.5 ml
- Buffer
  Mineral solution ---------- 37.5 ml
- Substrate --------- 500 mg

24-72 hours

In vitro rumen simulation



Batch culture



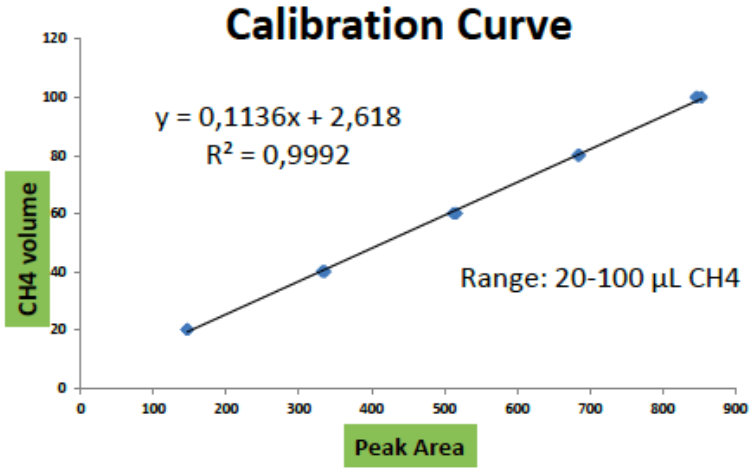
48 kPa

24-72 hours

Practical simulation

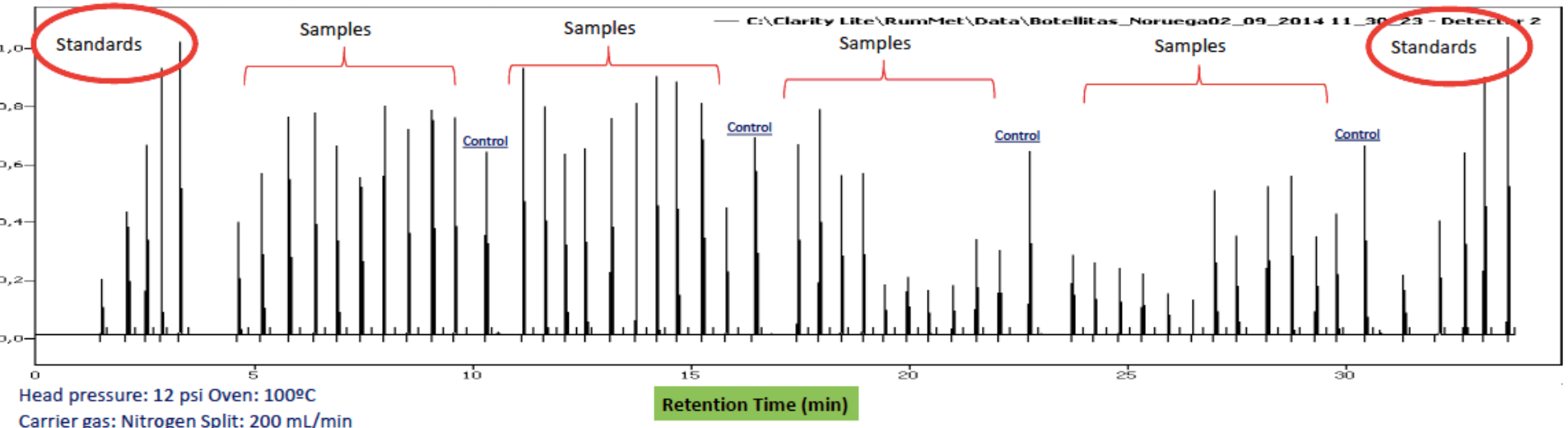
CH4 analysis in GC

Gas sampling bag
(for pure methane)

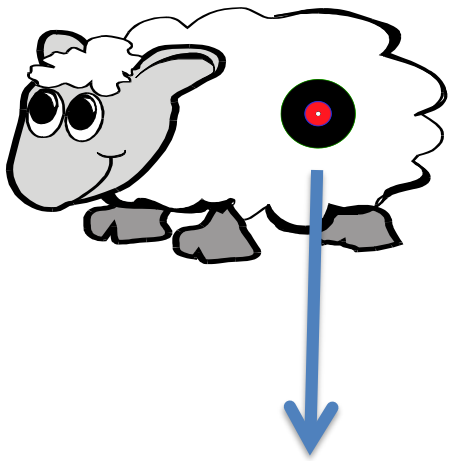


0.5 - 1 ml injection volume

8-12 % CH4 in gas



Head pressure: 12 psi Oven: 100ºC
Carrier gas: Nitrogen Split: 200 mL/min



Elements to consider:

- Donor animal species and number
- Diet fed to donor animals
- Adaptation period

- Collection procedure of inocolum
- Preservation and processing

- Substrate incubated
- Buffer and headspace gas compositions
- Inoculum:medium ratio
- Duration of incubation
- CH4 measurements and units

- In vitro vs. In vivo

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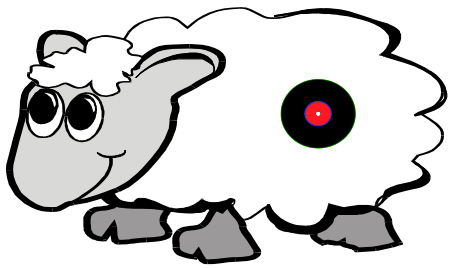
Review article

Design, implementation and interpretation of *in vitro* batch culture experiments to assess enteric methane mitigation in ruminants—a review

Yáñez-Ruiz D.R.[a,*], Bannink A.[b], Dijkstra J.[c], Kebreab E.[d], Morgavi D.P.[e], O'Kiely P.[f], Reynolds C.K.[g], Schwarm A.[h], Shingfield K.J.[i,j], Yu Z.[k], Hristov A.N.[l]

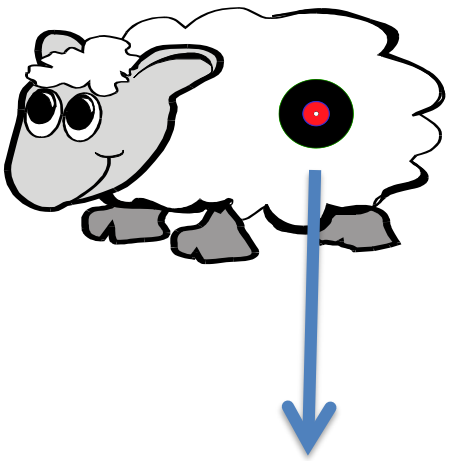


Elements to consider:

- Donor animal species and number
- Diet fed to donor animals
- Adaptation period

- Same **animal species** as target if possible (small / large ruminants)
- At least 3 **individual** animals as donors (do not pool from different ones)
- Rumen fluid to be collected **before morning** feeding
- Similar **diet** as tested in vitro
- Fed **restriced** amounts for better control of intakes and concentrate/forage

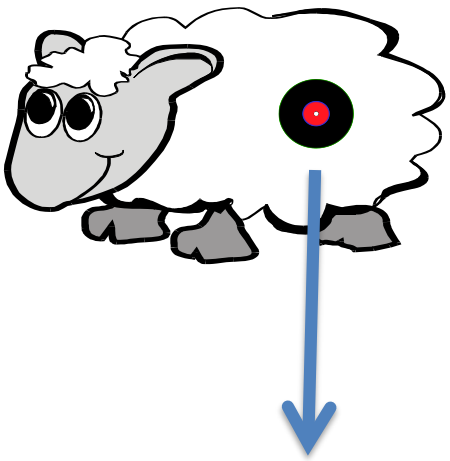
Elements to consider:



- Collection procedure of inocolum
- Preservation and processing

- Canula vs. stomach tubing - 250 µm pore size

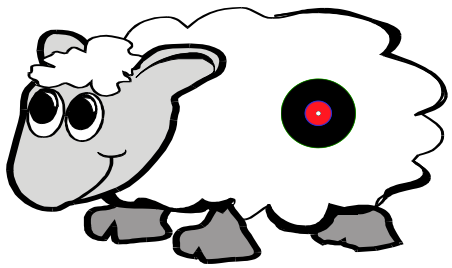
- Fresh rumen fluid at 39ºC (CO2) or 0-4ºC for up to 6 hours



Elements to consider:

- Feed relevant **diet** in commercial farms
- **Freeze drying** better than oven drying
- 1:2 or 1:3 Rumen **fluid:buffer** ratio
- **Bicarbonate** < 80 mM
- 3 **bottles** per unique treatment and run

- Substrate incubated
- Buffer and headspace gas compositions
- Inoculum:medium ratio
- Duration of incubation
- CH4 measurements and units



Vs.

1. Prediction of CH4 production per unit of feed ingested /digested

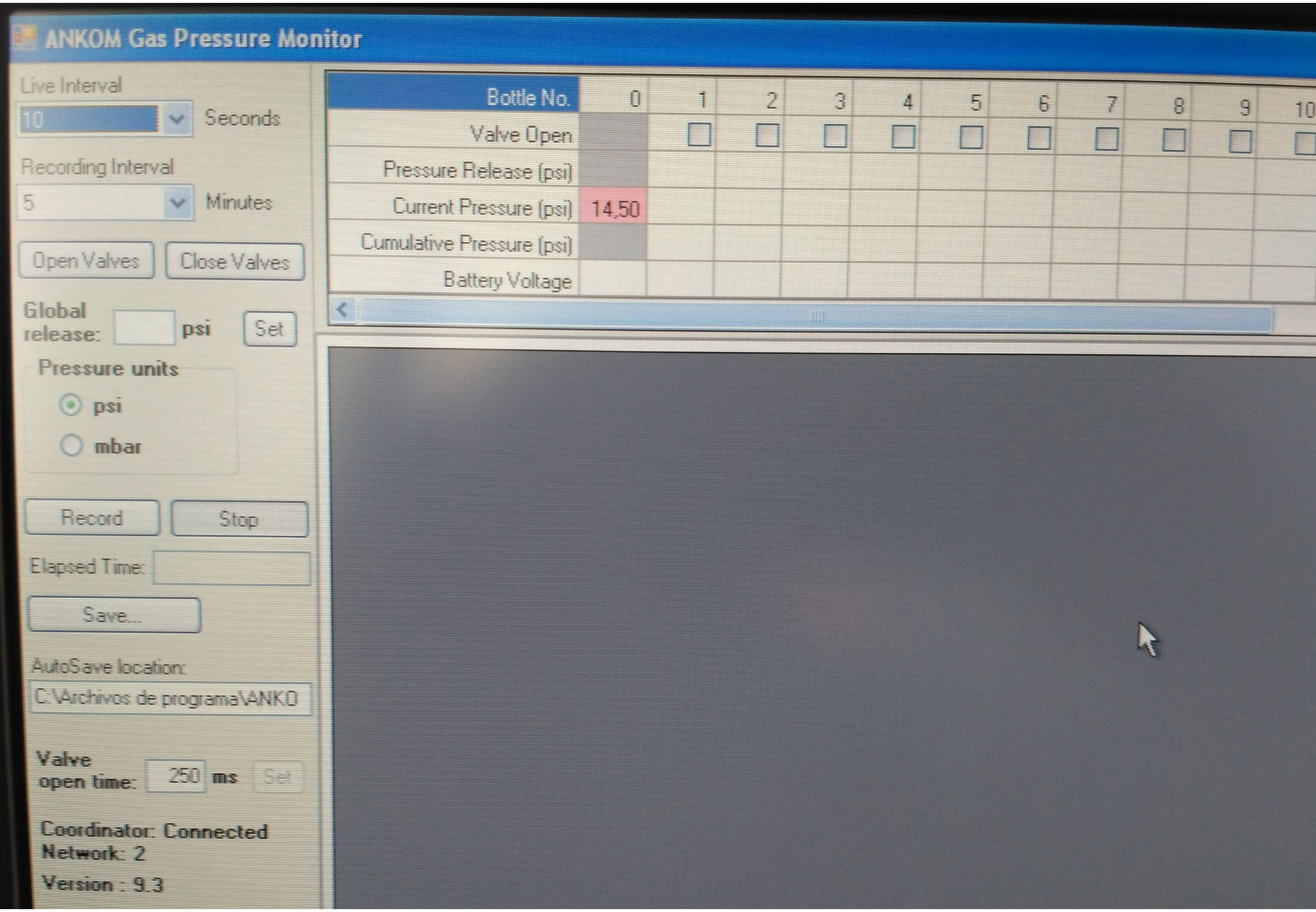
- Weak correlation but ability to rank feeds

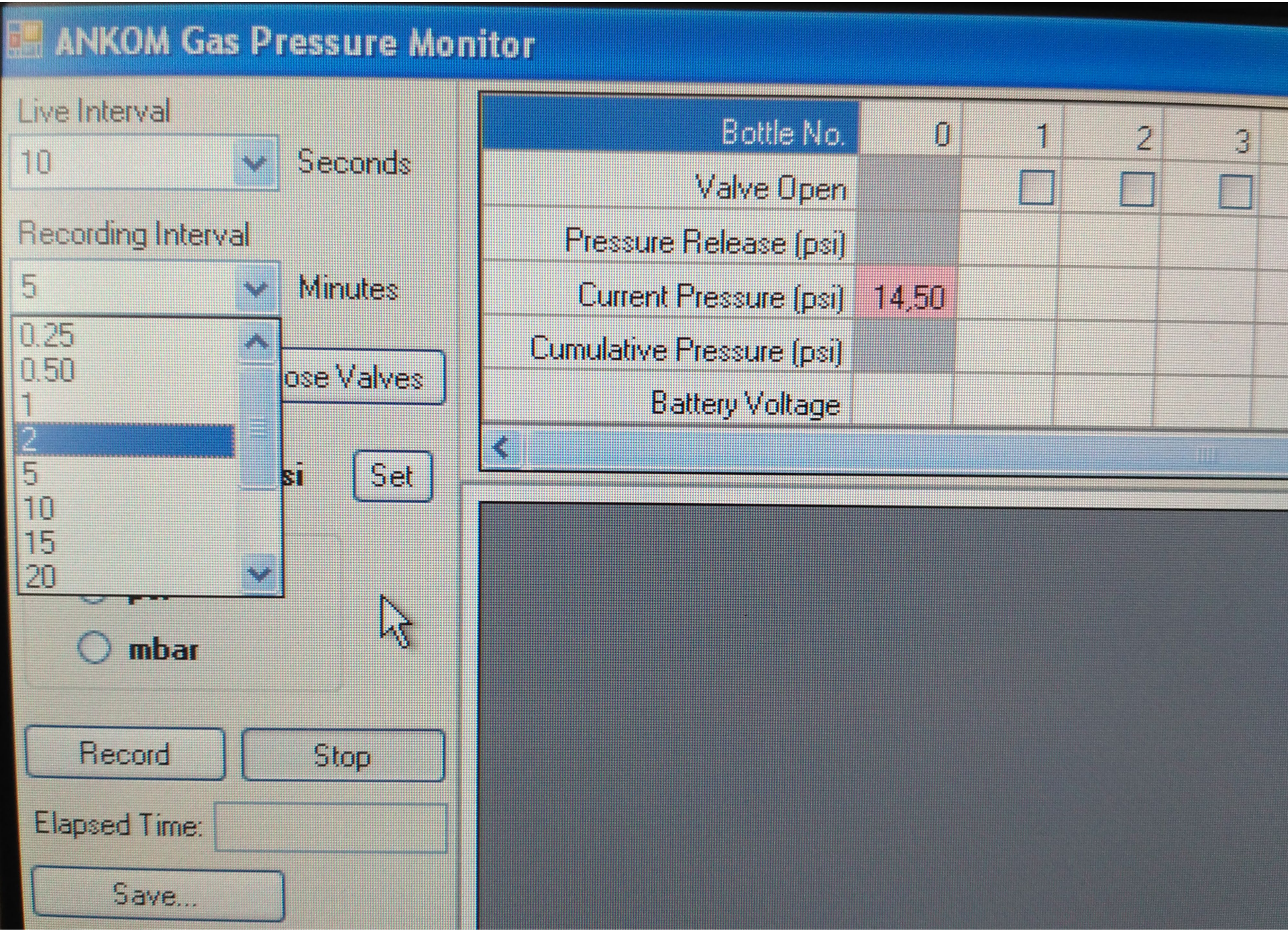
2. Direction of changes (not absoluate values)**: i.e. additives**

- 80.5 % reduction in vitro vs. **24.8 %** in vivo

Automatic remote control systems

ANKOM RF System ®


ANKOM Gas Pressure Monitor
Live Interval
10
Seconds
Recording Interval
5
Minutes
Open Valves
Close Valves
Global release:
psi
Set
Pressure units
psi
mbar
Record
Stop
Elapsed Time:
Save...
AutoSave location:
C:\Archivos de programa\ANKO
Valve open time:
250
ms
Set
Coordinator: Connected
Network: 2
Version : 9.3
Bottle No.
0
1
2
3
4
5
6
7
8
9
10
Valve Open
Pressure Release (psi)
Current Pressure (psi)
14,50
Cumulative Pressure (psi)
Battery Voltage


ANKOM Gas Pressure Monitor
Live Interval
10
Seconds
Recording Interval
5
Minutes
0.25
0.50
1
2
5
10
15
20
ose Valves
si
Set
mbar
Record
Stop
Elapsed Time:
Save...
Bottle No.
0
1
2
3
Valve Open
Pressure Release (psi)
Current Pressure (psi)
14,50
Cumulative Pressure (psi)
Battery Voltage

A	B	C	D	E	F	G	H	I	J
11/22/2010 10:42	0	1	2	3	4	5	6	7	8
0:00:01	13,64	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
0:05:01	13,64	0,29	0,47	0,44	0,47	0,55	2,58	0,00	0,29
0:10:01	13,64	0,66	0,84	0,80	0,80	0,84	2,95	0,00	0,62
0:15:02	13,64	0,91	1,16	1,13	1,06	1,16	3,31	0,00	0,91
0:20:02	13,64	1,16	1,42	1,38	1,31	1,42	3,57	0,00	1,13
0:25:02	13,64	1,35	1,60	1,56	1,42	1,60	3,75	0,00	1,24
0:30:02	13,64	1,46	1,82	1,78	1,60	1,78	3,97	0,00	1,46
0:35:02	13,64	1,67	2,07	1,97	1,82	1,97	4,18	0,00	1,64
0:40:02	13,64	1,82	2,26	2,18	2,04	2,15	4,37	0,00	1,75

A	B	C	D	E	F	G	H	I	J	K
11/22/2010 10:42	0	1	2	3	4	5	6	7	8	9
0:00:01	6,63	5,97	5,83	5,96	5,97	5,78	6,17	6,02	5,94	6,07
0:05:01	6,63	6,25	6,12	6,23	6,25	6,05	6,36	6,02	6,08	5,99
0:10:01	6,63	6,28	6,16	6,26	6,30	6,10	6,40	6,02	6,17	6,32
0:15:02	6,62	6,30	6,18	6,28	6,31	6,13	6,42	6,02	6,20	6,34
0:20:02	6,62	6,31	6,20	6,29	6,33	6,15	6,42	6,02	6,22	6,36
0:25:02	6,62	6,31	6,20	6,30	6,33	6,15	6,42	6,02	6,23	6,36
0:30:02	6,62	6,31	6,21	6,31	6,34	6,16	6,43	6,02	6,24	6,37
0:35:02	6,62	6,32	6,20	6,31	6,34	6,17	6,43	6,02	6,25	6,37
0:40:02	6,62	6,32	6,20	6,29	6,34	6,16	6,42	6,02	6,25	6,37
0:45:02	6,61	6,31	6,19	6,28	6,33	6,14	6,42	6,02	6,25	6,37
0:50:02	6,61	6,31	6,18	6,28	6,31	6,12	6,41	6,02	6,25	6,36
0:55:02	6,61	6,31	6,17	6,27	6,31	6,12	6,40	6,02	6,24	6,36
1:00:02	6,61	6,30	6,17	6,26	6,31	6,10	6,40	6,02	6,23	6,35
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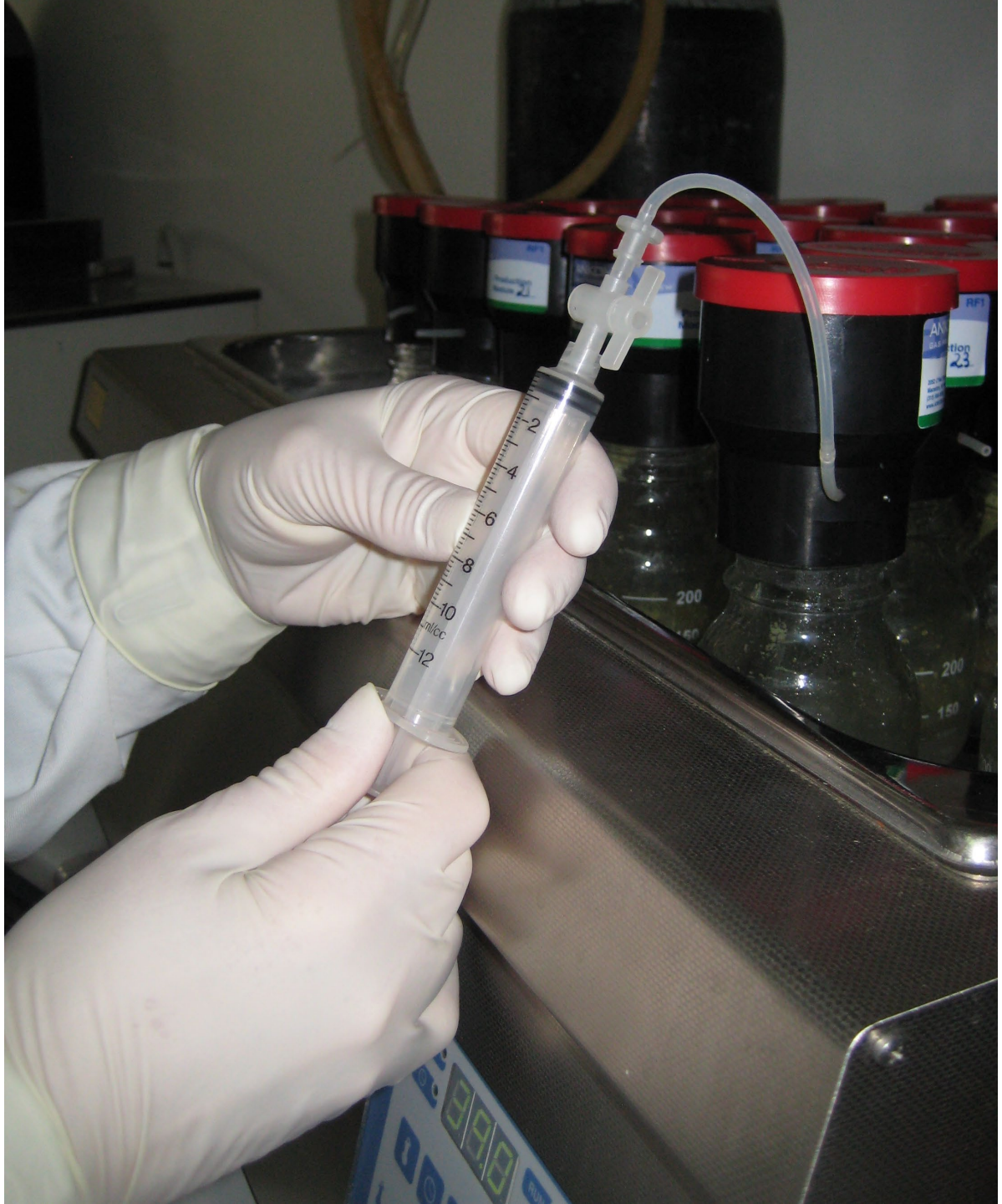
2
4
6
8
10
12
cm³/cc
200
200
150

Video Ankom

PROS

- Unexpensive system
- Animal ethics
- Ability to screen large number of samples
- Dose response

CONS

- Animal behaviour
- Short term measurement
- Dosage to be tested further in vivo
- False possitives

rednueva

network for updating emission values in spanish agriculture

livAGE

Ammonia & Greenhouse Gas Emission from Animal Production Housing