In vitro systems to measure methane production in ruminants

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CH4 production in the rumen

Feed (carbohydrate polymer)

Microbial hydrolysis

Monomer

Microbial fermentation

Acetate Propionate Butyrate

CO₂

H₂
Rumen microbiome

- **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

Video protozoa
Rumen content layers

- Saliva
- Water
- Feed
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

- **24-72 hours**
- **48 kPa**
Practical simulation
CH4 analysis in GC

Gas sampling bag (for pure methane)

0.5 - 1 ml injection volume

8-12 % CH4 in gas

Calibration Curve

\[ y = 0.1136x + 2.618 \]

\[ R^2 = 0.9992 \]

Range: 20-100 µL CH4

Retention Time (min)

Head pressure: 12 psi
Oven: 100°C
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 inoculum
- 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
Animal Feed Science and Technology

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 **restricted** amounts for better control of intakes and concentrate/forage
Elements to consider:

- Collection procedure of inoculum
- Preservation and processing

- Canula vs. stomach tubing - 250 μm pore size
- Fresh rumen fluid at 39°C (CO2) or 0-4°C 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
1. Prediction of CH4 production per unit of feed ingested /digested

- Weak correlation but ability to rank feeds

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

- 80.5 % reduction in vitro vs. 24.8 % in vivo
Automatic remote control systems

ANKOM RF System ®
<table>
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<th>Bottle No.</th>
<th>Valve Open</th>
<th>Pressure Release (psi)</th>
<th>Current Pressure (psi)</th>
<th>Cumulative Pressure (psi)</th>
<th>Battery Voltage</th>
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<td></td>
<td></td>
<td></td>
<td>14.50</td>
<td></td>
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</tr>
</tbody>
</table>

**Live Interval:** 10 Seconds

**Recording Interval:** 5 Minutes

**Global release:** psi

**Pressure units:**
- psi
- mbar

**Auto Save location:** C:\Archivos de programa\ANKO
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 possitves
Questions?