In vitro Techniques

- Simulation of the rumen fermentation
- Alternative to time consuming and expensive in vivo trials
- Quantification of the end products of fermentation and/or rumen dry matter digestibility

End products of fermentation

- **Gases** \((\text{CO}_2 \text{ and } \text{CH}_4)\)
- **Short chain fatty acids** (acetate, propionate, butyrate, valerate, isobutyrate, isovalerate)
- Ammonia
- Microbial protein
In vitro Incubation Systems

➢ Short time in vitro batch culture systems
  - Tilley and Terry System (determination of digestibility)
  - Hohenheim Gas Test (determination of gas production)
  - Reading Pressure Technique (determination of gas production)

➢ Continuous culture systems
  - Rumen Simulation Technique (RUSITEC)
  - Dual Flow Continuous Culture System (Hoover, 1964)
  - Hohenheim System (Single Flow Continuous Culture System)
Collection of Rumen Fluid

- Gas phase
- Feed mat
- Liquid phase
In vitro incubation 48 h

20,000 g
4°C, 10 min

Pepsin digestion 37°C

Apparent digestibility  

True digestibility
The Hohenheim Gas Test (HFT)

In vitro incubation

hours

0 6 12 18 24

Correct for Blank and for Standard GP

ME (MJ/kg) $= 2.2 \times 0.136GP + 0.057CP + 0.0029CL^2$

Digestibility (%DOM) $= 14.88 \times 0.146GP + 0.45CP + 0.65CA$
Determination of *in vitro* Digestibility from the HFT

**In vitro** incubation

- Apparent digestibility
- True digestibility
- Microbial biomass

Neutral Detergent Solution 100°C

Filtering ~ 50 µm pore size

20,000 g 4°C, 10 min

Determination of *in vitro* digestibility from the HFT.
End Product Quantification

- Gases
- Microbial biomass
- Undigested feed
- Short chain fatty acids
- Volume & Gas chromatography
- Microbial marker
- Neutral detergent solution
- Gas chromatography
Relationship between SCFA and Gas Production

Gases

Glucose = 2 acetate + 2 CO₂ + 8 H

Glucose = 1 butyrate + 2 CO₂ + 4 H

Glucose + 4 H = 2 propionate

CO₂ + 8 H = CH₄ + 2 H₂O

Short chain fatty acids
The Concept of the Partitioning of Nutrients

- Feed 1
- Feed 2
- Gases
- Microbial biomass
- Undigested feed
- Short chain fatty acids

Diagram shows the process of nutrient partitioning, including undigested feed, microbial biomass, gases, and short chain fatty acids.
## Microbial Biomass Marker

<table>
<thead>
<tr>
<th>Internal Marker</th>
<th>External Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell components</strong></td>
<td><strong>Stable or radioactive isotopes</strong></td>
</tr>
<tr>
<td>• Amino acids</td>
<td>• $^{15}$NH$_4^+$</td>
</tr>
<tr>
<td>• Nucleic acids</td>
<td>• $^{32}$PO$_4^{2-}$</td>
</tr>
<tr>
<td>• Phospholipids</td>
<td>• $^{35}$SO$_4^{2-}$</td>
</tr>
</tbody>
</table>
Microbial marker

Nucleic acids

\[ \text{PEG} \]

\[ \text{mg} \]

\[ \text{time (h)} \]

\[ \text{0} \]
\[ \text{6} \]
\[ \text{12} \]
\[ \text{18} \]
\[ \text{24} \]
\[ \text{30} \]
\[ \text{36} \]
\[ \text{42} \]
\[ \text{48} \]

\[ \text{mg} \]

\[ \text{15N Urea} \]

\[ \text{PEG} \]

\[ \text{mg} \]

\[ \text{time (h)} \]

\[ \text{0} \]
\[ \text{6} \]
\[ \text{12} \]
\[ \text{18} \]
\[ \text{24} \]
\[ \text{30} \]
\[ \text{36} \]
\[ \text{42} \]
\[ \text{48} \]
Reading Pressure Technique (RPT)

Gas phase Pressure: 0 – 700 mb

Incubation medium

Pressure Transducer

Receiver

Signal-Converter
Rate of Gas Production

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Fermentation of Individual Feed Components

<table>
<thead>
<tr>
<th>Components</th>
<th>TMR 1</th>
<th>TMR 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>grass silage</td>
<td>496</td>
<td>499</td>
</tr>
<tr>
<td>maize silage</td>
<td>367</td>
<td>369</td>
</tr>
<tr>
<td>milled wheat</td>
<td>40</td>
<td>65</td>
</tr>
<tr>
<td>rapeseed meal</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>soyabean meal</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>cane molasses</td>
<td>30</td>
<td>-</td>
</tr>
</tbody>
</table>

Gas production (ml/h) vs. time (h)
Kinetics of Gas Production

Gas production is described by:

\[ \text{Gas prod.} = A + B \times (1 - \exp(-c \times t)) \]

Barley straw:
\[ \text{Gas} = -13.7 + 127.6 \times (1 - \exp(-0.0428 \times t)) \]

Sesbania sesban leaves
\[ \text{Gas} = -12.3 + 104.3 \times (1 - \exp(-0.0955 \times t)) \]
Production of SCFA and Microbial Activity

SCFA concentration

- Barley straw
- S. sesban

RNA concentration

0,0
10,0
20,0
30,0
40,0
50,0
60,0
70,0
80,0
90,0
100,0
110,0
120,0
130,0
140,0
150,0
160,0
170,0
180,0
190,0
200,0

0,0
12
24
36
48

0,0
12
24
36
48

µM / ml

µg / ml
In vitro Quantification of the Effects of Tannins

Feed

Gas production

PEG (Polyethylene glycol)

Feed
Tannin Effect on Gas Production
### Corrections to be made

<table>
<thead>
<tr>
<th>Blank</th>
<th>In all batch culture systems a blank is needed to account for the substrate that comes along with the rumen fluid. Blank gas production is subtracted from substrate gas production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
</tbody>
</table>
| Stand | Hohenheim Gas Test  
Hay mixture with a known 24h gas production (calibrated) |
|       | The ratio of calibrated to measured gas production of the substrate is used to correct the gas production from the test substrates of the respective incubation for differences in microbial activity |
Implications

+ Short time *in vitro* incubation systems offer the possibility to quantify the kinetics and absolute amounts of end product formation

+ Partitioning of nutrients for the host animal can be determined

+ Rate and extent of the substrate degradation provides information about the potential intake of the substrate examined

+ They also can be used to assess the antinutritional effects

- Influence of host animal missing!

- No adaptation of the microbial community to the tested feeds!
Continuous in vitro Systems

- Continuous buffer inflow simulates the saliva flow
- Substrate administration
- Overflow is collected for analysis of end products of fermentation

Buffer

39°C

Rumen fluid

Overflow
Rumen Simulation Technique

- Substrate administration in nylon bags with defined pore sizes
- Substrate is completely removed and replaced at different time intervals

⇒ Semi continuous *in vitro* fermentation system

- no access of protozoa to feed particles
Single flow continuous *in vitro* system

- Substrate added directly to rumen fluid
- Formation of a feed mat
- Full access of larger microorganisms (protozoa) to the substrate

- no selective absorption of SCFA
Single flow continuous *in vitro* system
Single flow continuous *in vitro* system

- No selective absorption of SCFA
- Lack of the bacterial flora adherent to the rumen wall
- No method to mimic rumination
- No selective removal of small particles as in the rumen
Short time *in vitro* incubation systems can mimic the rumen fermentation to a certain extent and are therefore a good supplement to the estimation of feeding value by substrate composition.

Interpretation of data however has to be done very carefully because of the influence of the incubation time on the results.

Continuous *in vitro* incubation systems can be run on a steady state and allow the examination of adaptation of microbial communities to feeds. But they are time consuming and laborious.

*In vivo* trials are the only reliable method to determine the feeding value of different substrates, but they are laborious and expensive.
## Parameters for the nutritive value

### Substrate parameter
- Digestibility
- Energy content

### Animal performance
- Growth
- Milk, wool or egg production
- Reproduction
- Animal health
In situ digestibility trial: The Nylon Bag Technique

+ Feeding more than one feed to an animal at the same time
+ Conditions close to in vivo

- Fistulated animals required
- Influence of pore size: access of protozoa and bacteria, small particles escape
- Postrumen degradability
In vivo Production Trial
**In vivo digestibility trial: Monogastrics**

- **Enzymatic digestion**
- **Microbial fermentation**
- **Glucose, Lipids & Amino acids**
- **Endogenous components**
- **SCFA**
- **Gas**
- **Microorganisms**

**Apparent digestibility by mass difference**
Nitrogen flows in monogastric animals

Dietary-N

Amino acids

Undig. Diet-N

Microbial-N

Urea-N

Urine-N

Unferm. Diet-N

Microbial-N
**In vivo Digestibility Trial: Ruminants**

1. **Microbial fermentation**
   - SCFA
   - Glucose, Lipids & Amino acids

2. **Enzymatic digestion**
   - Endogenous components

3. **Microbial fermentation**
   - SCFA
   - Microorganisms

4. **Gas**

**Apparent digestibility by mass difference**
Balance Studies

- Basic metabolic rate
  - Digestion, absorption, excretion (maintenance of body functions)

- Measure Heat Energy

- Measure Feed Intake & Energy Content

- Measure Faeces & Urine Excretion & Energy Content

- CO₂ & CH₄
Balance Studies in Metabolic Cages

Urine collection

Faeces collection