In Situ Rumen Degradability Methods

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In situ rumen degradability (ISD)

- Determines the disappearance of feeds incubated in a porous bag within the rumen

- Measures degradability (≠ digestibility) (digested feeds > pore size not considered degraded)

- Estimates the extent & rate of degradation

- Basis of formulating rations to meet protein requirements of livestock in many countries
In situ degradability graph

Degradability (g/kg)

Time (h)
In situ degradability calculations

Degradability = \( a + b \left( 1 - e^{ct} \right) \)

\( a = \) zero time intercept \hspace{1cm} \( c = \) degradation rate
\( b = \) slowly degradable fraction \hspace{1cm} \( t = \) time

To account for feed outflow from rumen, (which reduces the actual rumen degradability) we use

‘Effective’ degradability = \( p = a + \frac{b \times c}{(c + k_p)} \)

Where \( k_p = \) rate of passage (\%/h)
When a, b & c values are generated for protein disappearance:

- **RDP** = \(a + b \left(\frac{c}{c+k_p}\right)\)

- **UDP** = \(b \left[\frac{k_p}{(c+k_p)}\right]\)

Calculate the RDP and UDP for these feeds

<table>
<thead>
<tr>
<th>Feed</th>
<th>a, %</th>
<th>b, %</th>
<th>c, /h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishmeal</td>
<td>13</td>
<td>77</td>
<td>0.01</td>
</tr>
<tr>
<td>Ryegrass silage</td>
<td>63</td>
<td>26</td>
<td>0.14</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>8</td>
<td>90</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Calculate the RDP and UDP for these feeds
Accuracy ($r^2$) of predicting digestibility & intake from ISD

<table>
<thead>
<tr>
<th>Factors</th>
<th>Digestibility</th>
<th>DM intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a + b$</td>
<td>0.82</td>
<td>0.77</td>
</tr>
<tr>
<td>$(a+b) + c$</td>
<td>0.86</td>
<td>0.88</td>
</tr>
</tbody>
</table>

(Khazaal et al., 1993)
Differences in the N degradability & a and b fractions of feeds

Grass silage

hay

Urea

Soyabean meal

fishmeal

Time (h)

N degradability

Time (h)

N degradability
Benefits of knowing feed N degradability

♦ Allows partitioning of protein sources according to whether they contain predominantly:

1. **Rumen degradable** protein (RDP)
   ♦ E.g. soybean meal, alfalfa,

2. **Undegradable** protein (RUP or UDP)
   ♦ E.g Fish meal, bone meal,
Benefits of knowing the N degradation of feeds

- Allows UDP supplementation at high performance levels
- Allows formulation of diets to ensure synchronous ruminal supply of energy and protein

1. Feed readily degradable N feeds with *readily fermentable* energy sources e.g.
   - Soybean meal and grass silage

2. Feed undegradable N feeds with feeds high in *slowly fermentable* energy e.g.
   - Hay / maize silage and fishmeal
Factors affecting degradability results

- Host animal spp & diet.
- Sample processing
- Particle size / form / fine particle losses
- Sample size to surface bag area ratio
- Bag pore size
- Data modelling
- Microbial N contamination of bags
- Incubation sequence
Effect of host animal on wheat silage degradation

- Host animal should be **identical** to those that will receive the test feed

(Adesogan et al., 1998)
Host animal diet

- Determines ruminal microbial composition

- Recommendations
  - Ensure diet is balanced
  - Feed it at level that supports target production level
  - Ensure diet & test feed are ........................................
Sample size to bag surface ratio

♦ Overfilling bags
  – Delays bacterial attachment & reduces digestion

♦ Underfilling bags
  – May leave insufficient residue for analysis

♦ Ideal ratio
  \[ = 10 - 15 \text{ mg/cm}^2 \text{ of bag surface area} \]

♦ Note: Count both bag sides (leave room for seal)
♦ e.g 4 g of DM weighed into an 8 x 14 cm bag = 4000 mg/224cm\(^2\) = 17.9 mg/cm\(^2\)
Pore size

- Varied sizes in literature (≤ 15 µm to 52 µm) due to:
  - Conflicting aims:
    - Maximising bacterial colonization & fluid ingress
    - Minimizing loss of undigested substrates

- Implications:
  - Variable particulate losses
  - Pressure build up which decreases digestibility.
Effect of washing procedure

<table>
<thead>
<tr>
<th>Forage type</th>
<th>Parameter</th>
<th>Washing procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Machine</td>
</tr>
<tr>
<td>Corn silage</td>
<td>a</td>
<td>0.48</td>
</tr>
<tr>
<td>Grass silage</td>
<td>a</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Implication: concentrates, starch-rich feeds are susceptible to fine particle losses which overestimate degradability
Microbial N contamination of bags

- Underestimates degradability in low N, high fiber feeds (can be up to 25%)
- Less important in concentrates (< 10%) high in CP
- Causes erroneous lag and rate estimates
- Solution: remove microbes by
  - Thorough washing / Dip in ice
  - DAPA / nucleic acids
  - Correction equations
  - Sonication / Stomaching
Cloth type & weave pattern

♦ Monofilamentous vs. multifilamentous
♦ In monofilamentous mesh types, pore sizes are more prone to be rearranged by stresses
♦ Polyester fabric is preferable to dacron
**In situ method - Summary**

Biologically it is the most meaningful & accurate method for estimating kinetics of digestion

However

- Difficult to standardize & laborious
- Has low reproducibility
- Inaccurate for soluble or small particulate feeds
- Requires fistulated animals
- Handles few samples & protracted
- Loss of soluble non-degradable matter
- Inaccurate for estimating the effect of anti-nutrients
## Recommend in situ procedures

<p>| | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td><strong>Diet</strong></td>
<td>60:40 hay:concentrate</td>
</tr>
<tr>
<td><strong>Feeding level</strong></td>
<td>Maintenance</td>
</tr>
<tr>
<td><strong>Bag material &amp; pore size</strong></td>
<td>Polyester, 40 -60 μm</td>
</tr>
<tr>
<td><strong>Sample size: bag area</strong></td>
<td>10-15 mg/cm²</td>
</tr>
<tr>
<td><strong>Particle size</strong></td>
<td>≥ 2 mm</td>
</tr>
<tr>
<td><strong>No. of replicate animals</strong></td>
<td>≥ 2</td>
</tr>
<tr>
<td><strong>Incubation sequence</strong></td>
<td>All in, sequential removal (with machine washing)</td>
</tr>
<tr>
<td><strong>Microbial correction</strong></td>
<td>Yes, if detectable</td>
</tr>
</tbody>
</table>

(Broderick & Cochran, 2000)
Other degradability methods contd.

- **Incubation in rumen fluid**
  - Labour intensive
  - Involves animal experimentation except if rumen fluid sourced from abattoirs
  - History of abattoir rumen fluid unknown
  - Batch culture

- **Incubation in**
  - Buffers
  - Proteolytic enzymes
Buffer degradability methods

- MacDougal’s buffer solubility
- Burrough’s buffer solubility
- Saline solutions
- TCA precipitation
- Tungstic acid precipitation
- Cold water solubility

Correlation between selected buffer methods and *in situ* degradability

<table>
<thead>
<tr>
<th>Method</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burroughs buffer</td>
<td>0.66</td>
</tr>
<tr>
<td>0.15M NaCl</td>
<td>0.47</td>
</tr>
<tr>
<td>Autoclaved rumen fluid</td>
<td>0.54</td>
</tr>
</tbody>
</table>
Buffer methods - summary

♦ Pros

- Simple to use
- OK for ranking e.g. effect of heat treatment
- OK for estimating ‘a’ fraction
- OK if good relationship b/w solubility & degradability

- albumin is soluble but not easily degradable
- Casein is degradable but not readily soluble
Buffer methods - summary

♦ Cons
  - Imprecise estimates of degradability especially for forages
  - Equations are species-specific,
  - Precipitation methods measure true protein, yet ruminants also use NPN
  - Do not accurately estimate degradation rates
Enzyme-based degradability

- Bacterial
  - *Bacteroides amylibilus*
  - *Streptomyces griseus*
  - *Bacillus subtilis*

- Plant proteases
  - *Papain & bromelain*

- Fungal proteases
  - *Aspergillus oryzae*

- Animal proteases
  - *Pancreatin & pepsin*
In-situ versus protease degradability

<table>
<thead>
<tr>
<th></th>
<th>$R^2$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Griseus (n= 21)</td>
<td>0.79</td>
<td>Aufrere &amp; Cartailler ‘88</td>
</tr>
<tr>
<td>Ficin (n=38)</td>
<td>0.85</td>
<td>Kosmala et al. (1996)</td>
</tr>
<tr>
<td>Bromelain (n=41)</td>
<td>0.53</td>
<td>Tomankeva et al. 1995</td>
</tr>
<tr>
<td>Bromelain (n=68)</td>
<td>0.55</td>
<td>Tomankeva et al. 1995</td>
</tr>
</tbody>
</table>

Little success in predicting the rate of degradation
Single proteases can’t fully simulate microbial activity of mixed rumen microbes.
Degradability references


