At the seminar “Laboratory methods to predict in situ degradation profiles” held in Uppsala November 17, 2004, methodological aspects on in sacco determination of rumen degradability were discussed by invited scientists and the NorFor Feed Table Group. One important goal with the seminar was to standardize the in sacco procedure as much as possible to minimize between-laboratory variation. Critical parts of the method were listed at the seminar and completed by literature review of other published standards (Madsen & Hvelplund, 1994; Madsen et al., 1995; VanZant et al., 1998; IAEA, 2000; NRC, 2001) and papers on methodological details (Lindberg, 1985; De Boer et al., 1987; Cherney et al., 1990; Varvikko and Vanhatalo, 1990; Uden, 1992; Madsen and Hvelplund, 1994; Wilkerson et al., 1995; Cobzentz et al., 1997; Huntington and Givens, 1997a; Huntington and Givens, 1997b; Huntington and Givens, 1997c). Preliminary proposals for the standard have been modified by the NorFor Feed Table Group after consulting scientists that attended the Uppsala seminar. The standard presented in Table 1 is the final agreement of the NorFor Feed Table Group.

The Feed Table Group in NorFor:
Torsten Eriksson, Swedish University of Agricultural Sciences
Erica Lindberg, Swedish Dairy Association
Odd-Magne Harstad, Norwegian University of Life Sciences (UMB)
Lars Bøvre, TINE, Norway
Bragi Líndal Olafsson, Agricultural Research Institute, Iceland
Martin Weisbjerg, University of Aarhus
Rudolf Thøgersen, Danish Agricultural Advisory Service, National Centre, Danish Cattle Federation

Table 1. NorFor In sacco standard

<table>
<thead>
<tr>
<th>Item</th>
<th>NorFor standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>Dry cow, dairy breed, representative animal</td>
</tr>
<tr>
<td>Type</td>
<td>Maintenance</td>
</tr>
<tr>
<td>Feeding level</td>
<td>(Hay+straw):concentrate 67:33. CP content of ration DM &gt;12%. The concentrate should contain a minimum of 3 sources of protein</td>
</tr>
<tr>
<td>Diet</td>
<td>Daily ration should be divided in 2 or more meals of equal size</td>
</tr>
<tr>
<td>Minimum adaptation period to diet</td>
<td>14 days but if the animal has been on pasture or otherwise been fed on a diet and level totally different from the standard, minimum adaptation period is 21 days</td>
</tr>
<tr>
<td>Replication</td>
<td>3 cows except for INDF determination where 2 cows is sufficient</td>
</tr>
<tr>
<td>Number of animals</td>
<td>Not specified</td>
</tr>
<tr>
<td>Bags per animal</td>
<td>1 (=days are not replicated)</td>
</tr>
<tr>
<td>Number of days when sample is replicated</td>
<td></td>
</tr>
<tr>
<td>Sample preparation</td>
<td></td>
</tr>
<tr>
<td>Drying</td>
<td>Freeze-drying preferable but oven drying at 45°C also allowed. For NDF determination, a drying temperature of 60°C is allowed</td>
</tr>
<tr>
<td>Grinding</td>
<td>Screen aperture 1.5 mm. Cutter mill preferable but hammer mill allowed during NorFor’s introduction phase</td>
</tr>
<tr>
<td>Sample size</td>
<td>1.0 – 2.0 grams dried sample.</td>
</tr>
<tr>
<td></td>
<td>See “Sample size to surface area” below</td>
</tr>
</tbody>
</table>
NorFor in sacco standard. September 10, 2007

Bags

Manufacturer and model of cloth
Saatifil PES 38/31 (Saatitech S.p.A., 22070 Veniano, Como, Italy)

Material
Polyester

Pore size
38 μm

Open surface area (pore area/total bag surface)
31%

Bag size
Bag size refers to internal measures when the bag is sealed and mounted on the actual carrying device. Specified to give 10 mg sample/cm² with the required sample size

Ratio internal length:internal width should preferably be 1:1.3. This means 8.1 x 6.2 cm for 1.0 g sample and 11.4 x 8.8 cm for 2.0 g sample.

Bags should have round corners.

Sample size to surface ratio
10 mg/cm² surface area. (INDF 10-20 mg/cm²).

Sealing method of filled bag
Free but standardized internal bag length must not be altered

Current methods are:
- Bag mounted on rubber stopper (DK, IS)
- Bag closed with rubber band (NO)
- Open end of bag inserted through a slit in a plastic tubing and strapped (SE)

Reusal of bags
Bags may be reused for a maximum of 20 incubations

Incubation conditions

Presoaking
Presoaking for 20 min in 39°C tap water without agitation of bags

Insertion/removal sequence
Bags for 2, 4 and 8 h should be inserted simultaneously 15-30 min prior to morning feeding

Insertion time for longer intervals is free

Ruminal location
Ventral rumen

Bag attachment device
Should allow "squeezing" of bags by contraction

Rinsing

First rinsing before washing machine
Cold tap water, no squeezing or manipulation

Store before rinsing and drying?
Bags may be rinsed immediately or freeze-stored and thawed before machine washing

Washing machine
During NorFor’s introduction phase existing lab equipment has to be used. As soon as possible this will be standardized so that identical washing machines are used at all labs with the same washing programme

Spinning
No

Number of bags washed simultaneously
Will be stated when washing machine model is selected.

Depends on attachment and carrier devices for bags. It is the amount of rumen fluid holding material (bag cloth, cheese cloth in attachment devices) that should be maximized

Water temperature
Thermostat adjusted to 25°C

Stomacher treatment of residue
At the present stage stomacher treatment is allowed to reduce microbial contamination of forage samples (used in DK)

Drying

Temperature
Oven drying at 45°C

Residue analysis

Samples for analysis
Free choice between quantitative analysis of residues from each cow or analysis of pooled residues from emptied bags

NDF
According to NorFor feed recommendations

Nitrogen
According to NorFor feed recommendations

Starch
Not included in standard
Incubation intervals

Protein
0,2,4,8,16,24,48 h; 96 h for forages and concentrates with low degradation rate.
As a rule, concentrates where less than 80% of (total N – N disappeared at 0h)
has disappeared at 48 h needs incubation for 96 h

NDF
0,2,4,8,16, 48,96 h.
INDF incubation for 288 h in bags with 10-15 my pore size

Starch
Not included in standard

Treatment of 0 h sample
Presoaking and washing as described for other incubation intervals

Calculation

Calculation method
Non-linear curve fitting by least squares method on untransformed values.

Particle loss correction
Correction of a and b fractions as described by Weisbjerg et al. (1990). Particle
loss of protein is assumed to be 0 h-value minus buffer soluble protein
determined separately (NorFor, 2006). See Appendix

Microbial contamination correction
None, except for stomacher treatment of forage samples (see this item under
“Rinsing”)

Lag phase
Degradation profiles should at the present stage be fit without lag phase

INDF determination
As described above for in sacco NDF degradation profile with the following
exceptions; 288 h incubation of 2 g sample in bags with 10-15 my pore size and
100-200cm² effective surface area. NorFor INDF standard cloth is Saatifil PES
12/6 (Saaititech S.p.A., 22070 Veniano, Como, Italy) with pore size 12 my and
open surface area 6%. Determination should be performed on at least 2
animals

References


Response to Spontaneous Heating in Alfalfa Hay by In Situ and Ficin Methods. J. Dairy Sci. 80: 700-713.


research project D3 10.22. IAEA, Vienna, 3 pp.

Lindberg, J. E. 1985. Estimation of rumen degradability of feed proteins with the in sacco technique and various in


Madsen, J., Hvelplund, T., Weisbjerg, M.R., Bertilsson, J., Olsson, I., Sporndly, R., Harstad, O.M., Volden H.,
Tuori, M., Varvikko, T., Huhtanen, P. and Olåsson, B.L. 1995. The AAT/PBV protein evaluation
system for ruminants - a revision. Norwegian Journal of Agricultural Sciences, Supplementum 19,
37 pp.


Uden, P. 1992. The influence of leaf and stem particle size in vitro and of sample size in sacco on neutral detergent


Varvikko, T. and A. Vanhatalo. 1990. The effect of differing types of cloth and of contamination by non-feed

Appendix. Guidelines for curve fitting and values to report

Curve fitting NDF and values to report

- Degraded NDF fraction at time \( t \) = \( \frac{\text{NDF amount}_0 - \text{NDF amount}_t}{\text{NDF amount}_0} \)

Where NDF amount is remaining NDF amount in bag at time \( t \)

Hence degraded fraction at time 0 is 0

- Include degraded fractions at times 0, 2, 4, 8, 16, 24, 48 and 96 in curve fitting. If incubation residue at time \( t \) is too small for analysis, NDF content from time \( (t-1) \) should be used
- Fit the equation Degraded fraction = \( b[1-\exp(-ct)] \)
- Restriction \( b \leq 1 \)
- Report \( b \) as DNDF curve fitting and \( c \) as kdNDF
- If DNDF\(_{288h}\) is available, report DNDF\(_{288h}\) as DNDF and correct kdNDF according to:

\[
\text{kdNDF}_{\text{corr}} = \text{kdNDF}_{\text{uncorr}} \times \frac{b}{\text{DNDF}}
\]

Curve fitting protein and values to report

- Include degraded fractions at times 0, 2, 4, 8, 16, 24, and 48 (96 if available) in curve fitting. If incubation residue at time \( t \) is too small for analysis, CP content from time \( (t-1) \) should be used
- Fit the equation Degraded fraction = \( a + b[1-\exp(-ct)] \)
- Restriction \( 0 \leq a \leq 1; \ 0 \leq b \leq 1; \ 0 \leq (a+b) \leq 1 \)
- Report \( c \) as kdCP
- \( sCP \) value from buffer solubility should be reported
- Correct “\( b \)” value for particle loss according to Weisbjerg et al. (1990) with \( sCP \) value and report as pdCP:

\[
pdCP = b + (a-sCP)\times \frac{b}{(1-(a))}
\]