Physico-chemical characteristics and degradation rate of soluble protein obtained from the washout fraction of feeds

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Abstract

The (water)-soluble fraction of feeds is often assumed to be completely and immediately degraded in the rumen. The objective of this study was to separate the washout fraction (fraction A) obtained usually by difference after submitting the nylon bags to the machine-washing program, to investigate the nature and the degradation of the soluble crude protein in the washout fraction. The washout fraction obtained in vitro (filtrate) of 10 feeds was collected in water using nylon mesh as a filter. The feeds used in the study were: two grass silages, soybean meal, three corn gluten feeds, lupine meal, rapeseed meal, wet brewers grain silage and corn gluten feed silage. Average N losses during filtration and from machine-washed nylon bags ranged from 15% (rapeseed meal) to 74% (grass silage) and were not different between procedures. N recovered in the filtrate ranged from 12% (soybean meal) to 60% (corn gluten feed silage) of sample N. The three fractions obtained from the filtrate were: soluble protein (TP), non-protein N (NPN) and fine particles (NS). The NS fraction was obtained after centrifugation of the filtrate and comprised 0% to 87% of N in the filtrate. Soluble protein (TP) in the supernatant was obtained after precipitation with trichloroacetic acid and N in the remaining supernatant was defined as non-protein N (NPN). Significant amounts of TP were found in soybean meal (58%), lupine meal (30%) and rapeseed meal (27%) as percent of total N in the filtrate. NPN ranged from 13% to 100% of N in the filtrate. The in vitro incubation of the protein N (NS + TP) showed that all fractions were not completely degraded, suggesting a potential participation as escape protein. Fine particles in the filtrate have similar degradation rates as the residue left in the filter. It is concluded that the washout fraction consisted of different crude protein fractions that were not always completely and immediately degraded in the rumen.

Keywords: Nylon bag; Washout fraction; Trichloroacetic acid; Soluble protein degradation

1. Introduction

In dairy cows, alpha-amino acids available for absorption in the small intestine originate largely from microbial protein and ruminally undegraded...
feed protein (UDP). The estimation of protein degradability and protein escape are necessary for an adequate diet formulation. The degradability of dietary proteins in the rumen as well as the estimation of escape protein is often assessed using the nylon bag technique. This technique plays a central role in several protein evaluation systems for dairy cows. In the nylon bag technique, the fraction A (Mehrez and Ørskov, 1977), which may contain soluble protein and protein in fine particles (e.g., <40 μm), leaves the bags. In most protein evaluation systems, this fraction A is assumed to be immediately and completely degraded in the rumen (AFRC, 1993; Tamminga et al., 1994; Madsen et al., 1995). This implies that only insoluble protein can leave the rumen. However, this assumption may be incorrect. Although the estimation of undegraded protein in the Dutch DVE/OEB and French PDI system use the factor 1.11 to correct the amount of undegraded feed protein, the results of Van Vuuren et al. (1998, 2000) showed that the estimation of undegradable protein fraction in some feeds, such as wet brewers grains could be higher than assumed by the system. This could be explained by the fact that the washout fraction (fraction A) is not, in most cases, immediately and completely degraded in the rumen (Messman et al., 1994; Giers et al., 2001). The washout fraction can be rather substantial for some feeds. For example, Madsen et al. (1995) reported a washing loss of N from bags ranging from 33.3% to 47.3% of the total N content for different concentrate feeds. Based on such results, Weisbjerg et al. (1990) suggested a correction for fine particle losses from the nylon bag, assuming the particle loss is degraded in a way similar to that of the remaining feed in the bag. However, besides fine particles, the amount of N washed out from the nylon bag may contain soluble protein, which may be resistant to rumen degradation and therefore, contribute to the escape protein. Thus, the rumen escape protein fraction estimated by nylon bag technique is underestimated.

Because the in situ technique does not account differently for the N compounds in the fraction A, nor can it be used to estimate the degradation rate of fraction A, the present study had two objectives: (1) to characterize and quantify the different N compounds in the washout fraction of 10 feeds and (2) to examine to what extent the fine non-soluble particle (NS) and trichloroacetic acid-precipitable proteins (TP) in the washout fraction were degraded using an in vitro method (Broderick, 1987). Consequently, the extent to what these fractions contribute to the feed protein escaping ruminal degradation was estimated.

2. Materials and methods

2.1. Sample description and preparation

Ten feeds of different origin and characteristics were utilized. Six concentrate feeds, two wet by-products and two samples of grass silage were used: soybean meal (SBM), lupine meal (LPM), rapeseed meal (RSM), three corn gluten feeds (CGF-1, CGF-2 and CGF-3), silage of wet brewers grain (WBGS) and silage of corn gluten feed (CGFS). The WBGS contained 5% of beet pulp (DM basis). Grass silages (GS-1 and GS-2) were taken from the sample collection of the Nutrition and Food Division in Lelystad (The Netherlands). They differed in DM content, as a result of different wilting times (Table 1). Both silages originated from a plot fertilized with 300 kg N/ha/year at the regional “Crandonck” experimental farm at Maarheeze (The Netherlands) and were harvested on June 17th, 1997 (second cut). The first silage was grass wilted to 25% DM; the second silage on grass wilted to 45% DM. The CP levels in these silages were high (Table 1), but not unusual for this level of N

<table>
<thead>
<tr>
<th>Feeds</th>
<th>DM, %</th>
<th>CP, g/kg DM</th>
<th>Fat, g/kg DM</th>
<th>Ash, g/kg DM</th>
<th>Starch, g/kg DM</th>
<th>DOM, % of OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass silage 1</td>
<td>25.1</td>
<td>295</td>
<td>NA</td>
<td>113</td>
<td>NA</td>
<td>80.3</td>
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<tr>
<td>Grass silage 2</td>
<td>52.9</td>
<td>288</td>
<td>NA</td>
<td>108</td>
<td>NA</td>
<td>79.5</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>87.8</td>
<td>544</td>
<td>21</td>
<td>72</td>
<td>ND</td>
<td>90.9</td>
</tr>
<tr>
<td>Corn gluten feed 1</td>
<td>89.3</td>
<td>227</td>
<td>37</td>
<td>58</td>
<td>173</td>
<td>86.5</td>
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<td>Corn gluten feed 2</td>
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<td>209</td>
<td>21</td>
<td>58</td>
<td>149</td>
<td>86.5</td>
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<td>93.5</td>
<td>199</td>
<td>53</td>
<td>135</td>
<td>124</td>
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<td>Lupine meal</td>
<td>91.7</td>
<td>322</td>
<td>58</td>
<td>27</td>
<td>173</td>
<td>89.1</td>
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<td>Rape seed meal</td>
<td>89.6</td>
<td>344</td>
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<td>72</td>
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<td>100</td>
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<td>59.0</td>
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<td>Wet brewers grain silage</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 1 Chemical analyses and feed evaluation of the 10 feeds

* DM= dry matter, CP= crude protein, DOM= digestible organic matter, NA= not analyzed, ND= not detected.
fertilization and harvesting date (Hindle et al., 2000). The grass had been chopped into 3 cm pieces for the present analysis. Because CGF-1 and CGF-2 were delivered in the form of pellets, they were milled through a 3 mm screen. Other samples were not treated prior to the study. All dry feeds were stored in a refrigerator at 4 °C and silages were stored at −12 °C.

2.2. Machine washing of nylon bags

As reference for the filtration procedure, the size of the washout fraction was determined using a conventional machine washing (AEG Tumamat 2800) of nylon bags (Nybolt 30/40) according to Hindle (1994) in four replicates of all 10 feed samples. The amount of water used was about 40 l and the centrifugation step was excluded. Each washing run took about 45–50 min. The loss of DM and total N was calculated from DM and the N content (Kjeldahl-N) of the original feed samples and the residues after washing and drying at 70 °C for at least 12 h. Total N loss during washing was assumed to be the difference in total N content of the feed sample before washing and the total N content of the residue after washing.

2.3. Filtration procedure

2.3.1. Development of the filtration procedure

A set of filtration trials was conducted. Initially, the recovery of the DM lost in the filtrate in the smallest possible volume of water was determined. Sample size and amount of rinsing water were varied, and loss of DM due to filtration was estimated as the difference between DM weight of the sample and DM weight of the residue after filtration (Table 2). In the first trial, lowering the amount of rinsing water to 45 ml decreased the DM loss significantly. In the second trial, no change in DM loss in filtration was observed when the sample size varied within the range of 1.2 g to 3.6 g of DM. The DM loss was, however, significantly lower when using 5.4 g DM of sample.

2.3.2. The filtration procedure as used in the study

For the concentrate ingredients, 3–5 g of fresh material was taken as sample size. For WBGS, CGFS and GS-2, 4 g of fresh material were taken. Because of its low DM content, the sample size for GS-1 was increased to 5 g. Samples of each feed were weighed into 250 ml beakers and soaked in 25 ml of distilled water during constant shaking (Gerhardt Schüttelmaschine RO20, Bonn, Germany) at 160 rpm/min for 1 h. Samples of GS-1 were, because of their bulky nature, soaked in 35 ml of water. After soaking, the samples were poured into the funnels containing a suspended filter. This filter was a piece of nylon mesh (41 μm pore size, Nybolt 30/40) that was used for the in situ trials. The filtrate was collected in a 100 ml centrifugation tube. The beakers were washed to get the remaining feed from the beakers into the funnels and the filter with residues placed into the oven (Hereaeus Electronics, type T50) for drying at 70 °C for determination of DM losses and N-content (Kjeldahl-N) of the residue.

Each filtrate was further divided into three different fractions. The fractions considered were the fine non-soluble particles (NS), soluble non-protein nitrogen (NPN) and trichloroacetic acid-precipitable protein (TP), as described in the following.

2.4. Nitrogen fractions in the filtrate

2.4.1. Fine non-soluble particles (NS fraction)

Firstly, each filtrate was centrifuged (BHG Hermple, Z 424) at 1500×g for 20 min and the supernatant was removed to a 100 ml centrifugation tube. The pellet (pellet 1) after this centrifugation was assumed to contain non-soluble particles and dried at 70 °C for the determination of DM weight and N content.

<table>
<thead>
<tr>
<th>Feed</th>
<th>Sample size, g DM</th>
<th>Rinsing water, ml</th>
<th>Loss of DM, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1: effect of amount of rinsing water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBM (n=4)</td>
<td>2.7</td>
<td>75</td>
<td>31.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBM (n=4)</td>
<td>2.7</td>
<td>45</td>
<td>27.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Trial 2: effect of sample size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBM (n=4)</td>
<td>1.2</td>
<td>75</td>
<td>31.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBM (n=4)</td>
<td>2.7</td>
<td>75</td>
<td>31.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBM (n=4)</td>
<td>3.6</td>
<td>75</td>
<td>31.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SBM (n=4)</td>
<td>5.4</td>
<td>75</td>
<td>29.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Different superscripts within the same trial indicate significant differences ($P<0.05$).
2.4.2. Trichloroacetic acid-precipitable proteins (TP fraction)

Secondly, an amount of a solution of 1000 g/l trichloroacetic acid (TCA) was added to the supernatant obtained as described above to achieve a final concentration of 100 g/l TCA in solution. The concentration of 10% TCA was used to precipitate all soluble protein in the supernatant (Greenberg and Ship, 1979; Marais and Evenwell, 1982), which was confirmed in a preliminary pilot experiment on quadruplicates of filtrates from SBM and CGF-2. The contents of the tubes were well mixed and the tubes were left for 2 h at 4 °C to allow complete precipitation of protein. These tubes were then centrifuged at 1500 × g for 20 min. The pellet (pellet 2), assumed to contain all water-soluble true protein, was dried and analyzed for DM and N content.

2.4.3. Non-protein nitrogen (NPN fraction)

The supernatant after precipitation was assumed to contain all soluble non-protein N and is defined as non-TCA-precipitable N. This supernatant was brought to a constant volume of 100 ml with distilled water. From this volume, an aliquot of 10 ml was taken for N determination. The DM content of the supernatant (DM_{SUP}) was calculated by difference as follows: DM_{SUP} = DM_{S} - DM_{RES} - DM_{NS} - DM_{TP}, where DM_{S}, DM_{RES}, DM_{NS} and DM_{TP} are the DM content in the sample, in the residue remaining after filtration, in the NS fraction and TP fraction, respectively. The pellets and the supernatants were stored at 4 °C prior to analysis.

2.5. Protein degradation in vitro

An inhibitor in vitro system, based on Broderick (1987), was used with few modifications as described below.

2.5.1. Preparation of rumen fluid

Rumen fluid was obtained 1 h after the morning feeding from two non-lactating cows. The cows received a standard diet of 9 kg hay fed twice daily (2 × 4.5 kg) and 1 kg concentrate fed once a day. Rumen fluid from both cows was combined and strained through nylon filter cloth of 40 μm. Equal amounts of McDougall’s buffer and the strained rumen fluid were poured into a pre-heated fermentor to obtain the inoculum, which was stirred continuous-ly and kept at 39 °C. All manipulations were performed under continuous flushing with oxygen-free carbon dioxide.

2.5.2. Pre-fermentation

The pre-fermentation steps were performed within 3 h and consisted of the addition of a carbohydrate mixture to decrease the ammonium concentration in the inoculum and to increase microbial activity. The carbohydrate mixture was based on maltose, starch, xylose and pectin, and 7.0, 3.5, 3.5 and 3.5 g/l of inoculum was added, respectively. Pectin was dissolved in 75 ml of warm McDougall’s buffer overnight. The inoculum was sampled at the start and every hour thereafter to monitor the pH. If necessary, a 3 N NaOH solution was used to adjust pH to 6.4 in the inoculum.

2.5.3. Sample preparation

Feeds used in this experiment were 5 out of 10 used before: LPM, CGF-2, CGFS, SBM and WBGS. The feeds were filtered according to the procedure described previously in order to obtain enough N in each fraction both for N determination and for the in vitro study. Four fractions of each feed were obtained for the study, which correspond to the fractions as obtained by filtration: (a) total filtrate (FIL), representing NPN, TP and NS, i.e., the washout fraction; (b) supernatant (SUP), representing the TP and NPN in the washout fraction; (c) pellet (PEL), representing the NS in the washout fraction; and (d) residue (RES), which remains after filtration, representing the potentially degradable fraction of feeds, including the indigestible fraction C from the in situ method. Fractions with less than 5% of the sample N content in anyone of the fractions were not considered for further analysis, as it was the case with the PEL fraction of SBM. After performing the filtration procedure, samples of each fraction were freeze-dried and an aliquot removed for N content determination. Samples of the RES were milled to pass through a 1 mm mesh. Other fractions were used without milling.

2.5.4. Incubation

Samples of each fraction were weighed into 250 ml flasks in order to ensure a sample N content of 0.20 mg/ml. Before starting the incubation, samples of the fractions were soaked in 30 ml McDougall’s buffer at 39 °C for 1 h. Twenty minutes before the incubation
started, inhibitors were added to the inoculum (0.2 g hydrazine sulfate, 0.5 g chloramphenicol and 310 μl mercaptoethanol per liter of inoculum) as described by Broderick (1987). The incubation was started by adding 120 ml of inoculum into the pre-heated incubation flasks. The flasks were flushed with oxygen-free carbon dioxide and sealed with Bunsen valves. Each flask represented a whole incubation set, with sampling times at 0, 180 and 240 min. Three flasks were used per feed fraction. Casein and bovine serum albumin were used as controls in each run. Flasks were placed into a water bath and shaken continuously at 150 rpm. At each sampling time, 10.0 ml of the inoculum was collected and transferred to centrifuge tubes, previously placed in ice and containing 2 ml of 65% (w/v) of trichloroacetic acid solution. Tubes were kept at least 15 min on ice before centrifugation at 3000 × g for 20 min at 4 °C. Nitrogen was determined in the supernatant and assumed to be NPN.

2.5.5. Calculations

The amount of TP incubated was determined from the amount of non-TCA-precipitable N at zero incubation time by difference. For each sampling time, undegraded N content was calculated as a proportion of the total amount of incubated sample N after subtracting the N content of blank flasks at each incubation time. Degradation rate \( (k_d) \) was computed as:

\[
h^{-1} = \frac{(\ln B_4 - \ln B_0)}{4 \text{ h}}\]

where \( B_0 \) and \( B_4 \) are the amount of TP after 0 and 4 h incubation time.

Estimated protein N escape was computed as:

\[
g \text{ kg}^{-1} \text{ total N} = B_0 \times \frac{k_p}{(k_p + k_d)}\]

where \( k_p \) is the ruminal passage rate and was estimated assuming a passage rate of the solid phase ranging from 2% to 8% h\(^{-1}\) and a passage rate of the liquid phase of 12% h\(^{-1}\). Undegraded fractions in RES were not corrected for the undegradable fraction C as well as fractions were not corrected for ammonia N contamination.

2.6. Chemical analysis

The total N content of feeds and fractions were determined by the Kjeldahl method (ISO, 1991). The samples were analyzed for non-TCA-precipitable N as performed by Neutze et al. (1993). Degraded N corresponded to the appearance of TCA-soluble N (NPN) in the supernatant after incubation, corrected for the TCA-soluble N content in the inoculum (blank tubes).

Feeds were sampled and analyzed for DM, CP (Kjeldahl-N × 6.25), crude fat, ash and starch, as described by Steg et al. (1990). Organic matter digestibility (DOM) was established in vitro (Tilley and Terry, 1963). The results of these chemical analyses are in Table 1.

2.7. Statistical analysis

Student’s \( t \)-tests were used to compare the effect of filtration and machine washing on DM and N content in residues. Fractions obtained by the separation procedure for each feed were submitted to analysis of variance and means separated by Tukey’s test at \( P<0.05 \). Due to the presence of zero values, data was transformed as \((y+0.5)^{0.5}\) for statistical analysis (Steel and Torrie, 1980). For the degradation study, fractions within feeds were submitted to analysis of variance and means separated by Tukey’s test at \( P<0.05 \).

3. Results

3.1. Filtration procedure

3.1.1. Nitrogen losses

The percentage of N remaining in the residue after machine washing ranged from 25.8% to 84.4% for GS-1 and SBM, respectively (Table 3). A similar range (24.2% to 89.1%) for the same feeds was obtained using the filtration procedure. The differences in %N in residues between the two methods were in the range of −5.5% (WBGS) to 10.6% units (CGFS) for all feed, and were significant for WBGS, CGFS and CGF-2. The difference between methods in N loss was the highest for CGFS \( (P<0.001) \).

3.2. Nitrogen in different fractions after filtration

The amount of N recovered in the different fractions of the filtration procedure is presented in Table
4. The recovery of N from the sample in the different fractions ranged from 80% for GS-1 to 103% for LPM. The amount of N in the washout fraction (filtrate) of feeds ranged from 9.0% (RSM) to 60.5% (CGFS). The washout fraction formed a considerable part of the feeds total N content for all of the feeds except for SBM, RSM and WBGS. However, the latter feeds are rich in N; thus, the absolute amount of N in the washout fraction is not negligible.

For GS-1, GS-2, CGF-1, CGF-2, CGF-3 and CGFS, the washout fraction consisted mainly of NPN, ranging from 80% (CGFS) to 100% (GS-1) of the filtrate. Significant amounts of TP were only present in LPM (12.4%), SBM (7.0%) and RSM (2.4%), as percentage of N in the sample. WBGS and LPM were distinguished by having substantial amounts of NS in the washout fraction, i.e., 22.4% and 21.8%, respectively (Table 4).

3.3. Degradation of crude protein in the different fractions

The amount of potentially degradable N incubated, i.e., TCA-precipitable N (TP) varied from 9.0 mg/g DM in the PEL fraction of CGFS to 118 mg/g DM in the PEL of LPM (Table 5). Depending on the feed, the fraction SUP has the lowest content of TP among fractions, contributing negatively to the accuracy of calculation of protein degradation.

After the 4 h incubation period, considerable degradation was observed in all fractions. However, undegraded N was present after 4 h in all feeds analyzed, allowing to estimate the escape protein for each fraction. At $k_p$ of 4% h$^{-1}$, escape protein estimates varied from 3.31% to 42.54% in FIL fraction, from 1.19% to 14.11% in SUP fraction, from 19.99% to 60.43% in PEL fraction and from 18.51% to 74.87% in RES fraction. In WBGS, only small amounts were degraded after 4 h incubation period, with high proportions of escape protein, especially RES fraction. Although results for all feeds analyzed are presented for the escape protein of RES at 12% h$^{-1}$, the largest proportion are expected to have a passage rate compatible with the solid phase.

RES and PEL showed similar degradation rate for the feeds analyzed. The degradation rate after 3 h incubation time showed less variation than the degradation computed after 4 h incubation time. Nevertheless,
similar protein nature seems to be present for degradation in the rumen for RES and PEL fractions. The SUP fraction was the fastest degraded in almost all feeds analyzed and higher degradation rate was observed in SUP than in PEL. The FIL fraction, containing both NS and TP fraction (FIL= SUP+PEL), was degraded depending on the highest amount of degradable CP present in SUP or PEL.

### 4. Discussion

The fraction A in the in situ method contains fine particles, soluble proteins and NPN, with variable amounts among feeds. Corrections for the loss of fine particles are proposed (Hvelplund and Weisbjerg, 2000) for the calculation of effective degradability, assuming a degradation rate ($c$) similar as to that of the fraction B.

For all feeds, except for SBM and RSM, N in the washout fraction was more than 25% of sample N (Table 4). De Boever et al. (1997) measured the washout fraction as the difference between N content in the sample and in the residue after machine washing. They observed a high %N lost in the washout fraction of silages of different qualities, ranging from 52.7% to 66.4%. For soybean meal, %N in the washout fraction was lower, 11.4% to 11.7%, and consistent within qualities. These results are in line with our observations (Table 3). For ensiled wet brewers grain, De Boever et al. (1997) found a %N in the washout fraction of 39.6%, which was high compared to our result (25.9%, Table 4). The N disappearance due to machine washing of corn gluten feed, soybean meal and grass silage observed by Van Straalen (1995) was 48%, 11% and 54% of sample N, respectively. These results are similar to ours for CGF-3, SBM and GS-1 obtained by filtration, being 50.5%, 12.0% and 56.1% of sample N, respectively.

The washout fraction of GS-1 was higher than that of GS-2. A higher NPN fraction in proportion to feed N in GS-1 accounted for the difference. The other difference between the two grass silages was the DM content. Wilting before ensiling is an efficient way of reducing proteolysis in the silo. According to Hristov and Sandev (1997), the contents of NPN were lower in grass silage when wilted to 475 g DM/kg before ensiling compared to when ensiled as fresh cut (250 g

<table>
<thead>
<tr>
<th>Feed N content, mg/g DM</th>
<th>TP</th>
<th>Deg. rate, h$^{-1}$</th>
<th>Escape, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lupine meal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIL 61.9</td>
<td>80.5b</td>
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<td>7.04</td>
</tr>
<tr>
<td>SUP 45.7</td>
<td>61.4a</td>
<td>0.333a</td>
<td>3.48d</td>
</tr>
<tr>
<td>PEL 118.0</td>
<td>94.4a</td>
<td>0.149a</td>
<td>11.18a</td>
</tr>
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<td>92.6a</td>
<td>0.160a</td>
<td>10.29b</td>
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<tr>
<td>S.E.</td>
<td>1.6</td>
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<td>0.10</td>
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<tr>
<td><strong>Soybean meal</strong></td>
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<tr>
<td>FIL 25.3</td>
<td>66.0b</td>
<td>0.184a</td>
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<tr>
<td>SUP 23.9</td>
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</tr>
<tr>
<td>PEL ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
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<td>RES 103.0</td>
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<td>S.E.</td>
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<td>S.E.</td>
<td>1.25</td>
<td>0.17</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Wet brewers grain silage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIL 40.6</td>
<td>63.0b</td>
<td>0.019b</td>
<td>32.12c</td>
</tr>
<tr>
<td>SUP 26.2</td>
<td>26.8c</td>
<td>0.173a</td>
<td>2.79d</td>
</tr>
<tr>
<td>PEL 81.6</td>
<td>89.8b</td>
<td>0.019a</td>
<td>45.54a</td>
</tr>
<tr>
<td>RES 31.1</td>
<td>89.2a</td>
<td>0.008b</td>
<td>64.50a</td>
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<td>S.E.</td>
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<td>0.02</td>
<td>1.80</td>
</tr>
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<td>FIL 51.0</td>
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<td>0.138e</td>
<td>1.87c</td>
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<td>SUP 53.9</td>
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<td>0.385a</td>
<td>0.63c</td>
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<tr>
<td>PEL 28.1</td>
<td>77.4b</td>
<td>0.068a</td>
<td>17.61c</td>
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<tr>
<td>RES 20.6</td>
<td>91.8b</td>
<td>0.044b</td>
<td>28.59a</td>
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<tr>
<td>S.E.</td>
<td>1.29</td>
<td>0.11</td>
<td>0.34</td>
</tr>
</tbody>
</table>

$B_2$: degradation rate calculated after 4 h incubation time.

ND = not determined.

FIL = filtrate fraction, corresponding to the whole fraction A as measured in the in situ method.

SUP = supernatant, corresponding to the true protein fraction, i.e., TCA-precipitable fraction in the fraction A.

PEL = pellet, corresponding to the insoluble small particles in the fraction A.

RES = residue, corresponding to the fraction B (and C) as assumed in the in situ method.

Mean values within columns and within each feed without a common superscript differ ($P<0.05$).

* Fractional passage rate (h$^{-1}$).
DM/kg). An extended proteolysis in the silo after ensiling might be the reason for the difference between GS-1 and GS-2, as observed also by Messman et al. (1994).

The recovery of N after filtration was high (>95%) for all concentrates, but lower in the grass silages and CGFS (80.5% to 91.0%). One reason for this could be a loss of highly volatile NPN such as ammonia during the filtration procedure. Another reason could be the retention of fine particles in the filter pores.

Only LPM, SBM and RSM had a substantial amount of TP in the washout fraction. The absolute amounts of TP in these fractions represent up to 40, 38 and 8 g CP/kg DM in sample, respectively. In the subsequent degradation study, TP in SUP was considered the potentially degradable protein N. TCA is assumed to precipitate peptides with a chain length >10 amino acids (Van Soest, 1994). Considering 10 amino acids as the TCA-cutoff, the use of TP as a criterion for what remains undegraded might be incorrect if peptides with less than 10 amino acids are prone to escape further degradation to ammonia in the rumen.

Fine particles were found especially in WBGS and LPM. The fine particles fraction of grass silage of ryegrass from Hvelplund and Weisbjerg (2000) accounted for 19.8% of N in sample. The nylon bag method is widely accepted as a method to estimate the degradability of feeds as they occur in the rumen. However, this method does not account for the rapid degradable fraction of feeds, i.e., the fraction A. Separation using buffer, saline or detergent solutions differs in the estimation of soluble proteins, since solubility is dependent on inherent physical and chemical characteristics of each protein. Solubility in water is a feature of albumins, which are present mostly in legume seeds, leaf proteins and dicotyledonous plants, and less in grasses (Van Soest, 1994), which may explain the detection of significant amounts of TCA-precipitable proteins in SBM, LPM and RSM.

In the present work, casein and bovine serum albumin were used in each incubation set to monitor the degradation of feed fractions. The degradation rate (k_d) after 4 h incubation time achieved 41.5 ± 0.06% h⁻¹ for casein and 6.4 ± 0.01% h⁻¹ for bovine serum albumin. The estimated escapes using a passage rate of 6% h⁻¹ were 9.8 ± 2.0% for casein and 47.6 ± 5.1% for albumin. Peltekova and Broderick (1996) found a degradation rate for casein of 0.486 h⁻¹ after 6 h incubation time and recent work of Broderick et al. (2004) reported a degradation rate of casein up to 0.318 h⁻¹ after adding different concentrations of inhibitors. For serum albumin, the degradation rate is lower, due to the disulfide bridges encountered in this protein source. Broderick (1987) found a degradation rate of 0.065 to 0.071 h⁻¹ after adding different amounts of maltose in the incubation medium. The degradation rates for casein and serum albumin in our study are consistent with the results found in literature.

The results presented for RES fraction show, however, a lower degradation rate in comparison to in situ results (Broderick, 1987; England et al., 1997; Reynal and Broderick, 2003). Values in literature report degradation rates for SBM ranging from 8% to 17% h⁻¹. In the present work, the degradation rate of RES in SBM was about 5.6% h⁻¹. Recent work of Broderick et al. (2004) with the inhibitor in vitro method showed a range of degradation rates varying from 0.01 to 0.12 h⁻¹ with different concentrations of inhibitors in the medium for solvent SBM. However, the variation observed by these authors may be related to an insufficient inhibitor concentration to prevent microbial uptake of the degraded N.

Based on in vivo measurements, Van Vuuren et al. (1998) suggested that the degradation of the washout fraction of brewers grain was incomplete. The washout fraction of WBGS consisted mainly of fine particles, which are insoluble in water. Nitrogen insoluble in water is often concealed in fibrous structures and thereby protected from degradation (Lindberg, 1981). Therefore, it is reasonable to believe the NS fraction is not degraded faster than the potentially degradable part of the feed. For the feeds in the present study, PEL and RES fractions did not differ in the degradation rate, either measured after 3 h or 4 h incubation time. Because degradation also is dependent on rumen passage rate (effective protein degradation; Ørskov and McDonald, 1979), the PEL fraction might be responsible for the incomplete degradation of the washout fraction as suggested by Van Vuuren et al. (1998, 2000). In line with this observation, De Boever et al. (1997) measured the washout fraction of brewers grain (by machine washing) to contain 39.6% of total feed N, which is in accordance with our observation.
A considerable part of the washout fraction comprised non-soluble particles (washout fraction–N soluble in borate-phosphate buffer). It was concluded that, because of this NS fraction, protein degradation of brewers grain measured in situ was seriously over-estimated. Our results are thus in line with observations of others and suggest that the NS proteins in the washout fraction may be the major determinant of (un)degradability of N in the washout fraction. In this regard, a correction is used in the Nordic AAT/PBV-system when calculating the rumen degradability of protein. In this system, loss of fine particles is estimated as the difference between total loss from bags and water-soluble N (Hvelplund and Weisbjerg, 2000). Water-soluble protein is measured as the washing loss over filter paper. The washout fraction corresponds to the true water-soluble fraction and the fine particle fraction is assumed to degrade in the same rate as the potentially degradable fraction (Madsen and Hvelplund, 1993). Madsen et al. (1995) compared the degradability of protein with the Nordic correction for the washout fraction and without the correction. With a rumen outflow rate of 0.05 h⁻¹, the uncorrected degradability ranged from 72.7% to 81.1% for different concentrate mixtures. For the same feeds, the corrected degradability markedly differed and ranged from 56% to 70%. The DVE/OEB-system considers the losses of the washout fraction as a part of the potentially undegradable fraction of feeds, using the correction factor of 1.11, which is taken from the French PDI System (Tamminga et al., 1994). This leads to the discussion of the necessity of a more extended correction for the washout fraction when calculating undegraded protein, because, to a varying degree, all protein evaluation systems are based on the prediction of the amount of undegraded protein reaching the duodenum.

The procedure reveals the presence of trichloroacetic acid-precipitable protein in the washout fraction for LPM, SBM and RSM, which is not fully and rapidly degraded in the rumen as assumed in the nylon bag method. Fine particles escaping the nylon mesh have similar degradation rates as the residue left in the filter. For some feeds, the fraction A may contribute substantially to the undegradable protein escaping the rumen. Corrections can be developed based on the technique presented in this study, which seems applicable for a wide range of other feeds.

5. Conclusion

The procedure reveals the presence of trichloroacetic acid-precipitable protein in the washout fraction for LPM, SBM and RSM, which is not fully and rapidly degraded in the rumen as assumed in the nylon bag method. Fine particles escaping the nylon mesh have similar degradation rates as the residue left in the filter. For some feeds, the fraction A may contribute substantially to the undegradable protein escaping the rumen. Corrections can be developed based on the technique presented in this study, which seems applicable for a wide range of other feeds.

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References


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