Individual variation of in vitro dry matter digestibility in moose

Ä. PEHRSON AND W.E. FABER
Grismö Wildlife Research Station, Department of Wildlife Ecology, Swedish University of Agricultural Sciences, S-730 91 Riddarhyttan, Sweden

Abstract

The in vitro technique for estimating relative digestion rates in ruminants on various forages has created conflicting results in a number of investigations. Some studies show both inter- and intraspecific variation in the ability of inocula to digest the same substrate, while the results of other studies do not verify this potential source of error. This study was designed to compare variation in in vitro dry matter digestibility (IVDMD) using inocula from 8 different moose. Further, samples were taken from 3 different parts of the rumen, cranial sac, ventral sac, and ventral blind sac, in order to look for potential variation in inoculum quality within the rumen. The moose were collected on 3 consecutive days in October 1990. Current year growth of Scots pine (Pinus sylvestris L.) collected immediately before the experiment was used as substrate. The results showed considerable differences between inocula from different animals in digestion of the substrate. Sex or age of inoculum donor did not account for the variability, and site of origin from within the rumen had a significant impact on IVDMD only in 1 animal. A botanical analysis of different parts of the rumen showed considerable variation in the proportion of pine and dwarf shrubs (Ericaceae), the main food types consumed. These feeding differences were not reflected in the IVDMD results.

Key Words: in vitro dry matter digestibility (IVDMD), moose, Scots pine, (Pinus sylvestris L.)

The in vitro rumen fermentation technique is a widely used tool for measuring nutritional value of forages in domestic ruminants. Much attention has been given to the possible sources of error. Differences in digestibility of the same substrate when inocula were taken from different ruminant species have been found in some studies (Horton et al. 1960, Blankenship et al. 1982, Clary et al. 1988), while in other studies such variation has not been observed (Welch et al. 1983, Brooks and Urness 1984, Striby et al. 1987). In addition, several workers have shown differences in digestibility even between individuals of the same ruminant species (Church and Petersen 1960, Bezaeu 1965, Nelson et al. 1972). As a possible explanation for the variation in digestibility it has been suggested that there are differences in the ruminal bacterial flora due to feed type. It is recommended that one use inocula from more than one donor animal combined with a standard reference forage as a correction factor (Clary et al. 1988).

In wildlife research, the in vitro technique is of special importance due to the difficulty in performing in vivo digestion studies. Even here, conflicting results are reported concerning inter- and intraspecific variation in digestibility when inocula from donor animals such as white-tailed deer (Odocoileus virginianus) (Robbins et al. 1975, Palmer et al. 1977, Campa et al. 1984), wapiti (Cervus elaphus) (Ward 1971, Brooks and Urness 1984), and mule deer (Odocoileus hemionus hemionus) (Striby et al. 1987) have been used. Cederlund and Nyström (1981) using in vitro trials showed that moose (Alces alces) and roe deer (Capreolus capreolus), occupying the same habitat, had consistent differences in digestibility during both summer and winter. Earlier unpublished in vitro dry matter digestibility (IVDMD) studies at Grimsö Wildlife Research Station showed conflicting results not attributable to methodological error. Therefore we designed a set of in vitro trials with moose to test for variations related to the role of donor animal and the collection site for inoculum within the rumen. Our intentions were to minimize eventual effects of season and forage substrate.

Materials and Methods

There are a variety of modifications of the original in vitro method described by Tilley and Terry (1963), e.g. the amount of substrate used, the ratio between buffer solution and inoculum, and length of digestion period. Our method was originally developed at the Swedish University of Agricultural Sciences for energy determinations in grass hay by den Braver and Eriksson (1967), and has been frequently used in Sweden. It includes incubation of 0.5 g test substrate with 1 ml of rumen fluid and 50 ml of buffer solution for 96 hours. Since such a small amount of rumen fluid is used it is not necessary to correct for the constituent amount of organic matter. The pepsin-hydrochloric digestion part of the Tilley and Terry method is excluded since the longer incubation times gives comparable or even better results (den Braver and Eriksson 1967). The procedure has also been adopted at Grimsö for studies on the digestibility of browse in moose and roe deer (Cederlund and Nyström 1981).

Rumen inocula were taken from each of 8 moose shot during 1 of 3 consecutive days (19-21 October 1990), at Grimsö Wildlife Research Area in southcentral Sweden. We attempted to collect 1 sample each from the cranial sac (Atrium ruminis), the ventral sac and the caudo-ventral blind sac. Distinct sampling sites within the rumen are difficult to define in a shot animal that has fallen to the ground. However, our intention was to determine if sampling of inoculum without careful mixing of the ruminal contents had any effect on the result. Since we do not compare the 3 sampling sites within the rumen between animals, a precisely defined sampling site is of minor importance here. Rumen material was brought immediately to the laboratory in warmed thermos flasks. It was filtered through a cheesecloth and immediately placed into the test tubes using a dosage syringe.

The in vitro substrate consisted of current-year twigs and needles of Scots pine (Pinus sylvestris L.) dried and milled through a 0.5-mm sieve. About 0.5 g of this material was weighed (0.0001 g precision) into each glass filter tube. The buffer solution (den Braver and Eriksson 1967), was saturated for 1 hour in advance with CO₂ and
moose warmed to 38°C. After 50 ml of buffer and 1 ml of inoculum had been added, the tubes were closed with gas valves and kept in a water bath at 38°C. The whole procedure from the time the moose was shot until the tubes were sealed never exceeded 1 hour. This time interval probably did not influence the digestion process (Schwartz and Nagy 1972). Each of the 3 rumen locations from the 8 moose was replicated 4 times.

The tests were run for 72 hours during which tubes were lightly shaken and swirled by hand once a day. Originally den Braver and Eriksson (1967) incubated for 96 hours but investigations at our laboratory have shown no influence on the digestibility when the time is reduced to 72 hours (unpubl. res.). After the digestion period each tube was filtered carefully with distilled water and acetone, dried for 48 hours at 50°C and weighed (0.0001 g precision).

A sample of 500 to 800 ml of rumen contents was taken from 7 moose for botanical analyses after thorough mixing of the entire ruminal contents. The sample was stored frozen at -24°C. After thawing, the material was rinsed under tap water through a 4-mm sieve. Rumen items were identified for assignment to one of the following categories: (a) conifers, mainly pine; (b) deciduous browse including birch twigs (Betula pendula Roth, B. pubescens Ehr.), and aspen twigs (Populus tremula L.); (c) dwarf shrubs including heather (Calluna vulgaris L.) and (Vaccinium spp.); and (d) grass and herbs. The identified material was dried for 48 hours at 50°C and weighed (0.1 g precision). The results are expressed as percent (% of dry matter in the sample.

Another sample of the rumen contents was collected in the same way as the sample taken for botanical analyses. These samples and a fecal sample from each individual moose were taken in order to examine some nutritional aspects of the ruminal contents and the undigested matter. These samples were dried for 1 week at 50°C. After drying, the material was ground (0.5 mm) and analyzed with near infrared reflectance (NIR) technique (Inframatic 8100; Pertem Instruments AB, Huddinge, Sweden). The analyses included nitrogen (N), acid detergent fiber (ADF) and lignin.

The results of the in vitro digestion of pine substrate were tested with one-factor ANOVA analyses. The Spearman rank correlation test was used to test for influence of rumen nutrient content on in vitro dry matter digestibility (IVDMD). Differences in IVDMD, rumen plant composition and nutrient content related to sex or age were tested with the Mann-Whitney U-test.

### Study Area

The Grimsvö Wildlife Research Area consists mainly of a fairly homogeneous coniferous forest dominated by Scots pine and Norway spruce (Picea abies L.). The deciduous trees, mainly birch and aspen, are relatively sparse due to intensive forest management favoring conifers. Clearcuts are mainly planted with pines which, within a height of 2 to 5 m, dominate the winter food for moose in this area.

For a more detailed description of the research area see Cederlund et al. (1980) and Cederlund and Nyström (1981).

### Results

Rumen content analyses showed that moose diets were dominated either by pine or dwarf shrubs, while deciduous browse and herbs constituted only smaller proportions (Table 1). One exception was female calf Number 60 where half the stomach content was pine and almost 15% consisted of deciduous browse. The intake of various food types was not related to sex or age of the animal (*P* > 0.05).

The 3 inocula samples taken from different parts of the rumen gave significantly different digestibilities from only 1 (No. 60) of the studied 8 moose (*P*= 0.04). Between individuals the average IVDMD for the 3 inocula samples differed significantly (*P*= 0.0001) (Table 2). This difference could not be explained by their relative intake of coniferous browse (*r* = 0.3).

### Table 2. In vitro dry matter digestibilities (% of dry matter) of Scots pine (Pinus sylvestris L.) twigs with inocula taken from 3 different locations in the rumens of 8 different moose.

<table>
<thead>
<tr>
<th>Location of rumen inoculum</th>
<th>Moose</th>
<th>Avian ruminis</th>
<th>Ventral sac</th>
<th>Caudo-ventral blind sac</th>
<th>X</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>55</td>
<td>46.4</td>
<td>44.4</td>
<td>41.2</td>
<td>44.3</td>
<td>5.23</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>30.0</td>
<td>30.2</td>
<td>49.9</td>
<td>30.0</td>
<td>4.86</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>37.5</td>
<td>35.5</td>
<td>37.3</td>
<td>36.7</td>
<td>5.58</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>49.7</td>
<td>44.4</td>
<td>48.7</td>
<td>47.6</td>
<td>4.82</td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>45.9</td>
<td>41.0</td>
<td>48.2</td>
<td>45.0</td>
<td>4.82</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>41.1</td>
<td>38.7</td>
<td>38.2</td>
<td>39.3</td>
<td>5.58</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>37.9</td>
<td>40.2</td>
<td>37.0</td>
<td>38.4</td>
<td>4.82</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>49.5</td>
<td>47.2</td>
<td>47.2</td>
<td>48.2</td>
<td>5.58</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>44.8</td>
<td>42.7</td>
<td>43.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>5.23</td>
<td>4.86</td>
<td>5.58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Concentration of nitrogen in the rumen contents was positively related (*r*= 0.88, *P* < 0.01, Table 3), while ADF (*r*= 0.62, *P* < 0.05) and lignin (*r*= 0.69, *P* < 0.05) were negatively related to IVDMD. There was also a negative relationship between lignin concentration of the feces and IVDMD (*r*= -0.71, *P* < 0.05) while nitrogen and ADF (*r* = 0.2 and *r*= -0.5 respectively) showed no relation to IVDMD. No differences in rumen nutrient concentrations were found that related to sex or age of the donor animals.

### Discussion

We found marked differences in in vitro dry matter digestibility due to inoculum taken from different animals. The range of 35-50% of the pine material digested by inoculum is similar to other in vitro studies performed at our laboratory, and also within the range of variation of in vivo results with captive moose (Pehrson unpubl.).

Although the sample size was small, food species consumed by moose in our study seemed to have no influence on the quality of the inoculum. The different proportions of the main food types, notably pine and Ericaceous dwarf shrubs, were not reflected in the digestibility figures. Inocula from stomach contents dominated by pine showed low as well as high digestibility figures. One possible expla-
nation for this observation may be that pine and the Ericaceous species are of similar nutritional value as a substrate for the rumen microbes. Logically moose would not jeopardize the functioning of the rumen microbes by changing between chemically very different plant parts.

Further, the concentration of different nutrients in the rumen contents could not be related to the proportions of different food species consumed. Yet nitrogen related positively to IVDMD, while ADF and lignin showed a weak negative relationship to the digestibility results. The nitrogen figures probably reflect different concentrations of bacterial and plant protein.

Our results agree with Cederlund and Nyström (1981) who found that organic matter digestibility related positively to nitrogen, and negatively to ADF in the plant material they tested with moose and roe deer inoculum. In our study neither digestibility nor rumen nutrient concentrations differed between animals according to sex or age.

Only 1 of the 8 investigated moose had significantly different IVDMD from the 3 different sample sites of inoculum. This suggests that experimental error is not inflated by sampling rumen contents properly from a shot animal. However, our handling of the rumens may have induced minor mixing and thus leveled out some potential differences.

We stress the necessity for careful mixing of rumen contents before in vitro experiments. A series of tests must include some sort of standard reference material that can be used for all separate inoculum samples. However, according to Cederlund (1989) individual moose inocula taken from different moose is related to the individual animal rather than the type of food consumed, or sex and age criteria. Regardless of what factor(s) controls the ability of moose inocula to digest plant substrate, there are significant differences between individuals. These differences must be taken into consideration when running in vitro experiments. A series of tests must include some sort of standard reference material that can be used for all separate inoculum involved. It also points to the fact that different investigations are difficult to properly compare to each other. For wildlife studies the value of such analyses best applies to the intentions of the individual laboratory or research project.

**Literature Cited**


