COMPARISON OF THREE IN VITRO METHODS FOR DETERMINING AND PREDICTING THE ORGANIC MATTER DIGESTIBILITY OF COMPLETE DIETS FOR RUMINANTS

Dragan V. Palić and Klaas-Jan Leeuw

In this study, the organic matter digestibility (OMD) of six complete diets for ruminants has been determined in-vivo in trials with sheep and in-vitro using two-stage Tilley and Terry (T&T) method, gas production (GP) technique and multi-enzyme incubation (EDOM) procedures. The mean OMD values obtained in vivo and using T&T, GP and EDOM techniques were 684, 716, 685 and 710 g OM/kgDM respectively and did not differ significantly (P>0.05). The obtained in vitro results were regressed against determined in-vivo values to derive prediction equations. Using the T&T technique, the prediction equation OMD (in_vivo) = \(-17.36 + 0.98 \times \text{OMD (in_vitro_T&T)}\), (R\(^2\) = 0.75; RMSE = 37.59) has been obtained. The equation OMD (in_vivo) = \(198.98 + 0.71 \times \text{OMD (in_vitro_GP)}\), (R\(^2\) = 0.21; RMSE = 66.36) has been derived for Gas production procedure, while the equation OMD (in_vivo) = \(102 + 0.82 \times \text{OMD (in_vitro_EDOM)}\), (R\(^2\) = 0.86; RMSE = 27.30) has been generated for multi-enzyme incubation technique. The results of this study showed that the OMD of complete diets for ruminants can be successfully determined, and in-vivo values predicted, using multi-enzyme incubation procedure, which is important because of the fact that rumen liquor, needed for the in-vitro two-stage T&T and GP techniques is not always available to analytical laboratories.

KEY WORDS: Ruminants, complete diets, organic matter digestibility, in vitro techniques, prediction

INTRODUCTION

Energy value of ruminant feeds and its bio-availability is of great importance for animal feed manufactures and end users. The amount of available energy in feeds for ruminants is described either by its metabolisable energy or by organic matter digestibility (1), since the value of organic matter digestibility is very close to the corresponding digestibility of energy (2). The most accurate way of obtaining information on digestibility of organic matter of feeds for ruminants is by conducting in vivo digestibility experi-

Dr. Dragan Palić, dragan.palic@fins.uns.ac.rs, Institute for Food Technology, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia; Klaas-Jan Leeuw, M. Sc., Agricultural Research Council, ARC-Animal Production Institute, Private Bag X2, Irene 0062, South Africa
ments. Since this method is expensive and time consuming, laboratory methods for routine prediction of the in vivo organic matter digestibility of ruminant feeds are an irreplaceable tool for routine quality control in the feed industry.

*In vitro* methods for determining organic matter digestibility (OMD) by the use of rumen liquor fermentation techniques have become well established despite the limitations on the use of rumen liquor for digestibility studies and the request for fistulated animals (which are not available to all laboratories) for the collection of fresh rumen liquor. Widely accepted *in vitro* rumen liquor fermentation procedures for determining the organic matter digestibility of ruminant feeds are the two-stage Tilley and Terry (T&T) method (3) and Gas Production (GP) technique developed by Menke and Steingass (4).

The alternative to rumen liquor is the use of incubation of feeds with exogenous enzymes, which has the aim to mimic the digestive processes in the animal. Enzymes can break down different parts of the plant constituents, which can be divided into those that make up the structure of the plant (cell-wall constituents) and the material within the cells (cell-content constituents). Cell content is essentially completely digestible in vivo, whereas cell-wall constituents vary in digestibility (5). Enzymes therefore need to remove the cell contents and to solubilise un lignified and moderately lignified cell-wall to a significant extent. Most enzymatic methods for organic matter digestibility determination have been developed for forage feedstuffs, with a few used for compound feeds (6). Hvelplund et al. (7) used a multi-enzymatic incubation method for estimating the enzymatic digestibility of organic matter (EDOM) of straws. Palic and Muller (8) demonstrated the ability of this method to determine the OMD of a variety of other feedstuffs for ruminants. Apart from the above-mentioned two references, more citations of this method in the literature have not been found.

The aim of this study was to compare the two rumen-fluid-based methods, i.e. the two-stage *in vitro* method (3) and gas production (4) with the under-utilized, multi-enzyme incubation procedure of Hvelplund et al., (7) for determining the OMD of compound feeds for ruminants and to develop equations for predicting the in vivo OMD of complete diets for ruminants using results obtained by *in vitro* T&T, GP and EDOM techniques.

**EXPERIMENTAL**

Six complete diets for ruminants were used in this study. The chemical composition of the diets was determined by the Official Methods of AOAC International (9).

The OMD of investigated diets was determined in the *in vivo* trial, as well as by three *in vitro* methods. *In vitro* procedures used for OMD determination were:

**Two-stage method** (3). This method has two main stages. In the first, 0.5 g of dried sample is incubated anaerobically with rumen liquor inoculum in a buffered solution, for 48 hours at 38°C in the dark. The second stage involves digestion with pepsin-HCl for 48 hours at 38°C. The OMD is calculated as the difference between the organic matter in the original sample and in the residue.

**Gas Production technique** (4). An amount of 200 mg of dried sample is introduced into a special piston-syringe, after which rumen liquor is added. The production of gas is measured during a 24-hour incubation at 39°C and the OMD is calculated from the following equation:
OMD (%) = 14.88 + 0.889 x gp + 0.45 x CP + 0.65 x CA

where:
gp = Gas produced (ml)  
CP = Crude protein (%)  
CA = Crude ash (%)

**EDOM procedure (7).** About 0.5 g of sample is incubated with pepsin-HCl solution for 24 hours at 40°C to dissolve protein, followed by incubation with a buffered enzyme solution (consisting of cellulase, cellobiase, hemicellulase and amyloglucosidase) first at 40°C for 19 hours and then at 60°C for 19 hours. The residue was dried and ashed, and the insoluble organic matter in the sample was determined as the difference.

**In vivo organic matter digestibility** was determined according to Steg et al. (10) using four castrated adult male sheep per diet. Water was freely available at all times, while the feed was standardized at 1000 g of dry matter daily for each animal. An 11-day adaptation period during which the animals were fed the trial ration was followed by a 10-day collection period during which the exact amounts of feed, residues and the faecal production were recorded. The organic matter contents of both feed and faeces were measured and their difference represented the digestible organic matter.

Data was analysed using the statistical programme GenStat (11).

**RESULTS AND DISCUSSION**

The chemical composition of studied diets is shown in Table 1.

**Table 1.** Chemical composition (%) of complete diets used in the study

<table>
<thead>
<tr>
<th>Diet</th>
<th>DM (%)</th>
<th>CP (%)</th>
<th>CF (%)</th>
<th>CA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89.39</td>
<td>9.44</td>
<td>1.45</td>
<td>6.37</td>
</tr>
<tr>
<td>2</td>
<td>85.35</td>
<td>13.99</td>
<td>1.09</td>
<td>7.00</td>
</tr>
<tr>
<td>3</td>
<td>86.24</td>
<td>13.45</td>
<td>1.29</td>
<td>8.69</td>
</tr>
<tr>
<td>4</td>
<td>87.45</td>
<td>14.52</td>
<td>2.69</td>
<td>6.72</td>
</tr>
<tr>
<td>5</td>
<td>86.07</td>
<td>21.33</td>
<td>1.07</td>
<td>7.20</td>
</tr>
<tr>
<td>6</td>
<td>87.04</td>
<td>9.40</td>
<td>1.34</td>
<td>8.08</td>
</tr>
</tbody>
</table>

DM = Dry matter  
CP = Crude protein  
CF = Crude fibre  
CA = Crude ash

The organic matter digestibility of complete diets for ruminants determined in *in vivo* trial and by three *in vitro* procedures is shown in Table 2.
**Table 2.** The OMD of complete diets for ruminants (g OM/kg DM) determined *in vivo* and by three *in vitro* methods

<table>
<thead>
<tr>
<th>Diet</th>
<th>Organic matter digestibility (g OM/kg DM)¹</th>
<th>In vivo</th>
<th>T &amp; T²</th>
<th>GP³</th>
<th>EDOM⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>595</td>
<td>625</td>
<td>665</td>
<td>622</td>
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<tr>
<td>2</td>
<td>716</td>
<td>756</td>
<td>686</td>
<td>781</td>
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<tr>
<td>3</td>
<td>674</td>
<td>724</td>
<td>618</td>
<td>726</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>710</td>
<td>776</td>
<td>708</td>
<td>708</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>781</td>
<td>749</td>
<td>609</td>
<td>801</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>628</td>
<td>664</td>
<td>599</td>
<td>624</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>684ᵃ</td>
<td>716ᵃ</td>
<td>685ᵃ</td>
<td>710ᵃ</td>
<td></td>
</tr>
<tr>
<td>SD²</td>
<td>66.7</td>
<td>58.9</td>
<td>43.1</td>
<td>75.8</td>
<td></td>
</tr>
</tbody>
</table>

¹Means of three replicates  
²Two-stage *in vitro* method (3)  
³Gas production method (4)  
⁴Multi-enzyme incubation method (7)  
⁵SD = Standard deviation  
ᵃMeans with same subscript in a row do not differ significantly (P>0.05)

The mean values obtained by *in-vivo*, T&T, GP and EDOM procedures were 684, 716, 685 and 710 g OM/kg DM respectively, and did not differ significantly (P>0.05). The values obtained by laboratory procedures were then regressed against determined *in vivo* values and the functions for each *in vitro* procedure for predicting the *in vivo* OMD of complete diets have been derived.

The following equation, shown also in Figure 1, to predict the *in vivo* OMD from the results of *in vitro* T&T method has been obtained:

\[
\text{OMD (in}_\text{vivo}) = -17.36 + 0.98 \times \text{OMD (in}_\text{vivo}_\text{T&T)},
\]

\[
R^2 = 0.75; \text{RMSE} = 37.59
\]

The regression of the OMD results obtained by Gas production method against the *in vivo* values, resulted in the following equation, shown also on Figure 2.

\[
\text{OMD (in}_\text{vivo}) = 198.98 + 0.71 \times \text{OMD (in}_\text{vivo}_\text{GP)}
\]

\[
R^2 = 0.21; \text{RMSE} = 66.36
\]

The equation, shown also on Figure 2, for predicting the *in vivo* OMD using Multi-enzyme incubation (EDOM) method, was as follows:

\[
\text{OMD (in}_\text{vivo}) = 102 + 0.82 \times \text{OMD (in}_\text{vivo}_\text{EDOM)}
\]

\[
R^2 = 0.86; \text{RMSE} = 27.30
\]
Fig. 1. Relationship between the OMD values of compound feeds for ruminants determined in vivo and by in vitro T&T method.

Fig. 2. Relationship between the OMD values of complete diets for ruminants determined in vivo and by in vitro GP method.
The organic matter digestibility of complete diets for ruminants in this study was predicted best by EDOM method, closely followed by the two-stage T&T procedure.

A low correlation between Gas production and in vivo results ($R^2 = 0.21$) was somehow unexpected, since Palic and Muller (8), investigating the OMD of feedstuffs for ruminants, established an $R^2 = 0.81$ for the relationship between the OMD values determined in vivo and by in vitro Gas production method.

The prediction of OMD of compound feeds for ruminants by the use of enzymes have been up to date applied mostly to forages. Enzymatic methods have been much less studied with energy and protein feeds (6), and in those seldom cases, the authors used single-enzyme incubations. Dowman and Collins (12), using incubation with pepsin-HCl, followed by the treatment of 12 complete diets with cellulase, reported a correlation of $R^2 = 0.87$ between in vivo and in vitro values, whereas Aufrere and Michalet-Doreau (6) found an $R^2 = 0.90$ using 24 energy-rich feeds. The procedure of Hvelplund et al. (7) used in this study, might have an advantage over the above-mentioned, as it uses multi-enzyme incubation and therefore may better mimic the digestion in the animal gastrointestinal tract.

**CONCLUSION**

Although conducted on a small number of samples, the results of this study yielded a clear reference point and showed that the organic matter digestibility (OMD) of complete diets for ruminants can be successfully determined, and the in vivo OMD successfully predicted, using a multi-enzyme incubation procedure. This is important because of the fact that rumen liquor, needed for the in vitro Tilley and Terry and Gas Production techniques, is not always available to analytical laboratories. Further work, with inclusion of more samples of complete diets, is needed to confirm the results of this study.
REFERENCES


ПОРЕЂЕЊЕ ТРИ IN VITRO МЕТОДЕ ЗА ОДРЕЂИВАЊЕ И ПРОЦЕНУ СВАРЉИВОСТИ ОРГАНСКЕ МАТЕРИЈЕ У ПОТПУНИМ СМЕШАМА ЗА ПРЕЖИВАРЕ

Драган Палић и Klaas-Jan Leeuw

У овом раду је одређена сварљивост органске материје (OMD) у шест потпуних смеша за преживаре и то in vivo, у огледима на овцама, и in vitro, коришћењем двостепене Tilley и Terry (T&T) (3) методе, технике мерења продукције гаса (GP) (4) и поступка мулти-ензимске инкубације (EDOM) (7). Средње вредности сварљивости органске материје добијене in vivo и коришћењем T&T, GP и EDOM in vitro
метода износило су 684, 716, 685 и 710 g органске материје/кт суве материје и нису се значајно разликовале (P>0,05). Регресионом анализом добијене су једначине за предвиђање in vivo OMD на основу резултата добијених in vitro методама. За T&T метodu добијена је једначина OMD (in vivo) = -17,36 + 0,98 x OMD (in vitro_T&T), (R²= 0,75; RMSE=37,59, за GP технику OMD (in vivo)=198,98 + 0,71 x OMD (in vitro_GP), (R²=0,21; RMSE=66,36), док је за EDOM методу изведена једначина OMD (in vivo)= 102 + 0,82 x OMD (in vitro_EDOM), (R²=0,86; RMSE = 27,30). Резултати овога рада показују да се сварљивист органске материје потпуних смеша за преживаре може успешно одредити, и њене in vivo вредности успешно предвидети, коришћењем мулти-ензимске инкубационе методе, што је веома важно с обзиром на чињеницу да буражни сок, неопходан за T&T и GP методе, није увек доступан аналитичким лабораторијама.

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