# Nutritive value of red clover and lucerne forages for ruminants estimated by *in vitro* and *in vivo* digestibility methods

P. Homolka[1,2], V. Koukolová[1], M. Podsedníček[1], A. Hlaváčková[2]

[1]Institute of Animal Science, Prague-Uhříněves, Czech Republic
[2]Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

**ABSTRACT**: The aim of this study was to determine the nutrient and energy levels of red clover and lucerne forage. Investigation of forage at different maturity stages of three growths was carried out by chemical analysis, *in vitro* and *in vivo* digestibility methods. Generally, maturation caused a significant increase in fibre fractions. With the increasing maturity of forage samples the *in vivo*, *in vitro,* and calculated *in vivo* (*in vivo*$_{calcul}$) digestibilities of organic matter (OM) linearly decreased. The *in vitro* and *in vivo*$_{calcul}$ digestibilities of OM averaged 0.754 and 0.708 for red clover and 0.717 and 0.667 for lucerne, respectively. The *in vivo* OM digestibility averaged 0.710 for red clover and 0.666 for lucerne. Gross energy (GE), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEL), and net energy for growth (NEG) averaged 18.12, 12.41, 9.60, 5.67, 5.50 and 18.09, 11.56, 9.01, 5.26, 4.99 MJ/kg of dry matter for red clover and lucerne, respectively. The effect of a vegetative stage on energy values of both forages was diverged for various growth times. When data were pooled across the estimated season, seven cutting-specific equations for descriptions of GE, DE, ME, NEL, NEG, *in vitro,* and *in vivo* OM digestibilities were obtained for red clover and lucerne, separately. The red clover model expression gave similar prediction equations for lucerne. It was possible to predict cutting-specific equations with coefficients of determination $R^2 > 0.719$ for red clover and $R^2 > 0.400$ for lucerne of the variation in GE, DE, ME, NEL, and NEG. The *in vitro* and *in vivo* OM digestibility equations were predicted with $R^2$ being 0.840 (*in vitro*) and 0.707 (*in vivo*) for red clover, and 0.979 (*in vitro*) and 0.937 (*in vivo*) for lucerne. The parameters of these specific equations were statistically preferable than the general model expression which included both forages together.

**Keywords**: ruminant; forage quality; organic matter digestibility; energy value

Dairy cattle nutrition can be defined broadly as the use of the feed components for the processes of maintenance, growth, reproduction, lactation, and health (Drackley et al., 2006). The net energy of lactation (NEL) of forages is important for formulating the diets of ruminants (Belyea et al., 1999). For optimal milk production from lactating dairy cows, therefore, knowledge of the efficiency of energy utilization by ruminants (Johnson et al., 2003) and, consequently, of the nutritional quality of forage is indispensable.

Forage quality is affected by a combination of numerous factors such as stage of maturity of forage, forage species, environmental conditions (locality of growth, temperatures, and precipitations), and agronomic treatments including storage conditions (Dubbs et al., 2003; Pozdíšek and Vaculová, 2008; Tyrolová and Výborná, 2008; Jančík et al.,

Supported by the Ministry of Agriculture of the Czech Republic (Projects No. MZE 0002701404 and No. QH81309) and by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6046070901).

2009). Low-quality forage often does not provide enough energy to adequately maintain cows during lactation (Baumann et al., 2004). Several authors (Buxton, 1996; Van Soest, 1996; Dubbs et al., 2003; Jančík et al., 2010 and others) mentioned temperature, light intensity, water availability, altitude, seasonal changes, weather, and the maturity stage of plants as factors influencing forage quality. Some authors (Hunt et al., 1989; Bal et al., 1997) have investigated the time of harvest for assessing the optimal maturity stage of the plant concerning nutrient content and digestibility.

Forage quality can also be subject to the fibre characteristics of forage (Scarbrough et al., 2001), because the nutritive value of forages for ruminants highly depends on the ratio between cell content and cell walls and on the ability of the rumen microorganisms to degrade the plant cell walls (Waldo, 1986). The fibre fraction makes up an important part of the ruminant diet and originates mainly from plant cell walls that consist of a variety of structural polysaccharides, often cross-linked with proteins and phenolic components, particularly with lignin, which is also prevalent in the cell wall. The main polysaccharides of the plant cell wall are cellulose, various hemicelluloses, and pectic polysaccharides. The noncarbohydrate phenolic polymer lignin can be described as a multibranched network consisting of phenyl propane units (Hindrichsen et al., 2006). With increasing maturity, the proportion of cell wall components (cellulose, hemicellulose, and lignin) of the forage increases, whereas the proportion of cell contents decreases (Bosch et al., 1992). The digestibility of forage is the highest in the vegetative stages due to variation in cell/wall content and the stem/leaf ratio with increasing maturity (Terry and Tilley, 1964). The rate of decline in digestibility with increasing maturity also depends on annual average temperatures (Wilson et al., 1991) and on the species (Bargo et al., 2003; Gallardo et al., 2005).

The aim of the present study was to determine nutritive data from the set of red clover and lucerne samples of different forage-maturity stages and to predict the equations for estimating of (1) fibre fractions in relation to the effect of maturing proceeding, (2) feed energy values, and (3) digestibility of red clover and lucerne. The nutritional value of red clover and lucerne was estimated by chemical analyses, *in vivo* and *in vitro* organic matter digestibility methods. It was hypothesized that the extent of organic matter digestibility would be in response to different maturity stages and growths.

## MATERIAL AND METHODS

### Forage sampling

Forage samples of one growing season originated from two fields (red clover field, lucerne field) of the Institute of Animal Science in Prague-Uhříněves, Czech Republic (50°2'17.996"N, 14°37'30.289"E). Twelve samples of red clover (*Trifolium pratense* L., variety Kvarta) were collected from the first growth ($n$ = 4), second growth ($n$ = 4), and third growth ($n$ = 4) of the same sward. Twelve samples of lucerne (*Medicago sativa* L., variety Palava) were collected during the same vegetative period also as the first growth ($n$ = 4), second growth ($n$ = 4), and third growth ($n$ = 4) of the same sward. The developmental stages and morphological descriptors for red clover and lucerne forages can be found in Tables 1 and 2.

The average annual temperature was 8.9°C and total annual rainfall made up to 626 mm (see Figure 1). The altitude of the field was 240 m. For soil fertilization, 40 kg/ha per year of $P_2O_5$ and 60 kg/ha per year of $K_2O$ were used.

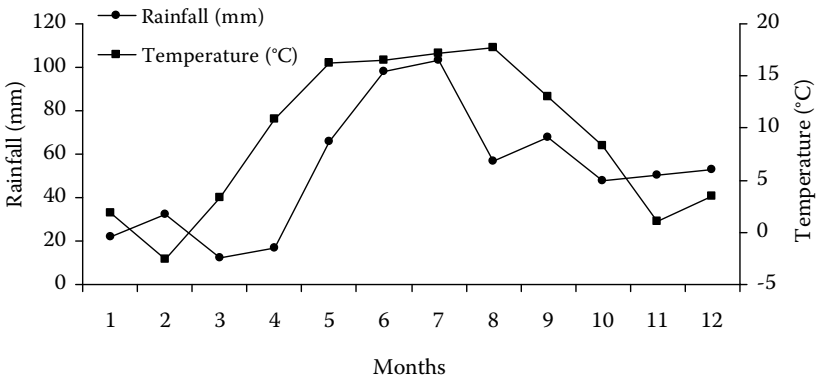


Figure 1. Total monthly mean temperatures and total monthly mean rainfall of observed sampling season (January–December)

Table 1. Developmental stages and morphological descriptors for red clover ($n$ = 12) divided into three growths

| Feed | VP | Phase | Red clover descriptors[1] |
|---|---|---|---|
| $1^{I}$ | 1 | vegetative | SL 25–30, no buds |
| $2^{I}$ | 2 | early bud | SL > 30, 1–2 nodes with buds, no flowers or seedpods |
| $3^{I}$ | 3 | late bud | SL > 40, > 3 nodes with buds, no flowers or seedpods |
| $4^{I}$ | 4 | late flower | SL > 60, full blooming (open flower on main and axillary stems) |
| $5^{II}$ | 1 | vegetative | SL 25–30, no buds, first overgrowing of vegetation after field cutting |
| $6^{II}$ | 2 | early bud | SL > 40, 1–2 nodes with buds, no flowers or seedpods |
| $7^{II}$ | 3 | late bud | SL 40–60, > 3 nodes with buds, no flowers or seedpods |
| $8^{II}$ | 4 | late flower | SL > 60, full blooming (open flower on main and axillary stems) |
| $9^{III}$ | 1 | vegetative | SL 25–30, no buds, second overgrowing of vegetation after field cutting |
| $10^{III}$ | 2 | early bud | SL > 30, 1–2 nodes with buds, no flowers or seedpods |
| $11^{III}$ | 3 | late bud | SL > 40, > 3 nodes with buds, no flowers or seedpods |
| $12^{III}$ | 4 | late flower | SL > 60, full blooming (open flower on main and axillary stems) |

VP = vegetation period, SL = stem length in cm

[I]first growth, [II]second growth, [III]third growth

[1]according to Fick and Mueller (1989) and Skinner and Moore (2007)

Table 2. Developmental stages and morphological descriptors for lucerne ($n$ = 12) divided into three growths

| Feed | VP | Phase | Lucerne descriptors[1] |
|---|---|---|---|
| $13^{I}$ | 1 | vegetative | SL > 30, no buds |
| $14^{I}$ | 2 | early bud | SL > 40, 1–2 nodes with buds, no flowers or seedpods |
| $15^{I}$ | 3 | late bud | SL > 50, > 3 nodes with buds, no flowers or seedpods |
| $16^{I}$ | 4 | early flower | SL > 50, 1 node with 1 open flower, no seedpods |
| $17^{II}$ | 1 | vegetative | SL > 30, no buds, first overgrowing of vegetation after field cutting |
| $18^{II}$ | 2 | early bud | SL > 40, 1–2 nodes with buds, no flowers or seedpods |
| $19^{II}$ | 3 | late bud | SL > 50, > 3 nodes with buds, no flowers or seedpods |
| $20^{II}$ | 4 | late flower | SL > 50, ± 2 nodes with open flowers, no seedpods |
| $21^{III}$ | 1 | vegetative | SL > 30, no buds, second overgrowing of vegetation after field cutting |
| $22^{III}$ | 2 | early bud | SL > 40, 1–2 nodes with buds, no flowers or seedpods |
| $23^{III}$ | 3 | late bud | SL > 50, > 3 nodes with buds, no flowers or seedpods |
| $24^{III}$ | 4 | late flower | SL > 50, ± 2 nodes with open flowers, no seedpods |

VP = vegetation period, SL = stem length in cm

[I]first growth, [II]second growth, [III]third growth

[1]according to Ohlsson and Wedin (1989) and Skinner and Moore (2007)

## Chemical analyses

Dry matter (DM) was obtained from drying of chopped fresh material at 45°C according to Harazim et al. (1999). Dried material was subsequently milled to pass through a one-millimetre sieve for laboratory analyses. Ash-free concentration of neutral-detergent fibre (NDF) was determined according to the methods described by Van Soest et al. (1991), and ash-free concentrations of acid-detergent fibre (ADF) and acid-detergent lignin (ADL) were determined according to AOAC Official Method 973.18 (AOAC, 2005). Crude protein (CP) was analyzed according to the Kjeldahl method, as N × 6.25 (AOAC, 2005). Ether extract (EE) was determined using Soxtec extraction with petroleum ether and crude fibre (CF) according to AOAC (2005). Ash was determined after 4.5 h of combustion at 550°C. Then, non-structural carbohydrates (NSC) were calculated as 1000 – (NDF + CP + EE + ASH) according to Van Soest et al. (1991).

### *In vitro* procedure

*In vitro* organic matter (OM) digestibility was determined using an enzyme cellulase technique (Homolka, 1994). The samples were weighted into the filtration crucibles of a 40 ml capacity. Samples were first treated with pepsin-HCl and incubated at 39°C for 24 h. Samples were mixed up after 6, 22, and 24 h during this incubation period. This was followed by the incubation of samples at 80°C for 30 min. Then, the incubated samples were vacuum-filtrated and washed with hot distilled water. Then followed a 24 h incubation at 39°C by cellulase *Trichoderma viride* (5 g per 1 l of buffer, activity 0.9 FPU/mg) in acetate buffer (Dowman and Collins, 1982); samples were mixed up after 6 and 22 h during this incubation period. The non-solubilized residues were subsequently washed with hot water and then with acetone in order to extract any fat before determining the insoluble organic residue as having a difference in residue before and after combustion.

After *in vitro* procedure, results of *in vitro* and calculated *in vivo* (*in vivo*$_{calcul}$) digestibilities of OM were obtained. *In vivo*$_{calcul}$ values of the digestibility of OM were calculated using predicted regression equations $y = 27.01 + (0.58 \times$ *in vitro* cellulase digestibility of OM) for red clover and $y = 10.07 + (0.79 \times$ *in vitro* cellulase digestibility of OM) for lucerne (unpublished equations originated from authors' databases).

### *In vivo* procedure

At each sampling, time chopped fresh samples ($n = 24$) were frozen until the *in vivo* trials. The *in vivo* metabolic trials were performed on four wethers (Merino breed, live weight 83 ± 9 kg) stabled in balance crates according to Vencl (1985). The duration of 17 days for each trial was divided into two periods: 10 days for the adjustment and 7 days for the main experimental period. The feed ration was offered twice a day, at 6 a.m. and at 6 p.m. The animals had free access to drinking water.

During the main *in vivo* experimental period, feeding intake and the amount of residual feedstuff and faeces were measured on a daily basis. The *in vivo* sheep digestibility of nutrients was calculated as

$$\text{Digestibility} = ((A - B)/A)$$

where:

A = average daily intake of nutrients

B = average quantity of undigested nutrients excreted

### Energy calculations

Total heating value (gross energy; GE) was measured using a calorimeter IKA C 5000 control (IKA-Werke GmbH & Co. KG, Staufen, Germany). Digestible energy (DE), metabolizable energy (ME), net energy of lactation (NEL), and net energy of growth (NEG) were calculated according to Sommer et al. (1994) by the following equations 1–4:

$$\text{DE (MJ/kg)} = \text{GE} \times \text{coefficient of } \textit{in vivo} \text{ sheep digestibility of energy} \quad (1)$$

$$\text{ME (MJ/kg)} = (\text{coefficient of } \textit{in vivo} \text{ sheep digestibility of CP} \times 0.00137) + (\text{coefficient of } \textit{in vivo} \text{ digestibility of OM} \times 0.01504) \quad (2)$$

$$\text{NEL (MJ/kg)} = \text{ME} \times (0.463 + 0.24 \times (\text{ME/GE})) \quad (3)$$

$$\text{NEG (MJ/kg)} = \text{ME} \times [((0.554 + (0.287 \times (\text{ME/GE}))) \times (0.006 + (0.780 \times (\text{ME/GE}))) \times 1.5)/((0.006 + (0.780 \times (\text{ME/GE}))) + (0.554 + (0.287 \times (\text{ME/GE}))) \times 0.5)] \quad (4)$$

### Statistical analysis

Statistical analysis of the experiment was performed using the statistical programme of SAS (Statistical Analysis System, Version 9.1, 2003). Correlation coefficients between variables were computed using PROC CORR and multiple regression analyses of determined variables were computed using the General Linear Models (GLM) standard procedure. The quality of the model for prediction equations to express fibre fractions in relation to the effect of vegetation period and growth was described with adjusted *R*-squared, multiple *R*-squared, and *P*-value. For comparison of the prediction equations it is better to use the modified value of adjusted *R*-squared, because it penalizes the number of variables used in the model.

Multiple regression equations for prediction of energy values (GE, DE, ME, NEL, and NEG) and digestibility of OM (*in vitro*, *in vivo*) were based on chemical analyses, type of forage (red clover or lucerne), and number of growth (first growth, second growth, third growth). Treatment means were compared by the Scheffe test at $P < 0.05$.

## RESULTS AND DISCUSSION

The influence of season (growth time) and maturity stage on chemical composition (Table 3)

Table 3. Chemical composition (g/kg DM) of red clover and lucerne forages

| Feed | DM | OM | EE | CP | CF | NSC | NDF | ADF | ADL |
|---|---|---|---|---|---|---|---|---|---|
| **Red clover** | | | | | | | | | |
| 1[I] | 154.7 | 854.7 | 24.2 | 218.8 | 181.5 | 234.2 | 377.5 | 258.0 | 66.7 |
| 2[I] | 190.1 | 896.4 | 24.1 | 211.1 | 181.4 | 286.1 | 375.1 | 276.8 | 70.1 |
| 3[I] | 180.3 | 908.8 | 22.3 | 179.9 | 218.8 | 285.7 | 420.9 | 310.0 | 77.8 |
| 4[I] | 172.9 | 892.7 | 21.3 | 177.4 | 237.1 | 276.0 | 418.0 | 293.8 | 49.5 |
| 5[II] | 130.3 | 872.5 | 24.3 | 207.8 | 219.2 | 202.2 | 438.2 | 308.7 | 88.4 |
| 6[II] | 137.3 | 864.8 | 22.7 | 213.9 | 202.9 | 232.8 | 395.4 | 272.3 | 67.1 |
| 7[II] | 159.4 | 901.2 | 23.7 | 197.6 | 230.0 | 271.7 | 408.2 | 296.2 | 83.1 |
| 8[II] | 157.0 | 861.0 | 22.2 | 181.6 | 236.4 | 245.8 | 411.4 | 307.7 | 62.5 |
| 9[III] | 141.4 | 879.0 | 19.8 | 202.7 | 260.9 | 211.5 | 445.0 | 351.0 | 97.5 |
| 10[III] | 140.4 | 871.4 | 22.1 | 209.2 | 240.7 | 211.3 | 428.8 | 317.7 | 68.5 |
| 11[III] | 196.1 | 885.0 | 21.3 | 187.9 | 258.4 | 231.2 | 444.6 | 346.0 | 93.7 |
| 12[III] | 241.0 | 878.4 | 23.5 | 180.7 | 247.6 | 257.5 | 416.7 | 352.2 | 89.2 |
| Average | 166.7 | 880.5 | 22.6 | 197.4 | 226.2 | 245.5 | 415.0 | 307.5 | 76.2 |
| Minimum | 130.3 | 854.7 | 19.8 | 177.4 | 181.4 | 202.2 | 375.1 | 258.0 | 49.5 |
| Maximum | 241.0 | 908.8 | 24.3 | 218.8 | 260.9 | 286.1 | 445.0 | 352.2 | 97.5 |
| SEM | 30.2 | 16.1 | 1.3 | 14.5 | 25.5 | 28.5 | 22.3 | 29.5 | 13.8 |
| **Lucerne** | | | | | | | | | |
| 13[I] | 150.5 | 861.2 | 20.1 | 201.0 | 230.5 | 257.8 | 382.3 | 281.1 | 54.7 |
| 14[I] | 247.6 | 865.3 | 18.5 | 197.7 | 237.7 | 328.3 | 320.8 | 251.3 | 69.8 |
| 15[I] | 195.2 | 873.3 | 19.6 | 177.2 | 275.7 | 299.0 | 377.5 | 299.5 | 91.3 |
| 16[I] | 237.1 | 890.3 | 20.6 | 145.5 | 313.4 | 280.1 | 444.1 | 344.8 | 83.7 |
| 17[II] | 177.6 | 875.3 | 19.1 | 217.2 | 243.8 | 259.9 | 379.1 | 306.8 | 77.4 |
| 18[II] | 215.1 | 875.2 | 18.3 | 201.9 | 275.0 | 247.2 | 407.8 | 353.3 | 68.3 |
| 19[II] | 183.5 | 897.0 | 15.2 | 167.7 | 330.1 | 256.8 | 457.3 | 402.0 | 93.6 |
| 20[II] | 198.3 | 887.0 | 16.5 | 201.7 | 303.6 | 235.6 | 433.2 | 345.4 | 89.6 |
| 21[III] | 197.0 | 876.0 | 20.2 | 200.3 | 279.6 | 258.7 | 396.8 | 333.1 | 76.8 |
| 22[III] | 195.6 | 873.3 | 16.4 | 175.2 | 316.5 | 234.5 | 447.2 | 368.3 | 93.1 |
| 23[III] | 261.9 | 892.7 | 18.4 | 165.7 | 318.9 | 274.9 | 433.7 | 353.8 | 98.7 |
| 24[III] | 230.3 | 895.3 | 17.1 | 165.9 | 347.5 | 232.3 | 480.0 | 360.7 | 88.1 |
| Average | 207.5 | 880.2 | 18.3 | 184.8 | 289.4 | 263.8 | 413.3 | 333.3 | 82.1 |
| Minimum | 150.5 | 861.2 | 15.2 | 145.5 | 230.5 | 232.3 | 320.8 | 251.3 | 54.7 |
| Maximum | 261.9 | 897.0 | 20.6 | 217.2 | 347.5 | 328.3 | 480.0 | 402.0 | 98.7 |
| SEM | 30.6 | 11.4 | 1.6 | 20.4 | 36.6 | 27.1 | 42.4 | 39.9 | 12.4 |

DM = dry matter, OM = organic matter, EE = ether extract, CP = crude protein, CF = crude fibre, NSC = nonstructural carbohydrates, NDF = neutral-detergent fibre, ADF = acid-detergent fibre, ADL = acid-detergent lignin, SEM = standard error of the mean

[I]first growth, [II]second growth, [III]third growth

was detected at the significance level $P < 0.05$. Changes in chemical composition of the investigated feedstuffs across the season (diverged to the first, second and third growth) were generally in agreement with the Czech feed table (Sommer et al., 1994). The DM within the range reported forages was from 130.3 to 261.9 g/kg. The OM varied from 854.7 to 908.8 and from 861.2 to 897.0 g/kg DM in red clover and in lucerne forages, respectively. On the average, in accordance with Belyea et al. (1999), the CP content was higher for the first two vegetative stages than for the third and fourth vegetative stages of the growths. Protein value of forages is related to the stage of maturity (Rinne

and Nykänen, 2000; Písaříková et al., 2007). The CP content in red clover feed varied from 177.4 to 218.8 g/kg DM and in lucerne feed it ranged from 145.5 to 217.2 g/kg DM. Content of NDF was between 375.1 and 445.0 g/kg DM in red clover and between 320.8 and 480.0 g/kg DM in lucerne. Content of ADF varied from 258.0 to 352.2 g/kg DM in red clover and from 251.3 to 402.0 g/kg DM in lucerne. The ADL generally increased with the growing maturity stage of growths for both forages (minimum values were 49.5 and 54.7 g/kg DM and maximum values were 97.5 and 98.7 g/kg DM for red clover and lucerne, respectively). In the present experiment, NDF (average across ma-

Table 4. Energy values (MJ/kg DM) of red clover and lucerne forages

| Feed | GE | DE | ME | NEL | NEG |
|---|---|---|---|---|---|
| **Red clover** | | | | | |
| $1^{I}$ | 18.08 | 11.81 | 9.04 | 5.27 | 5.01 |
| $2^{I}$ | 18.42 | 13.60 | 10.50 | 6.30 | 6.26 |
| $3^{I}$ | 18.02 | 12.90 | 10.37 | 6.23 | 6.22 |
| $4^{I}$ | 17.74 | 11.84 | 9.57 | 5.67 | 5.53 |
| $5^{II}$ | 17.56 | 12.69 | 10.11 | 6.08 | 6.07 |
| $6^{II}$ | 17.62 | 12.71 | 9.97 | 5.97 | 5.93 |
| $7^{II}$ | 18.88 | 13.17 | 9.87 | 5.81 | 5.61 |
| $8^{II}$ | 18.03 | 12.75 | 9.58 | 5.66 | 5.50 |
| $9^{III}$ | 18.07 | 10.91 | 8.64 | 4.99 | 4.66 |
| $10^{III}$ | 18.39 | 11.98 | 9.05 | 5.26 | 4.97 |
| $11^{III}$ | 18.26 | 12.04 | 9.25 | 5.41 | 5.16 |
| $12^{III}$ | 18.39 | 12.55 | 9.28 | 5.42 | 5.16 |
| Average | 18.12 | 12.41 | 9.60 | 5.67 | 5.50 |
| Minimum | 17.56 | 10.91 | 8.64 | 4.99 | 4.66 |
| Maximum | 18.88 | 13.60 | 10.50 | 6.30 | 6.26 |
| SEM | 0.36 | 0.69 | 0.55 | 0.40 | 0.51 |
| **Lucerne** | | | | | |
| $13^{I}$ | 17.46 | 11.86 | 9.75 | 5.82 | 5.75 |
| $14^{I}$ | 17.53 | 11.91 | 9.51 | 5.64 | 5.52 |
| $15^{I}$ | 17.87 | 11.98 | 9.40 | 5.54 | 5.36 |
| $16^{I}$ | 17.89 | 11.02 | 8.82 | 5.12 | 4.84 |
| $17^{II}$ | 18.18 | 11.58 | 8.94 | 5.19 | 4.90 |
| $18^{II}$ | 18.73 | 11.99 | 8.84 | 5.10 | 4.73 |
| $19^{II}$ | 18.75 | 11.28 | 8.57 | 4.91 | 4.50 |
| $20^{II}$ | 18.73 | 10.77 | 8.19 | 4.65 | 4.19 |
| $21^{III}$ | 17.19 | 11.43 | 9.37 | 5.56 | 5.45 |
| $22^{III}$ | 18.15 | 11.49 | 9.56 | 5.64 | 5.46 |
| $23^{III}$ | 18.30 | 11.83 | 8.88 | 5.14 | 4.83 |
| $24^{III}$ | 18.25 | 11.63 | 8.29 | 4.74 | 4.34 |
| Average | 18.09 | 11.56 | 9.01 | 5.26 | 4.99 |
| Minimum | 17.19 | 10.77 | 8.19 | 4.65 | 4.19 |
| Maximum | 18.75 | 11.99 | 9.75 | 5.84 | 5.75 |
| SEM | 0.50 | 0.37 | 0.49 | 0.37 | 0.49 |

GE = gross energy, DE = digestible energy, ME = metabolizable energy, NEL = net energy of lactation, NEG = net energy of growth, SEM = standard error of the mean

$^{I}$first growth, $^{II}$second growth, $^{III}$third growth

turities 415.0 and 413.3 g/kg DM), ADF (average across maturities 307.5 and 333.3 g/kg DM), and ADL (average across maturities 76.2 and 82.1 g/kg DM) concentrations increased with the increasing maturity of red clover and lucerne forages, respectively. Increasing concentrations of NDF, ADF, and ADL across maturities were similar to those found by several authors (Hoffman et al., 1993; Coblentz et al., 1998; Belyea et al., 1999; Elizalde et al., 1999; Koukolová et al., 2010).

The energy values in MJ/kg DM are presented in Table 4. Estimates of energy values (GE, DE, ME, NEL, and NEG) based on calorimeter determination were close to the typical tabulated values for energy in those feeds (Arieli et al., 1999). There was no difference between red clover and lucerne for GE, DE, ME, NEL, and NEG. The GE, DE, ME, NEL, and NEG values for red clover were on average 18.12, 12.41, 9.60, 5.67, and 5.50 MJ/kg DM and in the same order for lucerne they were 18.09, 11.56, 9.01, 5.26, and 4.99 MJ/kg DM. Energy is provided from the cell wall carbohydrate (fibre), non fibre carbohydrates (starch and sugar), protein, and fat (VandeHaar and St-Pierre, 2006). Metabolizable energy (ME) content of red clover and lucerne generally decreased as the growing season progressed (Table 4). This is in accordance with results of forage declared by Mountousis et al. (2011). Our results showed that the energy values of DE, ME, NEL, and NEG were negatively correlated with fibre concentration (NDF, ADF, ADL, and CF). These energy values (DE, ME, NEL, and NEG) were correlated mainly to the CF, with correlation coefficients ($r$) ranging from −0.666 to −0.708 ($P < 0.001$) (not tabulated results).

Values for *in vivo* sheep digestibility of individual nutrients (DM, OM, EE, CP, CF, and GE), *in vitro* cellulase OM digestibility, and calculated *in vivo* digestibility of OM (*in vivo*$_{calcul}$) of red clover and lucerne are given in Table 5. In general, the averaged digestibility of those individual nutrients was higher for red clover than for lucerne, except for CP. Digestibility of all the nutrients tended to decrease with the increasing maturity of red clover and lucerne, but significant ($P < 0.05$) influence of maturity stage on *in vivo* sheep digestibility was declared only for DM, CP, CF, and GE.

The average of *in vivo* sheep OM digestibility was 0.710 and 0.666 for red clover and lucerne, respectively. Comparing the previous *in vitro* method (*in vitro* cellulase method) with the *in vivo* sheep, OM digestibility seems to be in agreement with levels and tendencies for the effect of the maturity stage on OM digestibility. *In vitro* OM digestibility was generally higher than the *in vivo* OM digestibility, and noted the decreasing effect ($P < 0.05$) with increasing forage DM content.

*In vivo* digestibility of CP ranged from 0.689 to 0.864 and from 0.760 to 0.827 for red clover and lucerne, respectively. As in the experiment of Ribeiro et al. (2005), the digestibility of CP tended to be higher than the digestibility of OM. Values for *in vivo* digestibility of CF differ between the types of forage ($P < 0.01$). In red clover, a higher coefficient of digestibility for CF (0.531 on average) than in lucerne (0.422 on average) was found. *In vivo* digestibility of GE of red clover and lucerne varied from 0.604 to 0.738 and from 0.575 to 0.679, respectively.

The *in vitro* and *in vivo*$_{calcul}$ digestibilities of OM averaged 0.754 and 0.708 for red clover and 0.717 and 0.667 for lucerne, respectively (Table 5). The results of the *in vitro* technique were confirmed by the *in vivo* data of red clover and lucerne forages. *In vitro* cellulase OM digestibility results for red clover and lucerne ranged from 0.696 to 0.833 and from 0.648 to 0.797, respectively. *In vitro* cellulase OM digestibility achieved similar tendencies to *in vivo* OM digestibility, correlation coefficient $r = 0.686$ ($P < 0.05$) for red clover and $r = 0.790$ ($P < 0.05$) for lucerne (Table 6). The *in vivo* ($P < 0.1$) and *in vitro* ($P < 0.01$) techniques showed decreasing digestibility of OM and GE as the lucerne forage matured, and similar tendencies but with no significant effect were declared for red clover forage. The variation of *in vivo* and *in vitro* digestibility of OM and GE of red clover and lucerne are given in Figures 2 and 3, respectively.

For the different maturity stages of red clover and lucerne in this experiment, the correlations between the energy values and *in vivo* sheep digestibility of individual nutrients were determined (Table 6). Significantly declared interactions ($P < 0.05$) for GE versus *in vivo* sheep digestibility of nutrients (DM, OM, EE, CF and GE) were detected only in lucerne. Rather different correlations (also negative, but not significant) between GE and *in vivo* sheep digestibility of the above mentioned nutrients of red clover feed were observed, which probably caused the differences in the DM content of red clover and lucerne forages (Table 3). *In vivo* sheep digestibility of CP of red clover was significantly ($P < 0.05$) correlated to DE ($r = 0.787$), ME ($r = 0.654$), NEL ($r = 0.629$), and NEG ($r = 0.603$).

Table 5. *In vivo* sheep digestibility of individual nutrients and energy, *in vitro* and calculated *in vivo* digestibilities of organic matter of red clover and lucerne forages

| Feed | *In vivo* digestibility | | | | | | *In vitro*[A] | *In vivo*$_{calcul}$[B] |
|---|---|---|---|---|---|---|---|---|
| | DM | OM | EE | CP | CF | GE | | |
| **Red clover** | | | | | | | | |
| 1[I] | 0.657 | 0.687 | 0.495 | 0.716 | 0.472 | 0.653 | 0.813 | 0.742 |
| 2[I] | 0.729 | 0.762 | 0.430 | 0.792 | 0.536 | 0.738 | 0.833 | 0.753 |
| 3[I] | 0.697 | 0.745 | 0.551 | 0.753 | 0.559 | 0.716 | 0.787 | 0.726 |
| 4[I] | 0.655 | 0.700 | 0.461 | 0.724 | 0.521 | 0.668 | 0.754 | 0.707 |
| 5[II] | 0.702 | 0.753 | 0.578 | 0.784 | 0.616 | 0.723 | 0.783 | 0.724 |
| 6[II] | 0.671 | 0.749 | 0.629 | 0.793 | 0.572 | 0.721 | 0.777 | 0.720 |
| 7[II] | 0.652 | 0.711 | 0.637 | 0.864 | 0.530 | 0.698 | 0.741 | 0.700 |
| 8[II] | 0.705 | 0.725 | 0.648 | 0.785 | 0.542 | 0.707 | 0.725 | 0.691 |
| 9[III] | 0.598 | 0.639 | 0.513 | 0.689 | 0.497 | 0.604 | 0.696 | 0.674 |
| 10[III] | 0.637 | 0.674 | 0.589 | 0.752 | 0.497 | 0.652 | 0.734 | 0.696 |
| 11[III] | 0.648 | 0.681 | 0.654 | 0.730 | 0.495 | 0.660 | 0.714 | 0.684 |
| 12[III] | 0.676 | 0.689 | 0.599 | 0.717 | 0.534 | 0.682 | 0.696 | 0.673 |
| Average | 0.669 | 0.710 | 0.565 | 0.758 | 0.531 | 0.685 | 0.754 | 0.708 |
| Minimum | 0.598 | 0.639 | 0.430 | 0.689 | 0.472 | 0.604 | 0.696 | 0.673 |
| Maximum | 0.729 | 0.762 | 0.654 | 0.864 | 0.616 | 0.738 | 0.833 | 0.753 |
| SEM | 0.034 | 0.036 | 0.072 | 0.046 | 0.038 | 0.038 | 0.043 | 0.025 |
| **Lucerne** | | | | | | | | |
| 13[I] | 0.690 | 0.736 | 0.505 | 0.785 | 0.559 | 0.679 | 0.797 | 0.731 |
| 14[I] | 0.675 | 0.714 | 0.363 | 0.813 | 0.490 | 0.679 | 0.785 | 0.721 |
| 15[I] | 0.665 | 0.701 | 0.424 | 0.796 | 0.505 | 0.670 | 0.741 | 0.686 |
| 16[I] | 0.622 | 0.647 | 0.429 | 0.760 | 0.428 | 0.616 | 0.703 | 0.656 |
| 17[II] | 0.628 | 0.660 | 0.454 | 0.827 | 0.341 | 0.637 | 0.756 | 0.698 |
| 18[II] | 0.631 | 0.655 | 0.421 | 0.817 | 0.395 | 0.640 | 0.716 | 0.666 |
| 19[II] | 0.600 | 0.622 | 0.285 | 0.787 | 0.394 | 0.602 | 0.670 | 0.630 |
| 20[II] | 0.573 | 0.597 | 0.341 | 0.805 | 0.304 | 0.575 | 0.686 | 0.642 |
| 21[III] | 0.665 | 0.694 | 0.673 | 0.822 | 0.438 | 0.665 | 0.725 | 0.674 |
| 22[III] | 0.629 | 0.714 | 0.508 | 0.799 | 0.477 | 0.633 | 0.696 | 0.651 |
| 23[III] | 0.629 | 0.648 | 0.471 | 0.795 | 0.397 | 0.646 | 0.679 | 0.637 |
| 24[III] | 0.628 | 0.602 | 0.454 | 0.827 | 0.341 | 0.637 | 0.648 | 0.613 |
| Average | 0.636 | 0.666 | 0.444 | 0.803 | 0.422 | 0.640 | 0.717 | 0.667 |
| Minimum | 0.573 | 0.597 | 0.285 | 0.760 | 0.304 | 0.575 | 0.648 | 0.613 |
| Maximum | 0.690 | 0.736 | 0.673 | 0.827 | 0.559 | 0.679 | 0.797 | 0.731 |
| SEM | 0.031 | 0.044 | 0.093 | 0.019 | 0.072 | 0.030 | 0.044 | 0.035 |

DM = *in vivo* sheep digestibility of dry matter, OM = *in vivo* sheep digestibility of organic matter, EE = *in vivo* sheep digestibility of ether extract, CP = *in vivo* sheep digestibility of crude protein, CF = *in vivo* sheep digestibility of crude fibre, GE = *in vivo* sheep digestibility of gross energy, SEM = standard error of the mean

[A]*in vitro* digestibility of organic matter determined using the celullase, [B]predicted *in vivo* digestibility of organic matter using *in vitro* cellulase digestibility of organic matter

[I]first growth, [II]second growth, [III]third growth

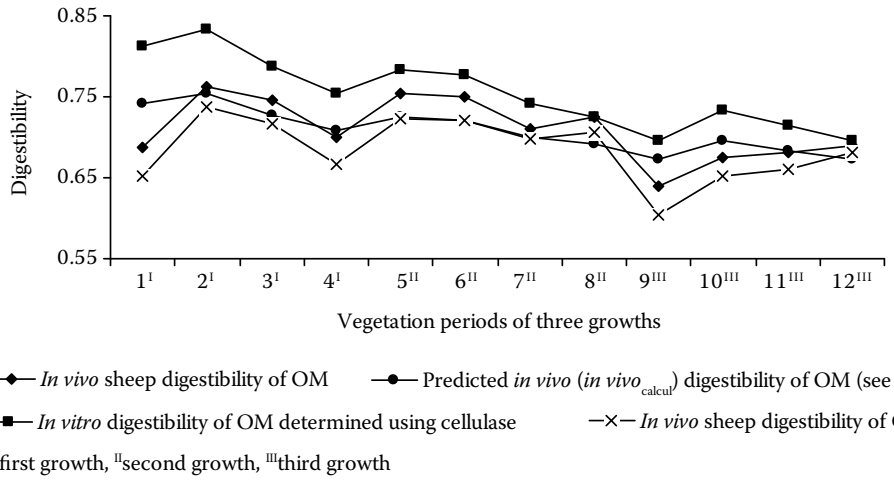


Figure 2. Digestibility of organic matter (OM) and gross energy (GE) of red clover

But in lucerne, the correlations (no significant results) between *in vivo* sheep digestibility of CP and ME, NEL, NEG were negatively correlated ($r$ = −0.112, −0.104, −0.098, respectively). Significant effect ($P < 0.05$) of *in vivo* sheep digestibility of CF on energy values (DE, ME, NEL, NEG) was found for red clover. A strong significant effect ($P < 0.0001$) of *in vivo* sheep digestibility of OM, and *in vivo* sheep digestibility of GE on energy values (DE, ME, NEL, NEG) was found for red clover. Also for lucerne, a strongly significant effect ($P < 0.0001$) of energy values (ME, NEL, and NEG) in relation to *in vivo* sheep digestibility of OM and CF (Table 6) was found.

Comprehension of the effect of the first, second, and third growth means that, in general, the observed variables of red clover and lucerne varied for different growths, although the significance of this variation is statistically unconfirmable for these dates (Table 7). A difference was found only for *in vitro* digestibility of OM ($P < 0.05$) for the first and the third growth in red clover feed. *In vivo* digestibility of OM of red clover exhibited a difference ($P < 0.05$) for the second and the third growth. In lucerne, a difference ($P < 0.05$) for GE in the first and second growth was found. In the study of Mountousis et al. (2011) GE was also affected ($P < 0.001$) by the growing season.

Prediction of the fibre fractions (NDF, ADF, and ADL) including the effect of vegetation period and growth (first growth, second growth, third growth) is in Table 8. This shows the effect of NDF, ADF, and ADL on the digestibility and utilization of nutrients. Only NDF covering the effect of vegetation period and growth variables was significantly ($P < 0.05$) different for lucerne and red clover. The prediction equations explain 50.5, 62.3, and 42.3% of the NDF, ADF, and ADL variablity, respectively. In this way it is possible to specify the fibre fractions of different growths for lucerne and red clover, separately (Table 8). These specific equations for the

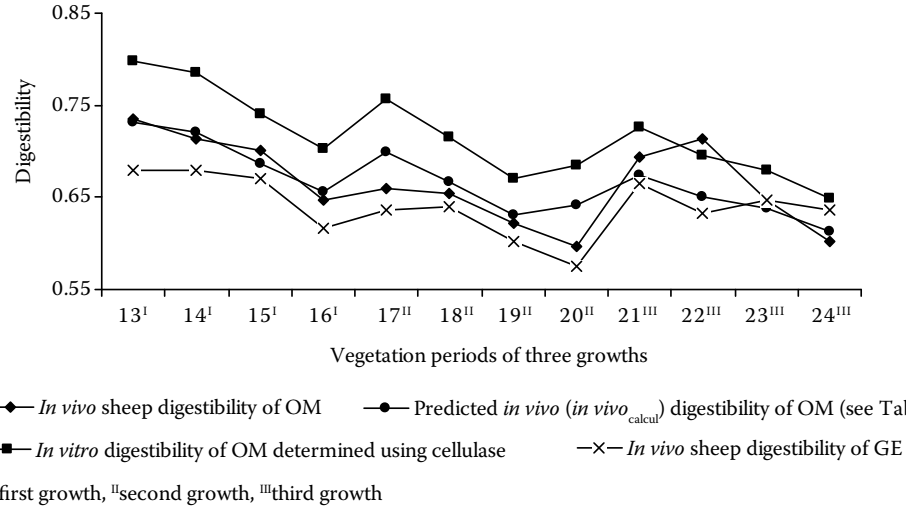


Figure 3. Digestibility of organic matter (OM) and gross energy (GE) of lucerne

Table 6. Correlation coefficients of red clover ($n$ = 12) and lucerne ($n$ = 12) forages

| Feed (units of DM) | *In vivo* sheep digestibility of nutrients | | | | |
|---|---|---|---|---|---|
| | DM | OM | EE | CP | CF |
| **Red clover** | | | | | |
| DM (g/kg) | 0.234 | –0.048 | –0.039 | –0.238 | –0.171 |
| OM (g/kg) | 0.125 | 0.202 | –0.219 | 0.237 | 0.125 |
| EE (g/kg) | 0.644* | 0.605* | –0.045 | 0.515 | 0.368 |
| CP (g/kg) | –0.086 | 0.077 | –0.201* | 0.162 | –0.059 |
| CF (g/kg) | –0.569* | –0.639 | 0.444 | –0.325 | –0.183 |
| NSC (g/kg) | 0.453 | 0.400 | –0.316 | 0.274 | 0.026 |
| NDF (g/kg) | –0.469 | –0.451 | 0.378 | –0.314 | 0.073 |
| ADF (g/kg) | –0.392 | –0.552 | 0.347 | –0.424 | –0.107 |
| ADL (g/kg) | –0.283 | –0.302 | 0.323 | –0.131 | 0.047 |
| GE (MJ/kg) | –0.139 | –0.276 | 0.134 | 0.294 | –0.465 |
| DE (MJ/kg) | 0.841** | 0.853*** | 0.131 | 0.787** | 0.573* |
| ME (MJ/kg) | 0.826** | 0.952*** | –0.099 | 0.654* | 0.726** |
| NEL (MJ/kg) | 0.826** | 0.961*** | –0.108 | 0.629* | 0.749** |
| NEG (MJ/kg) | 0.823** | 0.966*** | –0.113 | 0.603* | 0.771* |
| *In vitro*[A] | 0.588* | 0.686* | –0.534 | 0.300 | 0.249 |
| **Lucerne** | | | | | |
| DM (g/kg) | –0.124** | –0.284 | –0.120 | –0.007 | –0.225 |
| OM (g/kg) | –0.745** | –0.873** | –0.327 | –0.163 | –0.692** |
| EE (g/kg) | 0.656* | 0.497 | 0.556 | –0.157 | 0.446 |
| CP (g/kg) | 0.223 | 0.275 | 0.167 | 0.628* | –0.036 |
| CF (g/kg) | –0.652* | –0.678* | –0.163 | –0.173 | –0.485 |
| NSC (g/kg) | 0.524 | 0.442 | –0.172 | –0.208 | 0.486 |
| NDF (g/kg) | –0.669* | –0.670* | –0.080 | –0.208 | –0.497 |
| ADF (g/kg) | –0.725** | –0.636* | –0.132 | –0.134 | –0.521 |
| ADL (g/kg) | –0.618* | –0.514 | –0.231 | –0.131 | –0.432 |
| GE (MJ/kg) | –0.840** | –0.738* | –0.658* | 0.018 | –0.677* |
| DE (MJ/kg) | 0.721* | 0.551 | 0.190 | 0.293 | 0.512 |
| ME (MJ/kg) | 0.847** | 0.997*** | 0.484 | –0.112 | 0.906*** |
| NEL (MJ/kg) | 0.862** | 0.996*** | 0.503 | –0.104 | 0.907*** |
| NEG (MJ/kg) | 0.876** | 0.991*** | 0.527 | –0.098 | 0.902*** |
| *In vitro*[A] | 0.751** | 0.790* | 0.184 | 0.023 | 0.651* |

DM = dry matter, OM = organic matter, EE = ether extract, CP = crude protein, CF = crude fibre, NSC = non-structural carbohydrates, NDF = neutral-detergent fibre, ADF = acid-detergent fibre, ADL = acid-detergent lignin, GE = gross energy, DE = digestible energy, ME = metabolizable energy, NEL = net energy of lactation, NEG = net energy of growth

[A]*in vitro* digestibility of organic matter determined using the celullase

*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$

first, second, and third growth should be preferred over general equations for declared significant influences ($P < 0.05$) of the vegetation period and growths (first, second, and third growth).

Multiple regression produced the equations for each forage type (red clover, lucerne) to predict the coefficients of energy values (GE, DE, ME, NEL, and NEG) and digestibilities of OM (*in vitro*, *in vivo*)

Table 7. Scheffe test of different growth times differences for red clover ($n$ = 12) and for lucerne ($n$ = 12) forages

| Feed (units of DM) | Scheffe test | | | $R$-square | RMSE | $F$ | Prob > $F$ |
|---|---|---|---|---|---|---|---|
| | I | II | III | | | | |
| **Red clover** | | | | | | | |
| GE (MJ/kg) | 18.07 | 18.02 | 18.28 | 0.096 | 0.396 | 0.48 | 0.637 |
| DE (MJ/kg) | 12.54 | 12.83 | 11.87 | 0.334 | 0.655 | 2.26 | 0.160 |
| ME (MJ/kg) | 9.87 | 9.88 | 9.06 | 0.495 | 0.452 | 4.40 | 0.046 |
| NEL (MJ/kg) | 5.87 | 5.88 | 5.27 | 0.509 | 0.323 | 4.67 | 0.041 |
| NEG (MJ/kg) | 5.76 | 5.78 | 4.99 | 0.526 | 0.402 | 5.00 | 0.035 |
| *In vivo*[A] | 72.33[ab] | 73.46[a] | 67.07[b] | 0.588 | 2.692 | 6.43 | 0.018 |
| *In vitro*[B] | 79.67[a] | 75.63[ab] | 70.98[b] | 0.688 | 2.757 | 9.93 | 0.005 |
| **Lucerne** | | | | | | | |
| GE (MJ/kg) | 17.69[a] | 18.60[b] | 17.97[ab] | 0.589 | 0.367 | 6.44 | 0.018 |
| DE (MJ/kg) | 11.69 | 11.41 | 11.60 | 0.103 | 0.408 | 0.51 | 0.615 |
| ME (MJ/kg) | 9.87 | 9.88 | 9.06 | 0.379 | 0.444 | 2.75 | 0.117 |
| NEL (MJ/kg) | 5.87 | 5.88 | 5.27 | 0.404 | 0.326 | 3.04 | 0.098 |
| NEG (MJ/kg) | 5.37 | 4.58 | 5.02 | 0.437 | 0.423 | 3.49 | 0.076 |
| *In vivo*[A] | 69.95 | 63.34 | 66.44 | 0.378 | 3.999 | 2.74 | 0.118 |
| *In vitro*[B] | 75.64 | 70.70 | 68.72 | 0.437 | 3.815 | 3.49 | 0.076 |

GE = gross energy, DE = digestible energy, ME = metabolizable energy, NEL = net energy of lactation, NEG = net energy of growth, I = first growth, II = second growth, III = third growth, R-square = sum of square, RMSE = root mean square error, Prob = probability

[a,b]values designated by different letters in row differ significantly ($P$ < 0.05), [A]*in vivo* sheep digestibility of organic matter, [B]*in vitro* digestibility of organic matter determined using the celullase

(Table 9). For this, we assumed that all the laboratory variables are needed to characterize those energy and digestibility parameters. Data were averaged across the vegetation period and growth number, and feed was the main effect and the residual error term in the model. Significance was declared at $P$ < 0.05. There were predicted equations to estimate linear combinations of chemical composition (DM, OM, EE, CP, NDF, ADF, and ADL) and *in vitro* digestibility variance components in the model. The efficiency of the modelling for energy values (GE, DE, ME, NEL, and NEG) and digestibilities of OM (*in vivo*, *in vitro*) was estimated as 0.719, 0.742, 0.774, 0.761, 0.748, 0.707, and 0.840 for red clover forage and 0.695, 0.400, 0.912, 0.899, 0.882, 0.937, and 0.979 for lucerne forage, respectively. Prediction errors for these treatments were: for red clover 0.383 (GE), 0.706 (DE), 0.523 (ME), 0.390 (NEL), 0.508 (NEG), 0.039 (*in vivo*), and 0.030 (*in vitro*), and for lucerne 0.547 (GE), 0.577 (DE), 0.29 (ME), 0.232 (NEL), 0.335 (NEG), 0.022 (*in vivo*), and 0.011 (*in vitro*), respectively.

Quite a number of studies (e.g. Gosselink et al., 2004; Rinne et al., 2006) have compared various *in vitro* methods for predicting forage OM digestibility. A variable performance of the *in vitro* methods can be influenced by several factors. Laboratory methods for estimation of digestibility parameters are difficult to standardize, therefore, the relationships between the *in vitro* methods and *in vivo* OM digestibility should be established in every laboratory and separately for different types of forages (Weiss, 1994).

## CONCLUSION

This study confirmed that the vegetative stage of different growth times affects the digestibility and energy values of observed red clover and lucerne

Table 8. Prediction equations to express the fibre fractions in relation to the effect of vegetation period (VP) and growth. Units of fibre fractions are in g/kg DM

| Prediction equations | |
|---|---|
| NDF = 331.2+56.6 × I[**Feedredclover**] + 23.0 × **VP** + 26.8 × I[growth **II**] + 47.1 × I[growth **III**] – 22.0 × **Feedredclover** × **I[VP]** | |
| Adjusted *R*-squared = 0.5048, multiple *R*-squared = 0.6124, *P*-value = $2.56^{e-03}$ | |
| Equations expressing NDF for red clover and lucerne for different growths, separately: | |
| Red clover | $NDF^{I} = 387.8 + VP$ |
| | $NDF^{II} = 414.6 + VP$ |
| | $NDF^{III} = 434.9 + VP$ |
| Lucerne | $NDF^{I} = 331.2 + 23 \times VP$ |
| | $NDF^{II} = 358.0 + 23 \times VP$ |
| | $NDF^{III} = 378.3 + 23 \times VP$ |
| ADF = 266.5 – 9.5 × I[Feedredclover] + 11.1 × **VP** + 57.7 × I[growth **II**] +59.8 × I[growth **III**] – 46.1 × Feedredclover × I[growth II] – 2.7 × Feedredclover × I[growth III] | |
| Adjusted *R*-squared = 0.6232, multiple *R*-squared = 0.7215, *P*-value = $5.373^{e-04}$ | |
| Equations expressing ADF for red clover and lucerne for different growths, separately: | |
| Red clover | $ADF^{I} = 276.0 + 11.1 \times VP$ |
| | $ADF^{II} = 268.6 + 11.1 \times VP$ |
| | $ADF^{III} = 314.1 + 11.1 \times VP$ |
| Lucerne | $ADF^{I} = 266.5 + 11.1 \times VP$ |
| | $ADF^{II} = 324.2 + 11.1 \times VP$ |
| | $ADF^{III} = 326.3 + 11.1 \times VP$ |
| ADL = 55.9 + 20.4 ×I[Feedredclover] + 7.0 × **VP** + 8.3 × I[growth **II**]+17.8 × I[growth **III**] – 10.5 × **Feedredclover** × **I[VP]** | |
| Adjusted *R*-squared = 0.4231, multiple *R*-squared = 0.5485, *P*-value = $8.805^{e-03}$ | |
| Equations expressing ADL for redclover and lucerne for different growths, separately: | |
| Red clover | $ADL^{I} = 76.3 – 3.5 \times VP$ |
| | $ADL^{II} = 84.6 – 3.5 \times VP$ |
| | $ADL^{III} = 94.1 – 3.5 \times VP$ |
| Lucerne | $ADL^{I} = 55.9 + 7 \times VP$ |
| | $ADL^{II} = 64.2 + 7 \times VP$ |
| | $ADL^{III} = 73.7 + 7 \times VP$ |

NDF = neutral-detergent fibre, ADF = acid-detergent fibre, ADL = acid-detergent lignin
$^{I}$first growth, $^{II}$second growth, $^{III}$third growth
statistically significant variables ($P < 0.05$) are given in bold
I[variable = *x*] = variable is calculated if the value of variable is *x*
VP takes the values 1–4 (see Table 1)

forage. With increased maturity, a decreasing inclination for OM digestibility (*in vitro* and *in vivo*) as for energy measurements (GE, DE, ME, NEL, and NEG) was discovered for both forages. Specific fibre fractions (NDF, ADF, ADL) equations for the first, second, and third growth should be preferred over general equations for declared significances of the vegetation period and growth time. GE, DE, ME, NEL, NEG, and OM digestibility (*in vivo* and *in vitro*) in red clover and lucerne was better described by regression equations, different from growth times, than by overall forage equations.

## Acknowledgement

The authors are much obliged to Vlasta Hladká and Jana Forejtová for kind help and technical facility.

Table 9. Multiple regression equations to express energy values, *in vivo* organic matter (OM) digestibility, and *in vitro* OM digestibility based on chemical compositions and *in vitro* OM digestibility

| Feed (units of DM) | Chemical composition and *in vitro* digestibility (g/kg) | | | | | | | | | *R*-square | RMSE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Intercept | DM | OM | EE | CP | NDF | ADF | ADL | *in vitro*[B] | | |
| **Red clover model expression** | | | | | | | | | | | |
| GE MJ/kg) | 7.5216 | –0.0010 | 0.0186 | 0.1139 | 0.0145 | –0.0138 | 0.0063 | –0.0045 | –9.1979 | 0.7193 | 0.3825 |
| DE (MJ/kg) | –4.0568 | –0.0135 | 0.0184 | 0.4209 | –0.0218 | –0.0245 | 0.0191 | –0.0036 | 2.4983 | 0.7422 | 0.7057 |
| ME (MJ/kg) | –6.4692 | –0.0097 | 0.0130 | 0.1871 | –0.0224 | –0.0083 | 0.0091 | 0.0017 | 9.2139 | 0.7742 | 0.5232 |
| NEL (MJ/kg) | –5.2686 | –0.0069 | 0.0081 | 0.1265 | –0.0170 | –0.0051 | 0.0064 | 0.0015 | 7.3323 | 0.7607 | 0.3902 |
| NEG (MJ/kg) | –7.1331 | –0.0088 | 0.0084 | 0.1479 | –0.0227 | –0.0054 | 0.0075 | 0.0024 | 10.067 | 0.7481 | 0.5082 |
| *In vivo*[A] (g/kg) | 0.2730 | –0.0006 | 0.0001 | 0.0134 | –0.0018 | –0.0006 | 0.0007 | 0.0001 | 0.6970 | 0.7071 | 0.0392 |
| *In vitro*[B] (g/kg) | 0.0144 | 0.0005 | 0.0006 | 0.0038 | 0.0010 | 0.0009 | –0.0017 | 0.0001 | | 0.8403 | 0.0296 |
| **Lucerne model expression** | | | | | | | | | | | |
| GE (MJ/kg) | –14.948 | 0.0050 | 0.0230 | –0.1542 | 0.0111 | 0.0053 | 0.0064 | 0.0052 | 10.829 | 0.6950 | 0.5472 |
| DE (MJ/kg) | 44.879 | 0.0008 | –0.0258 | 0.0006 | –0.0069 | –0.0078 | 0.0011 | –0.0096 | –8.2093 | 0.4002 | 0.5774 |
| ME (MJ/kg) | 44.167 | 0.0007 | –0.0472 | 0.0376 | –0.0102 | –0.0010 | 0.0067 | 0.0117 | 6.5388 | 0.9117 | 0.2898 |
| NEL (MJ/kg) | 32.905 | 0.0001 | –0.0350 | 0.0328 | –0.0080 | –0.0012 | 0.0042 | 0.0074 | 3.5359 | 0.8993 | 0.2317 |
| NEG (MJ/kg) | 41.961 | –0.0004 | –0.0452 | 0.0539 | –0.0108 | –0.0018 | 0.0044 | 0.0088 | 3.4754 | 0.8824 | 0.3345 |
| *In vivo*[A] (g/kg) | 4.0512 | 0.0001 | –0.0044 | 0.0025 | –0.0009 | –0.0001 | 0.0005 | 0.0008 | 0.4920 | 0.9365 | 0.0221 |
| *In vitro*[B] (g/kg) | 1.7456 | –0.0004 | –0.0006 | 0.0016 | –0.0004 | –0.0006 | –0.0002 | –0.0007 | | 0.9791 | 0.0110 |

DM = dry matter, OM = organic matter, EE = ether extract, CP = crude protein, NDF = neutral-detergent fibre, ADF = acid-detergent fibre, ADL = acid-detergent lignin, GE = gross energy, DE = digestible energy, ME = metabolizable energy, NEL = net energy of lactation, NEG = net energy of growth, RMSE = residual standard error, *R*-square = multiple *R*-squared

[A]*in vivo* sheep digestibility of organic matter

[B]*in vitro* digestibility of organic matter determined using the cellulase

## REFERENCES


AOAC (2005): Official Methods of Analysis. 18th Ed. AOAC International, Gaithersburg, USA.

Arieli A., Shahar K., Mabjeesh S.J., Zamwel S., Sklan D. (1999): Estimation of the digestible energy of ruminant feedstuffs by the combined bag technique. Journal of Dairy Science, 86, 566–573.

Bal M.A., Coors J.G., Shaver R.D. (1997): Impact of the maturity of corn for use as silage in the diets of dairy cows on intake, digestion, and milk production. Journal of Dairy Science, 80, 2497–2503.

Bargo F., Muller L.D., Kelver E.S., Delahoy J.E. (2003): Invited review: Production and digestion of supplemented dairy cows on pasture. Journal of Dairy Science, 86, 1–42.

Baumann T.A., Lardy G.P., Caton J.S., Anderson V.L. (2004): Effect of energy source and ruminally degradable protein addition on performance of lactating beef cows and digestion characteristics of steers. Journal of Animal Science, 82, 2667–2678.

Belyea R., Restrepo R., Martz F., Ellersieck M. (1999): Effect of year and cutting on equations for estimating net energy of alfalfa forage. Journal of Dairy Science, 82, 1943–1949.

Bosch M.W., Tamminga S., Post G., Leffering C.P., Muylaert J.M. (1992): Influence of stage of maturity of grass silages on digestion processes in dairy cows. 1. Composition, nylon bag degradation rates, fermentation characteristics, digestibility and intake. Livestock Production Science, 32, 245–264.

Buxton D.R. (1996): Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Animal Feed Science and Technology, 59, 37–49.

Coblentz W.K., Fritz J.O., Fick W.H., Cochran R.C., Shirley J.E. (1998): *In situ* dry matter, nitrogen, and fiber degradation of alfalfa, red clover, and eastern gamagrass at four maturities. Journal of Dairy Science, 81, 150–161.

Dowman M.G., Collins F.C. (1982): The use of enzymes to predict the digestibility of animal feeds. Journal of the Science of Food and Agriculture, 33, 689–696.

Drackley J.K., Donkin S.S., Reynolds C.K. (2006): Major advances in fundamental dairy cattle nutrition. Journal of Dairy Science, 89, 1324–1336.

Dubbs T.M., Vanzant E.S., Kitts S.E., Bapst F.F., Fieser B.G., Howlett C.M. (2003): Characterization of season and sampling method effects on measurement of forage quality in fescue-based pastures. Journal of Animal Science, 81, 1308–1315.

Elizalde J.C., Merchen N.R., Faulkner D.B. (1999): *In situ* dry matter and crude protein degradation of fresh forages during the spring growth. Journal of Dairy Science, 82, 1978–1990.

Fick G.W., Mueller S.C. (1989): Alfalfa quality, maturity, and mean stage of development. Cornell Cooperative Extension, Information Bulletin, 217, 12.

Gallardo M.R., Castillo A.R., Bargo F., Abdala A.A., Maciel M.G., Perez-Monti H., Castro H.C., Castelli M.E. (2005): Monensin for lactating dairy cows grazing mixed alfalfa pasture and supplemented with partial mixed ration. Journal of Dairy Science, 88, 644–652.

Gosselink J.M.J., Dulphy J.P., Poncet C., Jailler M., Tamminga S., Cone J.W. (2004): Prediction of forage digestibility in ruminants using *in situ* and *in vitro* techniques. Animal Feed Science and Technology, 115, 227–246.

Harazim J., Pavelek L., Čerešňáková Z., Homolka P., Třináctý J., Jambor V., Pozdíšek J., Zeman L. (1999): Determination of degradability of feed crude protein and amino acids in the rumen using the method *in situ*, nylon bag. In: Proc. International Scientific Workshop Determination of the Use of Nutrients in Ruminants. Opava, Czech Republic, 115–118.

Hindrichsen I.K., Kreuzer M., Madsen J., Bach Knudsen K.E. (2006): Fiber and lignin analysis in concentrate, forage, and feaces: detergent versus enzymatic-chemical method. Journal of Dairy Science, 89, 2168–2176.

Hoffman P.C., Sievert S.J., Shaver R.D., Welch D.A., Combs D.K. (1993): *In situ* dry matter, protein, and fiber degradation of perennial forages. Journal of Dairy Science, 76, 2632–2643.

Homolka P. (1994): Prediction of bulk fodder organic matter digestibility by the enzyme cellulose. Czech Journal of Animal Science, 39, 599–604.

Hunt C.W., Kezar W., Vinande R. (1989): Yield, chemical composition and ruminal fermentability of corn whole plant, ear, and stover as affected by maturity. Journal of Production Agriculture, 2, 357–361.

Jančík F., Koukolová V., Homolka P. (2010): Ruminal degradability of dry matter and neutral detergent fibre of grasses. Czech Journal of Animal Science, 55, 359–371.

Jančík F., Koukolová V., Kubelková P., Čermák B. (2009): Effects of grass species on ruminal degradability of silages and prediction of dry matter effective degradability. Czech Journal of Animal Science, 54, 315–323.

Johnson D.E., Ferrell C.L., Jenkins T.G. (2003): The history of energetic efficiency research: Where have we been and where are we going? Journal of Animal Science, 81 (Suppl. 1), E27–E38.

Koukolová V., Homolka P., Koukol O., Jančík F. (2010): Nutritive value of *Trifolium pratense* L. for ruminants estimated from *in situ* ruminal degradation of neutral detergent fibre and *in vivo* digestibility of organic matter and energy. Czech Journal of Animal Science, 55, 372–381.

Mountousis I., Dotas V., Stanogias G., Papanikolaou K., Roukos C., Liamadis D. (2011): Altitudinal and seasonal variation in herbage composition and energy and protein content of grasslands on Mt Varnoudas, NW Greece. Animal Feed Science and Technology, 164, 174–183.

Ohlsson C., Wedin W.F. (1989): Phenological staging schemes for predicting red clover quality. Crop Science, 29, 416–420.

Písaříková B., Peterka J., Trčková M., Moudrý J., Zralý Z., Herzig I. (2007): The content of insoluble fibre and crude protein value of the aboveground biomass of *Amaranthus cruentus* and *A. hypochndriacus*. Czech Journal of Animal Science, 52, 348–353.

Pozdíšek J., Vaculová K. (2008): Study of wheat (*Triticum aestivum* L.) quality for feeding ruminants using *in vitro* and *in vivo* methods. Czech Journal of Animal Science, 53, 253–264.

Ribeiro C.V.D.M., Karnati S.K.R., Eastridge M.L. (2005): Biohydrogenation of fatty acids and digestibility of fresh alfalfa or alfalfa hay plus sucrose in continuous culture. Journal of Dairy Science, 88, 4007–4017.

Rinne M., Nykänen A. (2000): Timing of primary growth harvest affects the yield and nutritive value of timothy-red clover mixtures. Agricultural and Food Science in Finland, 9, 121–134.

Rinne M., Olt A., Nousiainen J., Seppälä A., Tuori M., Fraser M.D., Huhtanen P. (2006): Prediction of legume silage digestibility from various laboratory methods. Grass and Forage Science, 61, 354–362.

Scarbrough D.A., Coblentz W.K., Coffey K.P., Turner J.E., Davis G.V., Kellogg D.W., Hellwig D.H. (2001): Effects of calendar date and summer management on the *in situ* dry matter and fiber degradation of stockpiled forage from bermudagrass pastures. Journal of Animal Science, 79, 3158–3169.

Skinner H.R., Moore K.J. (2007): Growth and development of forage plant. In: Barnes R.F., Nelson C.J., Moore K.J., Collins M. (eds): Forages: The Science of Grassland Agriculture. 6th Ed. Wiley-Blackwell, Ames, USA, 53–66.

Sommer A., Čerešňáková Z., Frydrych Z., Králík O., Králíková Z., Krása A., Pajtáš M., Petrikovič P., Pozdíšek J., Šimek M., Třináctý J., Vencl B., Zeman L. (1994): Tables of nutrient requirements and nutritive value of feeds for ruminants. Czech Academy of Agricultural Sciences, Prague, Czech Republic. (in Czech)

Terry R.A., Tilley J.M.A. (1964): The digestibility of the leaves and stems of perrenial ryegrass, cockfoot, timothy, tall fescue, lucerne and saifoin, as measured by an *in vitro* procedure. Journal of the British Grassland Society, 19, 363–372.

Tyrolová Y., Výborná A. (2008): Effect of the stage of maturity on the leaf percentage of lucerne and the effect of additives on silage characteristics. Czech Journal of Animal Science, 53, 330–335.

Van Soest P.J. (1996): Environment and forage quality. In: Proc. 58th Cornell Nutrition Conference for Feed Manufacturers. Cornell University, Ithaca, USA, 1.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991): Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583–3597.

VandeHaar M.J., St-Pierre N. (2006): Major advances in nutrition: Relevance to the sustainability of the dairy industry. Journal of Dairy Science, 89, 1280–1291.

Vencl B. (1985): Metodology of *in vivo* digestibility determination and groups trials using ruminants. Research Institute of Animal Production Uhříněves, Prague, Czech Republic. (in Czech)

Waldo D.R. (1986): Effect of forage quality on intake and forage-concentrate interactions. Journal of Dairy Science, 69, 617–631.

Weiss W.P. (1994): Estimation of digestibility of forages by laboratory methods. In: Fahey Jr. G.C. (ed.): Forage Quality, Evaluation and Utilization. American Society of Agronomy, Madison, USA, 644–681.

Wilson J.R., Deinum B., Engels F.M. (1991): Temperature effects on anatomy and digestibility of leaf and stem of tropical and temperate forage species. Netherlands Journal of Agricultural Science, 39, 31–48.

Received: 2011–02–02
Accepted after corrections: 2012–05–22

*Corresponding Author*

Doc. Ing. Petr Homolka, CSc., Ph.D., Institute of Animal Science, Přátelství 815,
104 00 Prague-Uhříněves, Czech Republic
Tel. +420 267 009 650, fax +420 267 710 779, e-mail: homolka.petr@vuzv.cz