Comparison of organic matter digestibility determined by *in vivo* and *in vitro* methods

J. Forejtová[1], F. Lád[1], J. Třináctý[2], M. Richter[2], L. Gruber[3], P. Doležal[4], P. Homolka[5], L. Pavelek[6]

[1] University of South Bohemia, Faculty of Agriculture, České Budějovice, Czech Republic
[2]Research Institute for Cattle Breeding, Ltd. Rapotín, Department Pohořelice, Czech Republic
[3] Federal Research Institute for Agriculture in Alpine Regions, BAL Gumpenstein, Austria
[4]Mendel University of Agriculture and Forestry, Brno, Czech Republic
[5]Research Institute of Animal Production, Prague-Uhříněves, Czech Republic
[6]Central Institute for Supervising and Testing in Agriculture, Opava, Czech Republic

ABSTRACT: A total of 36 samples of feed – 19 samples of hays and 17 samples of silages were used for estimation of *in vivo* and *in vitro* digestibility. The organic matter digestibility (OMD) was estimated by *in vivo* and two *in vitro* techniques (method of Tilley and Terry and two-stage pepsin-cellulase method (Pepcel)). The regression equations were calculated from the results obtained in the experiment. Tilley and Terry method provided consistent results of OMD estimation for both the groups of roughages: *in vivo* OMD = 14.7 + 0.782 × [Tilley and Terry] (n = 18; R^2 = 0.76); *in vivo* OMD = 36.3 + 0.513 × [Tilley and Terry] (n = 16; R^2 = 0.75) for hays and silages, respectively. Different accuracy was found out in Pepcel method: *in vivo* OMD = 37.0 + 0.478 × [Pepcel] (n = 19; R^2 = 0.49); *in vivo* OMD = 37.8 + 0.484 × [Pepcel] (n = 17; R^2 = 0.87) for hays and silages, respectively.

Keywords: organic matter; digestibility; *in vitro; in vivo;* Tilley and Terry; pepsin-cellulase

Abbreviation key: R^2 = coefficient of determination, RSD = residual standard deviation, RVC = residual variation coefficient, Pepcel = pepsin-cellulase method, OMD = organic matter digestibility, d.m. = dry matter

Digestibility is an important factor of the nutritive value of feed. Digestibility determines the relation between contents of nutrients and energy that are available to ruminants. Chemical composition of feed provides information about physical properties and quality of feed and is used to derive digestibility and expected performance of the ruminant receiving the feeds (Expert Committee on Animal Nutrition, 1986).

Cell content includes carbohydrates, organic acids, lipids, proteins, nitrogenous substances and most of inorganic constituents. Digestibility of organic matter includes digestible cell content and digestible cell wall content. While cell content is digestible from almost 100%, the level of cell wall degradation is different. Digestibility of organic matter has a negative correlation with NDF, ADF and hemicelluloses. A significant negative correlation was found between digestible organic matter and NDF (%) in organic matter (Čerešňáková *et al*., 1996).

The nutritive value of forages for ruminants depends on the ability of rumen microorganisms to degrade the plant cell wall and to ferment available carbohydrates. Simple prediction methods are required for practical use. Many attempts have been made to predict the nutritive value from chemical composition, using enzyme or rumen liquor. Such data are only valuable if equations are used for the calculation based on *in vivo* digestibility data for the same type of feed grown in the same environment (Vencl, 1990). A technique using rumen liquor developed by Tilley *et al*. (1961) and by Tilley

Supported by the Ministry of Education, Youth and Sport of the Czech Republic (Grant No. 122200002) and Ministry of the Agriculture of the Czech Republic (Grant No. 1/3 44 037).

and Terry (1963) has been widely used because of the smallest error of digestibility prediction. But this method can show some deviations in silage samples. Alexander and McGowan (1969) presented a study on the relationship between *in vitro* and *in vivo* digestibilities of 18 silage samples. They confirmed the inference by Raymond and Terry (1966) that the drying process is contributory to the depressed correlations found.

Vencl (1990) compared chemical and *in vitro* methods for estimation of digestible organic matter prediction. He suggested that the cellulase method, for routine use, could replace *in vitro* method. This suggestion is in accordance with Aufrere and Doreau (1988) and Andrighetto *et al.* (1992). Míka *et al.* (1982) tested *in vitro* methods for digestibility of organic matter. They compared accuracy and reproducibility, Tilley and Terry was the best method. In opposite, the pepsin-cellulase method was the least accurate among *in vitro* methods.

There are many laboratory methods for predicting digestibility of organic matter. *In vivo* method is the best method for estimation of organic matter digestibility, but it is expensive, labour and time consuming. Simpler prediction methods are required for practical use.

The aim of this study was to compare the digestibility of organic matter of hays and silages using *in vivo* and *in vitro* (Tilley and Terry, Pepcel) methods.

MATERIAL AND METHODS

A total of 36 samples of roughages with known *in vivo* digestibility of organic matter were used for verification of two *in vitro* methods. Samples were divided into two groups – hays (19 samples – 3 lucerne hay, 2 grass hay and 14 meadow hay) and silages (17 samples – 1 lucerne, 2 lucerne + clover + grass, 1 barley (whole crop), 1 brome grass, 2 grass + clover, 1 meadow grass, 3 orchard grass, 3 red clover, 1 rye grass and 2 tall fescue). Samples with known values of *in vivo* digestibility were obtained from: Federal Research Institute for Agriculture in Alpine Regions, BAL Gumpenstein, Austria; Central Institute for Supervising and Testing in Agriculture, Opava, Czech Republic; Research Institute of Animal Production, Prague-Uhříněves, Czech Republic.

Two *in vitro* methods were tested: method of Tilley and Terry and two-stage pepsin-cellulase method (Pepcel). Regression equations describing the relation between *in vivo* and two *in vitro* methods were calculated from the results obtained in the experiment.

In vivo digestibility

Organic matter digestibility (OMD) of 36 feeds was realised on mature castrated wethers according to Van Es and Van der Meer (1989) (procedure similar to Schiemann, 1981).

In vitro digestibility

In vitro digestibility of organic matter was estimated by (a) modified Tilley and Terry method and (b) by two-stage pepsin-cellulase method (Pepcel)

(a) Modified Tilley-Terry method (personal communication – Resch, R., BAL Gumpenstein, Abteilung Grünland). The rumen liquor was strained through muslin into a flask and was kept at 38.5°C saturated with CO_2. The buffer solution was prepared according to the formula of McDougall (1948). Pepsin solution was made up by dissolving 20 g of 1 : 10 000 pepsin (Sigma Aldrich) in 1 000 ml of distilled water. The samples of forage (0.5 g) were weighed into 100 ml tubes, 40 ml of the buffer solution were added, followed by 10 ml of rumen liquor and gassed with CO_2. The tubes were sealed with Parafilm with several small holes and then incubated at 38.5°C in the dark for 48 h. Blank tubes were incubated together with samples. After the end of the first incubation period, the pH value was changed to 1.5 and then 5 ml of pepsin solution were added. The tubes were incubated for 48 h. After the end of the second incubation period, the samples were filtered through filter (Machery Nagel MN 640w), dried at 104°C and weighed. Then the samples on the filters were ashed at 450°C and weighed. OMD was calculated according to the following formulas:

weight of DM (g) = weight of sample/100 × DM
weight of OM (g) = weight of DM – ash
blank (g) = weight of filter with blank – weight of filter
rest of OM (g) = weight of filter with sample – weight of filter – blank – ash of rest
OMD (%) = 100 × (1 – rest of OM/weight of OM)

Three coincident parts of rumen liquor from three animals before feeding time were mixed in

order to spread the quality of rumen liquor variability. Three liquor withdrawals with one-month interval were done and for each of these intervals the digestibility of organic matter was determined. The mean values of OMD from three intervals were calculated.

(b) Two stage pepsin-cellulase method (Pepcel) (Aufrere, 1982).

The enzymes used in the experiment were cellulase from *T. viridae* and pepsin (1 : 10 000) (Sigma Aldrich). Acetate buffer (pH 4.8): 1.36 g of sodium acetate ($CH_3COONa \times 3H_2O$) was dissolved in 500 ml of distilled water, 0.6 ml of acetic acid was added and the solution diluted to 1 litre. The pH was adjusted to 4.8 by the addition of sodium hydroxide as necessary.

Pepsin: 2 g of pepsin (1 : 10 000) (Sigma Aldrich) were dissolved in 1 litre of 0.1 M hydrochloric acid. Daisy analyser (Ankom, USA) was used for the estimation of organic matter digestibility using the pepsin-cellulase method. 0.5 g of sample (in three duplicates) was incubated at 39.5°C with 40 ml of 0.2% pepsin in diluted hydrochloric acid for 24 h and it was stirred constantly. After incubation the sample was warmed for 30 min at 80°C and it was flushed with warm distilled water. In the second stage – 40 ml of cellulase solution was added (5 000 units cellulase, litre-1 buffer) and the sample was returned to the incubator for next 24 h at 39.5°C. After incubation, the sample was flushed with warm distilled water again and finally it was flushed with acetone. The residue was dried and weighed. The residue was ignited at 520°C, cooled and reweighed so that the percentage of indigestible organic matter could be determined.

The content of cellulase digestible organic matter (CDOM) was calculated according to the following formula:

$$\text{CDOM} = \frac{100 - 10\,000 \times (\text{indigestible d.m.} - \text{ash})}{\text{quantity of sample} \times \text{d.m. (\%)} \times (1 - 0.01 \times \text{ash in d.m.})}$$

Chemical analysis

The analysis of Weende chemical fractions (crude protein, ether extract – lipids, crude fibre and ash) was carried out according to AOAC (1984). Nitrogen free extract (NFE) was calculated as follows:

$$\text{NFE} = \text{d.m.} - (\text{CP} + \text{FAT} + \text{CF} + \text{ash})$$

Statistical analysis

Mean values of nutrient contents (OM, CP, CF, EE, ASH and NFE) in hays and silages were compared by the *t*-test and differences between methods in the case of OMD were analysed using the paired *t*-test (The QC.Expert/ADSTAT package, Trilobyte, Czech Republic). The same software was used to compute the regression analysis. Distant data were eliminated by this program. Where applicable, the results were expressed as means with standard deviations (SD). Coefficient of determination (R^2), residual standard deviation (RSD) and residual variation coefficient (RVC%) were used to compare equations with literature.

RESULTS AND DISCUSSION

The chemical composition of hays and silages is summarised in Table 1 and Table 2, respectively. An insignificant difference in mean values between hays and silages was found out only in the case of CF (30.3 and 28.9% of DM, respectively; $P > 0.05$). The largest difference was obtained for NFE mean values (45.5 and 36.3% of DM, respectively; $P < 0.01$). Andrighetto *et al.* (1992) published slightly higher values of NFE for hays than for silages (46.8 and 41.5% of DM, respectively). On the other hand, Aerts *et al.* (1977) did not find any differences. In our case the values of NFE were not correctly compared because the representation of single species of fodder crops of hays and silages was not equivalent.

Mean values of OMD obtained by *in vivo* and *in vitro* methods are given in Table 3. Differences in means between these methods were influenced by the type of roughage. In the case of hay no difference was found between *in vivo* and Tilley and Terry methods. Pepcel provided lower OMD than *in vivo* and Tilley and Terry methods (58.8, 65.2 and 64.5%, respectively; $P < 0.01$). Lower OMD determined by the pepsin-cellulase method than the values obtained by Tilley and Terry method was mentioned in hay in the paper of Adrighetto *et al.* (1992). Consistent mean values of OMD in both *in vitro* methods were found in silages. These values were significantly lower ($P < 0.01$) than those found by *in vivo* method. Similarly Aerts *et al.* (1977), Aufrere and Michalet-Doreau (1988), who compared *in vitro* and *in vivo* methods.

In the case of hay, coefficients (intercept and slope) and their confidence intervals in regression equations calculated to compare *in vivo* and Tilley

Table 1. Chemical composition of hays (% in d.m.)

Hays	Organic matter	Crude protein	Crude fibre	Ether extract	Ash	Nitrogen-free extract
Lucerne hay 1	90.0	18.6	31.1	1.6	10.0	38.7
Lucerne hay 2	90.1	13.8	37.0	1.3	9.9	38.1
Lucerne hay 3	88.4	16.4	29.1	1.1	11.6	41.8
Grass hay 1	91.2	8.5	29.7	2.1	8.8	51.0
Grass hay 2	90.5	14.1	31.1	1.8	9.5	43.5
Meadow hay 1	89.7	12.3	28.9	1.3	10.3	47.2
Meadow hay 2	92.0	10.5	34.0	1.1	8.0	46.4
Meadow hay 3	92.7	8.9	32.8	1.5	7.4	49.5
Meadow hay 4	91.9	13.7	27.4	2.0	8.1	48.8
Meadow hay 5	90.4	13.8	24.3	2.1	9.6	50.2
Meadow hay 6	92.9	10.6	38.4	2.0	7.1	41.9
Meadow hay 7	90.9	13.2	30.9	2.5	9.2	44.2
Meadow hay 8	90.4	17.6	26.1	2.7	9.6	44.0
Meadow hay 9	89.6	12.7	31.7	1.8	10.4	43.5
Meadow hay 10	91.4	14.7	30.2	1.6	8.6	44.9
Meadow hay 11	89.9	18.2	26.4	2.1	10.1	43.3
Meadow hay 12	89.8	14.8	26.6	1.8	10.2	46.6
Meadow hay 13	92.4	11.2	32.7	1.5	7.6	47.0
Meadow hay 14	92.6	9.8	26.8	1.9	7.4	54.1
Mean	90.9	13.3	30.3	1.8	9.1	45.5
SD	1.2	2.9	3.6	0.4	1.2	4.0

Table 2. Chemical composition of silages (% in d.m.)

Silages	Organic matter	Crude protein	Crude fibre	Ether extract	Ash	Nitrogen-free extract
Lucerne	87.9	21.9	27.7	3.1	12.1	35.2
Lucerne + clover + grass 1	87.9	23.7	25.0	3.5	12.1	35.7
Lucerne + clover + grass 2	86.8	14.8	32.2	2.3	13.2	37.5
Barley (whole crop)	93.6	9.6	31.5	2.3	6.4	50,2
Brome grass	85.1	15.9	27.3	3.7	14.9	38.2
Grass + clover 1	84.1	15.4	30.9	3.8	15.9	34.0
Grass + clover 2	89.0	16.9	29.0	2.8	11.0	40.3
Meadow grass	88.5	13.6	32.4	2.6	11.5	40.0
Orchard grass 1	89.9	13.5	34.8	3.4	10.1	38.3
Orchard grass 2	88.5	15.8	26.8	2.7	11.5	43.3
Orchard grass 3	85.0	17.2	32.3	3.7	15.0	31.8
Red clover 1	91.7	21.3	28.2	3.7	8.3	38.5
Red clover 2	92.3	21.8	25.6	3.3	7.7	41.6
Red clover 3	88.5	22.8	23.0	3.5	11.5	39.2
Rye grass	80.0	17.6	27.9	4.8	20.0	29.7
Tall fescue grass 1	84.6	17.5	29.6	2.6	15.4	34.9
Tall fescue grass 2	83.1	17.5	30.1	4.1	16.9	31.5
Mean	87.4	17.5	29.1	3.3	12.6	37.1
SD	3.5	3.8	3.1	0.7	3.5	4.9

Table 3. Digestibility of organic matter of hay and silage estimated by *in vivo*, Tilley and Terry, and Pepcel methods (%)

	In vivo	Tilley and Terry	Pepcel
Hays (n = 18)			
mean	65.2[b]	64.5[b]	58.8[a]
(s.e.)	(1.16)	(1.30)	(1.72)
Silages (n = 16)			
mean	69.6[b]	65.0[a]	65.9[a]
(s.e.)	(0.97)	(1.63)	(1.86)

Different superscripts indicate significant differences between means ($P < 0.01$)

and Terry methods (see Table 4) confirm the agreement of OMD mean values between *in vivo* and Tilley and Terry methods shown in Table 3. In the case of silages, the intercept (36.3) was significantly higher and the slope (0.513) significantly lower than 0 and 1, respectively ($P < 0.05$). The differences in regression analysis results in the case of silages were probably caused by drying process (Alexander and McGowan, 1969). Tilley and Terry method shows comparable correlation coefficients as well as coefficients of determination in hays and silages. The coefficient of determination calculated for hay (0.76) was lower than the value found by Aerts et al. (1977) being 0.87 (Table 5). This discrepancy can be caused by the lower number of samples (18 vs. 42).

Table 4. Regression equations relating *in vivo* digestibility of hays and silages and digestibility estimated by Tilley and Terry and Pepcel methods

	Regression equations	R^2	RSD	RVC (%)	n
Hays					
Tilley and Terry	*In vivo* = 14.7 (± 14.96) + 0.782 (± 0.231) × Tilley and Terry	0.76	2.48	3.80	18
Pepcel	*In vivo* = 37.0[a] (± 14.70) + 0.478[b] (± 0.250) × Pepcel	0.49	3.69	5.68	19
Silages					
Tilley and Terry	*In vivo* = 36.3[a] (± 11.04) + 0.513[b] (± 0.169) × Tilley and Terry	0.75	2.00	2.87	16
Pepcel	*In vivo* = 37.8[a] (± 6.96) + 0.484[b] (± 0.104) × Pepcel	0.87	1.47	2.11	17

Tilley and Terry – organic matter digestibility obtained by this method (%)
Pepcel – organic matter digestibility obtained by this method (%)
[a]coefficients followed by the superscript significantly differ from 0 ($P < 0.05$)
[b]coefficients followed by the superscript significantly differ from 1 ($P < 0.05$)

Table 5. Comparison of regression parameters estimated in our study with the published ones (in Tilley and Terry method)

	Regression equations	R^2	RSD	RVC (%)	n
Hay					
Our estimation	*In vivo* = 14.7 + 0.782 × Tilley and Terry	0.76	2.48	3.80	18
Aerts *et al.* (1977)	*In vivo* = 12.4 + 0.82 × Tilley and Terry	0.87	3.10	x	42
Aufrere *et al.* (1988)	*In vivo* = 14.6 + 0.789 × Tilley and Terry	0.91	4.40	x	23
Alexander and McGowan (1966)	*In vivo* = 5.05 + 0.97 × Tilley and Terry	x	x	x	43
Armstrong *et al.* (1964)	*In vivo* = 12.48 + 0.92 × Tilley and Terry	x	x	x	12
Silage					
Our estimation	*In vivo* = 36.3 + 0.513 × Tilley and Terry	0.75	2.00	2.87	16
Aerts *et al.* (1977)	*In vivo* = 20.6 + 0.72 × Tilley and Terry	0.73	6.20	x	56
Alexander and McGowan (1969)	*In vivo* = 28.62 + 0.62 × Tilley and Terry	0.27	3.63	x	12
	In vivo = 45.39 + 0.35 × Tilley and Terry	0.17	4.14	x	6

Tilley and Terry – organic matter digestibility obtained by this method (%); x – the values were not found

Table 6. Comparison of regression parameters estimated in our study with the published ones (in Pepcel method)

	Regression equations	R^2	RSD	RVC (%)	*n*
Our estimation – hays	*In vivo* = 37.0 + 0.478 × Pepcel	0.49	3.69	5.68	19
Our estimation – silages	*In vivo* = 37.8 + 0.484 × Pepcel	0.87	1.47	2.11	17
Vencl (1990)[1]	*In vivo* = 30.4 + 0.583 × Pepcel	0.74	4.49		97
Aufrere (1988)	*In vivo* = 22.6 + 0.699 × Pepcel	0.96	3.20		24

Pepcel – organic matter digestibility obtained by this method (%)

[1]Green forage + lucerne hay + haylage + grass silage

Table 7. Multiple regression estimation of *in vivo* digestibility of organic matter

	Regression equations	R^2	RSD	RVC (%)	*n*
Our estimation	*In vivo* = 31.6^a (± 11.99) – 4.57^b (± 2.13) × Hay[1] + 0.10 (± 6.37) × Silage[2] + 0.587^b (± 0.184) × Ti-Te	0.66	3.13	4.65	36
	In vivo = 39.2^a (± 9.17) – 2.84^b(± 2.26) × Hay[1] – 1.90 (± 5.91) × Silage[2] + 0.496^b (± 0.136) × Pepcel	0.71	2.90	4.31	36
Andrighetto (1992)	*In vivo* = 44.9 – 5.92Hay – 7.61Silage + 0.35 × Pepcel	0.54	3.43	5.95	66

Tilley and Terry – organic matter digestibility obtained by this method (%)

Pepcel – organic matter digestibility obtained by this method (%)

[a]coefficients followed by the superscript are significantly different from 0 ($P < 0.05$)

[b]coefficients followed by the superscript are significantly different from 1 ($P < 0.05$)

Hay[1] and Silage[2] are dummy variables, insert the following symbols: hay (1, yes; 0, no), silage (1, yes; 0, no)

Aufrere and Michalet-Doreau (1988) proved this method on 23 samples of feeds consisting of dry forage (n = 10), energetic feed (n = 8) and protein supplements (n = 5). Although the data set was relatively small, reliability of the equation was higher than that calculated in our study (0.91 vs. 0.76). The coefficient of determination in silages published by Aerts *et al.* (1977) was comparable with that found in our study (0.73 and 0.75, respectively).

Coefficients of regression equations for Tilley and Terry method used in hays and silages calculated in our study are in agreement with the other published results (see Table 5) because almost all published coefficients lie within corresponding confidence intervals. In the case of silage, no decline of the coefficient of determination against hay was found.

The relationship between OMD of hay and silage estimated by *in vivo* and Pepcel method is presented in Table 6. The equations for hay and silage had almost identical intercepts and slopes. These parameters were comparable with those reported by Vencl (1990). A large difference was found in the coefficient of determination (0.49 and 0.87 for hays and silages, respectively). This result indicates higher reliability of the equation for silages.

In the literature regression equations describing the relationship *in vivo* – Pepcel were not given separately for hays and silages. Vencl (1990) published a regression equation for roughage generally (see Table 6); Andrighetto *et al.* (1992) took into consideration the type of feed by multiple regression (see Table 7). It is presumable that the relationship between OMD of hay determined by *in vivo* and Pepcel methods is influenced by the fodder crop species. The ensilage process evidently diminishes these differences between species.

Andrighetto *et al.* (1992) compared chemical methods, *in situ* and *in vitro* methods for determination of OMD. By subjecting these methods to a multiple regression analysis they found out that the best equations were obtained with *in vitro* methods, especially the Pepcel procedure. The same type of regression equation for Tilley and Terry and Pepcel is presented in Table 7. Coefficients of the variables for Pepcel were comparable with the values of Andrighetto *et al.* (1992). Despite of the lower number of samples in our study (n = 36 v. 66), the coefficient of determination was higher than that calculated in the study cited above (0.71 and 0.54, respectively). Data acquired by Tilley and

Terry method was processed in the same way for the purposes of comparison. Parameters of the resultant regression equation were comparable, the coefficient of determination was lower for Tilley and Terry compared to Pepcel (0.66 and 0.71, respectively).

CONCLUSION

A good agreement was found between *in vivo* and Tilley and Terry methods in the case of hay. Parameters of regression equations for *in vivo* and Tilley and Terry methods were different for silage. Parameters of regression equations comparing *in vivo* and Pepcel methods were not different for hays and silages. In this case, a large difference was only in the coefficients of determination. Tilley and Terry method provided consistent results of OMD estimation for both the groups of roughages (hays and silages). Using the Pepcel method, more accurate estimation of OMD was obtained for silages than for hays. In the case of multiple regression equation, the higher coefficient of determination was obtained from Pepcel method than from that of Tilley and Terry.

REFERENCES

AOAC (1984): Official methods of analysis. 14th ed. Association of Official Analytical Chemists, Washington.

Aerts J.V., De Brabander D.L., Cottyn B.G., Buysse F.X. (1977): Comparison of laboratory methods for predicting the organic matter digestibility of forages. Anim. Feed Sci. Tech., *2*, 337–349.

Alexander R.H., McGowan M. (1966): The routine determination of *in vitro* digestibility of organic matter in forages – an investigation of the problems associated with continuous large-scale operation. J. Brit. Grassl. Soc., *21*, 140–147.

Alexander R.H., McGowan M. (1969): The assessment of the nutritive value of silage by determination of *in vitro* digestibility on homogenates prepared from fresh non-dried silage. J. Brit. Grassl. Soc., *24*, 195–198.

Andrighetto I., Gruber L., Cozzi G., Uray G., Guidetti G., Buchgraber K. (1992): Prediction of digestible organic matter in dry matter *in vivo* from the chemical composition, *in vitro* and in situ measurements on native mountain forages. Anim. Feed Sci. Tech., *39*, 323–333.

Armstrong D.G., Alexander R.H., McGowan M. (1964): The use of *in vitro* digestibilities of dried grass for the prediction of their energy values for ruminants. Proc. Nutr. Soc., *23*, xxvi–xxvii.

Aufrere J. (1982): Etude de la prévision de la digestibilité des fourrages par une méthode enzymatique. Ann. Zootech., *31*, 111–130.

Aufrere J., Michalet-Doreau B. (1988): Comparison of methods for predicting digestibility of feeds. Anim. Feed Sci. Tech., *20*, 203–218.

Čerešnáková Z., Chrenková M., Flak P. (1996): Relation of content and composition of cell walls to the *in vitro* digestibility of dry matter, organic matter and crude protein in roughages. J. Farm Anim. Sci., *29*, 153–158.

Expert Committee on Animal Nutrition (1986): Laboratory evaluation of farm grown forage. In: Proc. Third ECAN Workshop. Winnipeg, Man., Agriculture Canada, 24–27.

McDougall E.I. (1948): Studies on ruminant saliva. I. Composition and output of sheep's saliva. Biochem. J. *43*, 99–109.

Míka V., Paul Ch., Zimmer E., Kaufmann W. (1982): Stanovení stravitelnosti objemných krmiv. Živ. Výr., *27*, 409–416.

Raymond W.F., Terry R.A. (1966): Studies of herbage digestibility by an *in vitro* method. Outlook Agric., *5*, 60–68.

Schiemann R. (1981): Methodische Richtlinien zur Durchführung von Verdauungsversuchen für die Futterwertschätzung. Arch. Tierernahr., *31*, 1–19.

Tilley, J.M.A., Deriaz, E.E., Terry, R.A. (1961): The *in vitro* measurement of herbage digestibility and assessment of nutritive value. In: Proc. 8th Int. Grassl. Congr. 533-537.

Tilley J.M.A., Terry R.A. (1963): A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grassl. Soc., *18*, 104–111.

Van Es A.J.M., Van der Meer J.M. (1989): Methods of analysis for predicting the energy and protein value of feeds for farm animals. In: Workshop on Methodology of Analysis of Feeding Stuff for Ruminants, Lelystad, NL, May, 27–29.

Vencl B. (1990): Possibility of digestible organic matter prediction by chemical and *in vitro* methods. In: New Systems of Energy and Protein Evaluation for Ruminants. Prague. 187–199.

Received: 04–07–28
Accepted after corrections: 04–12–23

Corresponding Author

Ing. František Lád, CSc., University of South Bohemia, Faculty of Agriculture, Studentská 13, 370 05 České Budějovice, Czech Republic

Tel. +420 387 772 599, e-mail: lad@zf.jcu.cz