# Evaluating the Feeding Value of Forages for Horses

*F. Faurie*[1], *N. Miraglia*[2] *and J. L. Tisserand*[1]

[1] Laboratoire associé de recherches zootechniques INRA-ENSSAA Dijon, France
[2] Instituto di Produzione Animale, Perugia, Italie

*Key words: horse, feed value, in vitro, in sacco, enzymatic*

**Summary**

In order to perfect a speedy method to evaluate the digestibilities of forages by horses, we experimented on two techniques which we compared to the in vivo digestibility measure: – an in sacco method perfected by *Michalet-Doreau* et *al.* (1987) to evaluate nitrogen degradability in the rumen, – a pepsin-cellulase degradation recommended in the case of ruminants by *Aufrere* (1982). Moreover a first serie of studies comparing the in sacco and in vitro methods (derived from *Tisserand* et *Zelter*, 1965) do not show the superiority of this last method. The results show that the enzymatic test seems to be the best to anticipate the in vivo degistibilities of the dry matter and the organic matter in the forage but remains inefficient to predict crude fibre degradation.

**Bestimmung des Futterwertes in Futtermitteln für Pferde**

In der vorliegenden Untersuchung sollte ein Schnelltest zur Abschätzung der Verdaulichkeit von Futtermitteln für Pferde entwickelt werden. 2 Techniken, die bei Wiederkäuern entwickelt wurden, wurden überprüft: – eine in-sacco-Methode in Anlehnung an die Arbeit von *Michalet-Doreau* et *al.* (1987) zur Abschätzung der Abbaubarkeit N-haltiger Verbindungen im Pansen – und ein in vitro-Pepsin-Cellulase-Aufschluß, wie er für Wiederkäufer von *Aufrere* (1982) empfohlen wird. Diese Methode erwies sich bereits in früheren Untersuchungen (*Tisserand* und *Zelter*, 1965) nicht als der in-sacco-Methode überlegen. Die Ergebnisse zeigen, daß der enzymatische Pepsin-Cellulase-Test die bessere Methode zur Vorhersage der Verdaulichkeit der Trockenmasse und der organischen Substanz im Futter ist. Er eignet sich jedoch wenig für Aussagen zur Abbaubarkeit der Rohfaser.

## Introduction

For a few years, studies have been conducted to specify the particularities of horse digestion. These allow us to suggest nutritional recommendations for this species. Yet the problem of feed use and in particular that of fodder by horses has not been solved. Although the nutritional values of the feeds distributed to horses appear in nutritional value tables (*N. R. C.*, 1978; *Futtermittel-Tabellen*, 1984; *Jarrige* and *Martin-Rosset*, 1984) and can also be reckoned, in the case of fodders, thanks to estimates linking digestibility to chemical composition and, in particular, to cell wall content (*Axelsson*, 1949; *Olsson*, 1949; *Hintz*, 1969; *Vander Noot* and *Trout*, 1971; *Martin-Rosset* et *al.*, 1984), the variabilities of some categories of feeds, like fooders, are not always taken into account. The nutritional value of fodders is first linked to their digestibilities. The latter can be assessed through:
a) chemical methods (*Lancaster*, 1943; *Forbes*, 1950; *Raymond* et *al.*, 1953; *Kivimae*, 1969);
b) biological techniques like the in vitro test of *Tilley* and *Terry* (1963) and the nylon bag technique (*Applegate* and *Hershberger*, 1969; *Demarquilly* and *Chenost*, 1969) or „in sacco" measures.
This last method is already commonly used on ruminants to study fodder digestion and estimate their digestibilites (*Van Keuren* and *Heinemann*, 1962; *Hopson* et *al.*, 1963; *Johnson*, 1966; *Demarquilly* and *Chenost*, 1969; *Cote* et *al.*, 1982) but it is also used on horses more and more often (*Applegate* and *Hershberger*, 1969; *Koller* et *al.*, 1978, *Uden* and *Van Soest*, 1982 and 1984);
c) enzymatic techniques which, under some experimental conditions, use enzymes and, in particular, a pepsin-cellulase solution (*Lusket al.*, 1962; *Donefer* et *al.*, 1963; *Jones* and *Hayward*, 1975; *Mc Queen* and *Van Soest*, 1975; *Allison* and *Borzucki*, 1978; *Mac Leod* and *Minson*, 1978; *Terry*, 1978; *Adamson* and *Terri*, 1980; *Demarquilly* and *Jarrige*, 1981; *Aufrere*, 1982).
That is why we have tried to perfect a simple and reliable method to estimate the nutritional values of feeds by comparing an „in sacco" method with an enzymatic one by reference to the in vivo digestibility measure.

## Material and Methods

*Animals:* digestibility was measured in vivo through the total collection of the faeces of six adult saddle horses (500 kg BW) feed ad libitum according to a protocol which has been described (*Martin-Rosset* et *al.*, 1984).
Digestibility was measured with the nylon bag method on two male adult ponies with permanent cecum cannulas (*Tisserand* et *al.*, 1977).
*Fodders:* Thirteen fodders were studied: two kinds of straw, seven kinds of natural meadow hay of very different qualities harvested during the first cycle (n = 6) or the second (n = 1) and four kinds of alfalfa hay harvested during the first cycle (n = 1) or the second (n = 2) or the third (n = 1). All the fodders were tossed on the ground in good weather except for a second cycle of alfalfa which was ventilated in a barn.
During the in vivo digestibility measure period, representative fodder samples were taken so that their digestibilities could be measured later in sacco. The chemical composition of the fodders which were studied appear in table 1.
*Digestibility measurement with the nylon bag technique:* The ponies were each given 5 kg of middle-quality cocksfoot alfalfa hay daily. The experiment only started after an adaptation period to this fodder of fifteen days. 2.5 g of fodder crushed through a 0.8 mm grid were introduced in a nylon bag measuring 18 x 4 cm (nylon F 100, Tripette and Renaud, Paris). The diameters of the material stitches were

between 5 and 8 $\mu$. The bag was closed by electrical welding; glass balls were introduced in its lower part to make sure it was well immersed in the cecum.

It was continued in its upper part by a nylon thread which hung thanks to an eyelet. The fodder introduced in the bag was submitted to predigestion in a pepsin solution for 48 hours according to the second stage of *Tilley* and *Terry* technique (1963); after being rinsed and let to dry in the air for 24 hours, the bag was maintained in the cecum for 24 hours which corresponds to the average stay period observed in the cecum for fodder particles (*Applegate* and *Hershberger*, 1969); the measure was repeated five times for each fodder (on 6 consecutive days) and each pony, which meant 10 bags for each fodder. The dry matter and ash contents were respectively measured in an incubator at 103°C over a minimum of 12 hours and in an oven at 550°C over 5 hours. The cell-wall content was measured through measuring out crude cellulose with the Weende method.

The in sacco digestibilities of dry matter (D. M. d.), organic matter (O. M. d.) and crude fibre (C. F. d.) were calculated with the following formula:

$$\text{in casso degradability} = \frac{\text{disappeared D.M. (pepsin + cecum)}}{\text{D. M. at the start}}$$

The results obtained in sacco were compared to those of in vivo digestibility which were used as a reference. Simple correlation coefficients were calculated between the variables.

*Enzymatic degradability measurement:* The pepsin-cellulase method suggested by *J. Aufrere* (1982) was used. It includes:

- a pre-treatment of 500 mg of crushed fodder (0.8 mm grid) weighed in 90 ml filtering crucibles (fritted glass with a porosity of 2) lasting 24 hours, in a 40°C double-boiler with 50 ml of a pepsin solution (0.2 percent) in N hydrocloric acid,
- filtering and washing in distilled water,
- a 24 hour treatment in a double-boiler at a temperature of 40°C with 50 ml of a cellulase solution extracted from Trichoderma viride (Onozuka R10),

**Table 1:** Forage composition digestibility, in sacco and enzymatic degradability

| Forages | D.M. | % of D.M. | | | in vivo digestibility | | | degradability nylon bag | | | digestion pepsin-cellulase % | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | Crude protein | Crude fibre | Ashes | D.M. | O.M. | C.F. | D.M. | O.M. | C.F. | D.M. | O.M. |
| Wheat straw | 95.7 | 4.0 | 41.6 | 9.6 | 35.0±2.7 | 37.7±2.8 | 38.3±3.4 | 25.3±2.9 | 24.6±2.6 | 14.3±2.6 | 33.7±1.41 | 33.2±1.41 |
| Barley straw | 95.0 | 3.1 | 44.2 | 7.1 | 34.1±2.2 | 36.2±2.1 | 35.6±2.3 | 22.7±5.5 | 22.0±5.4 | 9.8±6.0 | 37.3±2.20 | 37.0±1.80 |
| Bad hay n' 1 | 94.5 | 4.0 | 40.3 | 9.7 | 37.5±1.9 | 37.9±1.9 | 30.3±2.6 | 41.9±1.3 | 41.3±1.2 | 32.9±3.0 | 43.2±2.24 | 41.8±2.18 |
| Bad hay n' 2 | 93.5 | 5.2 | 37.8 | 9.7 | 37.4±1.7 | 38.0±1.9 | 30.3±0.7 | 29.6±2.3 | 27.4±2.5 | 11.7±6.2 | 42.7±3.38 | 37.2±11.59 |
| Nat. mead. hay n' 1 | 94.2 | 8.8 | 32.3 | 7.6 | 49.3±2.4 | 50.4±2.2 | 39.9±3.0 | 50.1±3.8 | 57.9±3.4 | 39.3±4.3 | 57.4±1.50 | 55.9±1.33 |
| Nat. mead. hay 2nd cut n' 2 | 94.1 | 9.2 | 35.2 | 7.4 | 48.4±1.9 | 49.7±1.7 | 38.7±3.1 | 50.7±1.3 | 51.0±1.3 | 37.0±2.0 | 65.0±1.00 | 54.2±1.33 |
| Nat. mead. hay late harv. n' 3 | 94.8 | 6,3 | 36.8 | 6.1 | 44.6±2.3 | 45.8±2.4 | 40.1±3.0 | 45.6±1.2 | 45.7±1.0 | 28.3±3.4 | 55.8±0.65 | 54.3±0.71 |
| Nat. mead. hay n' 4 | 92.6 | 21.1 | 25.3 | 7.4 | 59.7±3.3 | 61.2±3.2 | 55.8±4.0 | 63.4±3.1 | 51.6±3.3 | 26.3±8.6 | 68.3±2.43 | 67.6±2.19 |
| Nat. mead. hay 2nd cut n' 5 | 91.3 | 21,2 | 30.0 | 12.7 | 61.7±0.4 | 63.3±0.4 | 60.2±1.3 | 73.1±2.5 | 72.9±2.6 | 55.9±5.3 | 76.1±0.77 | 74.8±0.79 |
| Lucerne hay 1st cut n' 1 | 94.3 | 14.3 | 35.5 | 10.2 | 54.8±2.7 | 56.6±2.7 | 35.3±1.1 | 60.8±2.7 | 59.0±2.6 | 34.6±6.6 | 57.0±1.10 | 53.4±0.88 |
| Lucerne hay 2nd cut n' 2 | 93.6 | 17.0 | 35.3 | 11.5 | 56.5±1.4 | 57.2±0.9 | 38.6±2.7 | 64.3±2.3 | 61.8±2.3 | 38.6±3.4 | 61.1±0.51 | 56.9±0.57 |
| Lucerne hay 3rd cut n' 3 | 92.9 | 19.8 | 27.9 | 12.9 | 58.6±1.3 | 58.6±1.2 | 40.6±1.8 | 71.8±2.6 | 69.5±2.9 | 40.6±5.9 | 65.9±0.80 | 62.0±0.70 |
| Barn hay drying lucerne hay n' 4 | 91.0 | 16,6 | 32.9 | 8.1 | 63.8±1.5 | 63.0±1.6 | 40.6±2.5 | 58.5±3.4 | 55.4±3.5 | 26.4±6.5 | 63.3±1.01 | 59.7±0.74 |

(1) Crude protein: N×6.25
(2) Crude fibre (Weender method)

**Table 2:** Correlation coefficient between the in vivo and the in sacco or enzymatic degradation (d) for Dry Matter (DM) and Organic Matter (OM)

| in vivo | DMd | OMd |
|---|---|---|
| in sacco | 0.904 | 0.883 |
| enzymatic | 0.942 | 0.926 |

- filtering and washing in distilled water,
- drying in an incubator at a temperature of a 103°C for 48 hours,
- an oven calcination at 550°C for 5 hours,
- the calculation of the soluble dry matter (D. M. d.) and organic matter (O. M. d.) percentages for the two treatments.

The analysis of each fodder was duplicated and repeated five times.

## Results and Discussion

Table 1 sums up the results concerning the composition, in vivo digestibilities, "in sacco" and enzymatic degradabilities of the fodders.

Its reading shows that as far as "in sacco" degradation is concerned O. M. d. is lower than D. M. d. (– 1.3 Â 1.0) as opposed to what is observed in vivo (+1.1 Â 0.9). This is likely to result from microbial contamination inside the bags. The "in sacco" crude fibre degradability varies a lot and is sometimes lower than in vivo digestiblity. This phenomenon could be explainded by a variation in the positions of the bags in the cecum. Moreover the nylon bag technique does not take into account the entire digestive process and, in particular, the effect of prececal fodder digestion on the digestion of the cell-walls, notably hemicellulose *(Keys et al., 1969)*, but also of the quite important degradation of cell-walls in the colon.

The variabilities of the digestibilities measured in sacco (D. M. d., O. M. d., C. F. d.) are slightly higher than those obtained with the same technique by *Applegate* and *Hersherberger* (1969), *Koller* et *al.* (1978). The small numbers of fodders studied (n = 3 to 4) by these researchers can partially explain the difference as well as the length of time spent by the bags in the cecum (24 hours and 48 hours; *Koller* et *al.*, 1978).

Concerning enzymatic degradability straws have the lowest values for DM and OM; conversely, the highest values are obtained with grass hay. Yet, whereas in vivo O.M.d. is almost always superior to D.M.d. we have observed the contrary with enzymatic degradations. There is a good correlation between the in vivo and the in sacco or the enzymatic results concerning DM and OM (table 2).

**Table 3:** In vitro and sacco degradability

| | in sacco | | in vitro | |
|---|---|---|---|---|
| Forages | 24 h | 8 h | 16 h | 24 h |
| Chopped hay | 60.8 | 32.4 | 35.2 | 32.2 |
| Chopped hay | 56.0 | 26.2 | 27.2 | 32.2 |
| Chopped hay | 55.8 | 27.5 | 29.8 | 33.4 |
| Pelleted hay | 55.2 | 33.5 | 35.0 | 33.7 |
| Pelleted hay | 60.0 | 35.0 | 35.4 | 35.0 |

Moreover a first series of studies dealing on a comparison between the in sacco method which has been described before and an in vitro degradation technique – adapted to the horse's cecum metabolism and based on the method perfected by *Tisserand* and *Zelter* (1965) for ruminants – seems to show that the latter does not constitute an improvement as compared to the in sacco method. As a matter of fact with 5 diets based on hay given to 3 ponies with permanent cecum cannulas we could not show any real relationship between the in sacco degradability and in vitro disappearance after 8, 16 or 24 hours of incubation (table 3).

## Conclusion

The nylon bag technique is interesting in the case of horses to assess and compare the horse's dry matter and organic matter digestibilities of fodder but not as far as crude fibre is concerned. Yet, as in the case of the in vitro methods, it calls for fistulated animals which is a serious handicap for routine estimate tests.

The pepsin-cellulase enzymatic test proposed by *J. Aufrere* (1982) to estimate ruminant fodder digestiblity can be applied to horses. This method which does not call for fistulated animals can be used in laboratories for routine tests. The results which we have obtained are encouraging; they ask for the reproductibility of the proposed technique.

Yet the analysis of many fodders should allow us, as is already the case for ruminants, to submit a regression equation to reckon horse fodder digestibilities with a satisfactory reliability.

## References

*Adamson, A. H., and Terry, G. R.*, 1980. The relationship between the in vivo digestibility of hay and its solubility in pepsin-hydrochloric acid and fungal cellulase solutions. J. Sci. Food Agric., **31**, 854 – 856.

*Allison, M., and Borzucki, R.*, 1978. Cellulase method for the efficient digestion of grasses and brassicas. J. Sci. Food Agric., **29**, 293 – 297

*Applegate, C. S., and Hershberger, T. V.*, 1969. Evaluation of in vitro and in vivo cecal fermentation techniques for estimating the nutritive value of forages for equine. J. Anim. Sci., **28**, 18 – 22.

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

*Axelsson, J.*, 1949. The ability of cattle, sheep, horses and swine to digest the nutrients of the feedingstuffs. Ann. R. Agric. Coll. Swed., 16, 84 – 100.

*Cote, M., Seoane, J. R., Gervais, P., and McQueen, R. E.*, 1982. Etudes sur la biodégradation de la matière sèche des fourrages par les méthodes in vitro et des sacs de nylon. Can. J. Plant Sci., **62**, 407 – 413.

*Demarquilly, C., and Chenost, M.,* 1969. Etude de la digestion des fourrages dans le rumen par la méthode des sachets de nylon; liaison avec la valeur alimentaire. Ann. Zootech., **18;** 419 – 436.

*Demarquilly, C., and Jarrige, R.,* 1981. Panorama des méthodes de prévision de la digestibilité et de la valeur énergétique des fourrages. XIe journées du Grenier de Theix, in: Prévision de la valeur nutritive des aliments des ruminants, I. N. R. A. Ed., 41 – 59.

*Donefer, E., Neimann, P. J., Crampton, E. W., and Lloyd, L. E.,* 1963. Dry matter disappearance by enzyme and aqueous solutions to predict the nutritive value of forages. J. Dairy Sci., **46,** 965 – 970.

*Forbes, R. M.,* 1950. Protein as an indicator of pasture forage digestibility. J. Anim. Sci., **9,** 231 – 237

*Futtermittel-Tabellen der D. L. G. für Pferde,* 1984. Ed. D. L. G. Verglas, D-6000 Frankfurt am Main, 128 p.

*Hintz, H. F.,* 1969. Review. Equine nutrition comparisons of digestion coefficients obtained with cattle, sheep, rabbits and horses. Veterinaria, **6,** 45 – 51.

*Hopson, J. D., Johnson, R. R., and Dehority, B. A.,* 1963. Evaluation of the dacron bag technique as a method for measuring cellulose digestibility and rate of forage digestion. J. Anim. Sci., **22,** 448 – 453.

*Jarrige, R., and Martin-Rosset, W.,* 1984. Le Cheval. INRA Paris, 689 p.

*Johnson, R. R.,* 1966. Techniques and procedures for in vitro and in vivo rumen studies. J. Anim. Sci., **25,** 855 – 875.

*Jones, D. I. H., and Hayward, M. V.,* 1975. The effect of pepsin pretreatment of herbage on the prediction of dry matter digestibility from solubility in fungal cellulase solutions. J. Sci. Food Agric., **26,** 711 – 718.

*Keys, J. F., Van Soest, P. J., Young, E. P.,* 1969. Comparative study of the digestibility of forage cellulose and herricellulose in ruminants and non-ruminants. J. Anim. Sci., **29,** 11 – 15.

*Kivimae, A.,* 1960. Estimation of the digestibility of grassland crops from their chemical composition. Proc. 8th. Int. Grassl. Congr., pp. 466 – 470.

*Koller, B. L., Hintz, H. F., Robertson, J. B., and Van Soest, P. J.,* 1978. Comparative cell wall and dry matter digestion in the cecum of the pony and the rumen of the cow using in vitro and nylon bag techniques. J. Anim. Sci., **47,** 209 – 215.

*Lancaster, R. J.,* 1943. Metabolism trials with New Zealand feeding stuffs. IV. The relative significance of lignin, cellulose and crude fibre in the evaluation of foods. N. Z. J. Sci. Tech., **25A,** 137 – 151.

*Lusk, J. W., Browning, C. B., and Miles, J. T.,* 1962. Small sample in vivo cellulase digestion procedure for forage evaluation. J. Dairy Sci., **45,** 69 – 73.

*Mc Leod, M. N., and Minson, D. J.,* 1978. The accuracy of the pepsin-cellulase technique for estimating the dry matter digestibility in vivo of grasses and legumes. Anim. Feed. Sci. Technol., **3,** 277 – 287.

*Mc Queen, R., and Van Soest, P. J.,* 1975. Fungal cellulase and hermicellulase prediction of forage digestibility. J. Dairy Sci., **58,** 1482 – 1492

*Martin-Rosset, W., Andrieu, J., Vermorel, M., and Dulphy, J. P.,* 1984. Valeur nutritive des aliments pour le cheval. In: R. Jarrige, W. Martin-Rosset. Le Cheval: reproduction-sélection-alimentation-exploitation, INRA Publication-Paris, p. 689.

*Michalet-Doreau, B., Verite, R., and Chapoutot, P.,* 1987. Méthodologie de mesures de la dégradabilité in sacco de l'azote des aliments dans le rumen. Bull. Tech. CRZV Theix INRA (69) 5 – 7.

*National Research Concil,* 1978. Nutrient requirement of horse. National Academy of Science. 4e Ed. Washington USA, Vol. 61.33.

*Olsson, N.,* 1949. The relation between the organic nutrients of rations and their digestibility in horses. Ann. R. Agric. Coll. Swed., **16,** 644 – 669.

*Raymond, W. F., Harris, C. E., and Baker, V. G.,* 1953. Studies on the digestibility of herbage. I. Technique of measurement of digestibility and some observations on factors affecting the accuracy of digestibility data. J. br. Grassl. Soc., **8,** 301 – 320.

*Terry, R. A., Mundell, D. C., and Osbourn, D. F.,* 1978. Comparison of two in vitro procedures using rumen liquor-pepsine or pepsine-cellulase for prediction of forage digestibility. J. br. Grassl. Soc., **33,** 13 – 18.

*Tilley, J. M. A., and Terry, R. A.* 1963. A two stage technique for in vitro digestion of forage crops. J. br. Grassl. Soc., **18,** 104 – 111.

*Tisserand, J. L., and Zelter, S. Z.,* 1965. Essai de normalisation d'une technique de mesure de la digestion des fourrages in vitro. Ann. Biol. anim. Bioch. Biophy. **5,** 101 – 111.

*Tisserand, J. L., Candau, M., Houiste, H., and Masson, C.,* 1977. Evolution de quelques paramètres physico-chimiques du contenu caecal d'un poney au cours du nycthémère. Ann.Zootech., **26,** 429 – 434.

*Uden, P., and Van Soest, P. J.,* 1982. Comparative digestion of timothy (Phleum pratense) fibre by ruminants, equines and rabbits. Br. J. Nutr., **47,** 267 – 272.

*Uden, P., Van Soest, P. J.,* 1984. Investigations of the in situ bag technique and a comparison of the fermentation in heifers, sheep, ponies and rabbits. J. Anim. Sci., **58,** 213 – 221.

*Vander Noot, G. W., and Trout, J. R.,* 1971. Prediction of digestive components of forages by equines. J. Anim. Sci., **33,** 351 – 355.

*F. Faurie*
*Laboratoire associe de recherches zootechniques INRA-ENSSAA*
*26, Bd. Docteur Petitjean*
*F-21000 Dijon*
*France*