The use of faeces as a source of inoculum for in vitro prediction of the energy value of feeds in horses

Ana Sophia Santos[1], José M. Abreu[2], Miguel A. M. Rodrigues[3] and Arnaldo A. Dias-da-Silva[3]

Department of Veterinary, Esc. Univ. Vasco da Gama, Quinta de S. Jorge, Castelo Viegas, Coimbra[1], CECA, Faculdade de Ciencias, Universidade do Porto[2] and CECAV – Department of Animal Science, Univ. de Trás-os-Montes e Alto Douro[3] Portugal

Introduction

The digestibility of feeds can be measured or estimated by means of different techniques. In vivo methods, particularly the total collection method (*Martin-Rosset* et al. 1984) are widely spread. The use of internal markers is also becoming popular. Several studies using acid insoluble ash (AIA) indicate that this marker can be utilised successfully to estimate digestibility (*Miraglia* et al. 1999). Digestibility can also be estimated from the chemical composition of feeds, as described by *Martin-Rosset* et al. (1996a), using crude protein (CP), neutral detergent fiber (NDF) and acid detergent lignin (ADL). In vitro methods are usually more precise than the use of chemical composition, but they require more complex techniques.

In horses, several attempts have been used to develop an in vitro method to estimate digestibility. *Trevor-Jones* et al. (1991) proposed an adaptation to the Tilley and Terry method used for ruminants and *Bush* et al. (2001) used caecal fluid to evaluate the fermentation of feeds using the gas production technique However, these methods have a common problem - the need for cannulated animals. An alternative to ruminal or caecal fluid this does not require surgical intervention while maintain the link to the animal (El Shaer et al. 1987). *Omed* et al. (2000) refers that both equine and bovine faeces have sufficient fermentable activity enabling its utilization as inoculum for in vitro fermentation studies.

In this study we evaluated the utilization of horse faeces as a source of inoculum to estimate in vitro digestibility of meadow hay and oat straw.

Material and methods

Two light horses fed close to the maintenance level of energy were used for faeces collection. The horses were fed natural meadow hay, and concentrate (oat grain and faba bean). The forage/concentrate ratio (dry matter basis) was 70/30, and the animals where fed, both forage and concentrate, three times a day (8.00, 14.00 and 20.00). Analyses were carried out on roughages for dry matter, organic matter, crude protein, ether extract and ash according to AOAC (1990). Fibre fractions (NDF, ADF and ADL) were determined by the detergent procedures described by *van Soest* et al. (1991).The first stage of the Tilley and Terry method, modified by *Marten* and *Barnes* (1980) was used. Two faecal samples, one of each horse, were taken, at the same hour (11.00 a.m.); 400 g of fresh faeces where collected by grab sampling. Once collected, the faeces were immediately carried to the laboratory mixed (1:2 w/v) with a CO_2-saturated nutritive medium and then filtered through a six-layer cloth to remove non digested materials. The nutritive medium consisted of a carbonate-phosphate buffer solution containing (g/l): 8.75 g $NaHCO_3$, 1.00 g NH_4CO_3, 1.43 g Na_2HPO_4, 1.55 g KH_2PO_4, 0.15 g $MgSO_4.7H_2O$, 0.52 g NaS, 0.017 g $CaCl_2.2H_2O$, 0.015 g $MnCl_2.4H_2O$, 0.002 g $CoCl_3.6H_2O$, 0.012 g $FeCl_3.6H_2O$ and 0.125 resazurin according to *Menke* et al. (1979) as modified by *Steingass* (1983). Faecal contents, solutions and containers were kept under a constant flow of CO_2 during inoculum preparation. Samples (20 ml) of the resulting inoculum were introduced in a 60 ml flasks containing 0.2 g of substrate. Control batches containing only 20 ml of faecal inoculum were also prepared. The batches were put into a waterbath at 39°C, and the insoluble residue after 48 hours of incubation was considered indigestible.

Results

Chemical composition of natural meadow hay and oat straw are presented in Table 1. These roughages can be described as having mid and low quality, respectively. Their chemical composition can be considered similar to those of the same roughages found in stables all over the north of Portugal. The

Table 1 Chemical composition of feeds (mean ± SD; values in $gkg^{-1}DM$).

	Natural meadow hay	Oat straw
Dry matter	871 ± 1.5	892 ± 0.5
Organic matter	909 ± 6.8	935 ± 5.8
Crude protein	75.4 ± 6.4	36.6 ± 2.8
NDF	692 ± 19.4	823 ± 22.2
ADF	415 ± 13.7	536 ± 14.1
ADL	56.6 ± 2.6	81.1 ± 3.7
Ether extract	8.6 ± 1.3	9.7 ± 1.4
AIA	25.5 ± 2.9	13.3 ± 1.7

NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, AIA: acid-insoluble ash

Table 2 Organic matter digestibility (mean ± SD; values in %) of natural meadow hay and oat straw obtained by in vitro fermentation, by chemical composition or found on the INRA tables.

	Natural meadow hay	Oat straw
In vitro fermentation		
Inoculum 1	60.6 ± 1.4	50.8 ± 0.7
Inoculum 2	58.5 ± 1.5	40.9 ± 0.5
Mean values	59.7± 1.8	46.9± 5.0
Chemical composition[a]	44.7	30.0
INRA Tables[b]	50	42

Each value is the mean of 10 observations
[a] – Martin-Rosset *et al*. (1996a); [b] – Martin-Rosset, 1990

estimations of organic matter digestibility of natural meadow hay and oat straw are presented in Table 2.

Values for OMD of both feeds studied, where statistically different when using inoculum from different animals (inoculum 1 or 2). The animal effect was significant ($p<0.05$) for natural meadow hay, and highly significant ($p<0,001$) for oat straw.

Discussion

In spite of the need to use homogenised faeces to reduce animal variation, as it is the case with in vitro methods using rumen liquor of at least two cannulated animals, we found high variation between the two horses in the case of oat straw. The figures for in vitro organic matter digestibility (OMD) of natural meadow hay and oat straw (59,7 and 46,9 %, respectively) were higher than those referred to in the INRA tables (*Martin-Rosset* 1990) or than those obtained by using the chemical composition (*Martin-Rosset* et al. 1996b) as shown in Table 2.

In a parallel study where the same two horses were part of a group used to measure the digestibility of mixed diets hay+concentrate and oat straw+concentrate (70:30) using AIA and ADL as markers, the figures for OMD of the total diet (56.5 and 45.2 for meadow hay and oat straw diets, respectively), were lower than those found in vitro for the corresponding roughages. This suggests that in vitro fermentation overestimated the digestibility of the roughages. As shown by others (*G. Le Golf* et al. 2003, *Wang* et al. 2004, *Omed* et al. 2000) the ratio feaces:buffer solution may have affected the results. Also incubation time can be a factor of variation as shown by *Omed* et al. (2000) who concluded that 36 hours may be more adequate to simulate the period of fermentation in the horse's ceacum-colon section.

Conclusions

The in vitro fermentation method of estimating the digestibility of feeds for horses, using a natural occurring residues (faeces), is an attractive idea since it allows the use of animals without surgical intervention and is easy to accomplish. In this preliminary study we conclude that the use of faeces for this purpose needs to be refined and tested on a large scale set of samples. More studies in this area are required, namely testing different faeces concentration, amount of substrate, and incubation time.

References

AOAC (1990): Official Methods of Analysis, 14th Edition, Vol. 1 AOAC, Washington DC, USA, 684

Bush J. A., Freeman D. E., Kline K. H., Merchen N. R. and *Fahey Jr. G. C.* (2001): Diatery fat supplementation effects on in vitro nutrient disappearance and in vivo nutrient intake and total tract digestibility. Journal of Animal Science 79, 232-239

El Shaer H. M., Omed H. M., Chamberlain A. G. and *Axford R. F. E.* (1987): Use of faecal organisms from sheep for the in vitro determination of digestibility. Journal of Agriculture Science. (Cambridge), 109, 257-259

Martin-Rosset W., Andrieu J. and *Jestin M.* (1996a): Prediction of the digestibility of organic matter (dMO) of forages in horses from the chemical composition, In: Book of Abstracts N.° 2 of the 47th Annual Meeting of the European Association of Animal Production, Lillehammer, Norway, 100-104

Martin-Rosset W. (1990): L'Alimentation des chevaux. Institute National de la Recherche Agronomique, Paris

Menke K. H., Raab L., Salewski A., Steingass H., Frtitz D. and Scneider W. (1979): The estimation of digestibility and metabolizable energy content of ruminal feedstuffs from the gas production when they are incubated with rumen liquor in vitro. Journal of Agriculture Science Cambridge. 93, 217-222

Miraglia N., Bergero D., Bassano B., Tarantola M. and *Ladetto G.* (1999): Studies of apparent digestibility in horses and the use of internal markers. Livestock Production Science 60, 21-25

Omed H. M., Lvett D. K. and *Axford R. F. E.* (2000): Faeces as a source of microbial enzimes for estimating digestibility, In: Forage Evaluation in Ruminant, Givens, D.I., Owen, E., Axford, R.F.E. e Omed, H.M. (Ed), CAB International, 135-154

Steingass H. (1983): Bestimmung des energetischen Futterwertes von wirtschaftseigenen Futtermitteln aus der Gasbildung bei der Pansenfermentation in vitro. Ph. D. Thesis, University of Hohenheim, Germany

Trevor-Jones P. J., Sriskandarajah N. and *Woong R. A.* (1991): Development of an in vitro technique for the evaluation of feeds for horses. Proceedings Nutrition Socitey Australia, 16, 54

Van Soest P. J., Robertson J. B. and *Lewis B. A.* (1991): Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583-3597

Wang J. F., Zhu Y. H., Li D. F., Wang Z. and *Jensen B. B.* (2004): In vitro fermentation of various fiber and starch sources by faecal pig inocula. Journal of Animal Science 82, 2615-2622

A. S. Santos
Department of Veterinary, Esc. Univ. Vasco da Gama
Quinta de S. Jorge,
Estrada da Conraria
3040-714 Castelo Viegas
Coimbra
Portugal
assantos@utad.pt