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Estimation of protein availability in ruminant feeds: A comparison of different methologies

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SUMMARY – Intestinal digestibility of undegraded feed protein is an important parameter in modern protein evaluation systems for ruminants. Different methods may estimate the intestinal digestibility of a wide range of feeds. Three methods based on either the acid detergent insoluble nitrogen (ADIN) content, the protein disappearance from the mobile bag and the *in vitro* intestinal digestibility using a preincubation stage in both sheep and goats were compared. Samples of different feedstuffs (olive cake from two different years; olive leaves with different treatments; a concentrate; sunflower cake with or without addition of quebracho tannin; barley; barley straw and alfalfa hay with or without addition of quebracho tannin) were used in the experiment. N post-ruminal availability of olive by-products was low and varied depending on factors such as year or treatment. Compared with the mobile bag technique, total N digestibility was overestimated by the *in vitro* method. If N disappearance from the mobile bag is accepted as a physiologically reasonable estimation for *in vivo* true protein digestibility, it could be concluded that acid detergent soluble nitrogen (ADSN) content and the *in vitro* method may give good estimates of digestibility of N although some differences between by-products and conventional feeds are observed.

Keywords: Nitrogen intestinal digestibility, ruminants, by-products, prediction methods.

RESUME – *"Estimation de la disponibilité des protéines dans les aliments pour ruminants : Comparaison de différentes méthodologies". La digestibilité intestinale de l'azote non dégradable est un paramètre important dans les nouveaux systèmes de valorisation protéique des aliments pour les ruminants. Différentes méthodes peuvent être utilisées pour estimer la digestibilité intestinale d'une grande variété d'aliments. Dans ce travail, trois méthodes sont comparées : le contenu en azote insoluble au détergent acide (ADIN) des aliments, la quantité d'azote disparue avec la méthode des sachets mobiles et la digestibilité intestinale* in vitro *en utilisant une période de préincubation dans le rumen de moutons ou de chèvres. Des échantillons de différents aliments (du grignon d'olive de deux années différentes, des feuilles d'olive soumises à différents traitements, un concentré, du tourteau de tournesol avec ou sans tannin de quebracho, de l'orge, de la paille d'orge et du foin de luzerne avec ou sans tannin de quebracho) ont été utilisés dans cet essai. La disponibilité post-ruminale de l'azote des sous-produits de l'olivier était basse et a varié en fonction de facteurs comme l'année ou le traitement. La digestibilité totale de l'azote était surestimée par la méthode* in vitro *quand on la compare avec la méthode des sachets mobiles. Si on considère que l'azote disparu des sachets mobiles est une estimation physiologiquement raisonnable de l'azote vraiment digestible* in vivo*, il est possible de conclure que le contenu en azote soluble au détergent acide (ADSN) et la méthode* in vitro *peuvent donner de bonnes estimations de la digestibilité de l'azote malgré quelques différences observées entre les sous-produits et les aliments conventionnels.*

Mots-clés : *Digestibilité intestinale de l'azote, ruminants, sous-produits, méthodes d'estimation.*

Introduction

Current feed evaluation systems recognise the need to estimate the protein value as the amount of amino acid truly absorbed in the small intestine [National Research Council (NRC), 1985; Nordisk Kontaktorgan for Jordbrugsforskning (NKJ), 1985; Agricultural and Food Research Council (AFRC), 1992]. Application of any of these systems requires data on the digestibility of rumen-undegradable protein in the small intestine. Despite that by-products may be an important part of the diet of ruminants in Mediterranean semi-arid lands and that their undegradable protein reaching the small intestine may be quantitatively important, very few intestinal digestibility data of bypass protein are available compared to conventional feeds. The nutritive value of by-products varies depending on factors such as maturity, season, industrial treatments and preservation methods. Therefore, easy and quick methods to accurately estimate the protein value of by-products are essential.

In vivo and *in vitro* techniques to measure intestinal digestion of proteins have been developed in the last few years (Hvelplund and Weisbjerg, 1998). The mobile bag method is easy to use and, good agreement between disappearance from the bags and those estimated on intragastric infusion have been obtained (Hvelplund *et al*., 1994), but requires access to ruminal and duodenal cannulated animals and is still time-consuming for routine feed evaluation. Calsamiglia and Stern (1995) developed a three-step enzymatic *in vitro* procedure, in which duodenal fistulated animals are not required. The technique has been successfully applied to numerous feeds (Calsamiglia *et al*., 1997). Acid detergent insoluble nitrogen (ADIN) is also assumed to be a measure of indigestibility and used to reveal heat damage of feedstuffs, but some authors presented evidence that the relationship between ADIN and indigestibility was very poor for some feeds (Waters *et al*., 1992; Kusumanti *et al*., 1996).

The aim of the present work was to compare the three methods using conventional and non-conventional feeds. The effect of two different rumen preincubation times (16 or 48 hours) was tested using both the mobile bag and the three steps *in vitro* methods.

Material and methods

Feeds samples

The feeds used in the present study were: olive cake (OC) from two different years (OC97 and OC99); olive cake 99 plus 2% of PEG (OC99+PEG); olive leaves (OL) dried, during 48 h, at room temperature (OL1), at 60°C (OL2), at 100°C (OL3) or freeze dried (OL4); sunflower cake (SC); sunflower cake plus 5% of quebracho (SC+Q); barley straw (BS); a concentrate composed by barley (2/3), olive cake (1/3) and mineral-vitamin mixture (CO); concentrate plus 2% PEG (CO+PEG); concentrate plus 5% of quebracho (CO+Q); barley (BA); alfalfa hay (AH); and alfalfa hay plus 5% of quebracho (AH+Q). The samples were ground through 2 mm screen for intestinal digestibility determination and, through 1 mm screen for chemical analysis.

Mobile bag technique

Total N disappearance of the feeds described above was measured with the mobile nylon bag technique (Hvelplund *et al*., 1992). For olive by-products also two different rumen preincubation times were compared (16 and 48 h). 6 Friesian non-lactating cows equipped with a ruminal cannula and T-shaped duodenal cannula were used. Approximately 1 g of the sample was weighed into each bag (11 × 11 µm pore size, external dimensions 6 × 6 cm), then the bags were heat sealed and preincubated in the rumen of cows. The bags were successively incubated in an HCl solution for 1 h at pH 2.4 and in an HCl-pepsin solution for 2 h at 39°C. Thereafter, the bags were introduced into the small intestine through the duodenal cannula, 1 bag per sample in each of six cows. After recovery from the faeces the bags were washed in a washing machine using cold water before determination of the nitrogen content in the residues.

Three step *in vitro* technique

The following feeds were used: OC97, OC99, OL1, OL2, SC, CO, CO+PEG and AH. The technique of Calsamiglia and Stern (1995) was followed but using also a rumen preincubation time of 48 h for olive by-products. Three Segureña wethers and three Granadina goats, fitted with permanent ruminal cannula, were used. Seven bags per sample (40 × 40 µm pore size, external dimensions 6 × 10 cm) with 1.5 g of sample were incubated in the rumen of each experimental animal. After rumen preincubation, the bags were washed and dried at 60°C for 48 h. Pooled residues containing 15 mg of N were incubated for 1 h in 10 ml of a 1 N HCl-pepsin solution. After incubation, pH was neutralised with 5 ml of 1 N NaOH and, 13.5 ml of a pH 7.8 phosphate buffer containing 37.5 mg of pancreatin were added to the solution and incubated at 38°C. After a 24 h incubation period, 3 ml of 100% (w/v) trichloroacetic acid solution were added to precipitate the undigested proteins and N content in supernatant was measured.

Chemical analysis

The feedstuffs and the residues were analysed for dry matter (DM) and nitrogen according to conventional methods (AOAC, 1990). Acid detergent fibre (ADF) was determined by the ANKOM technology 9/99, and the N in the ADF residue by the Kjeldahl procedure (ADIN). Acid detergent soluble nitrogen (ADSN) was calculated as 100-ADIN.

Statgraphics Plus was used for regression and variance analysis.

Results and discussion

Mobile bag technique

Apparent N disappearance from mobile bags are presented in Table 1 together with N and ADSN content of the different feeds. N disappearance from the mobile bag varied substantially among and within the different feeds. The N availability of the olive by-product was quite low, although it was improved by increasing rumen preincubation time, especially for olive leaves ($P < 0.001$). Although 16 h is the standard rumen preincubation time for the technique, some authors suggested, in accordance with our results, that at least 48 hours are necessary for estimating intestinal digestibility of rumen undegraded N (Mgheni *et al.*, 1994; Vanhatalo *et al.*, 1996) with roughages. Our results showed an important variability within the same by-product (OC) from two different years ($P < 0.001$). Heat treatment significantly increased N total digestibility ($P < 0.001$). However, differences in total digestibility between heated and non heated samples were much lower for 48 h of preincubation than for 16 h. Ahn *et al*. (1989) reported the same effect when comparing dried and freeze dried feeds. A reduction of the condensed tannins concentration as a consequence of heat treatment could explain the increasing N availability in heat treated samples observed in olive leaves. Total N digestibilities of OC and CO were not affected by PEG addition ($P > 0.05$).

Table 1. N content (% in DM), ADSN content (100-ADIN) and N total disappearance (% of total N) from mobile bags in the gastrointestinal tract for 16 and 48 hours of preincubation time

Feed	N	ADSN	16 h total	48 h total
Olive cake 1997	1.55	51.5	58.2	69.1
Olive cake 1999	1.68	51.9	32.8	47.7
Olive cake 1999 + PEG	1.73	53.1	34.7	50.1
Olive leaves, room dried	1.44	64.6	18.1	52.7
Olive leaves, 60°C	1.37	49.8	42.1	68.9
Olive leaves, 100°C	1.39	45.1	43.8	63.9
Olive leaves, freeze dried	1.38	70.3	14.1	46.3
Concentrate	1.84	82.6	86.6	90.4
Concentrate + 2% PEG	1.77	83.8	88.0	90.8
Concentrate + 5% quebracho	1.77	83.0	87.7	90.7
Sunflower cake	5.30	93.8	92.0	–
Sunflower cake + 5% quebracho	5.16	93.8	90.0	–
Barley grain	1.57	97.5	94.8	–
Barley straw	0.54	87.6	66.7	–
Alfalfa hay	2.98	93.8	91.0	–
Alfalfa hay + 5% quebracho	2.79	93.8	90.1	–

Acid detergent insoluble nitrogen

ADSN content (100-ADIN), as shown in Table 1, ranged from 45.1 to 97.5, being lower in the olive by-products. Heat treatment of olives leaves decreased ADSN content ($P < 0.001$), but total N availability based on mobile bag results was higher compared to non treated feeds. "Indigestible N"

(ADIN) may include lignin-bound N, Maillard reaction products and tannin-protein complexes (van Soest *et al.*, 1987).

Recent works have suggested that ADIN may not be entirely indigestible (Weiss *et al*., 1989; van Soest and Mason, 1991) in agreement with our results obtained with olive leaves. The lower differences between treated and non treated leaves when a 48 h period of rumen pre-incubation was used, suggest that ADIN digestibility may be due to degradation in the rumen where both cellulolytic and proteolytic enzymes are present.

Three step *in vitro* technique

Rumen degradability of the nitrogen, intestinal digestibility of rumen-undegraded N and total N digestibility estimated in both sheep and goats following the Calsamiglia and Stern (1995) procedure are shown in Table 2. Increased preincubation time had a significant effect on total N digestibility ($P < 0.01$) may be by increasing rumen degradability but also by increasing intestinal *in vitro* digestibility ($P < 0.01$), except for OC99. This is not in accordance with results reported by Hvelplund *et al*. (1992), in which, using the mobile bag method, extending rumen incubation time resulted in lower intestinal digestibilities for temperate forages and concentrates. This is probably due to the content of antinutritional factors in olive by-products. Drying at 60°C of olive leaves increased total N digestibility ($P < 0.001$) probably by improving rumen degradability, but it did not increase intestinal digestibility ($P > 0.05$). Concerning interspecies differences, goats showed a significantly ($P < 0.001$) better use of nitrogen from olive leaves than sheep at both *in situ* and *in vitro* stage. Those differences were also diluted for 48 h period of preincubation in the rumen.

Table 2. *In vitro* intestinal digestion (digested N/undegraded N, %) and total digestibility (digested N/original N feed content, %) of the different feedstuffs incubated for 16 and 48 h in the rumen of sheep and goats

Feed	Specie	16 h		48 h	
		Intestinal	Total	Intestinal	Total
Olive cake 1997	Sheep	38.4	87.6	45.6	91.0
	Goats	35.3	84.9	48.0	91.5
Olive cake 1999	Sheep	42.0	68.8	41.6	77.0
	Goats	41.5	71.0	42.5	78.4
Olive leaves, room dried	Sheep	34.5	58.4	53.3	88.1
	Goats	46.8	76.3	58.9	92.2
Olive leaves, 60°C	Sheep	35.4	72.1	55.2	92.2
	Goats	41.6	82.8	55.2	93.0
Concentrate	Sheep	84.1	97.3	–	–
	Goats	80.6	97.6	–	–
Concentrate + 2% PEG	Sheep	85.3	98.5	–	–
	Goats	81.8	98.8	–	–
Sunflower cake	Sheep	94.2	81.7	–	–
	Goats	96.6	84.5	–	–
Alfalfa hay	Sheep	92.8	99.2	–	–
	Goats	92.7	99.2	–	–

Comparison between the three methods

Values of ADSN content and digestibility estimated by the mobile bag method (for 16 h of preincubation time), were much closer for conventional feeds than for by-products, especially for non heated olive leaves. ADIN resulted in an underestimation of indigestible nitrogen of by-products (except for OC97) when compared with estimated digestibility of samples preincubated in the rumen for 16 h. Compared to the mobile bag method, N total digestibility were overestimated by the *in vitro*

method. The differences may be due to the different pore size of the nylon bags used in the two methods. There was also a good agreement between N disappearance from the mobile bag and ADSN values with the exception of olive leaves dried at room temperature or freeze dried. This relationship is in accordance with Kusumanti *et al*. (1996) who tested the same relationship on 45 different feeds representing a variety of feedstuffs used for ruminants. Nevertheless, the *in vitro* method gave better estimation of total digestibility obtained by the mobile bag procedure (Fig. 1) than ADSN values (Fig. 2).

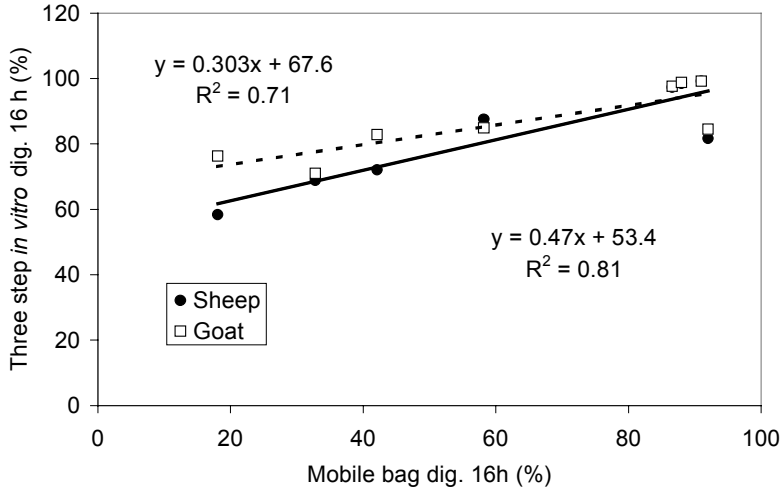

Fig. 1. Relationship between N digestibility estimated by the mobile bag and by the three step *in vitro* method.

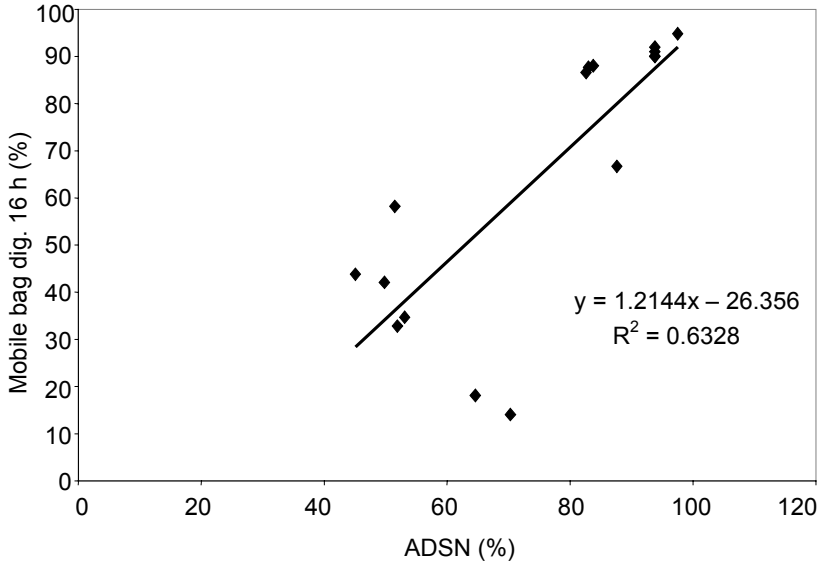

Fig. 2. Relationship between N digestibility estimated by the mobile bag and ADSN content.

Conclusions

The use of a 48 h rumen preincubation period seems to be satisfactory for estimating N post-ruminal availability of non conventional feeds.

The *in vitro* procedure is reasonably useful for protein evaluation of non conventional feeds, but more research is needed in order to validate it in a wide range of food.

References

Agricultural and Food Research Council (1992). Technical Committee on Responses to Nutrients, Report No. 9. *Nutrition Abstracts and Review*, *Series B*, 62: 787-835.

Ahn, J.H., Robertson, B.M., Elliot, R., Gutteridge, R.C. and Ford, C.W. (1989). Quality assessment of tropical browse legumes: Tannin content and protein degradation. *Anim. Feed Sci. Tech*., 27: 147-156.

AOAC (Association of Official Analytical Chemists) (1990). *Official Methods for Analysis*, 15th edn. AOAC, Washington DC, USA.

Calsamiglia, S., Ferret, A., Sensada, M., Miquel, N. and Doblas, A. (1997). Ruminal degradation and intestinal digestion of protein supplements commonly fed to ruminants. *J. Dairy Sci*., 80(Suppl. 1): 399 (abstract).

Calsamiglia, S. and Stern, M.D. (1995). A three step *in vitro* method procedure for estimating intestinal digestion of protein in ruminants. *J. Anim. Sci*., 73: 1459-1465.

Hvelplund, T., Hovell, F.D. DeB., Ørskov, E.R. and Kyle, D.J. (1994). True intestinal digestibility of protein estimated with sheep on intragastric infusion and with the mobile bag technique. *Proc. Soc. Nutr. Physiol*., 3: 645 (abstract).

Hvelplund, T. and Weisbjerg, M.R. (1998). *In vitro* techniques to replace *in vivo* methods for estimating amino acid supply. In: In Vitro *Techniques for Measuring Nutrient Supply to Ruminants.* Occasional Publication No. 22, British Society of Animal Science, pp. 131-144.

Hvelplund, T., Weisbjerg, M.R. and Andersen, L.S. (1992). Estimation of the true digestibility of rumen undegraded dietary protein in the small intestine of ruminants by the mobile bag technique. *Acta Agr. Scand*., *Sect. A, Anim. Sci.*, 42: 34-39.

Kusumanti, E., Weisbjerg, M.R. and Hvelplund, T. (1996). A comparison between protein disappearance from the mobile bag and acid detergent solubility of nitrogen as estimates of protein digestibility in ruminants. *Anim. Feed Sci. Tech*., 5: 337-345.

Mgheni, D.M., Hvelplund, T. and Weisbjerg, M.R. (1994). Intestinal digestibility of rumen undegraded dietary protein from tropical roughages estimated by the mobile bag technique. *Acta Agr. Scand.*, *Sect. A, Anim. Sci.*, 44: 230-235.

National Research Council (1985). *Ruminant Nitrogen Usage*. National Academy Press, Washington DC, USA.

Nordisk Kontaktorgan for Jordbrugsforskning (1985). Introduction of the Nordic protein system for ruminants into practice and further research requirements. *Acta Agric. Scand*., *Sect. A, Anim. Sci*., 25(Suppl.): 216-220.

Vanhatalo, A., Dakowski, P. and Huhtanen, P. (1996). Effects of stage of growth and duration of rumen incubation time on intestinal digestibility of rumen-undegradable nitrogen of grass by the mobile bag method in cows. *Acta Agric. Scand*., *Sect. A, Anim. Sci.*, 46: 1-10.

van Soest, P.J., Conklin, N.L. and Horvath, P.J. (1987). Tannins in foods and feeds. In: *Proc. Cornell Nutrition Conference for Feed Manufacturers*, Ithaca, NY (USA). Cornell University Press, Syracuse (NY), USA, pp. 115-122.

van Soest, P.J. and Mason, V.C. (1991). The influence of the Maillard reaction upon the nutritive value of fibrous feeds. *Anim. Feed Sci. Tech*., 32: 45-53.

Waters, C.J., Kitcherside, M.A. and Webster, A.J.F. (1992). Problems associated with estimating the digestibility of undegraded dietary nitrogen from acid-detergent nitrogen. *Anim. Feed Sci. Tech*., 39: 279-291.

Weiss, W.P., Erickson, D.O., Erickson, G.M. and Fisher, G.R. (1989). Barley distillers grain as a protein supplement for dairy cows. *J. Dairy Sci*., 72: 980-987.