General problems in assessing the nutritive value of Mediterranean forages

Susmel P., Stefanon B., Mills C.R.

in

Tisserand J.-L. (ed.), Alibés X. (ed.).
Fourrages et sous-produits méditerranéens

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 16

1991
pages 17-22

General problems in assessing the nutritive value of Mediterranean forages

P. SUSMEL, B. STEFANON, C.R. MILLS
ISTITUO DI PRODUZIONE ANIMALE
UNIVERSITA DEGLI STUDI DI UDINE
VIA. S. MAURO, 2 - 33010 PAGNACCO (UD) ITALY

SUMMARY - Some problems associated with feedingstuffs tables are presented. The authors discuss these problems in terms of the prediction of nutritive value and conclude that the in situ *nylon bag system has considerable potential for resolving many of the practical difficulties associated with producing feedingstuff tables. However, the in situ technique must be standardised to be valid. Enzymatic assays can be used to replace the nylon bag technique for the characterisation of protein.*

RESUME - "Problèmes généraux posés par l'appréciation de la valeur des fourrages méditerranéens". Les auteurs présentent des problèmes concernant les tables de prévision des aliments. Les méthodes et les équations pour l'évaluation de la digestibilité de la matière sèche, organique et azotée sont analysées. Les auteurs remarquent la possibilité d'utiliser les Méthodes "in sacco" *des sachets de nylon pour étudier la valeur énergétique et azotée des aliments, comment les sous-produits, qui varient beaucoup avec les diverses conditions géografiques et de climat. La nécessité d'une standardisation comme les méthodes "in sacco" est enfin soulignée.*

Introduction

In feedingstuffs tables, chemical and nutritive data are reported as well as some brief information to characterize the feed (NRC, 1982; INRA, 1980, 1988).

In the tables it is generally assumed that the same feed has an almost constant composition and nutritive value, but this is only true for most of the cereals, concentrates, vegetable oil extraction by-products and some animal meals. For forages and by-products, many factors, besides stage of maturity, are involved in determining both the chemical composition and the biological availability of nutrients, because rainfall, temperature, light and fertiliser applications all contribute to modifying plant structure. Even though tables can report detailed proximate analysis, fibre fractions, aminoacid and mineral and vitamin contents, the data relating to energy density and digestible or metabolizable protein, frequently derived from the former information using statistically derived equations, can often be useless for practical purposes, as forages with similar chemical composition can be utilised with different efficiencies by animals. For instance, it is well known that polyphenols, especially those classified as tannins, have an adverse effect on degradability and digestibility (Reed, 1987; Susmel *et al.*, 1989b; Waghorn *et al.* 1987) and their presence is not evident from proximate Van Soest analysis. By-products are even more variable with processing temperature and method and other factors contributing to a change in nutritive value; the formation of the Maillard products are one of the most frequent causes of reduction in digestibility (Van Soest, 1983).

Strictly speaking, the information obtainable from tables are useful only in the geographical area in which the forages and their by-products were grown. A possible solution to this problem would be to implement tables with chemical data highly related to nutritional values. The use of these chemical components in prediction equations for digestibility would be more useful if these were established for groups of feeds of similar origin and characteristics. For the Mediterranean feedstuffs, C.I.H.E.A.M. (1981, 1983) have proposed tables in which chemical and nutritive data derive directly from *in vivo* experiments, and this is probably the best way to estimate the nutritive value of feedstuffs.

The measurement of the nutritive value of feedstuffs

Energy

The nutritive value of feeds, forages and by-products of agricultural origin can be measured with digestibility trials, in which total faeces and urine are collected in a

Table 1. Previsional equations of organic matter digestibility based on chemical composition of feeds.

	EQUATION	r^2	rse
DRY MATTER DIGESTIBILITY:			
Von Keyserilngk and Mathison (1989)	97.29-0.701*(%Feed NDF) 98.76-1.174*(%Feed ADF)	0.86 0.60	4.35 7.29
Sauvant et al. (1985)	100.1-0.958*NDF 99.0-0.841*NDF-0.616*ADL	0.97 0.98	5.40 4.60
Girard and Dupuis (1988)	0.98*SOL+0.95*PDF	0.99	–
(maintenance; SOL = cell soluble; PDF= potentially digestible fibre)			
ORGANIC MATTER DIGESTIBILITY:			
Demarquilly and Jarrige (1981)	87.8+0.172*CP-3.796*ADL (grass) 73.4+0.601*CP-2.792*ADL (legumes)		2.30 2.79
Barber et al. (1984)	113.5-1.43*ADF (hay)	0.71	4.00
Gigier (1985)	86.4-2.09*KL (various feeds) 99.1-4.38*KL (14 forages)	0.68 0.93	6.60 3.20
NET ENERGY:			
Conrad et al. (1984)	1.98 - 1.73 * ADF	0.89	–
	2.28[CP*e-0.0172*ADIN+Fat+0.92* *(1-Ash-CP-NDF)+0.75*(NDF-L)* *(1-L2/3/(NDF)2/3)]-0.10	0.99	–

5-7 day period and related to intake. In these experiments, the apparent coefficients of digestibility for dry matter, organic matter, energy, crude protein, fat and fibre fractions (NDF, cellulose and hemicellulose) can be derived. It is well known that this method is time consuming and expensive. Recently, Tamminga *et al.* (1989) have proposed some fibrous feed components, considered indigestible (rumen indigestible NDF; cellulase indigestible ADF; KMnO4 lignin, soluble and insoluble KMnO4 lignin), as internal markers of forage dry matter digestibility. The use of internal markers such as acid detergent lignin and permanganate lignin, to estimate *in vivo* dry matter digestibility has been also applied by Krysl *et al* (1988) and Susmel *et al.* (1990).

It is generally accepted that simple proximate analysis cannot be used to estimate nutrient availability in forages; the fibre characterisation proposed by Goering and Van Soest has been used more successfully (table 1) to predict dry and organic matter digestibility for forages, hay and silage, some by-products and concentrates. It is likely that for feeds with high levels of fibre and lignin-like compounds, and especially those coming from countries with hot climates, the above equations would not function adequately.

Some *in vitro* techniques, such as the Tilley and Terry (1963) method, have been developed and then used widely to define the energy content of feeds, forages in particular. The Tilley and Terry method is the most accurate, but it has the disadvantage of requiring rumen liquid and thus either the availability of fistulated donors or cattle and sheep to be slaughtered. Some alternative methods use enzymes, generally bacterial or fungal cellulase, either alone or with pepsin (table 2), that simulate the whole digestive process. The agreement between *in vivo* and *in vitro* data for fresh forages and hay are generally high and statistical analysis gives good r2 and low residual standard errors (table 3). For this reason, enzymatic analysis has increased in the past few years and some energy systems suggest their use for the estimation of the nutritive value of feeds. Table 3 shows that the relationships are much more difficult to define for straw. Thus *in vitro* methods seem unable, in some cases, to reproduce accurately the conditions existing in the host animal and require continuous comparison with data obtained in the animal.

At the present time, the investigation of degradative processes directly in the ruminant represents one of the most promising methods available. Degradability values have been effectively used as predictors of intake

Table 2. Prediction of digestibility using enzymes

ENZYME	Statistical data	rsd	r	FEEDSTUFFS
IN VITRO DIGESTIBILITY (Tilley and Terry):				
Barber et al., 1984	y= 0.99 X + 5.9	2.6	0.71	89 fresh grasses
Barber et al., 1984	y= 1.01 X + - 0.3	2.0	0.89	53 hay
Barber et al., 1984	X	6.2	0.10	22 straw
CELLULASE OF TRICHODERMA VIRIDE:				
Dowman and Collins, 1977	y= 0.59 X + 31.27	2.3	0.89	silages
Dowman and Collins, 1982 (NDF)	y= 0.70 X + 16.73	1.9	0.84	grass silage
	y= 0.72 X + 11.25	1.7	0.72	maize silage
	y= 0.55 X + 28.49	1.6	0.70	dried grass
	y= 0.83 X + 7.91	2.6	0.88	hay
Rexen, 1977	y= 0.83 X + 33.24	3.6	0.91	alkali treated straw
Israelsen et al, 1978 (insoluble fibre)	y=-0.70 X + 87.2	3.2	–	39 compounds with alkali treated straw
PEPSIN CELLULASE:				
Jones and Hayward, 1975	y= 0.54 X + 35.0	2.9	0.93	19 grasses
Goto and Minson, 1977	y= 0.69 X + 20.3	2.7	0.94	45 tropical grasses
Aufrere, 1982	y= 0.87 X + 0.72	–	0.97	23 forages
Barber et al., 1984	y= 0.49 X + 39.2	2.5	0.73	88 fresh grasses
Barber et al., 1984	y= 0.68 X + 26.4	2.3	0.85	53 hays
Barber et al., 1984	X	5.8	0.20	17 straws
NDF (PEPSIN) CELLULASE				
McLeod and Minson, 1982	y= 0.74 X + 15.9	3.4	0.89	50 grass and 30 lucerne
Barber et al., 1984	y= 0.56 X + 30.2	2.5	0.76	131 fresh grasses
Barber et al., 1984	y= 0.84 X + 11.6	3.4	0.79	59 hays
Barber et al., 1984	X	4.9	0.16	24 straws

Table 3. Use of nylon bag technique to predict dry matter digestibility

Digestibility estimated:	E Q U A T I O N	rsd	r^2	
In vivo	1.258+0.0642*(a+b)	0.33	0.86	straw
	-2.595+0.06244*(a+b)+39.0*c	0.19	0.96	based
	-2.576+0.0554*a+0.0640*b+37.7*c (Orskov et al., 1988a)	0.20	0.95	diets
In vitro	0.60+0.41*DgOM-0.83*DgHem+0.51*DgNDF	0.03	0.73	forages
KMnO4-lignin (Susmel et al., 1990)	0.53+0.40*DgOM-0.88*DgHem+0.62*DgNDF	0.03	0.75	forages
In vivo	0.056 + 0.742* (a+b)	0.06	0.69	forages
In vivo (Von Keyserlingk and Mathison, 1989)	0.880 - 0.627*(24 h DM residue)	0.03	0.92	forages
In vivo (Bittante et al., 1988)	48 h DM degradability	–	0.93	22 by products
(Bittante et al., 1988)		–	0.92	various feeds
		–	0.96	15 diet

(Orskov *et al,* 1988b) and organic matter digestibility (Bittante *et al.,* 1988; Von Keyserlingk and Mathison, 1989; Susmel *et al.,* 1990). Degradability, digestibility and nutrients availability of forages and fibrous by- products depends mainly on the fibre composition (Van Soest, 1983; Susmel *et al,* 1990). The regression equations reported in table 4 shows that a large proportion of the OM digestibility can be predicted from the degradabilities of OM and fibre fractions. The potentially degradable fraction of dry matter, measured *in situ,* was highly correlated with the organic matter digestibility (Orskov et al., 1988a) in straw-based diets. Von Keyserlingk and Mathison (1989) also reported DM digestibility predictions based on either the potentially degradable fraction of the dry matter or on the bag residue after 24 hours, with an increasing level of r2. Susmel *et al.* (1990) observed higher r2 values using effective degradabilities, rather than the potentially degradable fraction, of fibre fractions (i.e. OM, hemicellulose and NDF). Bittante *et al.* (1988) concluded that a single incubation time of 48 hours could be sufficient to rank different class of feedstuffs according to their organic matter digestibility and metabolisable energy content.

Table 4. ***Correlation coefficients (r) between in situ*** **degradability of nitrogen and** ***in vitro*** **degradability.**

	variables in correlation						r^2
	In situ			*In vitro*			
Crawford et al. (1978)	2 h	Dg	VS	2 h	Buffer		0.66
Susmel et al. (1990)		Dg	VS	1 h	Enzyme		0.78
Aufrere and Cartailler (1988)		Dg	VS	Dg	Enzyme		0.95
Poos-Floyd et al., (1985)	12 h	Dg	VS	12 h	Enzyme		0.88
Krishnamoorthy et al. (1983)		Dg	VS	Dg	Enzyme		0.78
Stern and Satter (1984)	1 h	Dg	VS	1 h	Enzyme		0.79

Protein

Since the beginning of the 1980s, a large number of protein systems have been proposed (INRA, 1980, 1988; ARC, 1984; Madsen, 1985; NRC, 1985; Susmel and Stefanon, 1987) in which the nitrogen fractionation into degradable and undegradable has been taken into account. All the systems consider the relationship between energy and nitrogen availability in the rumen as the main factor in determining bacterial growth and, especially for forages, fibre and protein degradability and total digestibility. As shown above, rumen degradability can characterise the energy value of forages and, in turn, their protein value.

Nitrogen degradability can be measured by means of the in situ technique (Orskov and McDonald, 1979), but this method has the same limitations seen for *in vivo* digestibility. Antongiovanni et al. (1990) proposed a method for predicting the protein value of feedstuffs from the aminoacid profile of the feed before and after incubation in a tube according to Tilley and Terry methodology. The values are highly correlated to those obtained *in situ.* Alternatively, the use of proteolytic enzymes has been proposed to estimate nitrogen degradability, but the correlation coefficients obtained are not always adequate (table 5).

Table 5. Comparison of degradability coefficients and effective degradability either in situ or in rusitec.

Feed		a	b	c	Dg
NITROGEN:					
Soya	In situ	23.0	77.0	0.082	64.4
	Rusitec	57.8	40.8	0.106	84.6
		**	**	*	**
Oats	In situ	66.0	27.6	0.183	85.9
	Rusitec	79.1	10.4	3.455	89.2
		**	**	*	**
Wheat bran	In situ	29.1	70.7	0.100	70.6
	Rusitec	58.9	35.6	0.067	78.0
		**	**	*	**
Mean	In situ	29.5	43.8	0.091	55.2
	Rusitec	48.9	21.7	0.907	63.0
		ns	*	ns	ns

y=a+b*(1-e-ct); Dg = a+(b*c)/(c+k);
k in situ = 0.06; k rusitec = 0.055

In an experiment conducted by Susmel et al. (1990), the nitrogen degradability measured with protease from *Streptomyces griseus* at 1 and 24 hours and the *in situ* degradability coefficients (i.e. a, b, a+b) were generally poorly correlated, with r values less than 0.7. A highly significant ($P<0.001$) correlation was observed between the *in situ* effective degradability and the *in vitro* degradability value at 1 h. Good correlations between *in situ* nitrogen degradability values and short (from 1 to 4 hours) incubation times either in enzyme or buffer solutions were also reported by Crawford et al (1978), Krishnamoorthy et al (1983), Stern and Satter (1984) and Poos-Floyd et al. (1985). Aufrere and Cartailler (1988) reported a high correlation (r2 0.95) between the effective nitrogen degradability *in situ* and that obtained with an enzymatic technique with various incubation times.

The use of continuous fermentation devices to simulate rumen dry matter and nitrogen disappearance is also fairly common. In an experiment conducted at the Institute of Animal Production of Udine (Susmel, Mills and Stefanon, personal communication) the degradability of 4 feeds was measured simultaneously either *in situ* (4 cows, duplicate) or in a RUSITEC apparatus (triplicate bags). Incubation times were 0, 2, 4, 10 and 24 hours. The results reported in table 6 show that the *in vitro* data differed significantly from those obtained *in situ* for nitrogen. From these results, the difficulty of simulating the conditions found in the rumen is evident.

Conclusions

The results and the equations presented in this paper represent only a small part of a much larger number published elsewhere, but some conclusions can be drawn.

The prediction of energy and protein values for feeds needs to be stated for a specific feed type, since general equations can fail to give accurate estimates. In particular, straw, by-products and, probably, forages with high concentrations of fibre and lignin require additional care. From the above reported equations, it seems that for these feed types, the rumen degradability of dry matter, organic matter or fibre fractions are highly correlated with the digestibility and that the determination of fibre fractions according Van Soest or of other "indigestible" compounds is useful.

The *in situ* method has the advantage of comparative ease of use, together with the fact that it exposes test substrates to the entire rumen flora for an adequate period of time in the dark and under slightly reductive conditions. The *in situ* technique can be used either to study the degradability of a wide variety of incubated feedstuffs with one basal diet or to study the effect of a range of basal diets on one or more incubated feedstuffs. The adoption of degradability assays allows a simultaneous estimate of the degradability of both dry matter and nitrogen; in this way the protein value of a feed can be easily predicted (Susmel and Stefanon, 1987). Experimental work should be directed towards the estimation of the *in situ* degradability of standard forages and straw, such as lucerne hay and wheat straw, in reference laboratories in the Mediterranean area; on the same forages, chemical analysis should be performed. At the same time, *in vitro* determinations should be adopted to find close correlations with the *in vivo* data and with the chemical characterisation of the feeds.

The *in situ* technique needs to be standardized using appropriate basal diets and animals, although it is as yet difficult to apply results obtained from one species to another. This problem is particularly valid for the animal species found in developing countries, where the animals have adapted to the environment and probably have an enhanced ability to digest lignocellulose.

References

ANTONGIOVANNI,M., GRIFONI,F., PEZZATI,A., SUSMEL,P. STEFANON,B. (1989) Estimation of ruminal protein degradation and biosynthesis by an *in vitro* method. "Asian Journal of Animal Science", 2:343-345.

ARC 1984. The Nutrient Requirements of Ruminant Livestock. Suppl.1. Agricultural Research Council, Farnham Royal, Slough. UK.

AUFRERE,J. 1982. Etude de la prévision de la digestibilité des fourrages par une méthode enzymatique. "Ann. Zootech.", 31,111-130.

AUFRERE, J. and CARTAILLER, D. 1988. Mise au point d'une méthode de laboractoire de prévision de la degradabilité des proteines alimentaires des aliments concentrées dans le rumen. "Ann. Zootec.", 37,255-270.

BARBER,W.P., ADAMSON,A.H. and ALTMAN,J.F.B. 1984. New methods of forage evaluation. in Recent advances in animal nutrition. (Haresign,W. and Cole,D.J.A. Eds.). Butterworths, London.

BITTANTE,G., RAMANZIN,M., COZZI,G and SPANGHERO,M. 1988. Estimation of the organic matter digestibility and of the metabolizable energy content of feedstuffs and by-products by means of the fibre bag technique. "J. Anim. Sci.", 66, Suppl.1,495(A).

C.I.H.E.A.M. 1981. Options méditerranéennes. Tableaux de la valeur alimentaire pour les ruminants des fourrages et sous-produits d'origine méditerranéenne. Insitut Agronomique Méditerranéen de Zaragoza. Spain.

C.I.H.E.A.M. 1983. Options méditerranéennes. Tableaux de la valeur alimentaire pour les ruminants des fourrages et sous-produits d'origine méditerranéenne. Insitut Agronomique Méditerranéen de Zaragoza. Spain.

CONRAD, H.R., WEISS, W.P., ODWONGO, W.O. and SHOCKEY, W.L. 1984. Estimating net energy lactation from components of cells solubles and cell walls. "J. Dairy Sci.", 67,427-436.

CRAWFORD, R.J.,JR., HOOVER, W.H., SNIFFEN, C.J. and CROOKER, B.A. 1978. Degradation of feedstuff nitrogen in the rumen versus nitrogen solubility in three solvents. "J. Anim. Sci.", 46,1768-1775.

DEMARQUILLY,C. and JARRIGE,R. 1981. Panorama des méthodes de prévision de la digestibilité et de la valeur énergétique des fourrages. Prévision de la valeur nutritive des aliments des ruminants. (INRA Publ. 1981).

DOWMAN,M.G. and COLLINS,F.C. 1982. The use of enzymes to predict the digestibility of animal feeds. "J. Sci. Food Agric.", 33,689-696.

DOWMAN,M.G. and COLLINS,F.C., 1977. The prediction of the digestibility of silages using cellulase. "J. Sci. Food Agric.", 28,1071-1074.

GIGIER,S. 1985. Revue sur les méthodes de dosage de la lignine utiliséés en alimentation animale. "Ann. Zootech.", 34,85-122.

GIRARD,V. and DUPUIS,G. 1988. Effect of structural and chemical factors of forages on potentially digestible fiber, intake, and true digestiblity by ruminants. "Can. J. Anim. Sci.", 68,787-799.

GOTO,I. and MINSON,D.J. 1977. Prediction of the dry matter digestibility of tropical grasses using a pepsin-cellulase assay. "Anim. Feed Sci. Technol.", 2,247-253.

KRISHNAMOORTHY, U., SNIFFEN, C.J., STERN, M.D. and VAN SOEST, P.J. 1983. Evaluation of a mathematical model of rumen digestion and an in vitro simulation of rumen proteolysis to estimate the rumen undegraded nitrogen content of feedstuffs. "Br. J. Nutr.", 50,555-568.

KRYSL,L.J., GALYEAN,.M.L., ESTELL,R.E. and SOWELL,B.F. 1988. Estimating digestibility and faecal output in lambs using internal and external markers. "J. Agric. Sci. Camb.", 111,19-25. INRA 1980. Alimentation des ruminants. Ed. R. Jarrige. INRA, Paris

INRA 1988. Alimentation des bovins, ovins et caprins. Ed. R. Jarrige. INRA, Paris.

ISRAELSEN,M., REXEN,B. and THOMSEN,K.V. 1978. Cellulase insoluble fibre as a measure of unavailable organic matter in cattle compounds containing alkali-treated straw. "Anim. Feed Sci. Technol.", 3,227-234.

JONES,D.I.H. and HAYWARD,M.V. 1975. "J. Sci. Food Agric.", 26,711-718.

MADSEN, J. 1985. The basis for the proposed nordic protein system for ruminants. The AAT.PBV system. "Acta Agric. Scand.", Suppl.25,9-20.

McLEOD,M.N. and MINSON,D.J. 1982. "Anim. Feed Sci. Technol.", 7,83-92.

NRC 1982. United states-Canadian Tables of Feed Composition. National Academy Press, Washington, D.C.. USA.

NRC 1985. Ruminant Nitrogen Usage. National Research Council. National Academy Press, Washington D.C., USA.

ORSKOV, E.R. and McDONALD, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. "J. Agric. Sci. Camb.", 92,499-503.

ORSKOV, E. R., OJWANG, I. and REID, G. W. 1988a. A study on consistency of differences between cows in rumen outflow rate of fibrous particles and other substrates and consequences for digestibility and intake of roughages. "Anim. Prod." 47, 45-51.

ORSKOV, E. R., REID, G. W. and KAY, M. 1988b. Prediction of intake by cattle from degradation characteristics of roughages. "Anim. Prod." 46,29-34.

POOS-FLOYD, M., KLOPFENSTEIN, T. and BRITTON, R.A. 1985. Evaluation of laboratory techniques for predicting ruminal protein degradation. "J. Dairy Sci.", 68,829-839.

REED,J.D. 1987. Phenolics, fiber and fiber digestiblity in bird resistant and non bird resistant sorghum grain. "J. Agric. Food. Chem.", 35,461-464.

REXEN,B. 1977. Enzyme solubility - A method for evaluating the digestibility of alkali-treated straw. "Anim. Feed Sci. Technol.", 2,205-218.

SAUVANT,D., BERTRAND,D. and GIGIER,S. 1985. Variations and prevision of the *in sacco* dry matter digestion of concentrates and by-products. "Anim. Feed Sci. Technol.", 13,7-23.

STERN,M.D. and SATTER,L.D. 1984. Evaluation of nitrogen solubility and the dacron bag technique as methods for estimating protein degradation in the rumen. "J. Anim. Sci.", 58,714-724. -SEZIONE 2

SUSMEL P. and STEFANON B. 1987. Sistema per la valutazione delle proteine alimentari per i ruminanti. "Zoot. Nutr. Anim.", 13,567-582.

SUSMEL, P., STEFANON, B., MILLS, C. R. and SPANGHERO, M. 1990. Rumen degradability of organic matter, nitrogen and fibre fractions in forages. "Anim. Prod". In press.

SUSMEL, P., STEFANON, B., MILLS, C. R. and COLITTI,M. 1989a. The evaluation of PDI concentrations in some ruminant feedstuffs: a comparison of *in situ* and in vitro protein degradability. "Annal. Zootech." 38,269-283.

SUSMEL, P., STEFANON, B., OVAN, M. and PIASENTIER, E. 1989b. Effetto dell'aggiunta di urea e tannini sulla degradabilità *in situ* di alcune fonti proteiche. "Zoot. e Nutr. Anim." 15, 147-159.

TAMMINGA,S., ROBINSON,P.H., MEIJS,S and BOER,H. 1989. Feed components as internal markers in digestion studies with dairy cows. "Anim. Feed Sci. Technol.", 27,49-57.

TILLEY,J.M.A. and TERRY,R.A. 1963. A two-stage technique for the *in vitro* digestion of forage crops. "J. Br. Grassland Ass.", 18,104-111.

WAGHORN, G.C., ULYATT, M.J., JOHN, A. and FISCHER, M.T. 1987. The effect of condensed tannins on the site of digestion of amino acid and other nutrients in sheep fed on *Lotus corniculatus L.* "Br. J. Nutr.", 57, 115-126.

VAN SOEST,P.J. 1983. Nutritional ecology of the ruminant. Cornell University. O & B Books Inc., NW Kline Place, USA.

VON KEYSERLINGK, M.A.G. and MATHISON, G.W. 1989. Use of the *in situ* technique and passage rate constants in predicting voluntary intake and apparent digestiblity of forages by steers. "Can. J. Anim. Sci." 69,973-987.