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IN SITU RUMEN DEGRADATION KINETICS OF HIGH-PROTEIN FORAGE CROPS IN TEMPERATE CLIMATES

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The present study was conducted to evaluate the nutritional value and *in situ* degradation kinetics of eight high protein forage crops: alfalfa (*Medicago sativa* L.), forage oat (*Avena sativa* L.), mixed pasture, and ryegrass (*Lolium multiflorum* Lam.) pasture in early vegetative stages, two forage lupins (*Lupinus albus* L.) in early bloom stages, sugar beet (*Beta vulgaris* L.) and kale (*Brassica napus* var. *pabularia* (DC.) Rchb.) leaves at root maturity. Dry matter (DM) and crude protein (CP) degradation kinetics were evaluated by the nylon bag technique through the *in situ* procedure described by Ørskov and MacDonald (1979) using three ruminally cannulated sheep. Chemical composition of the forage crops showed on average 13.7% DM; 21.4% CP; 31.5% neutral detergent fiber (NDF); 17.7% crude fiber (CF), 80.6% digestibility of organic matter (DOMD) and 12.13 MJ kg^{-1} metabolizable energy (ME). The high total degradability of forage crops reported here (> 87% DM; > 93% CP) can be associated with the presence of large quantities of fraction *a* (> 34% DMa; > 29% CPa) and high degradability of fraction *b*, resulting in low amounts of undegradable fraction (U) (7.02% DM and 3.55% CP). Correlations between CPb and DMb degradability (r = 0.79) and CPc and DMc degradation rates (r = 0.78) were high, however differences in *c* were not explained by differences in CP or NDF contents, nor by the amounts of *a* or *b* fractions. Degradation for DM and CP during the first 6 h of incubation was strongly and inversely correlated to *b* (36 h) (r = 0.93) ($P < 0.0001$) regardless of forage type. The amounts of CPa and CPb influenced effective degradability of CP (r = 0.79; $P < 0.02$), EDp increased with increased CPa and decreased with increased CPb (r = 0.76; $P < 0.02$). Therefore, more than 75% of the forage crops degraded within the first 6 h of incubation, which was associated with the DM content and amount of the slowly degradable fraction present.

Key words: Rumen, forage, rate of passage, chemical composition, degradability.

The new methods used to estimate the protein value of food for ruminants are based on the characterization of nitrogenous fractions of the food and consider the rumen fermentation process. Protein fraction values allow for estimating the amount of dietary N available for microbial protein synthesis and the amount of N that escapes degradation in the rumen. Therefore, the real protein enzymatically digested and absorbed in the small intestine will be dependent on the dietary source and rumen fermentability of the feed (Kempton, 1980).

In southern Chile, pastures are the main source of nutrients for dairy, beef and sheep production. Since production systems work year-round, additional sources of feeds like silage and supplements are used in winter or under deficits of pasture availability to meet animal requirements. The nutritive value of the pasture changes in relation to fertilization, season, management, water availability, and stage of maturity (Chaves *et al*., 2006). As a result, crude protein (CP) content is high in early growth stages and decreases with maturity (Aufrère *et al*., 2000; 2003), which is related to decreasing CP content. The change in degradability is not consistent among forage species (Yu *et al*., 2004a). Immature forage crops contain more non-protein N, primarily composed of ammonia, nitrate, amides, and free amino acids that are rapidly degradable in the rumen, therefore having less by-pass protein. With advancing maturity, protein synthesis continues and the cell wall matrix becomes more complex, rendering forage protein less degradable (Van Soest, 1994; Cherney *et al*., 2003).

When early grass growth stages are combined with high N fertilization, which is a common situation for most forage resources used in intensive production systems, soluble CP and non-protein N (NNP) contents increase and favor higher degradability of CP (Van Soest *et al*., 1978; Tremblay *et al*., 2005). Since microbial population growth is highly dependent on the availability of fermentable carbohydrates, its insufficient supply with respect to protein should reduce microbial protein synthesis, increasing N loss through urine and reducing the supply of amino acids (Hristov *et al*., 2005). The most limiting factor for efficient N utilization is the type and amount of carbohydrates available in the rumen.

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Besides the reduced availability of readily fermentable carbohydrates and the faster rates of degradation of protein compared to dry matter (DM) (Nocek and Russell, 1988), a greater lipid content in early growth stages should also limit available rumen energy and the efficient use of N in the rumen (AFRC, 1992).

The present study was designed to evaluate and characterize protein and DM rumen degradation dynamics of high protein forage crops using the *in situ* nylon bag technique in order to optimize the use of the nitrogenous fraction in animal feed formulations.

MATERIALS AND METHODS

Three sheep fitted with rumen fistula (45 ± 5 kg) were used for this study. During the experimental period the animals were housed in individual pens. They were fed four times a day following a feeding schedule established in a previous study to keep rumen pH above 6.2. Hay was offered at 08:00 h, 16:00 h and a pellet mixture fed at 11:00 h, 19:00 h. The diet consisted of 40% pasture hay and 60% of a mixture of equal parts of alfalfa pellets, a digestible fiber concentrate based on sugar beet pulp (17% CP) and a starch concentrate based on cereal grains (17% CP); intake was kept at 1.8% of body weight.

In situ study

The *in situ* DM and CP disappearance of rumen-incubated feeds was conducted according to the method of Ørskov and MacDonald (1979). Polyester bags (14 × 9 cm external diameter) (ANKOM100, Turk Hill Park, Fairport, New York, USA) of known weight and controlled porosity (40-60 μm) (Ørskov *et al*., 1980; Nocek, 1988) were used. For each bag 3 g sample was added to allow a ratio of sample to effective bag area of 16 mg cm^{-2}. The bags were sealed with silicone paste after double sewing to avoid sharp edges and loss from stripping. Each forage species was evaluated by using five incubation periods (0, 2, 6, 12, 24, 36 h), considering two replicates per time and per animal, with a total of six observations per time. As forage crops were in the early growth stages, with presumably high digestibility, the use incubation periods beyond 36 h was not considered necessary. Bags were incorporated to the rumen in reverse order to incubation time and all were extracted at the same time. After rinsing in cold water several times, bags containing forage residues were oven dried to a constant weight (48 h, 60 ºC) before subsequent analysis (Mehrez and Orskov, 1977). CP and DM solubility was determined without incubation in the rumen (T0 h) by soaking the bags containing the samples in warm water (40 ºC) for 20 min, followed by drying as described.

Sample preparation and chemical analysis

The following forage species were studied: alfalfa (*Medicago sativa* L.), oat forage (*Avena sativa* L.), mixed pasture and rye grass (*Lolium multiflorum* Lam.) pasture, in early vegetative stages; two forage lupins (*Lupinus albus* L.) in early bloom stages, sugar beet (*Beta vulgaris* L.) and kale (*Brassica napus* var. *pabularia* (DC.) Rchb.) leaves at root maturity. Forage samples were freeze-dried and milled through a 5 mm screen for degradability studies and through a 1 mm screen for chemical analysis.

Forage species were analyzed for CP, DM, total ash (Ash), crude fiber (CF) (Cundiff, 1995), neutral detergent (NDF) and acid detergent fiber (ADF) and *in vitro* DM digestibility (DMOD) (Van Soest *et al*., 1991). Metabolizable energy (ME) was estimated by *in vitro* 48 h fermentation using rumen filtrated liquor and artificial saliva and then 48 h pepsin + HCl enzymatic digestion. Values for *in vitro* DOMD were associated to ME by regression (ME = 0.325 DOMD + 0.279; Garrido and Mann, 1981) (Table 1).

Table 1. Chemical composition of forages (DM basis).

Forage	DM	CP	CF	NDF	ME	DOMD	DM
		%			MJ kg^{-1}	%	
Vegetative kale leaves	11.3	22.5	12.4	19.1	13.0	87.3	4.8
Vegetative alfalfa	15.5	26.2	18.9	29.6	11.1	73.2	9.2
Lupin plant, early bloom (O)	13.9	17.1	18.7	29.0	12.0	81.2	4.9
Lupin plant, mid bloom (L)	12.5	14.9	19.3	27.7	12.2	80.8	4.9
Vegetative mixed pasture	16.2	27.4	19.4	37.7	12.1	80.5	11.4
Vegetative oat forage	14.7	20.8	23.0	40.5	12.5	83.6	8.2
Vegetative ryegrass pasture	13.9	22.6	20.1	39.1	11.9	79.1	8.9
Sugar beet leaves	11.2	19.6	9.8	29.5	11.9	79.0	13.6

DOMD: *in vitro* digestibility of organic matter; DM: dry matter; CP: crude protein; CF: crude fiber; NDF: neutral detergent fiber; ME: metabolizable energy.

In situ forage residue analysis

In situ DM and CP recovery data were fitted to the model of Ørskov and MacDonald (1979) using a nonlinear regression procedure: $p = a + b\ (1\text{-}e^{-ct})$, *p* being the percentage of CP or DM degradation. The formula considers two N fractions in the feed: where *a* is the soluble (or rapidly degraded) fraction, *b* is the slowly degradable (or potentially degradable) fraction and *c* is the rate of degradation of fraction *b* (h^{-1}) in the rumen.

The undegradable fraction *U* was calculated as: U = 100 – (a + b), at an exponential reducing rate (e^{-ct}). Effective degradability (ED) of CP and DM was calculated as; ED = a + [(b x c)/(c + k)] according to Ørskov and Ryle (1990), assuming constant passage rates from the rumen (k) of 0.02, 0.05, and 0.08 h^{-1} for each ingredient. Determination of degradability values and statistical analysis were performed by non-linear regression (Quasi-Newton) and the SYSTAT (1990) program. Regression analysis was used to establish the relationship between chemical composition and the proportions of the different fractions degraded over time and with *in situ* ED of CP.

RESULTS AND DISCUSSION

Forage chemical composition showed on average low DM (13.7%), high protein (21.4% CP), low fiber

(31.5% NDF, 17.7% CF) and high digestibility and energy contents (80.6% DOMD and 12.13 MJ ME kg^{-1} DM) (Table 1). The high protein and low fiber and DM content of the forage crops were in accordance with the early vegetative stages at which they were harvested. With the exception of lupin, the CP content of individual forage crops was above 20%. The two lupin forage crops had different CP contents, but similar NDF, ADF, CF, digestibility, ME, and ash contents. The high ash content in sugar beet leaves could be explained by soil contamination and the only affect could be a reduced DOMD value. The high digestibility of the different forage crops is attributed to the low fiber content due to low plant maturity or, in the case of kale and sugar beet, the high proportion of leaves.

Degradation kinetics

The kinetics of CP degradation was characterized by differences among forage crops in the soluble fraction (a) and slowly degradable fraction (b), with small differences in the undegradable fraction (*U*) and potential degradability (a + b) (Table 2). The high protein (95%) and DM (93%) degradability and the similar values among forage crops (CP SD = 1.89 and DM SD = 2.35) in early vegetative stages suggests that very low proportions of the two components were degraded beyond the rumen, as has also been supported by Nocek and Russell (1988), Elizalde *et al*. (1999), and Aufrère *et al*. (2000) (Figure 1). Similarities in DM and PC degradability were found between legumes (alfalfa, lupin) and grasses (ryegrass, mixed pasture, and oat forage), which is consistent with the results reported by Peteva-Vancheva *et al*. (1990), MAFF (1990), and Flachowsky *et al*. (1992).

It has been demonstrated that the growth stage affects nutrient degradability in the rumen (Aufrère *et al*., 2003; Yu *et al*., 2004b). Fresh forage is degraded extensively in the rumen but the distribution of morphological components is not the same, resulting in a differential degradation of nutrient fractions in the rumen. Peteva-Vancheva *et al*. (1990) and Vanzant *et al*. (1996) predicted high CP degradability for alfalfa (83.4% and 90%, respectively). Our results were slightly higher (95.5%), which could be due to the predominance of low lignin stems. Antoniewicz *et al*. (1995) reported values of 92.5% for early vegetative stage and 96.1% at full bloom of alfalfa. Other authors indicate that in general legumes can reach values of protein degradability up to 93% (López *et al*., 1991).

The high potential degradability of forage crops reported here (> 87% DM and > 93% CP) could be associated with the presence of large quantities of soluble fraction *a* (> 34% DMa and > 29% CPa) and high degradability of slowly degradable fraction *b*, resulting in low amounts of undegradable fraction *U* (7.02% DM and 3.55% CP) (Table 2). However, alfalfa and oat forage had higher than average DM *U* fraction, which could be due to reduced degradability of fraction *b* in both forage crops and to lower DOMD in alfalfa. Greater differences were found between soluble *a* and slowly degradable *b*

Table 2. Degradation fractions (a, b, U) and effective degradability (ED) of crude protein (CP) and dry matter (DM) of high protein forages (%) at ruminal outflow rates of 0.02, 0.05 and 0.08 h^{-1}.

Forage CP	a	B	U	a+b	c	EDp k = 0.02 h^{-1}	EDp k = 0.05 h^{-1}	EDp k = 0.08 h^{-1}
	%				% h^{-1}			
Vegetative kale leaves	31.22	66.40	2.38	97.62	0.162	90.32	81.96	75.67
Vegetative alfalfa	56.69	35.82	4.49	95.51	0.197	92.20	88.25	85.16
Lupin plant early bloom (O)	25.83	71.10	3.07	96.93	0.160	89.03	80.00	73.23
Lupin plant mid bloom (L)	29.45	67.58	2.99	97.01	0.142	88.67	79.42	72.66
Vegetative mixed pasture	33.39	64.03	2.58	97.42	0.314	93.58	88.62	84.42
Vegetative oat forage	68.47	28.40	3.13	96.87	0.294	95.06	92.75	90.80
Vegetative ryegrass pasture	49.05	47.66	3.29	96.71	0.157	91.33	85.20	80.62
Sugar beet leaves	47.26	46.26	6.48	93.52	0.164	88.50	82.72	78.36
CP Average	42.67	53.41	3.55	96.55	0.20	91.09	84.87	80.12
Standard Deviation	11.76	10.74	1.89	1.89	0.08	3.29	5.22	6.63
Forage DM						EDdm k = 0.02 h^{-1}	EDdm k = 0.05 h^{-1}	EDdm k = 0.08 h^{-1}
Vegetative kale leaves	34.91	62.00	3.09	96.91	0.166	90.25	82.56	76.75
Vegetative alfalfa	47.62	40.02	12.36	87.64	0.142	82.70	77.22	73.22
Lupin plant early bloom (O)	40.63	51.37	8.00	92.00	0.152	86.03	79.29	74.29
Lupin plant mid bloom (L)	38.73	55.36	5.91	94.09	0.121	86.24	77.90	72.08
Vegetative mixed pasture	34.63	59.47	5.90	94.10	0.182	88.21	81.28	75.94
Vegetative oat forage	46.62	43.82	9.56	90.40	0.232	86.98	82.67	79.21
Vegetative ryegrass pasture	44.16	50.74	5.10	94.90	0.123	87.80	80.23	74.90
Sugar beet leaves	53.68	40.12	6.20	93.80	0.165	89.46	84.47	80.70
DM Average	42.62	50.36	7.02	92.98	0.160	87.21	80.70	75.89
Standard deviation	4.94	5.36	2.32	2.35	0.05	1.26	2.13	3.01

a: soluble fraction, b: slowly degraded fraction, U: insoluble fraction, c: degradation rate of fraction b, EDp: effective degradability of protein, EDdm: effective degradability of dry matter.

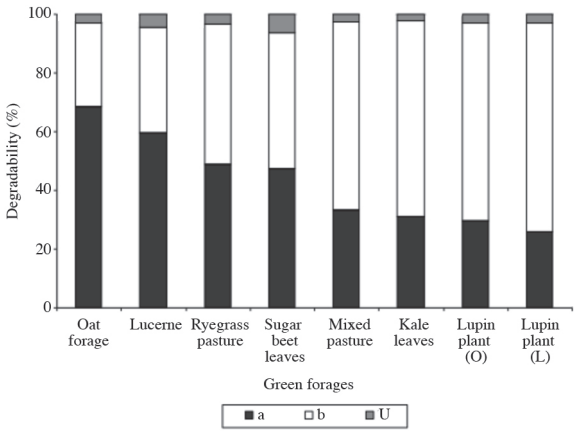

Figure 1. Crude protein degradable fractions of high protein green forages. a: soluble (■), b: slowly degradable (□) and U: insoluble (▒).

fractions between CP and DM. The soluble CP fraction (CPa) presented the highest standard deviation (11.8) among forage crops, ranging from 68.47% (oat forage) to 25.8% (lupin plant in early bloom) and was three times as high as DMa (4.94%).

The *U* fraction of DM for most forage crops was twice as high as that observed for CP and CPc (average 0.200 h^{-1}) and was on average 23% greater than the average DMc (0.16 h^{-1}), suggesting that little CP was bound to the cell wall and most of it was in the cell content as mitochondria and chloroplasts (Tamminga *et al*., 1991). This could explain the high CPb degradability rate *c* for oat forage (0.29 h^{-1}) considering its high DM U fraction (9.56%) in comparison to alfalfa, where rate of degradability decreased in the presence of high DM U fraction (12.4%) indicating that the proportion of CP bound to cell wall could be higher. Faster degradation rates than average for CPc were observed in oat forage (0.294% h^{-1}) and mixed pasture (0.314% h^{-1}). The DMc for oat forage DM (0.23 h^{-1}) was again the most rapidly degraded, lupin plant was the most slowly degraded (0.12% h^{-1}). High differences between CPc and DMc were observed in mixed pasture (+73%) and alfalfa (+39%), followed by ryegrass pasture and oat forage (+27%). Similar degradation rates for CP and DM resulted for lupin forage, kale leaves and sugar beet leaves (Table 2). Predicted *c* by Flachowsky *et al*. (1992) and López *et al*. (1991) for alfalfa (0.14% h^{-1}), lupin (0.12% h^{-1}) and white clover (0.12% h^{-1}) were similar to those determined in this study.

Nutrient composition (DM, CP and NDF) was not highly correlated with *c*. However, CPc and DMc rates were highly correlated (r = 0.78) (Figure 2A). This could be a consequence of low FDN and probably a low proportion of N bound to the cell wall. Protein degradation rates did not completely explain the amount of CPa or CPb fraction, as shown by the low correlation values. Meanwhile, the higher mean degradability for CP compared to DM (+3.7%) could be explained by the elevated degradation of CP (+23%). CP and DM degradability were positively correlated (r = 0.79) (Figure 2B), however differences in degradation rates were not explained by differences in CP or NDF contents, nor by the amounts of *a* or *b* fractions. This indicates that in grasses or legumes of high protein contents and high digestibility, the amount of NDF does not limit CP degradability. Therefore, factors explaining differences in CPc among forage crops should be further studied using a more discriminating approach with respect to N and carbohydrate fractions, as shown by Yu *et al*. (2004a).

Degradation of slowly degradable fraction *b* in time

Degraded proportions of fraction *b* among forage crops, along with incubation periods, were variable (Figure 2A and 2B). Most part of fraction *b* disappeared within the first 6 h of incubation (CPb: 57.7-84.8% and DMb: 52.3-75.2%) regardless of fraction size. After 6 h of incubation, more than 80% of CPb was degraded in mixed pasture and oat forage, which presented the highest degradation rates (0.314% h^{-1} and 0.294% h^{-1}). As well, the proportion degraded during this period correlated strongly and inversely ($P < 0.0001$) with the total amount of *b* (r = 0.93) for DM and CP, regardless of forage type. On average, 68% of CPb (Figure 2A) and 61% of DMb (Figure 2B) was degraded at 6 h of incubation. Correlations of CPc with CP, fraction *a* and *b* were low. Therefore, there might be other factors affecting early degradability of CP. Although forage crops responded differently in terms of CP compared to DM degradability of fraction *b*, the DMb degradability fraction is explained mainly by CPb degradability (r = 0.79) and the rate of degradation (r = 0.79) was positive and significant ($P < 0.02$). Differences in degradation rates could be due to amounts of intracellular components and digestibility, while N availability is due to its distribution among cell structures. In fact, it has been reported that neutral detergent insoluble N (NDIN) requires a greater lag time for degradation than the N pool, regardless of forage type and maturity (Coblentz *et al*., 1999). On the other hand, intracellular N is quickly available (1-2 h) and diminishes rapidly with time (Aufrère *et al*., 2000). Under these conditions, the high degree degradability observed during the first 6 h of fermentation could be associated with low NDIN in mixed pasture and oat forage, and therefore the higher content of NDF was not a limiting factor for degradation (Table 1). The slower rates of degradation found in lupin in early bloom and kale leaf could be influenced by a higher proportion of ADIN, despite its low NDF and higher *b* fraction. In general, the results indicate that as incubation time increased, differences in degradability among forage crops decreased, reaching nearly 90% of the degradable CPb and 85% of DMb fractions in just 12 h.

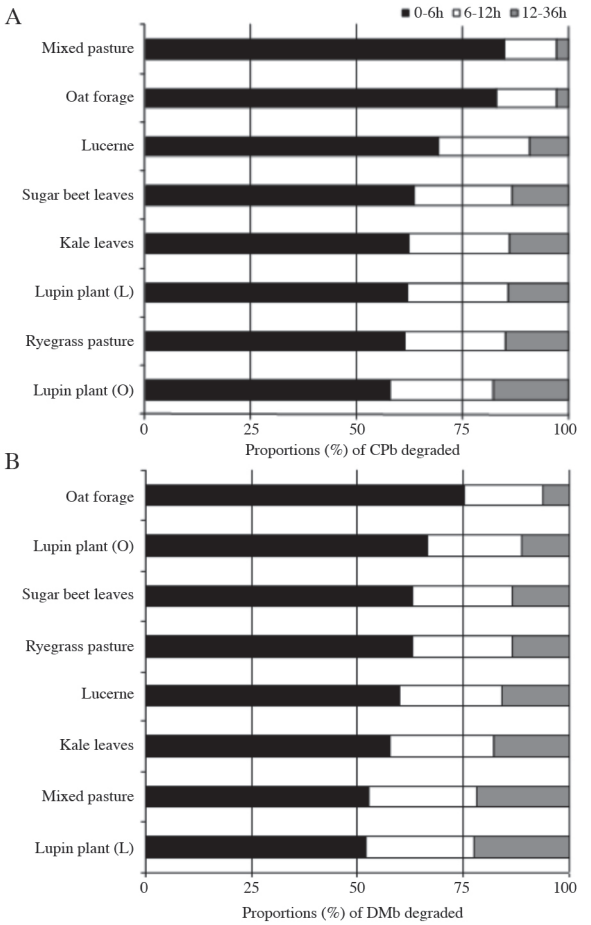

Figure 2. Characterization of slowly degradable fraction (b) in green forages. A) Proportions (%) of crude protein (CP) slowly degradable fraction (b) at different incubation times (■ 0-6 h, □ 6-12 h, and ■ 12-36 h). B) Proportions (%) of dry matter (DM) slowly degradable fraction (b) at different incubation times (■ 0-6 h, □ 6-12 h, and ■ 12-36 h).

Effective degradability (ED)

Differences in ED among forage crops can be explained by differences in the amount of fraction *a*, *b* and *c*. A significant correlation was found between CPa and EDp ($r = 0.79$; $P < 0.02$), showing that EDp increased by 0.23% units for each unit of increment in CPa content. Conversely, as the amount of CPb increased, EDp values decreased ($r = 0.76$; $P < 0.02$) at a rate of 0.21% units for each unit of increase *b* fraction. Therefore, forage crops showing higher *a* or lower *b* fraction displayed higher EDp values, and were less affected by the outflow rate. As an example, the highest EDp and the lowest effect of the outflow rate was observed with oat forage, with the highest proportion of fraction *a* (64.5%) and the lowest of fraction *b* (28.4%), with only a 6.3% reduction of total degradability. For this reason, the two lupin forage crops that showed the lowest amount of fraction *a*, the highest of fraction *b* and the lowest rates of *c* were the most affected by increasing the outflow rate, reducing total degradability by 25% (Table 2). The same pattern was observed for DM (EDdm).

CONCLUSIONS

Potential degradability was more uniform among forage crops than was degradation kinetics, which were more related to the proportions of soluble and insoluble degradable fractions. No consistent differences in degradability were found between legumes and grasses.

Degradability was high (> 90%) for DM and CP, but greater and more variable for CP. On average, degradability and degradation rates were higher for CP and most degradation occurred in the first 6 h of incubation. The proportion degraded during the first 6 h was highly, and inversely correlated to the slowly degradable fraction of DM and CP, regardless of forage type. Maximum degradability was reached between 24 and 36 h.

Effective degradability decreased as the rate of passage (*c*) and the slowly degradable fraction (*b*) increased, showing that degradability at a given rate of passage was more influenced by the magnitude of the insoluble degradable fraction. Forage crops rich in soluble CP or DM were the least affected by passage rate.

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Cinética de degradación ruminal *in situ* en forrajes de alto contenido proteico en clima templado. El presente estudio se desarrolló con el objetivo de evaluar el valor nutricional y la cinética de degradación *in situ* de ocho forrajes de alto valor proteico: alfalfa (*Medicago sativa* L.), avena (*Avena sativa* L.), pastos mixtos y pastos de ballica (*Lolium multiflorum* Lam.), en las primeras etapas vegetativas, dos lupinos forrajeros (*Lupinus albus* L.) en etapas inicio de la floración, hojas de remolacha azucarera (*Beta vulgaris* L.) y de col (*Brassica napus* var. *pabularia* (DC.) Rchb.) La cinética de degradación ruminal de la materia seca (DM) y proteína cruda (CP) se evaluaron mediante la técnica *in situ* de la bolsa de nylon descrita por Ørskov MacDonald (1979), con tres ovejas-fistuladas, en cinco tiempos de incubación. La composición química de los forrajes fue en promedio 13,7% DM, CP 21,4%; 31,5% de fibra detergente neutro (NDF), 17,7% fibra cruda (CF); digestibilidad de la materia orgánica (DOMD) 80,6% y 12,13 MJ kg^{-1} energía metabolizable (ME). La degradabilidad total de los forrajes fue alta (> 87% DM; > 93% CP) asociada a la presencia de grandes cantidades

de la fracción *a* (> 34% DMa; > 29% CPa) y a la alta degradabilidad de la fracción *b*, determinando cantidades bajas de la fracción no degradable (U) (7,02% DM y 3,55% CP). Las correlaciones entre la degradabilidad CPb y DMb (r = 0,79) y entre CPc y DMc (r = 0,78) fueron altas; sin embargo, las diferencias en *c* no se explican por las diferencias en CP o contenido de NDF, ni por la cantidad de fracciones de *a* o *b*. La degradación de DM y CP durante las primeras 6 h de incubación se correlacionó inversamente con *b* (36 h) (r = 0,93; P < 0,0001), independiente del tipo de forraje. La cantidad de CPa y CPb influenció la degradabilidad efectiva de CP (r = 0,79; P < 0,02), al aumentar CPa también lo hizo DEp y, disminuyó al aumentar la CPb (r = 0,76; P < 0,02). Por lo tanto, la PC y MS de estos forrajes se degradó en más del 75% en las primeras 6 h de incubación y esto puede asociarse con el contenido de DM y la cantidad de fracción lentamente degradable presente.

Palabras clave: rumen, forraje, tasa de pasaje, composición química, degradabilidad.

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