Comparison of the *in situ* degradation parameters of fresh and frozen-thawed ^{15}N-labelled alfalfa and ryegrass

R. Tahmasbi*, A. Khezri and O. Dayani,

Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, 76169-133, Kerman, Iran

* Corresponding author, E-mail address: rtahmasb@uk.ac.ir

Abstract Degradation of dry matter (DM) and nitrogen (N) in fresh or frozen-thawed alfalfa and ryegrass was studied by using *in situ* technique. The forages were labeled with ^{15}N during growth in a glasshouse, harvested at similar growth phase, and fresh (F) or frozen-thawed (FT) samples were incubated in the rumen of 3 sheep. There was no difference (P>0.05) between forage type (S) for the immediately soluble fraction (*a*) and the insoluble but slowly degradable fraction (*b*) estimates for DM and N disappearance over time. However, ^{15N} values for *a* and *b* were significantly (P<0.05) higher and lower, respectively, for ryegrass. There was a significant effect of forage type on the degradation rate of *b* (*c*) and potential degradability of DM, N and ^{15}N; degradation rate of *b* for DM, N and ^{15}N were higher for alfalfa. There was no effect due to sample preparation (M) in degradation rate (*c*) for DM and ^{15}N but FT had a significantly (P<0.05) higher estimate than F for N degradation rate. Potential degradability was higher for FT than F for DM and N but not for ^{15}N. Mean estimates of effective rumen degradable protein due to forage species were significantly (P<0.05) different, while there was no difference due to the preparation method. The undegraded protein estimates for alfalfa and ryegrass were 66.8 and 52.5 g/kg DM, respectively. The corresponding values for F and FT were 66.2 and 53.1 g/kg DM, respectively. There were significant (P<0.05) interactions between S and M for DM degradation rate and potential of degradability, where FT increased the rate of degradation and potential degradability in alfalfa but decreased these in ryegrass. A significant (P<0.05) interaction was also found between S and M for quickly degradable protein where the estimate for fresh alfalfa was ranked lowest while the FT alfalfa was ranked highest. because of differences in the prediction of degradation parameters for DM and N between preparation methods it is recommended that for *in situ* degradation determination the same preparation method be used throughout.

Keywords: fresh forages; *in situ*; degradation; dry matter; nitrogen; labeled nitrogen

Received: 10 Oct. 2013, *accepted:* 4 Mar. 2014, *published online:* 7 May. 2014

Introduction

Quantification of the degradation characteristics of feeds consumed by ruminants is normally determined by the *in situ* methods. Many factors influence feed degradation rate in the rumen, making it necessary to standardize the *in situ* methods in terms of size and porosity of bags, fineness of sample grinding, procedure for washing bags, animal feeding and so on (Lopez *et al.* 1995). Standards for dry feed samples have been proposed but there is still limited information on the *in situ* degradation characteristics of fresh forages.

The manner in which samples are prepared can affect degradability estimates (AFRC 1992; Huntington and Givens 1995). Forage sample preparation methods have included oven or freeze drying, chopping, grinding and mincing (Stern and Satter 1984; Abdalla *et al.* 1988; Waghorn and Caradus 1994) while the use of masticated preparations has been less common (Playne *et al.* 1978; Acosta and Kothmann 1978; Dove and McCormack 1986). Oven preparation is known to lower N degradability (Lopez *et al.* 1995); however, while using fresh preparations is ideal, obtaining uniform samples is hard to achieve. There is also a need to make measurements of degradability as soon as possible after harvesting in order to avoid the affects of wilting on the loss of soluble fraction (Olubobokun *et al.* 1990; AFRC 1992; Michalet-Doreau and Ould-Bah 1992). Dried preparations are more convenient for comparing the forages, especially when large numbers of feeds are to be compared. Consistency between samples is also relatively easy to achieve for dried forages. In their *in vitro* exper-

iment Tahmasbi *et al.* (2012) found that freezing and storing samples before *in vitro* evaluation could lead to false conclusions on the nutritive value of fresh forage ingested by grazing animals. The effect of preparation method of fresh clover on estimates of rumen degradable protein and undegraded dietary protein was compared by Cohen and Doyle (2001), assuming that cows were producing >15 L milk/d. They concluded that no sample preparation method would be ideal and that the preferred method for *in situ* studies would vary for fresh forages compared with conserved forages and concentrates.

The isotope of nitrogen, ^{15}N, has been employed in many plant physiology studies (Broadbent and Carlton 1980; Danso *et al*. 1988; Harris and Hesterman 1990), but it has only infrequently been used to study ruminal metabolism of forages (Varvikko and Lindberg 1985; Vanhatalo and Varvikko 1995). The ^{15}N method has some advantages in comparison with radioisotopes such as its constant character, simplicity of usage and less contamination of tissues and wastes compared to other markers such as ^{32}P and ^{32}S (Broderick and Merchen 1992). The principle of the *in situ* method is that microbes should enter the bag and degrade the feed in a similar way to that if the feed was consumed directly by the animal. Natural labelling of forage N with ^{15}N is an appropriate technique for studying metabolism of plant N (Hristov *et al*. 2001).

The objective of this study was to compare the degradation characteristics of two species of forages (alfalfa and ryegrass) using the *in situ* method, and to quantify the extent to which sample preparation method (fresh *vs*. frozen-thawed) affected degradability estimates, including the estimates of effective degradable protein (ERDP) and undegraded dietary protein (UDP).

Material and Methods

Experimental design, sheep and basal diet

The experimental design was a 2×2 factorial (2 forage species and 2 preparation methods) with each treatment being replicated in 3 rumen cannulated wethers (~3 years old, weighing 45.3±1.73 kg) on 4 occasions. The wethers were housed in metabolism crates in a controlled- environment room under continuous lighting. All wethers received a basal diet of chopped alfalfa hay (600g, 92% DM, 19% CP) and oaten chaff (400 g, 91.5% DM, 7.5% CP) daily at about 0900 h and had free access to drinking water.

Labeling of forages

Alfalfa (*Medicago sativa* L.) and perennial ryegrass Alfalfa (*Medicago sativa* L.) and perennial ryegrass (*Lolium perenne* L.) were grown in 4-L pots in a glasshouse. The potting mix was almost free of N, so that the required N for plant growth was provided from either fertilizer (non-labeled) added monthly, or from the ^{15}N provided by hand watering with $^{15}NH4^{15}NO_3$ solution twice weekly (14mg of ^{15}N per pot per month, 99.2% enriched, Shanghai Research Institute of Chemical Industry). It was assumed that all plant tissue N was labeled when the samples were placed in nylon bags – approximately 90 days after initial watering with the label.

Sample preparation

After 90 days, alfalfa was harvested at early bloom (second cut, 22% DM; 22.5% CP) and immediately transferred to plastic bags, sealed and stored at -20°C. The same procedure was applied to third-cut ryegrass (17% DM; 13.8% CP). On the day of the experiment, frozen samples were removed from the freezer and thawed for 15 min at room temperature. There was no visible leakage of fluid from the thawed material. The period between cutting fresh growing plants in the glasshouse and placing the prepared samples in bags in the rumen was less than 30 min. Fresh (F) or frozen and thawed (FT) forages (about 1 g DM) were chopped by scissors to approximately 5-mm length and added to 24 pre-weighed polyester bags (7 cm × 4 cm, pore size of 44 μm × 44 μm; Swiss Screen, Seven Hills, NSW 2147). A marble was placed into each bag to ensure that it remained in the liquid phase of the rumen contents during incubation and to facilitate its removal from the rumen. The bags were then re-weighed and tied up with monofilament fishing line (9kg breaking strain). Seven bags were chosen at random and placed in the rumen of each of the 3 wethers at the same time. Three other bags, chosen at random, were usedas 'zero time' samples. The bags in the rumen (n=7) were removed successively after 1, 3, 6, 9, 12, 24 and 48 h incubation.

The procedure for *in situ* degradability determination was similar to that recommended by the Agricultural and Feed Research Council (AFRC, 1992),with the exception that the sheep were offered feed once-daily.

Chemical analysis

Dry matter was determined by weighing approximately 15-20 g of sample into a dry weighed aluminum tray and then placed in a fan-forced oven for 24 h at 105 ºC. Total N content of the feed samples was determined in finely ground sample DM (1-mm sieve) either using an N-analyzer (Leco FP 2000) or, when N was required for ^{15}N analysis, by micro-Kjeldhal digestion followed by stea-

m-distillation and titration of the resulting ammonia.
Immediately after removal from the rumen, the bags were put into a bucket of cold water and feed particles were washed off by hand. Each bag was then rinsed separately under running cold tap water for about 2 min. The bags and contents were then transferred to another bucket and cleaned under running cold tap water. Care was taken to ensure coarse particles trapped around the mouth of the bag were removed. Bags that were not incubated in the rumen were washed and dried in a similar manner. After washing, bags were gently squeezed, placed in an aluminum tray and dried in a forced-draught oven at 65 °C for 48 h. The dried bags and contents were cooled in a desiccator, weighed (for DM calculation) and stored pending N and ^{15}N analysis. Concentrations of N in bag residues were determined in dry samples ground to pass through a 1-mm sieve (Foss Tecator, 1093 Cyclotec sample mill). Samples for ^{15}N were analyzed using an automatic N/carbon/sulfur analyzer and in-line isotope-ratio mass spectrometer (Carlo Erber Instruments; Model NA1500; Tracermass Inc.).

Rumen degradability and statistical analysis

The percentage disappearance of DM, N and ^{15}N from bags at different times during rumen incubation were fitted by equation 1 (Ørskov and McDonald 1979) using 'NEWAY' Chen (1997):

$$P_t = a + b(1 - \exp^{-ct}) \quad (1)$$

where P_t indicates the proportion (or percentage) of the material degraded through the bag at time *t*; *a* is the immediately soluble fraction that represents the zero time measurement of degradability; *b* is the insoluble but slowly or potentially degradable fraction; *c* is the rate of degradation of *b*.
The effective degradability (*ED*) of forage DM, N and ^{15}N and the rumen degradability of crude protein (CP; ERDP) were determined according to equations 2 and 3 below:

$$ED = a + (bc)/(c + k_p) \quad (2)$$

$$ERDP = 0.8a + (b * c)/(c + r) \quad (3)$$

where *t* is time (h), *r* is the particle flow rate constant assumed to be 0.05/h (AFRC 1992).
The metabolizable protein system (AFRC 1992) was used to calculate the following degradability parameters in sheep consuming either alfalfa or ryegrass prepared as F or FT:

Quickly degradable protein (QDP, g/kg DM)=a[CP]* (4)

where [CP] is CP concentration in alfalfa or ryegrass (g CP/kg DM).

*Slowly degradable protein (SDP, g/kg DM) = [(b*c) / (c+r)]*[CP]* (5)

*ERDP (g/kg DM)=[(0.8*QDP)+SDP)]* (6)

Rumen degradable protein (RDP, g/kg DM) = QDP + SDP (7)

Undegraded dietary protein (UDP, g/kg DM)=[CP]-RDP (8)

Degradation parameters were analyzed using the general linear model (GLM) procedure of (SAS 2003) with the following statistical model:

$$Y_{ijk} = \mu + S_i + P_j + (SP)_{ij} + \varepsilon_{ijk} \quad (9)$$

where Y_{ijk} = dependent variable; μ= overall mean; S_i = main effect of forage species P_j =main effect of preparation method; $(SP)_{ij}$= average effect of interaction of forage species *i* and preparation method *j*; ε_{ijk}= residual error.

Results

There were no differences (P>0.05) between sheep and periods for any of the parameters measured; therefore, the mean degradabilities of fresh and frozen-thawed alfalfa and ryegrass are presented in Table 1.
There was no effect (P>0.05) due to forage species on the soluble potentially degradable (*a*) and potentially degradable (*b*) fractions for DM and N. However, alfalfa had a higher *a* fraction (38.7±3.47 *vs* 14.8±1.13) but a lower *b* fraction than ryegrass (60.5±3.61 *vs* 90.5±2.49) for ^{15}N (P<0.05). There was a significant effect due to forage species on degradation rate constant of fraction *b* (*c*) for DM, N and ^{15}N (0.09±0.006 *vs.* 0.5±0.005; 0.11±0.007 *vs.* 0.08±0.005; 0.12±0.01 *v.* 0.07±0.008), respectively (P<0.05). Effective degradabilities (ED) were consistently higher for alfalfa DM, N and ^{15}N than for ryegrass (Table 1).
There were no effects of sample preparation method on *cDM*, $a^{15}N$, $b^{15}N$, $c^{15}N$ and ED of ^{15}N but FT samples had higher (P<0.05) mean *aDM*, *aN* and *cN* and lower mean *bDM* and *bN* than F samples (Table 1). An interaction (P<0.05) between forage species and sample preparation method for *cDM*, *bN* and ED of DM was found (Table 1). Mean calculated degradability parameters (QDP, SDP, ERDP and UDP in g/kg DM) for N from the forages incubated *in situ* and prepared as either fresh or frozen-thawed are shown in Table 2.
There were significant (P<0.05) differences for QDP, ERDP, SDP, QDP and RDP, between forage species, with the values being greater for alfalfa than for ryegrass. The QDP, ERDP and RDP were smaller in fresh samples compared with the frozen-thawed samples

Table 1. Effect of forage species (L and F) and sample preparation method (F and FT) on mean (±se) dry matter (DM), nitrogen (N) and isotope of nitrogen (15N) degradability coefficients (a, b and c %) and effective degradability of nitrogen (ED%) incubated *in situ*.

Treatment	Contrast	a	b	c	ED
DM					
LF		9.42a(± 1.434)	84.28a(± 1.622)	0.07a(± 0.001)	59.91a(±0.514)
LFT		21.82b(± 1.760)	65.46b(± 2.041)	0.10b(± 0.006)	65.04b(±0.776)
RF		11.98a(± 0.597)	82.70a(± 0.380)	0.06ac(± 0.001)	56.00c(±0.650)
RFT		20.09b(± 1.439)	77.38a(± 4.760)	0.04c(± 0.010)	54.77c(±1.220)
LSD		4.49	8.87	0.02	2.72
Significance.	S	0.77	0.09	0.0003	<0.0001
	M	<0.0001	0.002	0.46	0.047
	S.M	0.16	0.10	0.02	0.005
N					
LF		3.38c(± 1.382)	95.63a(± 1.673)	0.09b(± 0.004)	65.85b(± 0.325)
LFT		18.73a (± 2.029)	79.15b(± 2.658)	0.12a(± 0.004)	74.75a(± 0.370)
RF		7.59bc(± 1.879)	89.14a(± 0.834)	0.07c(± 0.003)	59.73c(± 1.604)
RFT		13.82ab(± 2.731)	82.23b(± 2.298)	0.08bc(± 0.009)	64.20bc(± 2.685)
LSD		6.73	6.49	0.018	5.16
Significance.	S	0.87	0.42	0.0004	0.00076
	M	0.0008	0.0004	0.01	0.0029
	S.M	0.06	0.04	0.17	0.20
^{15}N					
LF		43.70a(± 4.871)	56.08a(± 6.301)	0.11ab(± 0.017)	81.58a(± 2.094)
LFT		33.71a(± 3.390)	64.84a(± 2.524)	0.13a(± 0.007)	80.83a(± 0.918)
RF		14.25b(± 1.298)	94.33b(± 2.106)	0.06c(± 0.004)	63.80b(± 1.188)
RFT		15.40b(± 2.094)	86.59b(± 3.395)	0.08bc(± 0.014)	67.36b(± 3.063)
LSD		10.48	12.84	0.04	6.53
Significance	S	<0.0001	<0.0001	0.001	<0.0001
	M	0.21	0.9	0.09	0.50
	S.M	0.12	0.07	0.99	0.31

Values within columns followed by different letters indicate significant differences between treatments at P=0.05. Degradability coefficients include: *a*, immediately soluble fraction; *b*, insoluble but potentially degradable fraction; *c*, fractional degradation rate of *b*/h. ED was calculated at a ruminal outflow rate of 0.05/h.

but the opposite was found for SDP and UDP. There were significant ($P<0.05$) interactions between forage species and sample preparation method for QDP, UDP and RDP, with fresh samples having lower values compared to frozen-thawed samples.

Discussion

As predictive models for estimating the nutrient requirements of grazing ruminants have become increasingly complex in their approach, they have highlighted the need for a more accurate characterization of the degradation kinetics of different feed fractions. This current experiment compared two forage species commonly grazed by ruminant livestock in Australia – alfalfa and ryegrass. Higher rates of degradation (*c*) were found for DM, N and ^{15}N for alfalfa than for ryegrass. Average disappearance of ^{15}N for labeled forages was faster than that for total N and the difference was greatest for ryegrass (independent of sample preparation method). The disappearance of microbially corrected N was, accordingly, always faster than the uncorrected DM, N and ^{15}N.

Immediate solubility is an important contributor to the calculation of N degradability using the equation of Ørskov and McDonald (1979) and also in the estimation of ERDP and QDP (AFRC 1992). Sample preparation method resulted in ERDP estimates for FT samples being higher than the values for F samples. Therefore, care needs to be taken in interpreting estimates of N degradability of highly digestible fresh forages where these are determined using the *in situ* technique. As expected, ERDP estimates for alfalfa were higher than for ryegrass, because of higher N content.

Understanding the extent to which rapidly released N meets the requirements of rumen microorganisms is important not only for optimization of rumen microbial protein synthesis, but also because high rates of ammonia production may result in inefficient utilization of di-

Table 2. Effect of forage species (L and F) and sample preparation method (F and FT) on mean (±se) calculated degradability parameters (QDP, SDP, ERDP and UDP in g/kg DM) for nitrogen incubated *in situ*.

Treatment	Contrast	QDP	SDP	ERDP	UDP	RDP
LF		7.60b(± 3.109)	140.56a(± 3.750)	146.64b(± 1.302)	76.84a(± 0.732)	148.16(± 0.732b)
LFT		42.14a(± 4.566)	126.06b(± 5.353)	159.77a(± 1.718)	56.80b(± 0.833)	168.20a(± 0.833)
RF		10.48b(± 2.593)	71.95c(± 1.818)	80.33c(± 1.872)	55.58bc(± 2.214)	82.33c(± 2.214)
RFT		19.07b(± 3.768)	69.53c(± 3.047)	84.79c(± 3.251)	49.40c(± 3.706)	84.79c(± 3.706)
LSD		11.69	12.13	7.06	7.27	7.27
Significance	S	0.023	<0.0001	<0.0001	0.0002	<0.0001
	M	0.0003	0.05	0.0036	0.0004	<0.0001
	S.M	0.007	0.14	0.08	0.01	0.01

Values within columns followed by different letters indicate significant differences between treatments at P=0.05. The parameters, QDP (quickly degradable protein), SDP (slowly degradable protein), ERDP (effective rumen degradability protein) and UDP (undegraded dietary protein) were calculated as described in the Materials and methods. ERDP was calculated at a ruminal outflow rate of 0.05/h.

etary N (Beever 1993). Plant proteases play an important role in degradation of fresh forage N in the initial stages of digestion before they are subject to the action of microbial proteolytic enzymes in the rumen (Kingston-Smith and Theodorou 2000).

It is known that oven drying reduces N degradability (Lopez *et al*. 1995) and freezing and thawing increases the rate of hydrolysis of disaccharides to monosaccharides (MacRae 1970). The results presented here support those of MacRae (1970) who found reductions in soluble N due to freezing and thawing of samples before incubation in nylon bags in the rumen. Subsequent work by MacRae *et al.* (1975) showed that this was caused by the rupturing of the vacuolar membrane of the plant cell during freezing, thereby facilitating a mixing of the vacuolar and cytoplasmic contents and consequent precipitation of proteins. A similar precipitation of proteins due to freezing was also noted by Kohn and Allen (1992). They also showed that freezing forage samples (smooth brome grass and alfalfa) affected the neutral detergent fiber, acid detergent fiber, lignin and ash content in different ways depending on the forage type and duration of freezing.

In our experiment fresh samples were chopped into ~5 mm-length as recommended by AFRC (1992). However, Cohen and Doyle (2001) found that mincing may better reflect what happens *in vivo*, especially in relation to quickly degradable protein (QDP = *a* * [CP]). Chopped, milled or pulverized forages may not effectively mimic mastication by the animal (Bailey 1982; Playne *et al.* 1978). Mastication decreases particle size and exposes more surface area and more area of digestible tissue within a given particle size and causes the feed to become more crushed, crimped and cracked (Pond *et al.* 1984). Mincing fresh samples (to 2 mm) before *in situ* incubation resulted in significantly higher degradation parameter estimates than chopping fresh samples to 10 mm-length (Cohen and Doyle 2001).

Conclusions

Results of feed degradation parameters *a* and *b,* determined using the *in situ* technique, showed there were no detectable differences between alfalfa and ryegrass. However, the degradation rates for DM, N and ^{15}N represented by parameter *c* were faster for alfalfa than for ryegrass. Sample preparation method affected the prediction for DM and N degradation given by *a* (fresh < frozen-thawed) and *b* (fresh > frozen- thawed), as well as a faster degradation rate (*c*) for N (but not DM).

Because sample preparation method influences estimation of *in situ* degradation parameters, it is recommended that the same preparation method be used consistently when making comparisons between forages. A faster rate of degradation would mean that forage would have a shorter residence time in the rumen allowing animals to maintain a higher intake. Also, higher effective degradabilities of DM and CP of fresh *v.* frozen-thawed forages in the rumen indicated there was more intensive microbial digestion of fresh material and that freezing has a disruptive effect on plant cell structure. Therefore results obtained with this technique have to be interpreted with care.

References

Abdalla, H.O., Fox, D.G., Van Soest, P.J., 1988. An evaluation of methods for preserving fresh forage samples before protein fraction determination. *Journal of Animal Science* 66, 2646–2649.

Acosta, R.A., Kothmann, M.M., 1978. Chemical composition of esophageal-fistula forage samples as influenced by drying method and salivary leaching. *Journal of Animal Science* 47, 691–698.

AFRC, 1992. Nutritive requirements of ruminant animals: protein. Report No. 9 of the Agricultural and Food Research Council Technical Committee on Responses to Nutrients. *Nutrition Abstracts and Reviews, Series B.* 62, 787–835.

Bailey, C.B., 1982. Rates of digestion of swallowed and unswallowed dried grass in the rumen. *Canadian Journal of Animal Science* 42, 49-54.

Beever, D.E., 1993. Ruminal animal production from forages: present position and future opportunities. Proceedings of the XVII International Grassland Congress. Palmerston North, New Zealand.

Broadbent, F.E., Carlton, A.B., 1980. Methodology for field trials with nitrogen-15-depleted nitrogen. *Journal of Environmental Quality* 9, 236–242.

Broderick G.A., Merchen N.R., (1992) Markers for quantifying microbial protein synthesis in the rumen. *Journal of Dairy Science* 75, 2618-2632.

Chen X.B., Neway Excel: A utility for processing data of feed degradability and in-vitro gas production (version 6.0). UK: Rowett Research Institute; 1997.

Cohen, D.C., Doyle, P.T., 2001. Effect of sample preparation on *in situ* estimates of protein degradability for white clover herbages. *Australian Journal of Experimental Agriculture* 41, 619-624.

Danso, S.K.A., Hardarson, G., Zapata, F., 1988. Dinitrogen fixation estimates in alfalfa-ryegrass swards using different nitrogen-15 labeling methods. *Crop Science* 28, 106–110.

Dove, H., Mccormack, H.A., 1986. Estimation of the ruminal degradation of forage rape after incubation in nylon bags in the rumen of sheep. *Grass and Forage Science* 41, 129–136.

Harris, G.H., Hesterman, O.B., 1990. Quantifying the nitrogen contribution from alfalfa to soil and two succeeding crops using nitrogen-15. *Agronomy Journal* 82,129–134.

Hristov, A., Broderick, G.A., 1994. *In vitro* determination of ruminal protein degradability using [^{15}N] ammonia to correct for microbial nitrogen uptake. *Journal of Animal Science* 72, 1344-1354.

Huntington, J.A. Givens, D.I., 1995. The *in situ* technique for studying the rumen degradation of feeds. *Nutrition Abstracts and Reviews 65*, 63–93.

Kingston-Smith, A.H., Theodorou, M.K., 2000. Post-ingestion metabolism of fresh forage. *New Phytologist* 148, 37-55.

Kohn, R.A., Allen, M.S., 1992. Storage of fresh and ensiled forages by freezing affects fibre and crude protein fractions *Journal of the Science of Food and Agriculture* 58, 215-220.

Lopez, S., Hovell, F.D.D., Manyuchi, B., Smart, I., 1995. Comparison of sample preparation methods for the determination of the rumen degradation characteristics of fresh and ensiled forages by the nylon bag technique. *Animal Science* 60, 439-450.

MacRae, J.C., 1970. Changes in chemical composition of freeze-stored herbage. *New Zealand Journal of Agricultural Research* 13, 45–50.

MacRae, J.C., Campbell, D.R., Eadie, J., 1975. Changes in the biochemical composition of herbage upon freezing and thawing. *The Journal of Agricultural Science (Cambridge)* 84, 125-131

Michalet-Doreau, B., Ould-Bah, M.Y., 1992. *In vitro* and *in sacco* methods for the estimation of dietary nitrogen degradability in the rumen: a review. *Animal Feed Science and Technology* 40, 57–86.

Olubobokun, J.A., Craig, W.M., Pond KR 1990. Effect of mastication and microbial contamination on the ruminal *in situ* forage disappearance. *Journal of Animal Science* 68, 3371–3381.

Ørskov, E.R., Mcdonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. *The Journal of Agricultural Science (Cambridge)* 92, 499–503.

Playne, M.J., Khumnalthong, W., Echevarria, M.G., 1978. Factors affecting the digestion of oesophageal fistula samples and hay samples in nylon bags in the rumen of cattle. *The Journal of Agricultural Science (Cambridge)* 90, 193–204.

Pond, K.R., Ellis, W.C., Akin, D.E., 1984. Ingestive mastication and fragmentation of forages. *Journal of Animal Science* 58, 1567-1574.

SAS (2003) Statistical Analysis System (SAS Institute Inc.: Cary, NC, USA)

Stern, M.D., Satter, L.D., 1984. Evaluation of nitrogen solubility and the dacron bag technique as methods for estimating protein degradability in the rumen. *Journal of Animal Science* 58, 714–724.

Tahmasbi, R., Nolan, J.V., Dobos, R.C. 2012. *In vitro* determination of the characteristics of fresh and froze-

n-thawed alfalfa and ryegrass. *Journal of Livestock Science and Technologies* 1, 11-18

Vanhatalo, A., Varvikko, T., 1995. Effect of rumen degradation on intestinal digestion of nitrogen of ^{15}N-labeled rapeseed meal and straw measured by the mobile-bag method in cows. *Journal of Agricultural Science (Cambridge)* 125, 253–261.

Waghorn, G.C., Caradus, J.R., 1994. Screening white clover cultivars for improved nutritive value- development of a method. Proceedings of the 56th New Zealand Grassland Association, Hanmer Springs, New Zealand.

Communicating editor: *Mehdi Dehghan-Banadaky*

مقایسه پارامترهای تجزیه پذیری به روش کیسه های نایلونی علوفه های تازه و یخ گشایی شده پس از انجماد یونجه و رای گراس نشان دار با ^{15}N

ر. طهماسبی[۱]، ا. خضری[۱] و ا. دیانی[۲]

نویسنده مسئول، پست الکترونیک: rtahmasb@uk.ac.ir

چکیده در این آزمایش تجزیه پذیری ماده خشک (DM) و نیتروژن (N) در گیاهان یونجه و رای گراس به صورت تازه یا یخ گشایی شده پس از انجماد با استفاده از تکنیک کیسه های نایلونی مطالعه گردید. علوفه ها با استفاده از ایزوتوپ N^{15} در هنگام رشد در گلخانه نشان دار و در مراحل مشابه رشد برداشت شدند و به صورت تازه یا یخ گشایی پس از انجماد در درون شکمبه سه راس گوسفند فیستولا گردیدند. از نظر آماری هیچگونه تفاوت معنی دار (P<۰/۰۵) بین نوع علوفه برای پارامترهای بخش سریع التجزیه (a) و بخش محلول اما کند تجزیه (b) در مورد ناپدید شدن ماده خشک و نیتروژن در طول دوره مشاهده نگردید، اگر چه مقادیر مربوط به ^{15}N برای a و b معنی دار (P<۰/۰۵) و برای رای گراس در مقایسه با یونجه به ترتیب بالاتر و پایین تر بدست آمد. اثر نوع گیاه بر نرخ تجزیه بخش b (c) و پتانسیل تجزیه پذیری DM ، N و ^{15}N معنی دار (P<۰/۰۵) بدست آمد و نرخ تجزیه بخش b برای DM ، N و ^{15}N در مورد یونجه نسبت به رای گراس بالاتر بود. نحوه آماده سازی نمونه ها روی نرخ تجزیه (c) ماده خشک و ^{15}N معنی دار نبود، اما نرخ تجزیه نیتروژن در علوفه یخ گشایی شده پس از انجماد تخمین های بالاتری نسبت به علوفه تازه دارا بود (P<۰/۰۵). پتانسیل تجزیه پذیری در مورد علوفه یخ گشایی شده پس از انجماد در مقایسه با علوفه تازه در مورد ماده خشک و نیتروژن بالاتر بود. میانگین تخمین های پروتئین تجزیه پذیر موثر با توجه به گونه گیاه معنی دار بدست آمد (P<۰/۰۵)، در حالی که هیچ اثری درباره روش آماده سازی مشاهده نگردید. تخمین های پروتئین تجزیه نشده در مورد یونجه و رای گراس به ترتیب ۶۶/۲ و ۵۳/۱ g/kg ماده خشک بدست آمد. اثر متقابل معنی داری (P<۰/۰۵) بین نوع گیاه و روش آماده سازی روی نرخ تجزیه ماده خشک و پتانسیل تجزیه پذیری مشاهده شد، اما یخ گشایی علوفه پس از انجماد باعث افزایش نرخ تجزیه و پتانسیل تجزیه پذیری موثر در یونجه و کاهش آن در رای گراس گردید. تاثیر نوع گیاه و روش آماده سازی روی پروتئین سریع التجزیه معنی دار بود (P<۰/۰۵) و در مورد یونجه تازه کمترین و در مورد یونجه یخ گشایی شده پس از انجماد بیشترین مقدار بدست آمد. به دلیل تفاوت های موجود در پیش بینی پارامترهای تجزیه پذیری ماده خشک و نیتروژن به دلیل تاثیر نوع روش آماده سازی نمونه ها، پیشنهاد می شود که برای تعیین تجزیه پذیری با استفاده از روش کیسه های نایلونی، روش آماده سازی یکسان در کل آزمایش استفاده گردد.