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Short Communication

Determination of forage escape protein value with *in situ* and enzyme techniques

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This study was carried out to compare *in situ* and *in vitro* enzyme techniques for determining estimates of forage escape proteins. Eight forages (vetch hay, wheat silage, corn silage, wheat hay, grass hay, lentil straw, triticale hay and alfalfa hay) were used as feed materials. During 70 h, incubation with enzyme method had similar escape protein values for vetch hay, wheat hay, grass hay, triticale hay and corn silage with *in situ* methods (P>0.05). On the contrary, enzyme method failed to estimate escape protein values of wheat silage, lentil straw and alfalfa hay comparison with *in situ* values at all the incubation times. Further research is required to improve *in vitro* enzyme technique for determining accurate escape protein estimates of forages.

Key words: *In situ* and *in vitro* techniques, escape protein, enzyme, forages.

INTRODUCTION

In vivo, *in vitro*, and *in situ* techniques are available for forage nitrogen (N) evaluation systems in ruminant nutrition, but none of them is completely satisfactory. *In vivo* technique has been accepted as the standard technique, but separation of microbial protein and endogenous protein from feed protein has associated errors. Additionally, accurate measurement of digesta flow due to markers, sampling and cannulas is the other concerns (NRC, 1985). The *in situ* technique is the most common technique used for determining ruminal escape protein values of feedstuffs. In this technique, the protein under study is added to artificial fiber bags (usually made of dacron or nylon bag) which then are suspended in the rumen and the time course of dry matter (DM) and N loss from the bags is used to estimate rate and extent of degradation (Mehrez and Orskov, 1977). *In vitro* systems include use of enzymes such as ficin (Poos-Floyd et al., 1985), protein solubility (Krishnamoorthy et al., 1982), ammonia release (Britton et al., 1978), and an *in vitro* inhibitor system (Broderick, 1987). The *in vitro* system offers a fast and inexpensive way to estimate degradability of feed proteins. French authors (Aufrere et al., 1991) adopted enzymatic *Streptomyces griseus*: SGP) method as an acceptable method for their metabolizable protein system. More recently, researchers at Kansas State University (Abdelgadir et al., 1997) studied the SGP strictly for forages. They over estimated escape protein values of alfalfa hay and prairie hay.

The purpose of this study was to obtain accurate estimates of forage escape proteins via *in vitro* enzyme techniques and comparison of them with *in situ* values.

MATERIALS AND METHODS

Feeds and animals

Eight forages (vetch hay, wheat silage, corn silage, wheat hay, grass hay, lentil straw, triticale hay and alfalfa hay) were chosen for feed materials. The chemical compositions of the feeds were presented in Table 1. In *in situ* trial, four rams lambs (average BW 55 to 60 kg) fitted with ruminal cannula were housed in a stall. The rams had free access to water. A diet consisted of alfalfa hay (75% of diet) and concentrate feed (25%) was formulated to meet 1.25 X maintenance requirement of ram lambs (Bhargava and Orskov, 1987). Concentrate feed was consisted of 50% barley grain, 25% cotton seed meal, 21% wheat bran, 1% salt, 1% DCP, 1% $CaCO_3$ and 1% mineral and vitamin mixture.

In situ assay

Representative samples of forage samples were evaluated using

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Table 1. Nutrient composition of the forages (%).

Forages	DM	Ash*	CF*	ADF*	NDF*	Crude protein*
Vetch hay	87.81	9.13	25.55	34.15	45.56	13.84
Wheat silage	46.21	7.70	31.25	38.81	55.13	8.20
Wheat hay	94.24	8.24	31.97	41.61	65.02	11.60
Lentil straw	95.18	12.96	30.45	44.22	57.07	8.31
Grass hay	90.58	10.82	35.07	43.77	66.19	15.89
Triticale hay	93.75	6.99	32.46	40.30	63.13	10.11
Alfalfa hay	88.78	10.87	22.40	32.76	45.13	17.87
Corn silage	32.10	9.79	30.15	39.74	59.78	9.79

*DM basis.

Table 2. Escape protein values of forages estimated two different methods (% of crude protein.

Treatment	Forage							
	Vetch hay	Wheat silage	Wheat hay	Lentil straw	Grass hay	Triticale hay	Alfalfa hay	Corn silage
In situ	32.89^{c}	40.35^{c}	28.95^{c}	31.13^{d}	38.85^{c}	41.18^{bc}	27.44^{d}	40.02^{d}
In vitro, (1 h)	54.26^{a}	67.29^{a}	52.35^{a}	63.01^{a}	58.83^{a}	55.41^{a}	53.91^{a}	71.07^{a}
In vitro, (6 h)	52.71^{a}	64.42^{a}	50.61^{a}	60.31^{a}	53.18^{a}	56.03^{a}	51.11^{a}	65.50^{b}
In vitro, (24 h)	41.16^{b}	52.59^{a}	38.41^{b}	43.96^{b}	45.29^{b}	44.56^{b}	43.45^{b}	50.99^{c}
In vitro, (70 h)	31.91^{c}	32.36^{a}	31.04^{c}	34.82^{c}	34.36^{c}	37.98^{c}	33.84^{c}	38.99^{d}
SEM	0.88	1.14	0.69	1.06	1.85	1.40	1.58	1.56

^{a,b,c,d} Means with different superscripts in same column different (P<0.05).

15 × 10 cm dacron polyester bags with an average of 40 micron pore size. A 3 g sample was weighed into each bag and tied well with robber bands. Bags which include one samples of each treatment were tied to plastic hose (25 cm length) and a plastic hoses with bags for each incubation time (4, 16 and 96 h) were suspended in the rumens of four rams lambs (one hose for each ram) approximately 2 h after the morning feedings. At the end of the incubations, bags were removed from the rumen and washed with water and then squeezed until the runoff was clear. After that the bags were dried at 60°C for 48 h and weighed. The N remaining in the bags was determined by the macro-Kjeldahl procedure (AOAC, 1990). Disappearance of N from the dacron bag vs time in the rumen was expressed as a percentage of the original N. The rate of N disappearance (rate of digestion -kd) was estimated as the slope of the regression of natural logarithm of the N remaining vs incubation time in the rumen (that is, 4 to 16 h). The potential digestible or degradable fraction (B) of total crude protein was calculated using the y- intercept of the rate of digestion equation. N residue of 48 h incubated bags was used as a completely indigestible fraction (C) of total CP. Escape protein (EP) values were calculated by the complementary equation (Orskov and McDonald, 1979; NRC, 1985; Mass, 1997; Can and Yilmaz, 2002).

$$EP = B \times [kp/ (kd+kp)] + C$$

A rate constant for passage of (kp) of 5% h was used for soybean meal (SBM) and treated SBM (Nelson et al., 1984).

Enzyme assay

N solubility was measured after 1, 6, 24 and 72 h of incubation with enzymes in the 0.1 M borate/phosphate buffer at pH 8.0 as described by Aufere et al. (1991). In 1 L buffer, 20 mg protease from *Streptomysenes griseus* (type XIV, Sigma P-5147) and to prevent microbial growth 1 mg/L tetracycline (Sigma T-3258) was added to buffer. Following incubations, samples were filtered using filter paper (Whatman 41), residue was washed with deionize water, and N is determined on the residues and assumed as an EP value (Cone et al., 1996).

The DM, crude protein and crude ash were estimated by oven-drying and oven-ashing at 105 and 550°C, respectively (AOAC, 1990). Crude fiber (CF) was determined according to Crampton and Maynard (1938). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were analyzed according Van Soest et al. (1991) and expressed including residual ash.

Statistical analyses

Data were analyzed using a model for completely randomized design (CRD) using GLM procedure and means were compared using least significant differences (LSD) using SAS program (SAS, 1989).

RESULTS AND DISCUSSION

Escape protein values of forages estimated two different methods (*in situ* vs enzyme) are presented in Table 2. During 70 h incubation, the enzyme method had similar escape protein values for vetch hay, wheat hay, grass hay, triticale hay and corn silage when compared with the *in situ* method (P>0.05). On the contrary, enzyme method failed to estimate escape protein values of wheat silage, lentil straw and alfalfa hay comparison with *in situ* values

at the any incubation times. Abdelgadir et al. (1996) reported that EP contents determined with enzyme procedure for alfalfa and praire hay (*S. griseus*) were overestimated comparison to *in vivo* EP values. However, pre-treatment with carbohydrase had resulted in lower escape protein estimates that were closer to the *in vivo* values. In the current study, enzyme of *S. griseus* did not estimate EP value of Alfalfa hay. Cone et al. (1996) concluded that the enzymatic method using protease from *S. griseus* offers rapid and satisfactory estimates of the percentage of EP mainly from concentrate feedstuffs as determined by the *in situ* method. Similarly, in this study escape protein values of 5 forages out of eight were estimated similarly with 70 h incubation with protease of *S. griseus*. Aufrère et al. (1991) improved the relationship between escape protein *in situ* and *in vitro* by using correction factors for groups of related feedstuffs. According to this study, only correction factor might be needed to estimate escape protein values of wheat silage, lentil straw and alfalfa hay.

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