INFLUENCE OF GRASS-CLOVER MATURITY STAGE ON RUMINAL DEGRADABILITY OF SILAGE

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Abstract: The aim of this experiment was the evaluation of the rumen degradation kinetics of the dry matter (DM) and crude protein (CP) of grass-clover silage harvested at three phenological stages of grass-clover mixture. Samples were collected from cultivated grass-clover plots established in Zagreb (Croatia) in 2002. The kinetics of degradation of dry matter and crude protein was determined by the technique of nylon bags using 4 Charolais sheep provided with a rumen cannula. Significant differences (P<0,01) were obtained among phenological stages in rapidly soluble and potentially degradable fractions, degradation rates of DM and CP and in the effective degradability (ED) of DM and CP.

Keywords: Silage, dry matter, crude protein, in sacco degradability, phenological stage

Introduction

The yield of arable crops may be influenced by many factors such as water, temperature and nutrient deficiency (Bertić et al., 2007). Grass-clover silage (GCS) varies greatly in terms of chemical and biological composition due to the impact of factors such as the maturity stage at harvesting, sward botanical composition, level of fertilisation, climate and ensiling techniques upon the fermentation process in the silo and on nutritive value (Szabó and Pepó, 2007; Knežević *et al.,* 2007). For grass and clovers in general, the substantial decrease in the feeding value that accompanies advanced maturity is due to chemical and physical changes in the plants. The proportion of cell walls increases in the plant material and the increased lignin content is correlated with reduced forage intake and degradability of the cell wall material in the rumen (Jung, 1989). The present study was carried out under the hypothesis that feeding early-cut silage would significantly increase DM and CP degradability in comparison with the medium- and late-cut silage.

Materials and methods

Sward and silage making

The GCS was made from a semi-permanent, predominately orchardgrass meadow harvested on 18 May, 25 May and 06 June (late vegetative, internode elongation and flowering growth stages of orchardgrass respectively) for silages designated early, medium and late, respectively. Botanical composition was determined from 30 forage samples by manual separation of sward components (grasses, clovers, forbs). The sward contained 80.6% orchardgrass, 13.7% legumes out of which 11.2% white clover (*Trifolium repens L.*) and 2.5% red clover (*Trifolium pratense L.*), 2.3% other grasses and 3.4% forbs on a DM basis.Herbage for silage was cut with a disc mower and wilted for 8 h in the swath before harvesting with a round baler.

Chemical analysis

The DM contents of GCS were determined by oven drying to a constant weight at 60 ºC in a fan-assisted oven (ELE International). Total N concentrations of GCS were determined by the Kjeldahl method (AOAC 1990, ID 954.01) using a Gerhardt nitrogen analyzer. Additionally, N concentration was expressed as crude protein (CP) (total N x 6.25) g kg^{-1} DM. Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were measured using the procedure of Van Soest *et al.* (1991). Silage pH was determined in a water extract from 10 g of fresh silage and 100 ml distilled water using the pH meter 315i (WTW).

In sacco degradability studies

The nylon bag method (AFRC, 1992) was used to determine the rate of degradability of DM from the feeds when suspended in the rumens of four rumen-fistulated Charolais sheep, 4 year old, of approximately 60 kg live weight. The animals were fed a ration of meadow hay and pellet concentrate for sheep in a ratio of 75:25 (DM basis), which was calculated to provide maintenance. The bag size was 10 cm x 20 cm with a pore size 50±15μm (ANCOM Technology Corp., USA). All silage samples were dried and milled trough a 1.5 mm sieve. Then, 5 g of each sample was put in nylon bags and incubated in the rumen for 0, 3, 6, 12, 24, 48 and 72 h. In each sheep, two bags were used for each time interval. After withdrawing the bags from the rumen, they were put into icy water, then washed in a washing machine for 15 min using cold water and then kept in a freezer. After all the bags had been taken from the rumen, they were dried for 2 days at 60 °C. The value of degradability at time 0 was obtained by washing two bags in a washing machine for 1 h using cold water. For each bag, the residue was analyzed for DM. The degradability at each time interval was calculated by taking the mean value obtained from the eight bags. The percentage of degradability (Y) of DM and CP at time (t) was obtained from an exponential curve of the type:

$Y= a + b\ (1-e^{(-ct)})$

which was fitted to the experimental data by iterative regression analysis (Ørskov and McDonald, 1979). In this equation, e is the base of natural logarithms, constant 'a' represents the soluble and very rapidly degradable component and 'b' represents the insoluble but potentially degradable component, which degrades at a constant fractional rate (c) per unit time. The effective degradability (ED) of DM and CP in each GCS was then estimated (Ørskov and McDonald, 1979) by the following equation:

Effective degradability (%) = $a + bc / c + k$

In this equation, k refers to the fractional outflow rate of small particles from the rumen. The value of 4% fraction/h for k was used (Alvir *et al.*, 1998). The results on DM and CP degradability were corrected using the NEWAY 5.0 software (Ørskov and McDonald, 1979). The results on GCS chemical composition were analyzed using mixed model procedures while those on *in sacco* degradability using the GLM procedure (SAS, 1999).

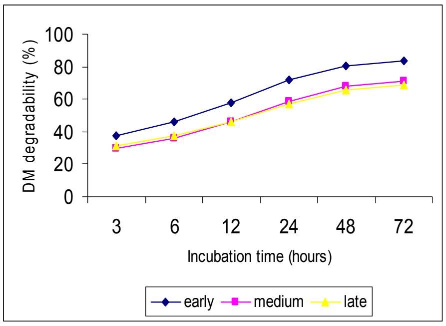
Results and discussion

Chemical composition of grass silages

Silage at a more advanced stage of maturity was harvested 19 days later than the early-cut material and the orchardgrass was stemmy and in full flower, in marked contrast to the early-cut herbage which had no emerged seed heads. The chemical composition of GCS is given in *Table 1*. Advancing maturity was evidenced by a linear increase in cell-wall carbohydrate as ADF ($P<0.01$) and by a linear decrease in the CP content ($P<0.01$) in GCS. Higher CP concentration and lower NDF and ADF concentrations in the early-cut silage than in the medium- and late-cut silage can be explained by a higher leaf to stem ratio (Jung, 1989). The average pH values varied from 4.4 in early-cut silage to 5.2 in medium-cut silage.

Degradability characteristics of DM

The mean *in sacco* DM and CP degradability at each time interval is given in Figure 1. and 2. The release of DM and CP from feeds during 3 to 72 h of incubation in the rumen indicates differences in degradation between the forages as well as differences in the final maximum release after 72 h incubation. Differences were the greatest between the early- and the late-cut silages, as expected. Characteristics of the DM and CP degradation of the silages are given in Table 2. Medium- and late-cut silages had a significantly lower (P<0.01) soluble component than the early cut silage. This indicates that early-cut silage may be rich in soluble compounds. The insoluble but fermentable component (b fraction) and its rate of fermentation in the early- and medium-cut silages was significantly (P<0.01) higher than that in the late-cut silage, suggesting lower degradability of ADF and NDF in the latter feed. This superiority of degradable ADF and NDF of the early- and medium-cut GCS may be caused by lower lignin content in these feeds compared to that of the late-cut silage. There was a significant (P<0.01) difference between all feedstuffs in the DM and CP ED, with the highest ED of early-cut silage and the lowest ED of late-cut silage. This study and that of Long *et al.* (1999) support the view that the herbage maturity stage at harvest is one of the main factors affecting the nutritive value of forages. According to Long *et al.* (1999), the 48 h *in sacco* DM degradability varies from 621-778 g kg^{-1}DM. The *in sacco* DM degradability of grass silages in the present study varied within these ranges. The advancing maturity of grass led to decreased degradation of silage DM as determined using the polyester bag procedure in the rumen (Vanhatalo *et al.*, 1996).



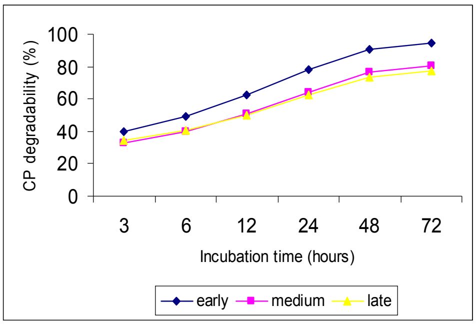


Figure 1 and 2. Dry matter and crude protein disappearance of early (-◊-), medium (□) and late-cut (Δ) grass clover silage incubated in sacco for different periods of time

Table 1. Chemical composition of silages at different stages of grass maturity (g kg^{-1} DM unless otherwise stated)

Item	Stage of grass maturity for silage I	II	III	SEM	Sig.
Dry matter (g kg^{-1})	396[a]	408[a]	463[b]	13.7	**
Organic matter	900[a]	912[b]	913[b]	1.13	**
Crude protein	120[a]	98[b]	90[c]	1.40	**
NDF[a]	677[a]	672[a]	705[b]	11.7	*
ADF[b]	372[a]	423[b]	429[b]	3.94	**
Starch	16.2[a]	17.9[b]	14.7[a]	1.10	*
pH	4.2[a]	4.5[b]	4.7[b]	0.17	*

S.E.M.: standard error of the mean. * P<0.05, ** P<0.01, NS P>0.05

Table 2. Dry matter and crude protein degradation (%) parameters of silages at different stages of grass maturity

Stage of grass maturity for silage	a (%)	b (%)	c (h-1)	ED (%)
Dry matter degradability				k=0,04 (h-1)
Significance	**	**	**	**
I-early cut	34.8a	49.9a	0.067a	62.6a
II-medium cut	28.9b	43.9b	0.055b	51.2b
III-late cut	30.7b	39.9c	0.055b	50.7c
SEM	0.46	0.57	0.007	0.49
Crude protein degradability				
Significance	**	**	**	**
I-early cut	38.9a	58.3a	0.059a	68.6a
II-medium cut	32.5b	51.8b	0.049b	56.9b
III-late cut	34.3b	46.9c	0.048b	55.9c
SEM	0.49	0.58	0.008	1,26

(a) - rapidly degraded fraction (%); (b) - slowly degraded fraction (%) and (c) - rate of degradation (fraction/h) ED (%), effective degradability (out flow rate: 4% h); * $P<0.05$, ** $P<0.01$, NS $P>0.05$

Conclusions

In conclusion, the results of this work demonstrate that the increasing maturity of grass ensiled had evident effects on the silage chemical composition, DM and CP degradability. Early harvest ensured higher DM and CP degradability of silages, which is necessary when a high production level is to be achieved with forage-based diets.

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