Intake, Digestibility *In vivo*, N Utilization and *In sacco* Dry Matter Degradability of Grass Silage Harvested at Three Stages of Maturity

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ABSTRACT : The objective of this experiment was to study the effects of grass maturity at harvest on the nutritive value of grass silage (GS) in relation to voluntary intake, digestibility, nitrogen (N) utilization and *in sacco* dry matter (DM) degradability. Silage was cut from a sward dominated by orchardgrass (*Dactylis glomerata L.*) at the late-vegetative (early-cut), internode elongation (medium-cut) and flowering (late-cut) stages of growth. The DM yield at harvest was the lowest for early-cut silage (5.4 t/ha) and increased to 6.5 and 7.0 t/ha for the medium and late-cut silage respectively. As the crop matured, the crude protein (CP) concentration decreased significantly ($p<0.05$) and there was a marked increase in acid detergent fiber (ADF) concentration ($p<0.001$). The three different silages were offered to four 18-month old Charolais wether sheep to measure the voluntary intake, *in vivo* digestibility and N retention over four 21-day periods in an incomplete changeover design. Silage degradability characteristics were determined using four fistulated sheep to measure DM degradability over 3, 6, 12, 24, 48 and 72 h. There was a linear decrease in the voluntary intake of silage fresh matter, DM, organic matter (OM) and neutral detergent fiber (NDF), digestibility of DM, OM, NDF, ADF and CP, and digestibility of OM in DM (D-value) ($P_L<0.01$) as harvesting of grass was delayed. Nitrogen intake, N output in urine, N output in faeces and N balance also linearly decreased ($P_L<0.01$) with postponed harvesting of grass for silage. DM degradability and effective degradability (ED) significantly decreased with increasing maturity of grass at harvest. The results suggest that harvesting date has a significant influence on the nutritive value of GS in terms of intake, digestibility, N balance and *in sacco* degradability in the rumen. It was concluded that early harvest GS ensured higher intake, digestibility, N intake and DM degradability in comparison with the medium and the late cut GS as a result of improved rumen N efficiency and utilization probably due to a better balance of available energy and protein. (**Key Words :** Grass Silage, Intake, N Retention, *In vivo* Digestibility, *In sacco* Degradability)

INTRODUCTION

Grass silage (GS) is the primary form of conserved forage for winter-feeding of ruminants in Europe. It 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 (Rahman et al., 2008), climate and ensiling techniques (Shaoa et al., 2005) upon the fermentation process in the silo and on nutritive value. Climate conditions in the continental part of Croatia in late April/early May are unfavourable for the production of high quality GS.

Furthermore, the widely grown grass in Croatia, orchardgrass (*Dactylis glomerata L.*), may turn from

vegetative to generative growth in a couple of days, which greatly influences its quality (Fowler et al., 2003). For grass 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 digestibility of the cell wall material (Jung, 1989; Iiyama and Lam, 2001; Touqir et al., 2007). On the one hand, the low nitrogen (N) content in mature forage plants may limit animal production (Adams et al., 2002; Kaiser et al., 2007) but, on the other hand, the efficiency of N capture in the rumen of N-rich silages harvested at an early stage of growth is poor (Tamminga, 1992; Aibibula et al., 2007).

Many studies have shown that the intake and performance of GS are higher when silage digestibility is higher (Steen et al., 1998; Sung and Okubo, 2001; Adams et al., 2002) probably due to a better balance of available

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energy and protein (Raza and Rowlinson, 1995; Kim et al., 2000). Information on the nutritive value of GS dominated by orchardgrass, and its dependence on different maturity stages at harvest, might be useful to farmers that constantly produce GS of lower quality.

The hypothesis tested in this study was that feeding early-cut silage would significantly increase feed intake, digestibility and N retention by sheep in comparison with the medium- and late-cut silage.

MATERIALS AND METHODS

Sward and silage making

The GS was made from a semi-permanent, predominately orchardgrass meadow harvested on 18 May, 25 May and 06 June 2002 (late vegetative, internode elongation and flowering growth stages of orchardgrass respectively) for silages designated early, medium and late, respectively.

Two applications of a commercial inorganic fertilizer were provided during the growing season. In February 2002, 450 kg/ha N-P-K fertilizer (8:26:26) was applied. This was followed by 150 kg/ha of ammonium nitrate thirty-five days prior to first harvest.

Green and dry matter yield (t/ha) were determined at mowing using 30 forage samples randomly taken by a quadratic frame (0.25×0.25 m). Botanical composition was determined from the same samples by manual separation of sward components (grasses, clovers, forbs). The sward contained 80.6% orchardgrass, 13.7% legumes (11.2% white clover (*Trifolium repens L.*) and 2.5% red clover (*Trifolium pratense L.*), 3.4% forbs and 2.3% other grasses 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. Five bales per treatment were wrapped in 4 layers of 500 mm-wide white plastic film. No additive was applied. The weather at harvest was warm and sunny on all three harvesting dates.

Chemical analysis

The DM contents of feed offered, feed refused and faeces were determined by oven drying to a constant weight at 60°C in a fan-assisted oven (ELE International). Ash was measured by igniting samples in a muffle furnace (Nabertherm) at 550°C for 16 h. Total N concentrations of feed offered, feed refused, faeces and urine 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×6.25) g/kg DM for feed offered, feed refused and faeces. 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).

Starch content of the feed offered, feed refused and faeces was determined by the method of Theander (1991). Silage volatile fatty acids (VFA) were measured by gas liquid chromatography and lactic acid was determined enzymatically on an Express Auto biochemical analyzer using juice expressed from the silage.

In vivo digestibility, voluntary intake and N balance

The nutritive value of the three grass silages (early, medium and late cut) was assessed by determining the voluntary intake, apparent digestibility and N balance when offered to wether sheep. Four Charolais wethers were selected on the basis of their live weight (mean body weight 43.5 kg, s.d. 3.8 kg) and condition. All animals were treated for internal parasites prior to the start of the experiment. The sheep were subjected to artificial light from 08:00 to 20:00 h daily.

Each sheep was randomly allocated to treatment sequences in an incomplete changeover design with four periods. A 10-day acclimatization period was followed by an 11-day measurement period (4-day *ad libitum* intake was followed by 7-day digestibility and N retention measurements) where feed offers and refusals were measured and total urine and faeces were collected. The animals were housed in individual pens (1.5×2.2 m) over the acclimatization period and in individual crates (1.36× 0.53×1.49 m) during the measurement period. Diets were offered twice a day (8:30 and 16:00 h) in equal amounts, calculated to ensure a refusal margin of 10-15% on each day. During the measurement period, the fresh weights and DM contents of feed offered and feed refused were recorded daily. Subsamples of the feed "as offered" were taken daily and stored at -20°C until the end of the experiment, when they were bulked prior to chemical analysis. Daily subsamples of refusals were bulked on an individual animal basis and stored at -20°C prior to chemical analyses.

Daily production of urine and faeces were collected separately. Daily output of urine from each animal was preserved by acidification (100 ml of 2 mol/L sulphuric acid to achieve a pH value of 2-3) and its volume was measured. Daily subsamples of urine from individual animals were then bulked over the measurement week and stored at -20°C until analysis.

Total daily faecal production of each animal was stored frozen until completion of the collection period. The bulked faecal output from each animal was then weighed and subsampled prior to subsequent analysis.

The sheep were weighed on the 10th, 14th and 21st day of each period and the mean weight was used to calculate

the daily voluntary intake of fresh matter (FM), DM and OM expressed per unit of metabolic weight, *i.e.*, g/kg $M^{0.75}$.

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 rumen of four rumen-fistulated Charolais sheep (4 years old, 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×20 cm with a pore size 50±15 μm (ANCOM Technology Corp., USA). All silage samples were dried at 60°C and milled through a 1.5 mm sieve. Then, 5 g of each sample was put into a nylon bag 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, 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. For each incubation time, 6 bags were incubated in the rumen of each animal. All 4 sheep were used to measure degradability of each diet (3 diets×2 replicates = 6 bags per sheep). Blanks were used in the calculation.

The percentage of degradability (Y) of DM 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 in each GS was then estimated (Ørskov and McDonald, 1979) by the following equation:

$$\text{Effective degradability (\%)} = \frac{a+bc}{c+k}$$

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 for DM degradability were corrected using the NEWAY 5.0 software (Ørskov and McDonald, 1979).

Statistical analysis of data

The results for GS chemical composition were analyzed using mixed model procedures and those for *in sacco* degradability analysed using the GLM procedure (SAS, 1999). Mean separation was calculated using the LSD values if the F-test was significant at p = 0.05. Linear and quadratic effects of GS maturity at harvest on silage chemical composition, *ad libitum* intake, digestibility and N utilization were examined using the CONTRAST statement of SAS. The model applied:

$$Yij = μ+Ti+Pj+eij$$

where Y is the overall model, μ = grand mean, T = treatment, P = period, e = experimental error, I = number of treatments, and j = number of periods.

RESULTS AND DISCUSSION

Chemical composition of grass silages

In the current experiment, grass was cut at late vegetative, internode elongation and flowering growth stages of orchardgrass (*Dactylis glomerata*).

The herbage DM yields (t/ha) were 5.4, 6.5 and 7.0 from the early-, medium- and late-cut grass, respectively. Delaying the harvest of silage from 18 May to 6 June increased the DM yield by 30%, but the quality of the GS was reduced. Silage at a more advanced stage of maturity was harvested 19 days later than the early-cut material.

The chemical composition of bulked samples of silages, as offered during the measurement periods of the feed evaluation experiment, is given in Table 1. There was no production of effluent from silages and the DM concentrations in silages were high. 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 GS. Higher CP concentration and lower NDF and ADF concentrations in the early-cut silage compared to the medium- and late-cut silage can be explained by a higher leaf to stem ratio (Jung, 1989).

The average pH values varied from 4.2 in early-cut silage to 4.7 in the late cut silage. Silages were well fermented, as indicated by low concentrations of ammonia N and fermentation acids.

Butyric acid was not present in the silages while relatively high pH values are normal for big-bale silages due to a higher DM content at harvesting. The average DM content of silages matches the recommended DM values of 300-400 g DM/kg fresh silage specified by O'Kiely and Muck (1998).

The average CP content of GS used in this experiment (90-120 g/kg DM) fell within the range reported by Vranić et al. (2005) (77-167 g/kg DM) in a survey of 19 family

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

Item	Stage of grass maturity for silage			SEM	Sig.	Significance of effect	
	Early	Medium	Late			L	Q
Dry matter	396[a]	408[a]	463[b]	13.7	***	<0.01	NS
Organic matter	900[a]	912[b]	913[b]	1.13	***	<0.01	NS
pH	4.2[a]	4.5[b]	4.7[b]	0.17	*	NS	NS
Neutral detergent fibre	677[a]	672[a]	705[b]	11.7	*	NS	NS
Acid detergent fibre	372[a]	423[b]	429[b]	3.94	***	<0.01	NS
Starch	16.2[a]	17.9[b]	14.7[a]	1.10	*	NS	NS
Crude protein	120[a]	98[b]	90[c]	1.40	***	<0.01	NS
Ammonia-N (g/kg TN)	76.0	98.7	128.6	ND			
Lactic acid concentration	60.7	72.1	78.8	ND			
Acetic acid concentration	1.2	36.3	36.9	ND			
Butyric acid concentration	NP	NP	NP	ND			

TN = Total nitrogen. NP = Not present. ND = Not determined. SEM = Standard error of the mean.
L = Linear effect of grass maturity at harvest. Q = Quadratic effect of grass maturity at harvest.
Values within the same row with different superscripts differ significantly (p<0.05). NS, p>0.05; * p<0.05; *** p<0.001.

farms in Croatia in 2004. The average CP contents of the early-cut and late-cut GS were similar to those reported by Fowler et al. (2003) (166 g/kg DM and 106 g/kg DM for early- and late-cut grass respectively).

Feed intake, digestibility and N-balance

The mean weights of silage consumed in each treatment and its digestibility by sheep are given in Table 2. In this table, the means have been adjusted for residual effects. Voluntary intake of silage DM, organic matter (OM) and NDF was affected by the chemical changes in silage composition and linearly decreased (p<0.01) with increasing maturity of grass ensiled.

The effect of delayed harvest was most pronounced between the early and late cut harvests. This is in agreement with the results reported by Thomas et al. (1988) and is associated with higher NDF and ADF concentrations with advanced grass maturity. Dove and Milne (2006) reported higher NDF content, lower CP content and decreased intake in lambs grazing a stemmy sward compared to a leafy sward.

A linear decrease in DM, OM, CP, NDF and ADF digestibility (p<0.01) resulted in linear decrease in silage DM, OM and NDF intake (p<0.01) with a postponed date of grass harvesting. Huhtanen et al. (2001) have already confirmed the positive relationship between feed intake and digestibility. The importance of forage digestibility in sheep production might be stressed from the work of Masters et al. (2006) who reported that faster growth rate in sheep is consistent with a higher forage digestibility.

The mean decrease in apparent digestibility of diet OM was 0.011 units per day (P_L<0.01) as the grass matured. The decrease was most pronounced between the first and the third cuts, as expected. Fowler et al. (2003) reported 74.2%

Table 2. Voluntary intake and digestibility by sheep fed silages at different stages of grass maturity

Item	Stage of grass maturity for silage			SEM	Significance of effect	
	Early	Medium	Late		L	Q
Voluntary intake						
Fresh matter (kg/d)	3.22[a]	2.48[b]	2.37[b]	0.067	<0.01	NS
Dry matter (kg/d)	1.28[a]	0.97[b]	1.08[c]	0.032	<0.01	NS
Organic matter (kg/d)	1.15[a]	0.88[b]	0.99[c]	0.04	<0.01	NS
Neutral detergent fibre (kg/d)	0.88[a]	0.76[b]	0.55[c]	0.035	<0.01	NS
Fresh matter (g/kg $M^{0.75}$ d)	179.9[a]	134.3[b]	129.2[b]	3.79	<0.01	NS
Dry matter (g/kg $M^{0.75}$ d)	72.5[a]	57.8[b]	59.0[b]	2.03	<0.01	NS
Organic matter (g/kg $M^{0.75}$ d)	64.6[a]	54.5[b]	48.1[c]	1.64	<0.01	NS
Neutral detergent fibre (g/kg $M^{0.75}$ d)	49.5[a]	41.7[b]	30.2[c]	1.28	<0.01	NS
Digestibility (g/kg DM)						
Neutral detergent fibre	754[a]	592[b]	515[c]	17.5	<0.01	NS
Acid detergent fibre	698[a]	571[b]	455[c]	15.8	<0.01	NS
Crude protein	596[a]	512[b]	489[b]	20.0	<0.01	NS
Dry matter	668[a]	534[b]	487[c]	13.1	<0.01	NS
Organic matter	691[a]	551[b]	494[b]	17.6	<0.01	NS
D-value	625[a]	524[b]	476[c]	16.2	<0.01	NS

D-value = Digestible organic matter in the dry matter; L = Linear effect of grass maturity at harvest.
Q = Quadratic effect of grass maturity at harvest; SEM = Standard error of the mean; $M^{0.75}$ = Metabolic body weight.
Values within the same row with different superscripts differ significantly (p<0.05). NS, p>0.05.

Table 3. Nitrogen utilization by sheep fed silages at different stages of grass maturity

Item	Stage of grass maturity for silage			SEM	Significance of effect	
	Early	Medium	Late		L	Q
N intake (g/d)	24.7[a]	15.3[b]	16.1[b]	0.51	<0.01	NS
N output in faeces (g/d)	9.9[a]	7.5[b]	8.3[b]	0.28	<0.01	NS
N output in urine (g/d)	7.3[a]	4.5[b]	4.9[b]	0.29	<0.01	NS
N balance (g/d)	7.5[a]	3.3[b]	2.9[b]	0.63	<0.01	NS
Faecal N/N intake (%)	40.4[a]	50.1[b]	52.9[b]	1.91	<0.01	NS
Urinary N/N intake (%)	30.3[a]	30.2[a]	32.0[a]	2.08	NS	NS
Absorbed N (g/d)	14.7[a]	7.8[b]	7.8[b]	0.46	<0.01	NS

N = Nitrogen; L = Linear effect of grass maturity at harvest; Q = Quadratic effect of grass maturity at harvest.
SEM = Standard error of the mean; Values within the same row with different superscripts differ significantly (p<0.05). NS, p>0.05.

OM digestibility of *Dactylis glomerata* at earlier and 68.2% at later vegetative growth stages.

The OM digestibility of early-cut silage in this study (69.11%) harvested at the late vegetative growth stage was between the two values previously reported.

Digestibility of OM in DM (D-value) in grasses declines at a rate of 2 units per week during early vegetative growth and at a rate of 3-5 units per week from the vegetative growth stage to blooming (Hopkins, 2000). D-value linearly decreased (p<0.01) with advanced stage of grass maturity. As the date of cutting was delayed 19 days (from the late vegetative to the flowering stage), the D-value declined at a rate of 5.6 units per week (from 62.55% in the early-cut silage to 47.6% in the late-cut silage). Our data are in agreement with Hopkins (2000), who reported a decline in D-value of *Dactylis glomerata* from 74% at the early vegetative growth to 49% at the full blooming stage.

Nitrogen utilization by sheep fed silages at different stages of grass maturity at harvest is given in Table 3.

The N content decreased by 1.6 g/kg DM per day when the harvest was delayed, resulting in a lower N intake of animals consuming the late-cut silage. Consequently, N output in faeces, N output in urine and N balance decreased in a linear manner (p<0.01) as the grass matured. Absorbed

N (intake N-fecal N) decreased in a linear manner (p<0.01) with the decreasing level of N intake. The proportionate values for faecal N linearly increased (p<0.01) with decreasing level of N intake. Feeding treatment did not influence the proportionate values for urinary N. This pattern of change in faecal and urinary N excretion is consistant with observations made in other studies utilizing different dietary protein concentrations. For example, when dietary protein was increased from from 13 to 17% (Kauffman and St-Pierre, 2001) and from 15 to 16.5% (Broderick, 2003), both feacal and urinary N excretion increased. However, when dietary protein was changed at higher levels, from 16.7 to 18.4% (Broderick, 2003), from 16.5 to 19.4% (Davidson et al., 2003) and from 16.5 to 17.7% (Wattiaux and Karg, 2004), only urinary N excretion increased. This suggests that feeding protein in excess of requirements not only increases total N excretion, but also increases the risk of N loss to the environment, because urine is more labile than fecal N (Varel et al., 1999).

Our data indicate that the Charolais wethers generally had a positive N balance in all three feeding treatments. Nitrogen retention increased (p<0.01) with the increasing level of N intake. Nitrogen retention has been shown to be lower with forage of lower CP concentration due to decreased DM intake and CP digestibility (Ko et al., 2006), which may account for the linear decrease in N balance from the early- to late-cut material. Our data are consistent with the results reported by Bunting et al. (1987), who fed a low CP (8.7%) and a high CP (15.4%) diet to lambs. A higher percentage of urea produced in the body was degraded in the digestive tract with the low protein diet, resulting in a lower percentage of nitrogen being excreted in the urine.

Degradability characteristics of DM

The mean *in sacco* DM degradability at each time interval is given in Figure 1. The release of DM 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-
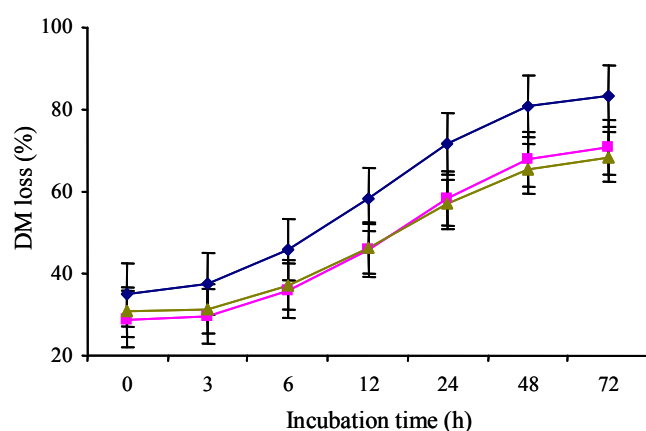

Figure 1. Dry matter disappearance of early (♦), medium (■) and late-cut (▲) grass silage incubated in sacco for different periods of time.

Table 4. Dry matter degradation (%) parameters of silages at different stages of grass maturity

Item	Stage of grass maturity for silage			SEM	Significance
	Early	Medium	Late		
a	34.8[a]	28.9[b]	30.7[b]	0.46	**
b	49.9[a]	43.9[b]	39.9[c]	0.57	**
a+b	84.7[a]	72.8[b]	70.5[c]	0.89	**
c (f/h)	0.067[a]	0.055[b]	0.055[b]	0.007	**
ED	62.6[a]	51.2[b]	50.7[c]	0.49	**

a = Rapidly degraded fraction (%); b = Slowly degraded fraction (%) and c = Rate of degradation (fraction/h) are constants in the exponential equation ($p = a+b(1-e^{-ct})$); ED (%), effective degradability (out flow rate: 4% h); SEM = Standard error of the mean. Means in the same row with different superscripts (a-c) letters are significantly different (** $p < 0.01$).

and the late-cut silages, as expected.

Characteristics of the DM degradation of the silages are given in Table 4. The degradation of silage DM incubated *in sacco* in sheep followed the pattern of the results obtained *in vivo*. Medium- and late-cut silages had a significantly lower soluble component than the early cut silage ($p < 0.01$). 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 higher than in the late-cut silage ($p < 0.01$), suggesting lower degradability of ADF and NDF in the latter feed.

There was a significant ($p < 0.01$) difference between all feedstuffs in the DM 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 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).

CONCLUSIONS

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

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