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ScienceDirect
Livestock Science 114 (2008) 84–92
LIVESTOCK SCIENCE
www.elsevier.com/locate/livsci

Effects of replacing grass silage harvested at two maturity stages with maize silage in the ration upon the intake, digestibility and N retention in wether sheep

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Received 25 January 2006; received in revised form 2 April 2007; accepted 13 April 2007

Abstract

The objective of this experiment was to study the effects of interactions between medium quality grass silage (GS1) and maize silage (MS) as well as between low-quality grass silage (GS2) and MS on *ad libitum* intake, digestibility and N retention in wether sheep. Two grass silages (GS1 and GS2) were ensiled in round bales, without additives, from the primary growth of orchard grass (*Dactylis glomerata* L.) harvested at two different maturity stages. The study consisted of seven feeding treatments incorporating GS1, GS2 and MS fed alone and forage mixtures of GS1 and MS as well as GS2 and MS (67:33% and 33:67%, respectively, DM (dry matter) basis).

Delayed harvesting lowered ($P<0.05$) the crude protein (CP) concentration in GS2 compared to GS1. The DM content (g kg^{-1} fresh sample) and starch concentration (g kg^{-1} DM) of MS were 264 and 211, respectively.

Inclusion of MS in the GS1-based ration had positive linear effects on CP and starch digestibility ($P<0.05$ and $P<0.01$, respectively) and N intake ($P<0.01$) while a negative effect on neutral detergent fibre (NDF) and acid detergent fibre (ADF) digestibility ($P<0.05$ and $P<0.01$, respectively). A positive associative response of GS1 and MS was observed for DM *ad libitum* intake (g kg^{-1} $M^{0.75}$ day^{-1}) (quadratic, $P<0.05$), CP digestibility (quadratic, $P<0.01$), N intake (quadratic, $P<0.01$) and N balance (quadratic, $P<0.05$). Inclusion of MS into the GS2-based ration had a positive linear effect on the ration fresh matter *ad libitum* intake (kg day^{-1} and g kg^{-1} $M^{0.75}$ day^{-1}) ($P<0.01$ and $P<0.001$, respectively), NDF *ad libitum* intake (kg day^{-1} and g kg^{-1} $M^{0.75}$ day^{-1}) ($P<0.01$), digestibility of DM ($P<0.01$), organic matter (OM) ($P<0.01$), ADF ($P<0.05$), starch ($P<0.001$), digestibility of OM in DM (*D*-value) ($P<0.001$), and N intake ($P<0.01$). Positive associative effects of GS2 and MS were observed on all the intake and digestibility parameters measured, N intake (quadratic, $P<0.001$) and N balance (quadratic, $P<0.05$). It was concluded that, as expected, a positive associative response of GS2 and MS was recorded for all the measured parameters while that of GS1 and MS for a limited number of parameters, probably due to lower quality of MS (lower starch concentration) than required for improved utilization of the GS1-based ration.

Keywords: Grass silage; Maize silage; Intake; Digestibility; N retention

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1871-1413/$ - see front matter
doi:10.1016/j.livsci.2007.04.011

1. Introduction

Grass silage (GS) production in Croatia does not have a long tradition, which may partly account for its variable quality. Monitoring the grass silage quality over 2 years on 19 farms, large variations were observed for dry matter (DM) content (123–825 g kg^{-1} fresh sample), crude protein (CP) (50–217 g kg^{-1} DM) and neutral detergent fibre (NDF) concentration (300–605 g kg^{-1} DM), pH value (3.5–6.2), digestibility of organic matter (OM) in DM (*D*-value) (44–73%) and metabolizable energy (ME) concentration (7.1–12.4 MJ ME kg^{-1} DM) (Vranić et al., 2004, 2005a). According to Chamberlain and Wilkinson (1996), the average quality of GS produced on family farms in Croatia is of medium to low quality (less than 150–175 g CP kg^{-1} DM, less than 11 MJ ME kg^{-1} DM, and more than 50 g NH_3–N kg^{-1} total N).

Grass silage has a great potential in sheep nutrition, especially over 4–5 months of winter-feeding when sheep are kept indoors. There are certain concerns about improving the quality of the ration based on GS in sheep nutrition by its partial replacement with other forage, possibly maize silage (MS), which is widely produced in Croatia.

When GS or MS of nearly equal DM content (213 and 219 g kg^{-1} fresh sample, respectively) were fed as a sole feed, sheep preferred MS to GS rations (O'Doherty et al., 1997). Previous investigations with sheep have shown increased intake and digestibility when GS was partially replaced with molassed sugar beet feed (Rouzbehan et al., 1996) as well as increased N retention when MS was partially replaced with red clover hay (Margan et al., 1994).

The hypothesis of this study was that replacement of GS with MS would have positive associative effects on feed intake, digestibility of DM, OM, NDF, acid detergent fibre (ADF), CP, starch, *D*-value, and N retention in sheep. The objectives of the experiment were to examine the effects of interactions between the medium and low quality GS dominated by orchard grass and MS, with no supplementary feed provided, on feed intake, digestibility and N retention in wether sheep.

2. Materials and methods

2.1. The sward and silage making

Grass silage was made in 2002 from predominately orchard grass (*Dactylis glomerata* L.) meadow harvested at the late vegetative (18 May) and early flowering (6 June) stages of orchard grass (GS1 and GS2, respectively). Two applications of a commercial inorganic fertilizer were provided during the growing season: 450 kg ha^{-1} of N–P–K fertilizer (8:26:26) in February 2002 and 150 kg ha^{-1} of ammonium nitrate thirty-five days prior to harvesting. Green and DM yield (t ha^{-1}) was determined at mowing by calculating the weight of 30 forage samples randomly taken by quadratic frame (0.25 × 0.25 m^2). Botanical composition was determined from the same samples by manual separation of grasses, clovers and forbs. The sward contained 80.6% orchard grass, 13.7% legumes, 2.3% other grasses and 3.4% forbs on a DM basis. Forage DM content at harvest was 169 and 276 g kg^{-1} fresh sample, while DM yield amounted to 5.4 and 7.0 t ha^{-1} of GS1 and GS2, respectively.

The crop was mown and allowed to wilt for 24 h before harvesting with a round baler. Bales were wrapped in four layers of 500-mm-wide white plastic film. The weather at harvest was warm and sunny. No additive was applied.

The maize crop (*Zea mays* L., cultivar BC 566) was sown on March 8, 2002 into a ploughed and rolled seedbed. The crop was sown with a row space of 75 cm and the establishment target was 70,000 plants ha^{-1}. Whole crop maize was harvested on September 23, 2002 to a nominal stubble height of 25 cm above ground (pre-harvest DM of 275 g kg^{-1} fresh weight). The DM yield of forage maize at harvest was 13.5 t ha^{-1}, while the cob DM to total DM ratio was 6:1. The forage was chopped at harvest to a chop length of 1.9 cm, ensiled immediately into a clamp silo, without any additive, and rolled thoroughly before being sheeted with plastic and covered with rubber tyres to ensure exclusion of air.

2.2. Dietary treatments

The treatments consisted of either GS1, GS2 or MS alone, a forage mixture (DM-based) of GS1 and MS of 670 g kg^{-1} GS1 and 330 g kg^{-1} MS (GS1MS), and 330 g kg^{-1} GS1 and 670 g kg^{-1} MS (MSGS1), or a forage mixture (DM-based) of GS2 and MS of 670 g kg^{-1} GS2 and 330 g kg^{-1} MS (GS2MS), and 330 g kg^{-1} GS2 and 670 g kg^{-1} MS (MSGS2). Seven feeding treatments were examined in all. Just before the experiment started the MS for the experimental needs was compressed into 8 plastic containers (approximately 200 L each) and stored in a cold chamber maintained at a temperature of 4 °C.

The GS1 and GS2 were chopped to approximately 3–5 cm using a commercial chopper. The chopped material was compressed into plastic bags (approximately 10 kg

GS per bag) under continuous CO_2 flushing and stored in a cold chamber (4 °C).

The forage was mixed weekly and kept in plastic bags in a cold room (4 °C) prior to feeding to prevent warming. No supplementary feeds were provided.

2.3. Animals and design

Ten Charolais wethers were selected based on live weight (mean body weight 43.5 kg, S.D. 3.8 kg) and condition score. All the animals were treated for internal parasites prior to the start of the experiment. The sheep were subjected to artificial lightening from 08:00 to 20:00 hours 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^2) over the acclimatization period and in individual crates (136 cm × 53 cm × 148.5 cm) during the measurement period. Rations were offered twice a day (8:30 and 16:00 h) in equal amounts, designed to ensure a refusal margin of 10–15% each day. During the measurement period, fresh weights and DM contents of feed offered and feed refused were recorded daily. Subsamples of offered feed 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. The daily output of urine from each animal was preserved by acidification (100 ml of 2 mol l^{-1} 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. Bulked faecal output from each animal was then weighed and subsampled prior to subsequent analyses. The sheep were weighted 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, OM and NDF expressed per unit of metabolic weight, i.e., g kg^{-1} M$^{0.75}$.

The experiment conducted followed the Council Directive issued by the European Economic Community (EEC) (1986) on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes.

2.4. 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 CP (total N × 6.25) g kg^{-1} DM for feed offered, feed refused and faeces.

The procedure of Van Soest et al. (1991) was applied for ADF and NDF determination. 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 liquid gas chromatography and lactic acid was determined enzymatically on an Express Auto biochemical analyzer using juice expressed from the silage.

2.5. Statistical analysis

Results were analyzed using mixed model procedures (SAS, 1999). Mean separation was calculated using the LSD values if the *F*-test was significant at $P = 0.05$. Also, orthogonal contrasts of *ad libitum* intake, digestibility and N utilization of GS1 versus GS2 were made using the CONTRAST statement of SAS. Linear and quadratic effects of the level of MS inclusion in GS1 and GS2 on *ad libitum* intake, digestibility and N utilization were examined using the CONTRAST statement of SAS. Model applied: $Yij = \mu + Ti + Pj + eij$ 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.

3. Results

3.1. Ration chemical composition

The chemical composition of grass silages, maize silage and forage mixtures are presented in Table 1.

The DM content of GS1 was lower than that of GS2 ($P < 0.05$). MS was much lower in DM than GS1 and GS2

Table 1
Chemical composition of silage from early cut grass (GS1), silage from late cut grass (GS2) and maize silage (MS) (g kg^{-1} DM, unless otherwise stated)

Feeding treatment	DM g kg^{-1} fresh sample	Organic matter	Crude protein	NDF	ADF	Starch	pH	Butyric acid	Acetic acid	Lactic acid	NH_3–N g kg^{-1} total N
GS1	396^b	901^c	119.6^a	697^a	372^b	16.2^a	4.4^b	NF	1.2	60.7	76.0
GS2	463^a	914^b	90.3^b	705^a	429^a	14.6^a	4.7^a	NF	36.9	78.7	128.6
MS	264^c	955^a	61.6^c	582^c	322^c	211.0^b	3.7^c	NF	67.1	93.7	165.2
S.E.M.	6.4	1.47	1.28	12.4	5.09	6.0	0.06	ND	ND	ND	ND
Significance	***	***	***	***	***	***	**				

NF: not found. ND: not determined. S.E.M.: standard error of the mean.
Values within the same column with different superscripts differ significantly (**$P<0.01$; ***$P<0.001$).

($P<0.05$). As expected, CP declined from the early to late cutting ($P<0.001$). MS was much lower in CP than GS1 and GS2 ($P<0.001$). MS contained larger quantities of OM and starch than GS1 and GS2 ($P<0.001$).

Lactic acid was the major organic acid in the silages. GS1 was well fermented with the ammonia level below 100 g NH_3–N kg^{-1} total N while GS2 and MS contained more than 100 g NH_3–N kg^{-1} total N.

3.2. Intake and digestibility

Table 2 shows FM, DM, OM and NDF *ad libitum* intake and the total tract apparent digestibility of GS1, GS2, MS and mixtures of GS1 and MS as well as GS2 and MS fed to wether sheep.

3.3. Effect of MS supplementation to GS1 on ad libitum intake and the total tract apparent digestibility

Ration DM intake (g kg^{-1} $M^{0.75}$ day^{-1}) responded quadratically ($P<0.05$) to increasing levels of MS. Addition of MS linearly increased the apparent digestibility of CP ($P<0.05$) and starch ($P<0.01$) and decreased that of NDF ($P<0.05$) and ADF ($P<0.01$). Digestibility of CP responded quadratically ($P<0.01$) as the proportion of MS increased in the ration.

3.4. Effect of MS supplementation to GS2 on ad libitum intake and the total tract apparent digestibility

Ration FM intake (g kg^{-1} $M^{0.75}$ day^{-1}) increased linearly ($P<0.001$) and so did NDF intake ($P<0.01$) as the proportion of MS in the ration increased. Ration FM intake (g kg^{-1} $M^{0.75}$ day^{-1}) responded quadratically ($P<0.01$) to increasing levels of MS and so did the ration DM ($P<0.01$), OM ($P<0.001$) and NDF ($P<0.001$) intake. Addition of MS linearly increased the apparent digestibility of DM ($P<0.01$), OM ($P<0.01$), ADF ($P<0.05$), starch ($P<0.001$) and *D*-value ($P<0.001$). Digestibility of DM, NDF, ADF, CP, starch and *D*-value responded quadratically ($P<0.01$) and so did OM digestibility ($P<0.05$) as the proportion of MS increased in the ration.

Also, higher FM intake and digestibility of all measured parameters in diets based on GS1 compared to GS2 ($P<0.001$) was observed.

3.5. Nitrogen balance

Table 3 shows nitrogen utilization of GS1, GS2, MS and mixtures of GS1 and MS as well as GS2 and MS fed to wether sheep.

3.6. Effect of MS supplementation to GS1 on nitrogen utilization

Total dietary N intake increased linearly and quadratically ($P<0.01$) as the proportion of MS increased in the ration. There was a linear decrease ($P<0.01$) in N output in faeces and a quadratic response to N balance ($P<0.05$) as the proportion of MS increased in the ration.

3.7. Effect of MS supplementation to GS2 on nitrogen utilization

Nitrogen intake and N output in faeces were linearly affected ($P<0.01$) by the inclusion of MS in the ration based on GS2. Nitrogen intake responded quadratically ($P<0.001$) to increasing levels of MS and so did N balance ($P<0.05$). Negative N balance was found in sheep fed MS only.

Higher N intake, N output in urine and N balance in diets based on GS1 compared to GS2 ($P<0.001$) was observed.

4. Discussion

4.1. Chemical composition

There was a reasonable degree of variation in the quality of GS offered when assessed in terms of the DM content, fibre concentration, fermentation characteristics

Table 2
Fresh matter, dry matter, organic matter *ad libitum* intake and total tract digestibility of silage from early cut grass (GS1), silage from late cut grass (GS2), maize silage (MS) and mixtures of grass silage and maize silage fed to wether sheep

Voluntary intake (g kg^{-1} $M^{0.75}$ day^{-1})	GS1	GS1MS	MSGS1	MS	S.E.M.	Significance of		GS2	GS2MS	MSGS2	MS	S.E.M.	Significance of		GS1 vs. GS2 [a]
						L	Q						*L*	Q	
Fresh matter	179.9	185.2	242.9	206	14.4	N.S.	N.S.	129	189	216	206	8.89	***	**	***
Dry matter	72.53	66.7	84.3	49.6	6.8	N.S.	*	59.0	80.7	79.2	49.6	5.65	N.S.	**	N.S.
Organic matter	64.7	59.9	73	49.8	5.7	N.S.	N.S.	54.5	71.9	70.6	49.8	3.57	N.S.	***	N.S.
NDF	49.5	43	48.6	31.3	6.5	N.S.	N.S.	42.2	54.5	50.3	31.3	3.15	**	***	N.S.
Digestibility (g kg^{-1})															
Dry matter	668	685	699	631	24	N.S.	N.S.	487	628	669	631	24.6	**	**	***
Organic matter	691	699	708	651	24	N.S.	N.S.	495	644	684	651	31.3	**	*	***
Neutral-detergent fibre	754	711	686	595	40	*	N.S.	514	650	667	595	30.9	N.S.	**	***
Acid-detergent fibre	698	663	669	562	25	**	N.S.	454	604	630	562	32.1	*	**	***
Crude protein	596	678	658	469	32	*	**	489	570	568	469	30.6	N.S.	**	***
Starch	966	988	995	998	5.6	**	N.S.	948	990	995	998	4.5	***	**	***
D-value (g kg^{-1} DM)	627	635	647	617	21	N.S.	N.S.	476	594	637	617	19.4	***	**	***

GS1MS = silage from early cut grass 670 g/kg DM, maize silage 330 g/kg DM, MSGS1 = maize silage 670 g/kg DM, silage from early cut grass 330 g/kg DM, GS2MS = silage from late cut grass 670 g/kg DM, maize silage 330 g/kg DM, MSGS2 = maize silage 670 g/kg DM, silage from late cut grass 330 g/kg DM.

S.E.M.: standard error of the mean. *L*: linear effect of maize silage in the diet. *Q*: quadratic effect of maize silage in the diet. *D*-value: digestible organic matter in the dry matter. $M^{0.75}$ = metabolic body weight.

N.S.: not significant. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

[a] Orthogonal contrast.

Table 3
Nitrogen utilization of silage from early cut grass (GS1), silage from late cut grass (GS2), maize silage (MS) and mixtures of grass silage and maize silage fed to wether sheep

Nitrogen balance (g day^{-1})	GS1	GS1MS	MSGS1	MS	S.E.M.	Significance of		GS2	GS2MS	MSGS2	MS	S.E.M.	Significance of		GS1 vs. GS2 [a]
						L	Q						*L*	Q	
N intake	24.6	21.9	25.6	9.46	2.0	**	**	16.2	20.0	18.3	9.46	1.18	**	***	***
N output in faeces	9.9	7.4	8.6	5.8	0.68	**	N.S.	8.3	8.6	7.9	5.8	0.55	**	N.S.	N.S.
N output in urine	7.3	8.0	4.2	3.9	1.49	N.S.	N.S.	4.9	4.0	5.8	3.9	1.12	N.S.	N.S.	***
N balance	7.45	6.6	12.85	−0.28	2.0	N.S.	*	2.9	7.5	4.5	−0.28	1.69	N.S.	*	***

GS1MS = silage from early cut grass 670 g/kg DM, maize silage 330 g/kg DM, MSGS1 = maize silage 670 g/kg DM, silage from early cut grass 330 g/kg DM, GS2MS = silage from late cut grass 670 g/kg DM, maize silage 330 g/kg DM, MSGS2 = maize silage 670 g/kg DM, silage from late cut grass 330 g/kg DM.
S.E.M.: standard error of the mean. *L*: linear effect of maize silage in the diet. *Q*: quadratic effect of maize silage in the diet.
N.S.: not significant. *$P<0.05$; **$P<0.01$; ***$P<0.001$.
[a] Orthogonal contrast.

and digestibility, which reflected earlier and late cutting dates of herbage from which the silages were produced. The average CP content of GS (119.6 and 90.3 g kg^{-1} DM for GS1 and GS2, respectively) was between the average minimum and maximum CP concentrations (77–168 g kg^{-1} DM) determined for grass silages from 19 farms in Croatia (Vranić et al., 2005a).

The DM content of maize silage used in this experiment (264 g kg^{-1} fresh weight) was much lower compared to the average 2-year DM content of maize silages in Croatia (372.38 g kg^{-1} fresh weight) (Vranić et al., 2005b). When viewed in conjunction with its medium starch content of 211 g kg^{-1} DM, this is indicative of less mature maize silage, caused by an unusually wet summer in 2002 when the maize silage was produced.

The first priority in feeding ruminants is to ensure that there are no nutrient deficiencies in the ration for ruminal microbial growth by providing easily digestible high-energy feeds and N as well. Bondi (1987) suggested that feeds containing less than 60 g CP kg^{-1} DM promote negative N balance caused by protein malnutrition. Both GSs used (GS1 and GS2) as N sources had CP concentrations higher than 60 g kg^{-1} DM. They were supplemented with MS as energy source to study the possible interactions of forages in terms of voluntary intake, digestibility and N balance.

4.2. Effect on feed intake

Despite the lower DM content of MS compared to GS1 and GS2 and the known negative relationship between forage moisture content and forage DM intake (Steen et al., 1998; Mulligan et al., 2002), there was a positive associative effect of MS and GS1 in voluntary DM intake (g kg^{-1} M$^{0.75}$ day^{-1}) and of MS and GS2 in voluntary intake of all parameters measured. This is not surprising given that sheep develop preferences for feeds that are richer in energy (Provenza, 1995), prefer MS to GS diets (O'Doherty et al., 1997) and that associative effects depend on GS quality and MS maturity (Hameleers, 1998). Also, positive associative responses of GS2 and MS for intake (FM, DM, OM, NDF) and digestibility (DM, OM, CP, NDF, ADF, starch, *D*-value) might be explained by the lower quality of GS2 than that of included MS (Weller et al., 1991). This, however, was not the case of GS1 and MS and it resulted in a limited associative response of the two forages with regard to intake and digestibility. Besides DM and energy content, forage NDF is also suggested to be important in the regulation of forage intake (Van Soest et al., 1991). Mertens (1994) noted that at high

NDF concentrations in rations, the rumen fill limited DM intake, whereas at low NDF concentrations, energy intake feedback inhibitors limited DM intake. Allen (2000) summarized 15 studies and showed a general decline in DM intake with increasing NDF concentrations in rations when diets exceeded 25% NDF. At any particular NDF concentration in the ration, however, a considerable range of DM intake was observed, suggesting that the source of NDF in the rations, affected by the particle size, digestibility and rate of passage from the reticulorumen, influenced the DM intake.

4.3. Effect on digestibility

A positive associative effect of MS and GS2 on fibre digestibility (NDF, ADF) (Fig. 1A) was probably a result of the higher NDF content (715 g kg^{-1} DM) and lower NDF digestibility (514 g kg^{-1}) of GS2 compared to the NDF content (582 g kg^{-1} DM) and NDF digestibility (595 g kg^{-1}) of MS when silages were fed as the sole diet. Besides NDF concentration, also maturity influences the NDF digestibility of GS, which declines with advancing maturity mainly due to advancing lignification of cell walls (NDF) (Jung and Allen, 1995). For this reason, despite the same NDF concentration in GS1 and GS2 ($P>0.05$), higher NDF digestibility was observed in GS1 than in GS2 (by 31.8%). Although GS1 contained a higher NDF concentration (697 g kg^{-1} DM) than MS, no associative effect but a negative linear effect of GS1 and MS was recorded for fibre digestibility (NDF, ADF) (Fig. 1), probably due to lower NDF digestibility of MS (595 g kg^{-1}) than GS1 (754 g kg^{-1}) when silages were fed as the sole diet. Also, with higher quality forages, feeding starch-based energy supplements was shown to cause negative associative effects on fibre digestibility (Pordomingo et al., 1991). A better way of

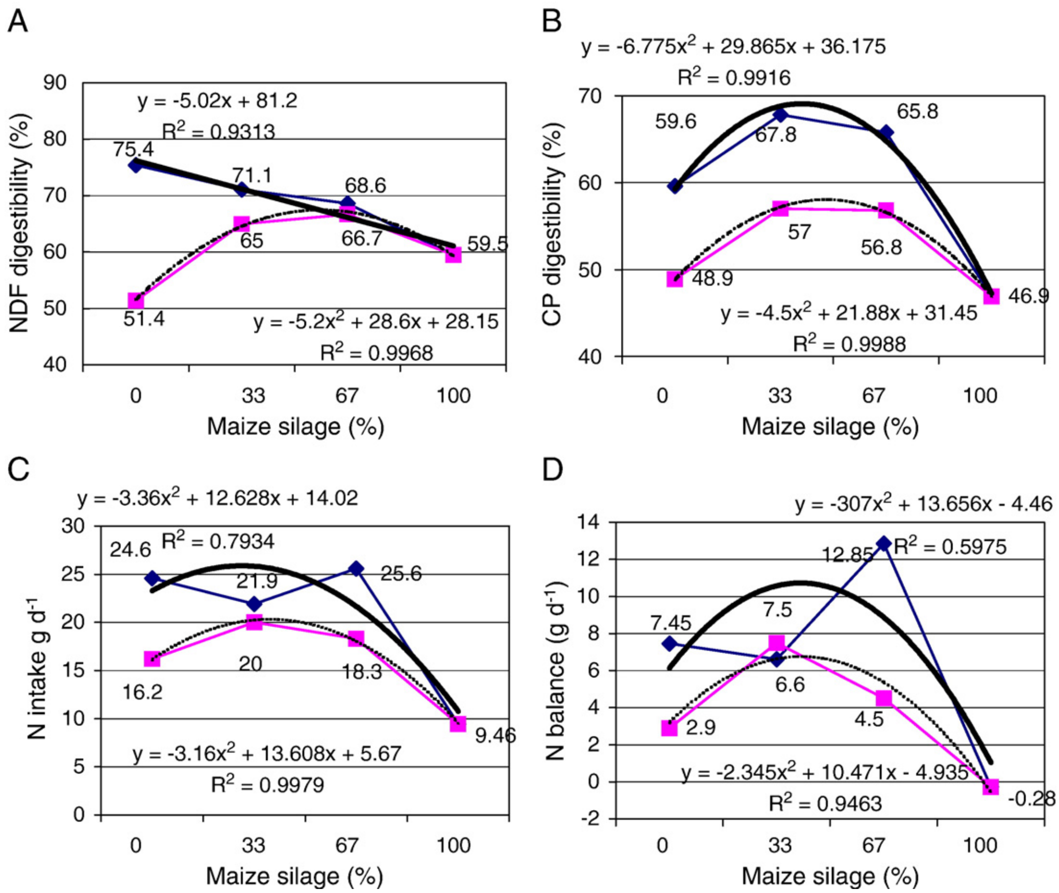

Fig. 1. Digestibility of NDF (A), crude protein (B), N intake (C) and N balance (D) of silage from early cut grass (GS1), silage from late cut grass (GS2), maize silage (MS) and mixtures of grass silage and maize silage fed to wether sheep, (♂) GS1; (■) GS2; (—) regression curve GS1; (- - -) regression curve GS2.

increasing digestibility is to supplement low quality forage with higher quality forage, which is corroborated by the current results as well as by research reported by Matejovsky and Sanson (1995).

The positive associative effect of MS inclusion (33% vs. 67% for GS1 and GS2, respectively) on CP digestibility (Fig. 1B) is probably related to higher intake and simultaneous increase of total N consumption owing to energy supplementation. Cottrill et al. (1982) reported similar beneficial effects of increased energy levels on N digestibility in young cattle.

In this experiment, starch digestibility in the MS ration was high (998 g/kg DM) and close to the value of 990 g/kg DM reported by Anil et al. (2000) for starch digestibility in MS of similar quality determined in wether sheep. This supports the linear increase in digestibility of the ration based on GS1 and GS2 with the increasing level of MS, since reduced starch digestibility accounts for approximately one-half of the depression in MS digestibility (Joanning et al., 1981).

4.4. Effect on N balance

The positive associative effect on N intake observed with MS supplementation to GS1 and GS2 (Fig. 1C) is largely a reflection of higher DM intake and N intake of the forage mixture, as suggested by Adesogan et al. (2002). A linear increase in N intake with increasing MS supplementation to GS1 and GS2 suggests that the intake of N was affected by the energy level of the diet.

The positive associative effect on N balance with MS supplementation to GS1 and GS2 (Fig. 1D) could be partly attributed to improved microbial protein synthesis of rumen-degraded GS nitrogen in the presence of maize starch (Hvelplund et al., 1987) and an increased supply of non-ammonium N to the abomasum and small intestine (Beever et al., 1986).

The highest proportion of N output in urine of N consumed was recorded for the MS ration (41.2%), indicating an inefficient microbial capture of rumen degradable N and contributing, along with the low N content in MS, to negative N balance for lambs offered the MS ration (Bondi, 1987; Fraser et al., 2000). This showed that 62 g CP kg^{-1} DM was not enough to meet the N requirements of wether sheep, which is in agreement with the results reported by Bondi (1987). A positive associative response of GS1 and MS as well as of GS2 and MS to N retention agrees with the findings that the improvement in N balance with the addition of MS to GS may be related to the presence of more readily fermentable carbohydrate, improving microbial N use in the rumen (Moss et al., 1992). It is known that the yield of microbial CP does not depend solely on the solubility of dietary CP, but also on the supply of fermentable energy sources and the degree of ruminal synchronization of CP and carbohydrate catabolism (Beever, 1993). Energy and nutrient supplies to rumen microorganisms are of major importance because they influence bacterial lysis (Meng et al., 1999), predation of bacteria by protozoa (Clark et al., 1992) and the share of nutrient consumption for maintenance of rumen microbes (Hespell and Bryant, 1979). It therefore appears more likely that rumen degradable CP, i.e., N supply from GS1, was higher than the available energy from MS for optimal N utilization. That was not the case of GS2 and MS where rumen energy demands were met, resulting in positive associative effects of the two forages on all the measured parameters.

5. Conclusions

The results of this research might be of general interest, especially in parts of Europe where lower quality GS is produced but where production of MS is possible. A positive associative response of GS2 and MS was, as expected, recorded for all the measured parameters while that of GS1 and MS was found for a limited number of parameters, probably due to lower quality of MS (lower starch concentration) than required for better utilization of the GS1-based ration.

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