

Feeding value of low quality grass silage supplemented with maize silage for sheep

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The objective of this experiment was to study the effects of interactions between low quality grass silage (GS) dominated by orchardgrass and maize silage (MS) on ad libitum intake, digestibility and nitrogen retention in wether sheep. The study consisted of four feeding treatments involving GS and MS alone and GS and MS mixtures in a ratio of 67:33 or 33:67 (dry matter (DM) basis) fed twice daily. The GS was high in DM (463 g kg⁻¹), neutral detergent fibre (715 g kg⁻¹DM) and acid detergent fibre (429 g kg⁻¹DM) while low in crude protein (90.1 g kg⁻¹DM). The DM content (g kg⁻¹) and starch concentration (g kg⁻¹DM) of MS were 264 and 211, respectively. The inclusion of MS into diet had positive linear effects on fresh matter ad libitum intake (kg d⁻¹ and g kg⁻¹M0.75d⁻¹) (P < 0.01 and P < 0.001 respectively), digestibility of DM (P < 0.01), organic matter (P < 0.01), acid detergent fibre (P < 0.05), starch (P < 0.001), digestibility of organic matter in DM (D-value) (P < 0.001), nitrogen intake (P < 0.01) and nitrogen output in faeces (P < 0.01). A positive associative effect of low quality GS and MS was observed for ad libitum intake (kg d⁻¹ and g kg⁻¹M0.75d⁻¹) of fresh matter (quadratic, P < 0.01), DM (quadratic, P < 0.001 and P < 0.01 respectively) and organic matter (P < 0.001), for digestibility of DM, neutral detergent fibre, acid detergent fibre, crude protein, starch and D-value (quadratic, P < 0.01), digestibility of organic matter (quadratic, P < 0.05), nitrogen intake (quadratic, P < 0.001) and nitrogen balance (quadratic, P < 0.05). It was concluded that differences between low quality GS and MS resulted in positive associative responses of GS and MS for all parameters measured (intake, digestibility and nitrogen retention).

Key-words: grass silage, maize silage, intake, digestibility, nitrogen retention

Introduction

Many sheep producers in Croatia utilize conserved forages such as grass silage (GS) in their winter-feeding program. However, GS produced at family

farms is often of low nutritive value due to its high concentration of fibre, low digestibility and low concentration of crude protein (CP) (Vranić et al. 2005a). Improvement of digestibility and intake are the two major factors for raising the nutritive

value of low quality forage for ruminants. One way of improving utilization of low quality GS is to increase microbial activity in the rumen by supplementing the diet with feeds high in rumen degradable organic matter and thereby increase microbial protein synthesis and short chain fatty acids production. Maize silage (MS) may be used as supplemental forage to GS because it complements grass silage well. Previous investigations with sheep have shown increased intake and digestibility when GS was partially replaced with a supplemental energy source (Rouzbehan et al. 1996). Margan et al. (1994) observed positive associative effects of MS and red clover hay for voluntary intake, digestibility of nitrogen (N), organic matter (OM) and N balance.

When fed in combination, associative effects depend on the quality of GS and are also related to the maturity of MS (Hameleers 1998). Positive responses could be expected when the GS to be replaced was of lower quality than the included forage substitute (Weller et al. 1991). As sheep prefer maize to grass silage diet (O'Doherty et al. 1997) and the GS in this study was of low quality, the combination of GS and MS was offered under the hypothesis that feeding a mixture of these supplements would have positive associative effects on food intake, digestibility and N retention in sheep. The objective of this experiment was to examine the effects of interactions between the low quality GS dominated by orchardgrass and MS on feed intake, digestibility and N retention in wether sheep.

Green and dry matter (DM) yield (t ha^{-1}) was determined at mowing by calculating the weight of 30 forage samples randomly taken by a quadratic frame ($0.25 \times 0.25 \text{ m}$). Botanical composition was determined from the same samples by manual separation of sward components (grasses, clovers, forbs).

The sward contained 80.6% orchardgrass (*Dactylis glomerata* L.), 13.7% legumes (11.2% white clover and 2.5% red clover), 2.3% other grasses and 3.4% forbs on a DM basis. Forage DM content at harvest was 276 g kg^{-1} fresh sample and DM yield was 7.01 t ha^{-1} . The crop was allowed to wilt for 24 h before harvesting with a round baler. Bales were wrapped in 4 layers of 500 mm-wide white plastic film. The weather at harvest was warm and sunny. No additive was applied.

Forage maize crop (*Zea mays* L., cultivar BC 566) was sown on 8 March 2002 into a prepared (ploughed and rolled) seedbed. The crop was sown with a row space of 75 cm and the establishment target was $70000 \text{ plants ha}^{-1}$. Whole crop maize was harvested on 23 September 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 standard chop length, ensiled into a clamp silo immediately, without any additive, and rolled thoroughly before being sheeted with plastic and covered with rubber tyres to ensure exclusion of air.

Material and methods

Sward and silage making

The GS was made from a semi-permanent, predominantly orchardgrass (*Dactylis glomerata* L.) meadow harvested on 6 June 2002, primary growth, late bloom stage. During the growing season two applications of a commercial inorganic fertilizer were provided. In February 2002, 450 kg ha^{-1} N-P-K fertilizer (8:26:26), and thirty-five days prior to harvesting 150 kg ha^{-1} of ammonium nitrate were applied.

Dietary treatments

The treatments consisted of either GS or MS alone, or a forage mixture (DM based) of GS and MS of 670 g kg^{-1} GS and 330 g kg^{-1} MS (GGM) or 330 g kg^{-1} GS and 670 g kg^{-1} MS (MMG). Just before the experiment started, the MS for 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 GS was chopped to approximately 3–5 cm using a commercial chopper. The chopped material was compressed into plastic bags (approximately

20 kg GS per bag) under continuous CO₂ flushing and stored in a cold chamber (4°C). Prior to feeding, the forage was mixed weekly and held in plastic bags in a cold room (4°C) to prevent heating. No supplementary feeds were provided.

Animals and experimental design

Ten Charolais wethers were selected on the basis of their live weight (mean body weight 43.5 kg, s.d. 3.8 kg) and condition score. All animals were treated for internal parasites prior to the start of experiment. The sheep were subjected to artificial lightening from 0800 to 2000 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 offer 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 (136 cm × 53 cm × 148.5 cm) during the measurement period. Diets were offered twice a day (0830 and 1600) in equal amounts, designed to ensure a refusal margin of 10–15% 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 analysis.

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⁻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 across 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 peri-

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

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.

Acid detergent fibre (ADF) and neutral detergent fibre (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 polarimetry (Ministry of Agriculture, Fishers and Food 1982).

Statistical analysis

Results were analyzed using mixed model procedures (SAS 1999). Mean separation of chemical composition of grass and maize silage and their mixtures was calculated using the LSD values if the F-test was significant at P = 0.05. Linear and quadratic effects of the level of MS inclusion in GS on *ad libitum* intake, digestibility and N utilization were examined using the CONTRAST statement of SAS. Model applied: $Y_{ij} = \mu + T_i + P_j + e_{ij}$, 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

Diet chemical composition

The chemical composition of GS, MS and mixtures of the two forages is presented in Table 1.

Inclusion of MS into GS (33 vs. 67%) reduced the DM content of the diet ($P < 0.001$) due to much lower DM in MS than GS ($P < 0.001$). Maize silage was lower in CP than GS ($P < 0.001$), which progressively lowered the CP concentration in the diet with both levels of MS inclusion (33 vs. 67%) ($P < 0.001$). Forage mixture of 330 g kg⁻¹ GS and 670 g kg⁻¹ MS had a lower CP concentration ($P < 0.001$) compared to GGM.

Grass silage contained less OM than MS ($P < 0.001$) but a higher concentration of NDF ($P < 0.05$) and ADF ($P < 0.001$). In contrast, MS contained more non-structural carbohydrates, such as starch ($P < 0.001$), than GS. Therefore, with increasing the MS inclusion in forage mixtures, a reduction was expected in NDF ($P < 0.05$) and ADF ($P < 0.001$) concentration and an increase in starch concentra-

tion ($P < 0.001$). Lactic acid was the major organic fermentation acid in the silages and pH ranged from 3.7 to 4.6.

Intake and digestibility

Table 2 shows FM, DM and OM *ad libitum* intake and total tract apparent digestibility of GS, MS and their mixtures fed to wether sheep. Silage FM intake (kg d⁻¹ and g kg⁻¹ M^{0.75} d⁻¹) increased linearly ($P < 0.01$ and $P < 0.001$ respectively) as the proportion of MS in the diet increased. Diet FM intake (kg d⁻¹ and g kg⁻¹ M^{0.75} d⁻¹) responded quadratically ($P < 0.01$) to increasing levels of MS and so did diet DM ($P < 0.001$ and $P < 0.01$ respectively) and OM intake ($P < 0.001$).

Addition of MS linearly increased apparent digestibility of DM ($P < 0.01$), OM ($P < 0.01$), ADF ($P < 0.05$), starch ($P < 0.001$) and digestibility of OM in DM (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 diet.

Table 1. Chemical composition of grass and maize silage and their mixtures (g kg⁻¹DM, unless otherwise stated).

	Grass silage	GGM	MMG	Maize silage	SED	Significance
Dry matter (DM) (g kg ⁻¹ fresh weight)	463 ^a	412 ^b	345 ^c	264 ^d	0.78	***
DM composition (g kg ⁻¹ DM)						
Organic matter	914 ^d	923 ^c	933 ^b	955 ^a	1	***
Crude protein	90.1 ^a	84.7 ^b	79.7 ^c	62.0 ^d	1.2	***
Neutral detergent fibre	715 ^a	694 ^a	674 ^b	582 ^c	13	*
Acid detergent fibre	429 ^a	407 ^b	374 ^c	321 ^d	6	***
Starch	14.7 ^d	46.7 ^c	96.5 ^b	211 ^a	7.7	***
Fermentation characteristics (g kg ⁻¹ DM)						
Lactic acid	78.7	84.5	92.4	93.7	ND	
Acetic acid	36.9	43.2	49.8	67.1	ND	
Butyric acid	NF	NF	NF	NF	ND	
Ammonium nitrogen, g kg ⁻¹ total N	128.6	132.0	145.0	165.2	ND	
pH	4.6 ^a	4.2 ^b	4.1 ^b	3.7 ^c	0.08	***

GGM = grass silage 670 g kg⁻¹ DM, maize silage 330 g kg⁻¹ DM, MMG = maize silage 670 g kg⁻¹ DM, grass silage 330 g kg⁻¹ DM. NF = not found, ND = not determined, SED = standard error of difference
Values within the same row with different superscripts differ significantly (*, $P < 0.05$; ***, $P < 0.001$).

Nitrogen balance

Table 3 shows N utilization of GS, MS and their mixtures. Nitrogen intake and N output in faeces were linearly affected ($P < 0.01$) by the MS inclusion in the diet. 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.

Discussion

The average CP content of GS used in this experiment was between 77 and 167.5 g kg⁻¹ DM determined as minimum and maximum average values for grass silages produced at 19 family farms in Croatia in 2004 (Vranić et al. 2005a). Relatively high DM content of GS was a result of advanced grass maturity and 24-hour wilting prior to harvest.

The DM content of MS used in this experiment (264 g kg⁻¹) was much lower than the average two-year DM content of maize silages for Croatia (372.38 g kg⁻¹) (Vranić et al. 2005b), and when viewed in conjunction with its medium starch content of 211 g kg⁻¹DM is indicative of less mature maize silage. The reason was an unusually wet summer in 2002, which prolonged the growth of maize crop and resulted in lower DM and starch concentration at harvest. Expected differences in the carbohydrate components of the two crops were apparent, with GS containing more ADF and NDF than MS. Lower pH for MS was probably related to lower DM concentration and lower buffering capacity of MS compared with GS. In restrictively fermented GS the water soluble carbohydrates can be at the level of the fresh grass, whereas in extensively fermented silage they have been mainly exhausted (Jaakkola et al. 2006).

Table 2. Fresh matter, dry matter, organic matter *ad libitum* intake and total tract digestibility of grass silage, maize silage and their mixtures fed to wether sheep.

	Grass silage	GGM	MMG	Maize silage	SEM	Significance of	
						L	Q
Voluntary intake							
Fresh matter (kg d ⁻¹)	2.36	3.52	4.06	3.62	0.22	**	**
Dry matter (kg d ⁻¹)	1.08	1.45	1.42	0.93	0.08	NS	***
Organic matter (kg d ⁻¹)	0.99	1.34	1.32	0.87	0.07	NS	***
Fresh matter (g kg ⁻¹ M ^{0.75} d ⁻¹)	129	189	216	206	8.89	***	**
Dry matter (g kg ⁻¹ M ^{0.75} d ⁻¹)	59.0	80.7	79.2	49.6	5.65	NS	**
Organic matter (g kg ⁻¹ M ^{0.75} d ⁻¹)	54.5	71.9	70.6	49.8	3.57	NS	***
Digestibility (g kg ⁻¹)							
Dry matter	487	628	669	631	24.6	**	**
Organic matter	495	644	684	651	31.3	**	*
Neutral detergent fibre	514	650	667	595	30.9	NS	**
Acid detergent fibre	454	604	630	562	32.1	*	**
Crude protein	489	570	568	469	30.6	NS	**
Starch	948	990	995	998	4.5	***	**
D-value (g kg ⁻¹ DM)	476	594	637	617	19.4	***	**

GGM = grass silage 670 g kg⁻¹ DM, maize silage 330 g kg⁻¹ DM, MMG = maize silage 670 g kg⁻¹ DM, grass silage 330 g kg⁻¹ DM. SEM = standard error of the mean. L = Linear effect of maize silage in the diet, Q = Quadratic effect of maize silage in the diet, NS = not significant, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. D-value = digestible organic matter in the dry matter. M^{0.75} = metabolic body weight.

Table 3. Nitrogen utilization of grass silage, maize silage and their mixtures fed to wether sheep.

	Grass silage	GGM	MMG	Maize silage	SEM	Significance of	
						L	Q
Nitrogen balance (g d ⁻¹)							
Nitrogen intake	16.2	20.0	18.3	9.46	1.18	**	***
Nitrogen output in faeces	8.3	8.6	7.9	5.8	0.55	**	NS
Nitrogen output in urine	4.9	4.0	5.8	3.9	1.12	NS	NS
Nitrogen balance	2.9	7.5	4.5	-0.28	1.69	NS	*

GGM = grass silage 670 g kg⁻¹ DM, maize silage 330 g kg⁻¹ DM, MMG = maize silage 670 g kg⁻¹ DM, grass silage 330 g kg⁻¹ DM. SEM = standard error of the mean, L = Linear effect of maize silage in the diet, Q = Quadratic effect of maize silage in the diet. NS = not significant, * P < 0.05; ** P < 0.01; *** P < 0.001

Voluntary DM intakes across the four feeding treatments approached or exceeded the upper limit of the intake range of 800–1100 g d⁻¹ for 50 kg intact male lambs (AFRC 1993). The increasing level of MS linearly increased FM intake while a positive associative effect of the two forages was observed for FM, DM and OM intake. It has been suggested that forage NDF content (Van Soest et al. 1991) and digestibility, especially NDF digestibility (Anil et al. 2000) are important in the regulation of forage intake. Also, for low quality forages, intake is regulated predominantly by physical factors, principally the physical fill in the rumen. Therefore, higher NDF content (715 g kg⁻¹DM) and lower NDF digestibility (514 g kg⁻¹) of GS in comparison with the NDF content (582 g kg⁻¹DM) and NDF digestibility (595 g kg⁻¹) of MS resulted in a linear increase in diet FM intake with the increasing level of MS and in positive associative effects of the two forages for voluntary intake. Although GS and MS used in this experiment were both low in CP content, which resulted in limited N supply to rumen microorganisms, the supplemented energy in the form of MS improved microbial activity by developing a better environment for rumen fermentation and reduced indigestible materials of the diets (Matsui et al. 1998).

The *in vivo* digestibility of total diets, when determined with wether sheep, quadratically increased for all parameters with the increasing level of MS. This was a reflection of the higher *in vivo* digestibility obtained with MS for all parameters except

CP when silages were fed as the sole diet. Higher CP digestibility in the GS diet than MS diet may be due to the fact that MS has a lower CP level and thus the impact of metabolic faecal nitrogen in causing apparent CP digestibility is lower with the MS than the GS diet (O'Mara et al. 1998).

Digestibility of starch was much higher than that of NDF, which is consistent with the results of Firkins et al. (2001) that, on average, the apparent digestibility of starch is almost twice as high as that of NDF. In this experiment, starch digestibility in MS diet was high (998 g kg⁻¹ DM) and similar to the value of 990 g kg⁻¹ DM reported by Anil et al. (2000) for starch digestibility in MS of similar quality determined in wether sheep. This further supports the linear increase in diet digestibility with the increasing level of MS, since reduced starch digestibility accounts for approximately one-half of depression in the MS digestibility (Joanning et al. 1981).

The intake of N was affected by the energy level of the diet and the sheep fed higher energy diets (GGM, MMG) consumed more N than the sheep fed GS diet. The intake of N increased linearly as the NDF:starch ratio increased, as a result of a positive associative effect of the two forages in the intake of forage mixtures. Higher N output in urine and faeces (9.7 g d⁻¹) than N input (9.46 g d⁻¹) was recorded in lambs offered the MS diet, which led to negative N balance. Nitrogen output in urine and faeces for diets containing GS was estimated between 63 and 81.4% of N intake (GGS and GS

diet, respectively), indicating an inefficient microbial capture of rumen degradable N. This is partly supported with the results of Fraser et al. (2000) that nitrogen excretion in faeces and urine accounts for a high proportion of N intake, which may be more than 70% of the daily N consumption. Despite a similar urinary and faecal N loss in the diets containing GS, a positive associative effect of two forages on N retention was recorded due to a positive associative effect on N intake. Nitrogen retention has been shown to be lower with the forage of lower CP concentration due to decreased DM intake and CP digestibility (Ko et al. 2006), which may account for the low nitrogen balance of GS diet. Conversely, higher N balances recorded for GGM and MMG in comparison with GS diet were due to positive associative effects of the two forages.

Positive associative effects have been noted when different forage sources, such as grasses and legumes, are fed in combination (Hunt et al. 1985). These effects are usually only observed when one forage source supplies a nutrient, most often protein, which is deficient in the other forage source. Positive associative responses in intake and digestibility are commonly noted when protein supplements are provided to ruminants fed low quality forage (Hannah et al. 1991). Thus, Margan et al. (1994) reported positive associative effects for voluntary intake, digestibility of N, OM and N balance of MS and red clover hay that contained as much as 231 g CP kg⁻¹ DM. Both GS and MS used in this experiment were low in CP content, but MS was much higher in starch as an important source of energy for ruminants. These differences resulted in positive associative responses of GS and MS for all parameters measured (intake, digestibility and nitrogen balance).

The results of this experiment might be useful to producers who find it difficult to consistently produce maize silage with DM contents close to or above 300 g kg⁻¹, since they indicate that moderate quality maize silage has a potential to increase diet quality when it replaces low quality grass silage.

In conclusion, this study shows that replacing low quality grass silage with 33 or 67% of maize silage linearly increases the diet FM intake and digestibility of DM, OM, ADF, starch, D-value and

N intake in wether sheep. These linear effects on intake and digestibility suggest that there were no interactions between the forages, but the presence of quadratic effects in the diet FM, DM, and OM intake, digestibility of DM, OM, NDF, ADF, CP, starch and D-value as well as in N intake and N balance proved the existence of associative effects. A positive associative response of the two forages was recorded for all the measured parameters as expected, probably due to the fact that MS complements GS well and that the replaced GS was of lower quality than the included MS.

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