The effect of supplementing highly wilted grass silage with maize silage, fodder beet or molasses on degradation of the diets and the efficiency of microbial protein synthesis in the rumen of sheep

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Abstract

This study aimed at determining the efficiency of microbial protein synthesis (EMPS) in diets based on highly wilted grass silage [(GS) 539 g dry matter (DM) kg–1] with the supplementation of starch or water-soluble carbohydrates (WSC) rich feeds, i.e. maize silage [MS, 391 g kg–1 DM intake (DMI)], fodder beet (FB, 173 g kg–1 DMI) or molasses (M, 137 g kg–1 DMI). All the diets were made isonitrogenous by urea supplementation (14.0, 4.0 and 2.0 g kg–1 DMI in GS-MS, GS-FB and GS-M diets). In sacco determined crude protein (CP) and organic matter (OM) degradabilities were 756, 800, 778 and 814 (P<0.05) and 563, 577, 593 and 618 g kg–1 (P<0.05) in GS, GS-MS, GS-FB and GS-M diets, respectively. Synchrony indexes that describe the synchrony of CP and OM degradation in the rumen were 0.75, 0.67, 0.67 and 0.79 in GS, GS-MS, GS-FB and GS-M diets, respectively. The EMPS, assessed by means of urinary purine derivative excretion, did not differ significantly (P>0.05) among the diets (36.6, 35.1, 34.7 and 34.0 g microbial nitrogen kg–1 OM apparently digested in the rumen in GS, GS-MS, GS-FB and GS-M diets, respectively). The estimated metabolizable protein supply from GS, GS-MS, GS-FB and GS-M diets amounted to 98, 90, 93 and 87 g kg–1 DMI, respectively. Apparently, highly wilted GS containing a high concentration of WSC (91 g kg–1 DM) supports high EMPS in the rumen and this cannot be improved by the supplementation with starch or water-soluble carbohydrates (WSC) rich feeds.

Introduction

Animal production systems suffer from low nitrogen (N) use efficiency. Nitrogen utilization is especially poor for ruminants fed grass silage (GS)-based diets. Based on a large number of observations from the literature (998 treatment means) Huhtanen et al. (2008) reported the efficiency of transferring dietary N to milk N in GS-based diets to range from 16.45% to 40.2%. High protein concentration (Huhtanen and Shingfield, 2005), high protein degradability (Rinne et al., 1997) and low efficiency of microbial protein synthesis in the rumen (EMPS) (Givens and Rulquin, 2004) are believed to be the major causes of these low efficiency values. The main factor limiting EMPS in GS-based diets is fermentation in the silo. Some fermentation end products, like acetic or butyric acid, cannot serve as a source of energy for microbial growth in the rumen while the potential of lactic acid is considerably lower than the potential of the water-soluble carbohydrates (WSC) from which it was derived (Chamberlain, 1987). In GS-based diets there are also other limiting factors, such as asynchronous supply of energy and N in the rumen (Chamberlain and Choug, 1995) and low outflow rate caused by low intake or presence of biogenic amines in badly preserved silages, which affect microbial growth in the rumen (Kriszan and Randby, 2007; Phuntsok et al., 1998).

The EMPS in the rumen in GS-based diets may be improved by proper diet supplementation. In regions suitable for maize growing, diets are often supplemented with maize silage (MS), which is a good source of energy and characterised by high EMPS (as summarized by Givens and Rulquin, 2004). In many regions of Europe, however, the proportion of maize in crop rotation is limited with the aim of controlling the spread of western corn rootworm (2003/766/EC) or Fusarium sp. (2006/583/EC). Thus, there are demands to lower the area under maize and to find alternatives for MS in ruminant diets.

One of the alternatives to replace MS as a supplement to GS might be fodder beet (FB). It is characterized by a high concentration of WSC (537 g kg–1 DM; DLG, 1997) that may have a specific role in stimulating microbial protein synthesis (MPS) in the rumen (Chamberlain et al., 1993). Fodder beet is also a rich source of pectin (Eriksson, 2003), which has the highest degradation rate among the complex carbohydrates (Van Soest, 1994). In contrast to sugars and rapidly degradable starch, which may depress digestion of cellulose in the rumen, it seems that pectin has no such effect (Van Soest, 1994). Therefore, theoretically, it could stimulate MPS in the rumen. However, experimentally, sucrose and pectin showed higher microbial protein yield and efficiency of MPS than starch only during the first hours of fermentation, while curves converged after 10 h and the starch then gave both higher yield and efficiency than the other substrates (Hall and Herejk, 2001). Using an in vitro method, Eriksson et al. (2004a) found that supplementation of GS with FB resulted in higher MPS and higher EMPS. However, the same authors were not able to confirm their in vitro results in an in vivo trial (Eriksson et al., 2004b). Some other traits that can indirectly indicate a beneficial effect of FB on rumen function can be found in the literature. These include improved dry matter intake, milk yield and milk composition (Roberts, 1987; Murphy et al., 1993; Gruber, 1994; Birkenmaier et al., 1996). On the basis of the existing evidence, the effect of FB as a supplement to GS on the EMPS cannot be defined.

Beneficial effect on rumen function could also be expected in the case of GS diet supplementation by molasses (M). Compared to FB, M is characterised by higher concentration of WSC (600-650 g kg–1 DM; Kling and Wöhlbier, 1983), which are more or less instantly released in the rumen. This could imply readily available energy supply and consequently higher EMPS in the rumen. However, the literature data dealing with the effect of M supple-
Determination of digestibility, nitrogen balance and microbial protein synthesis in the rumen

Digestibilities of OM, CP, starch and N balance were determined using the total faeces and urine collection method. Microbial protein synthesis was assessed on the basis of the excretion of urinary purine derivatives (allantoin, uric acid, xanthine and hypoxanthine) using the model of Chen et al. (1990).

Four different diets containing GS only, a mixture of grass and maize silage (GS-MS, 60:40 on DM basis), a mixture of GS and fodder beet (GS-FB, 83:17 on DM basis) and a mixture of GS and molasses (GS-M, 86:14 on DM basis) were composed. The proportion between GS and MS was chosen on the basis of practical experiences, i.e., this is a very frequent diet on farms in regions where maize is grown. The proportions between GS, FB and M were chosen on the basis of literature data where GS was supplemented by FB or M (Sabri et al., 1988; Stokes et al., 1991; Debrabander and Bouque, 1992; Givens et al., 1992; Murphy et al., 1993; Gruber, 1994; Phipps et al., 1995; Birkenmaier et al., 1996; Murphy, 1999; Ordonez-Tercero et al., 2003). In order to equalise the diets with respect to N concentrations 14.0, 4.0 and 2.0 g of urea per kg of diet DM was supplemented to GS-MS, GS-FB and GS-M diets. In addition, 0.45, 0.15 and 0.06 g of sulphur per day were provided to wethers that were given urea supplemented diets. With the aim of supplying animals with the required quantities of minerals 0.6 g of Ca, 1.0 g of Na, 110 mg of Mn, 81 mg of S, 35 mg of Zn, 0.8 mg of I and 0.15 mg of Se was provided daily as a mineral mix. Vitamin suspension (0.03 mL) containing 375 and 750 µg of vitamin D and A was also added to the diet of each individual animal.

The experiment was carried out with four animals x four periods. During the whole experiment the daylight was extended in the morning from 5.00 to 8.00 and in the evening from 16.00 to 22.00 to 17 h. Animals had free access to fresh water and they were given two equal meals at 7.30 and 19.30. Each experimental period lasted 28 days. During the first 21 days wethers were fed individually, adapted to the experimental diets and kept on sawdust litter. Feeds were offered at 0.9 of the ad libitum intake that was determined during the pre-experimental period. In the last week of each period (from the day 22 to 28) animals were moved to metabolism cages for faeces and urine collection. Cages were located in the same room. Feed residues were removed daily, weighed and dried. Urine was collected by the use of separators placed under the metabolism cages. In order to maintain the urine pH below 3 and to prevent precipitation of uric acid, 250 mL of 1M H2SO4 and 500 mL of water were prepared in the vessels in advance. Daily urine amounts were diluted to 5 L with water, mixed and sampled. Fifty mL of diluted samples were stored at −20°C. Before analyses they were pooled over the collection period. Faeces were collected daily, weighed and stored at −20°C. At the end of collection period, faeces was bulked and sampled for further analyses. True CP and OM digestibilities were calculated by taking into account the fact that neutral detergent insoluble protein and neutral detergent fibre were the only truly undigested feed CP or OM fractions which appeared in the faeces (Van Soest, 1994).

Determination of organic matter, protein and starch degradability in the rumen

Organic matter, protein and starch degradabilities (d_{0.03}, d_{0.5}, d_{0.870}) of experimental feeds were determined using the nylon bag technique as described by Orskov et al. (1980). Three adult Zezersko-Sočavska wethers (68±9.6 kg) each fitted with a ruminal cannula (40 mm diameter) were used. The animals had free access to water. During a three-week pre-experimental period the animals were gradually adapted to the experimental diet. During the experiment, which lasted 17 days, they were offered a diet, which consisted of 510 g of hay, 255 g of GS and 255 g of maize silage on a DM basis per day. The hay contained 830 g DM, 106 g crude protein and 62 g of ash per kg DM. The grass and maize silage were the same as in the in vivo trial; their chemical composition is presented in Table 1. Diets were supplemented with urea (5 g per day) and mineral vitamin mix which provided 0.1 g P, 0.45 g Na, 0.50 g Cl,
DM, g kg⁻¹
538±11
361±8
134±11
885±10
CP
173±3
70±2
111±9
149±0
OM
898±3
958±2
878±7
898±0
Crude fat
37±2
26±2
3.6±0.4
0.25±0.1
NDF
444±12
478±2
183±15
-
ADF
272±7
224±2
114±8
-
ADL
19±1
21±2
8±2
-
N-NH₃, g kg⁻¹ total N
38
93
-
-
NDIN, g kg⁻¹ total N
176
236
127
-
ADIN, g kg⁻¹ total N
55
101
112
-
WSC
91±13
53±2
527±53
706±6
Starch
-0.360±2
-
-
-
pH
5.9
3.9
-
-
Lactic acid
3.2
41.4
-
-
Acetic acid
1.4
7.3
-
-
Butyric acid
0.00
0.00
-
-
Propionic acid
0.00
0.00
-
-
Valeric acid
0.00
0.00
-
-
Ethanol
16.4
2.4
0.47
-

DM, dry matter; CP, crude protein; OM, organic matter; WSC, water-soluble carbohydrates; SD, standard deviation; -, not analysed; N-NH₃, pH and volatile fatty acids analysed on a pooled sample; NDIN and ADIN analysed on a pooled lyophilised sample; x, average value.
daily ratios of effective degradable protein to OM (y, in g N kg⁻¹ degradable OM) were calculated (eq. 4).

\[ y = \frac{CP \times EDG_{OM}}{OM \times EDG_{OM}} \quad \text{eq. 4} \]

Synchrony indexes were calculated according to the equation proposed by Verbič et al. (1999):

\[ I_s = \frac{\sum (y - y_{avg})}{y} \quad \text{eq. 5} \]

n = hours between two feedings

**Chemical analyses**

Feed and faeces samples for the determination of hygroscopic moisture, N, ash, crude fat, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and starch were dried in a ventilated oven at 60°C. Samples for determination of neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were lyophilised. After drying, all samples were ground with a laboratory hammer mill to pass a 1 mm screen. Analyses of N in silages, faeces and urine as well as lactic and volatile fatty acids (acetic, butyric, propionic and valeric), ammonia, ethanol, pH value and WSC in silages were carried out on fresh samples. Hygroscopic moisture was determined by drying at 103°C according to the EU directive 73/47/EEC (European Commission, 1973). Crude protein (CP, N x 6.25) was analysed according to the Kjeldahl method described by the standard ISO 5893-2 (ISO, 2005). The analyses of crude fat and ash were performed according to the methods described in EU directive 98/64/EC (European Commission, 1998) and standard ISO 5894 (ISO, 2002). Neutral detergent fibre, ADF and ADL were determined according to Goering and Van Soest (1970). For the analysis of NDIN and ADIN the procedure described by Licitra et al. (1996) was applied. The ammonia concentration in feeds was determined by steam distillation as a volatile base (Naumann and Bassler, 1976). Water-soluble carbohydrates were determined in water extracts according to the method described in EU directive 71/250/EEC (European Commission, 1971) and standard ISO 6498 (ISO, 1998). Starch was analysed by the use of polarimetric method described by the standard ISO 6493 (ISO, 2000). The concentrations of lactic and volatile fatty acids (acetic, butyric, propionic and valeric) were determined by gas chromatography according to Holdemann and Moore (1975). Ethanol was determined by high-pressure gas chromatography using Hewlett Packard 6890 equipment, capillary column (CP-WAX-57CB, 50 m x 0.25 mm, Varian CP97723), N as carrier gas and flame ionization detector. The concentrations of purine derivatives (uric acid, allantoin, xanthine and hypoxanthine) in urine were determined by the HPLC method described by Diez et al. (1992) with UV detection at 205 nm.

The concentration of metabolizable energy (ME, in MJ kg⁻¹ DM) of the diets was estimated on the basis of digestible crude fat (DCF), digestible crude fibre (DCFi), digestible organic matter (DOM) and crude protein (CP) content (all in g kg⁻¹ DM). The in vivo digestibilities of nutrients in the diets were determined by the total faeces collection method (results not presented). For the calculation of ME the regression equation (eq. 6) proposed by the German Society of Nutrition Physiology (GfE, 1995) was used.

\[ \text{ME} = 0.0312 \times \text{DCF} + 0.0136 \times \text{DCFi} + 0.0147 \times \text{DOM} \]

\[ - 0.0156 \times \text{CP} + 0.1147 \times \text{CP} \]

\[ + 0.00234 \times \text{CP} \quad \text{eq. 6} \]

**Statistical analysis**

Statistical analysis was performed by means of SAS (2001) using the ANOVA procedure. In vitro data (dry matter intake, microbial protein synthesis, digestibilities, N balance) were analysed using the model Yijk = μ + Di + Aj + Pk + eijk, where Yijk is the dependent variable, μ – the overall mean, Di – effect of diet (i=1 to 4), Aj – effect of period (j=1 to 4), Pk – effect of animal (k=1 to 4), eijk – residual error.

Data concerning the in sacco degradability characteristics and synchrony index included 12 observations (4 diets x 3 animals) which were analysed using the model Yijk = μ + Di + Aj + Sk + eijk where Yijk is dependent variable, μ is the overall mean, Di is the effect of diet (i=1 to 4), Aj is the effect of animal (j=1 to 3) and eijk is the residual error.

**Results and discussion**

**Composition of feeds and diets**

The chemical composition of feeds used in the experiment is presented in Table 1. With regard to the concentration of acetic acid, butyric acid and ammonia both silages can be considered as well preserved (Dulphy and Demarquilly, 1981). GS was highly wilted and contained 539 g DM kg⁻¹ on average. This value is higher than the average DM concentration of parental material (521 g kg⁻¹) restricted the intensity of fermentation. Silage contained a relatively high amount of residual WSC. The value was similar to highly wilted GS as reported by McEniry et al. (2007) (from 86 to 103 g kg⁻¹ DM for variants with restricted air infiltration), but higher than in the highly wilted GS examined by Eriksson et al. (2004a) (69 g kg⁻¹ DM). The concentration of residual WSC in GS from this study was similar or higher than in silages prepared from perennial ryegrass varieties that were selected for elevated levels of WSC (Merry et al., 2003; Merry et al., 2006, Yanez-Ruiz et al., 2006). This was obtained despite the considerable proportion of grasses (smooth meadow grass, rough meadow grass etc., see section M&M) characterised by a lower WSC concentration than perennial ryegrasses (Wilson and Collins, 1980; Haugland et al., 1998; Jeangros et al., 2001; Miller et al., 2001; Mooby et al., 2006) and the fact that common ryegrass varieties were used in the present experiment. High residual WSC in silage from the present experiment can be attributed to relatively favourable wilting conditions that allowed an increase of DM up to 521 g kg⁻¹ in less than 24 h and to low lactic acid bacteria activity in the silo.

The composition of maize silage (Table 1) was in all aspects similar to the composition of silages produced in farm practice (Verbič, 2008). The composition of fodder beet (Table 1) was in the majority of components comparable to data from DLG tables (DLG, 1997) only the protein concentration was slightly higher. The composition of experimental diets is presented in Table 2. Diets were made more or less isonitrogenous by the supplementation of urea. Also, the differences between diets in estimated concentration of metabolizable energy were small (less than 5% between two extreme values). On the other hand, diets varied widely in the concentration of WSC and starch (considered to be negligible in diets without MS). The concentrations of WSC in all diets exceeded some recommendations for dairy cow diets (50 to 70 g kg⁻¹ DM; Murphy, 1999; Hall, 2002; Broderick and Radloff, 2004; Molinary and Villa, 2006).

**Degradability of feeds and diets in the rumen**

Characteristics of OM and CP degradation of GS, MS and FB in the rumen are presented in Table 3. Both silages expressed similar EDGOM and EDGCP. Effective degradabilities of FB were considerably higher. The EDGOM for GS during the period from 2000 to 2008 (440 g kg⁻¹) but still within the range observed in practice. Very low concentrations of lactic and acetic acid indicate that high DM concentration of parental material (521 g kg⁻¹) restricted the intensity of fermentation. Silage contained a relatively high amount of residual WSC. The value was similar to highly wilted GS as reported by McEniry et al. (2007) (from 86 to 103 g kg⁻¹ DM for variants with restricted air infiltration), but higher than in the highly wilted GS examined by Eriksson et al. (2004a) (69 g kg⁻¹ DM). The concentration of residual WSC in GS from this study was similar or higher than in silages prepared from perennial ryegrass varieties that were selected for elevated levels of WSC (Merry et al., 2003; Merry et al., 2006, Yanez-Ruiz et al., 2006). This was obtained despite the considerable proportion of grasses (smooth meadow grass, rough meadow grass etc., see section M&M) characterised by a lower WSC concentration than perennial ryegrasses (Wilson and Collins, 1980; Haugland et al., 1998; Jeangros et al., 2001; Miller et al., 2001; Mooby et al., 2006) and the fact that common ryegrass varieties were used in the present experiment. High residual WSC in silage from the present experiment can be attributed to relatively favourable wilting conditions that allowed an increase of DM up to 521 g kg⁻¹ in less than 24 h and to low lactic acid bacteria activity in the silo.

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was comparable to the results of Moss et al. (1995) and Moss and Givens (2002) (580 and 558 g kg⁻¹, respectively). The EDGOM of MS was between the values reported by Arieli et al. (1989) and Kaswari et al. (2007) (504 and 740 g kg⁻¹, respectively). We were unable to find any data in the literature on EDGOM for FB. The effective degradability of starch (EDGstarch) in MS (not shown in the tables) amounted to 801 g kg⁻¹. Maize silage in the present experiment was made of flint type grain hybrid, however, the EDGstarch was between the values reported by Verbić et al. (2005) for flint (740 g kg⁻¹) and dent (913 g kg⁻¹) type grain hybrids.

The EDGstarch for GS was similar to the results presented by von Keyserlingk et al. (1996) (734 g kg⁻¹ for GS containing 380 g DM kg⁻¹) and Verbić et al. (1999) (750 g kg⁻¹ for GS containing 521 g DM kg⁻¹). Considerably higher values can be found in the literature for direct cut silages (855 g kg⁻¹, Verbić et al., 1999; 840 to 880 g kg⁻¹, Hvelplund and Weiβbjerg, 2000). The relatively low EDGstarch in silage from the present experiment was probably the result of high DM concentration, which was found to be an important factor controlling the shift of protein degradability during the ensiling process (Tamminga et al., 1991). In MS, protein degradation in the rumen depends on the type of grain. Higher degradabilities are expected in silages made from dent type grain hybrids than in those characterised by the flint type grain (Verbić et al., 2005). Despite the fact that in the present experiment MS was made of flint type hybrid, EDGstarch was closer to the value reported for silage from dent type hybrid (Verbić et al., 2005). The EDGstarch of FB was slightly lower than what was reported by Hvelplund and Weiβbjerg (2000) (880 g kg⁻¹).

Forages differed in degradation dynamics in the rumen. Soluble organic matter and protein fractions (aOM and aCP) were higher in MS while insoluble but potentially degradable fractions (bOM and bCP) and their degradation rates (cOM and cCP) were higher in GS. Contrary to GS and MS, where OM and CP were degraded more or less simultaneously, degradation of OM and CP in FB was very asynchronous (Table 3). The protein in FB was characterized by its high solubility (aCP) while OM was characterized by low solubility but high insoluble and potentially degradable fraction (bOM). The differences in degradation characteristics also resulted in the variation of synchrony indexes (Is), which describe the synchrony of CP and OM degradation in the rumen. It was the highest in GS followed by MS and the lowest in FB (Table 3). In GS the direct supply of rumen degradable N seems to be sufficient over the entire period during the two feedings (Figure 1). In MS and FB the direct supply of rumen degradable N was insufficient or sufficient only during the first few hours after feeding. Later, there was a constant and significant lack of degradable N (Figure 1), indicating that rumen microorganisms had to rely on N recycled into the rumen by the rumino-hepatic cycle. The lack of N in MS and FB for a certain period between two feedings is first of all the result of low protein concentration (Table 1) and low average daily ratio between effectively degraded CP (EDGCP) and effectively degraded OM (EDGOM) (Table 3). Extremely low ratios between EDGCP and EDGOM in FB during the period from 3 to 12 h after feeding (Figure 1) can also be attributed to a great extent to un-synchronous release of CP and OM in the rumen. Degradation characteristics of experimental diets in the rumen are presented in Tables 4 and 5. Supplementation of

Table 2. Composition of experimental diets in the in vivo trial.

| Feed/Diet | Diet, g of feed DM per kg DMI | Diet composition* |
|-----------|-----------------------------|-------------------|
|           | GS                          | MS                | FB                |
| OM        | CP                          | OM                | CP                | cp | ME |
| GS        | 1000                        | 0                 | 0                 | 0  | -  | 173 | 10.45 |
| GS-MS     | 595                         | 391               | 0                 | 0  | 14.0 | 143 | 551 | 172 | 10.89 |
| GS-FB     | 823                         | 0                 | 173               | 0  | 4.0  | -   | 397 | 166 | 174 | 10.71 |
| GS-M      | 681                         | 0                 | 0                 | 137| 2.0  | -   | 382 | 175 | 176 | 10.79 |

* Diet composition was derived by calculation on the basis of feed composition presented in Table 1 and the amount of feeds offered to animals; NDF, WSC, CP expressed as g kg⁻¹ DM; ME energy, expressed as MJ kg⁻¹ DM; GS-MS, grass and maize silage diet (60:40 on a DM basis); GS-FB, grass silage and fodder beet diet (83:17 on a DM basis); GS-M, grass silage and molasses diet (86:14 on a DM basis); DMI, dry matter intake.

Table 3. Characteristics of the in sacco organic matter and crude protein degradation of individual feeds.

| Parameter | GS | MS | FB |
|-----------|-----------------------------|-----------------------------|-----------------------------|
| OM        | CP                          | OM                          | CP                          |
| a, g kg⁻¹ | 256                         | 440                         | 478                         |
| b, g kg⁻¹ | 601                         | 513                         | 350                         |
| PDG, g kg⁻¹ | 857                     | 952                         | 829                         |
| c, g kg⁻¹ | 0.052                       | 0.089                       | 0.027                       |
| EDG, g kg⁻¹ | 563                   | 756                         | 584                         |
| ADR, g N kg⁻¹ | 14.1              | 15.1                        | 22.4                        |
| Is         | 0.75                        | 0.63                        | 0.35                        |

Figure 1. Diurnal variation in the ratio between effectively degraded CP (EDGCP) and OM (EDGOM) of individual feeds.
GS with MS, FB or M increased EDGOM. It indicates that improved energy supply for microbial growth in the rumen could be expected due to the supplementation of GS diet.

Effective protein degradabilities in GS-MS, GS-FB and GS-M were significantly higher than in GS (Table 5). Values for EDGCP depend largely on urea supplementation, which was also taken into account in the calculations of EDGCP. In the case of theoretical diets without urea supplementation (data not shown) there was only a minor effect of the addition of MS, FB or M to GS on the EDGCP of the diet (756 in GS vs. 753, 766 and 779 in GS-MS, GS-FB and GS-M, respectively).

In comparison to GS, GS-M improved the index of synchrony of CP and OM degradation in the rumen (Table 6). In the case of GS supplementation with MS or FB the IS was impaired in both cases. In all diets, the ratio between the EDGCP and EDGOM within the first 3 hours after feeding was higher than EMPS expressed per kg of EDGOMI (Table 8). GS and GS-M diets maintained the adequate EDGCP, GS-MS and GS-FB the ratio decreased to a suboptimal level already 3 hours after feeding (Figure 2). The observation is a result of the fact that GS was a considerably better source of slowly degradable protein fraction than MS and FB (Table 3).

It has to be emphasized that in the present experiment urea was used to maintain the diets isonitrogenous. On the basis of CP intake, EDGCP, and EMPS it can be estimated that the GS-MS, GS-FB and GS-M diets provided enough rumen degradable N even without urea supplementation. Urea, as a source of immediately available N, certainly affects the IS and it would be interesting to know if MS, FB or M can improve the synchrony of CP and OM release in diets with no urea inclusion. Data (not presented in the tables) showed that MS or M improved the synchrony of CP and OM degradation in the rumen (IS=0.85 and 0.81) in comparison to GS diet (IS=0.75) in cases when a diet was not supplemented with urea. Fodder beet showed no such potential (IS=0.72).

**Total tract digestion of nutrients**

The digestibilities of OM, fibre fractions, starch and CP are presented in Table 7. As expected, supplementation of GS with MS, FB or M increased the apparent OM digestibility of the diets. With the exception of MS supplementation, the differences were significant (P<0.05). Supplementation of the GS diet with FB and M also improved the true digestibility of OM (P<0.05). In case of MS supplementation there was a slight and insignificant

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**Table 4. Parameters of the in sacco organic matter degradation of grass silage-based diets.**

| Parameter | GS | GS-MS | GS-FB | GS-M | SEE | Significance |
|-----------|----|-------|-------|------|-----|--------------|
| aOM, g kg⁻¹ | 258ᵇ | 364ᵃ | 273ᵇ | 360ᵇ | 2.76 | P<0.05 |
| bOM, g kg⁻¹ | 601ᵈ | 476ᵃ | 590ᵇ | 518ᵇ | 2.65 | P<0.05 |
| PDGOM, g kg⁻¹ | 857ᵇ | 840ᵃ | 863ᵇ | 879ᵇ | 3.75 | P<0.05 |
| cCP, g kg⁻¹ | 0.052ᵃ | 0.043ᵇ | 0.059ᵇ | 0.052ᵇ | 0.001 | P<0.05 |
| EDGOM, g kg⁻¹ | 563ᵇ | 577ᵇ | 593ᵇ | 618ᵇ | 2.31 | P<0.05 |

SEE, standard error of estimate; ᵒᵇᵃᵇᶜᵈmeans with different superscripts are significantly different (P<0.05).

**Table 5. Parameters of the in sacco crude protein degradation of grass silage-based diets.**

| Parameter | GS | GS-MS | GS-FB | GS-M | SEE | Significance |
|-----------|----|-------|-------|------|-----|--------------|
| aCP, g kg⁻¹ | 440ᵇ | 618ᵈ | 508ᵇ | 544ᵇ | 6.64 | P<0.05 |
| bCP, g kg⁻¹ | 513ᵇ | 328ᵃ | 446ᵇ | 451ᵇ | 6.17 | P<0.05 |
| PDGCP, g kg⁻¹ | 953ᵇ | 945ᵃ | 955ᵇ | 995ᵈ | 0.71 | P<0.05 |
| cCP, g kg⁻¹ | 0.089ᵇ | 0.084ᵇ | 0.089ᵇ | 0.089ᵇ | 0.002 | P<0.05 |
| EDGCP, g kg⁻¹ | 756ᵇ | 800ᵃ | 778ᵇ | 814ᵈ | 1.09 | P<0.05 |

⁽ᵃᵇᶜᵈ⁾means with different superscripts are significantly different (P<0.05).

**Table 6. Average daily ratio between EDGCP and EDGOM (ADR in g N kg⁻¹ EDGOM) and index of synchrony.**

| Parameter | GS | GS-MS | GS-FB | GS-M | SEE | Significance |
|-----------|----|-------|-------|------|-----|--------------|
| ADR | 41.3ᵇ | 40.9ᶜ | 40.7ᵇ | 41.1ᶜ | 0.11 | P<0.05 |
| IS | 0.75ᵇ | 0.67ᵃ | 0.67ᵇ | 0.79ᶜ | 0.014 | P<0.05 |

⁽ᵃᵇᶜᵈ⁾means with different superscripts are significantly different (P<0.05).

**Table 7. Apparent and true in vivo digestibility of nutrients in grass silage-based diets.**

| Parameter | GS | GS-MS | GS-FB | GS-M | SEE | Significance |
|-----------|----|-------|-------|------|-----|--------------|
| Digestibilities, g kg⁻¹ | | | | | | |
| OM (apparent) | 748ᵇ | 758ᵃᵇ | 774ᵇ | 775ᵇ | 0.10 | P<0.05 |
| OM (true) | 876ᵇ | 861ᵃ | 890ᵇ | 895ᵇ | 0.099 | P<0.05 |
| Starch | - | 996 | - | - | - |
| NDF | 749 | 717 | 752 | 749 | 0.018 | ns |
| ADF | 753ᵇ | 698ᵃ | 758ᵇ | 760ᵇ | 0.017 | P<0.05 |
| CP (true) | 955 | 956 | 955 | 958 | 0.001 | ns |

*Calculated by taking into account that neutral detergent insoluble nitrogen (NDIN) and neutral detergent fibre (NDF) were the only truly undegraded feed residues that appeared in the faeces. Values refer to silage protein including urea. ᵒᵇᵃᵇᶜᵈmeans with different superscripts are significantly different (P<0.05); ns, not significant.

![Figure 2. Diurnal variation in the ratio between the effectively degraded CP (EDGCP) and OM (EDGOM) in grass silage-based diets supplemented with MS (GS-MS), FB (GS-FB) and molasses (GS-M).](image-url)
(P>0.05) decrease in the true OM digestibility. A minor decrease was probably due to a decrease in digestibility of fibre fractions (NDF and ADF). The phenomenon can be attributed to a relatively poor digestibility of non-grain parts of maize plant (Argillier et al., 1996; Barriere et al., 1997), which contribute more than 50% of the total DM yield of maize (Verbić et al., 1995). The considerable proportion of starch in the GS-MS diet (Table 2) and its high digestibility (Table 7) certainly diminished the effect of low fibre digestibility and ensured the true OM digestibility on a level that was similar to GS. True protein digestibilities were almost complete and not affected by the diets (Table 7).

**Efficiency of microbial protein synthesis in the rumen**

Data on microbial protein synthesis (MPS) in the rumen are shown in Table 8. When the MPS was expressed on a DMI basis, the differences between diets were small. The values ranged from 15.4 to 16.0 g microbial N (MN) kg⁻¹ DMI. In the case of expression per kg of organic matter intake apparently digested in the rumen (OMADRI) or fermentable organic matter intake (FOMI) there was a tendency towards lower EMPS in diets containing high levels of WSC (GS-FB, GS-M) than in diets containing moderate levels of WSC (GS, GS-MS), however, the differences were not significant (P>0.1).

Values for the EMPS of all diets from the present study were higher than the average value for GS-based diets summarized by Givens and Rulquin (2004) on the basis of 17 published studies (30.1 g MN kg⁻¹ OMADRI). The values were also considerably higher than the mean value of 23 g MN kg⁻¹ OMADRI reported for silages by ARC (1984). This indicates that well-preserved highly wilted GS from the present experiment supported high EMPS. True protein digestibilities were almost complete and not affected by the diets (Table 7).

| Parameter          | GS          | GS-MS      | GS-FB      | GS-M       | SEE  | Significance |
|--------------------|-------------|------------|------------|------------|------|--------------|
| DMI, g day⁻¹       | 1031        | 1040       | 1048       | 1033       | 12.7 | ns           |
| Microbial nitrogen, g | 16.5       | 16.6       | 16.4       | 15.9       | 0.86 | ns           |
| per day            | 31.0        | 30.9       | 29.3       | 27.3       |      |              |
| per kg DMI         | 36.6        | 35.1       | 34.7       | 34.0       | 1.99 | ns           |
| per kg OMADRI      | 27.9        | 27.3       | 25.5       | 24.9       | 1.53 | ns           |
| per kg FOMI        | 31.0        | 30.9       | 29.3       | 27.3       |      |              |
| per kg EDGOMI      |             |            |            |            |      |              |

**Table 8. Dry matter intake and in vivo microbial protein synthesis in grass silage-based diets.**

There are only limited data on the EMPS for various feeds from the same laboratory using the same methodology and the same breed of sheep as in the present experiment (Verbić, 2002). These data support our speculations on the high potential of the present GS for MPS in the rumen. The EMPS in GS was comparable to the diets containing maize silage (26.4 to 34.7 g MN kg⁻¹ FOMI) or grass-clover herbage (23.2 to 31.8 g MN kg⁻¹ FOMI), but considerably higher than in diets containing silages from permanent grasslands (18.4 to 22.2 g MN kg⁻¹ FOMI). The aim of the present study was to investigate if the EMPS in diets based on highly wilted silage can be improved by feeds containing high amounts of WSC or starch. Several studies indicate that an increased concentration of WSC in grass silages by means of wilting (Narasimhalu et al., 1989), addition of efficient silage additives (Jaakkola et al., 2006) or using grasses that were selected for elevated WSC concentration (Merry et al., 2006) improved the EMPS. However, in all of these studies the WSC concentrations were increased from relatively low (3 to 30 g kg⁻¹ DM) to moderate (73 to 92 kg g⁻¹ DM) concentrations. The WSC concentration in GS from the present experiment (91 g kg⁻¹ DM) was close to the upper value of moderate concentration and thus the question arises as to whether further increase of WSC concentration may lead to further improvement of EMPS. The results from the present study indicate that it may not (Table 8). In the literature, we were not able to find any evidence obtained on naturally fermented GS-based diets to confirm or reject this observation. However, there are some results obtained on fresh grass supporting it. Lee et al. (2002), for instance, reported that EMPS in a diet containing perennial ryegrass selected for elevated levels of WSC (243 g WSC kg⁻¹ DM) was similar to the control (161 g WSC kg⁻¹ DM). One of the factors that might limit the MPS in diets containing excessive levels of WSC is a low pH value of rumen fluid that is expected to reduce the digestibility of fibrous plant tissues and to divert the energy in the rumen to non-growth functions, i.e. maintaining neutral pH in the bacterial cells (Strobel and Russell 1986). In the experiments reported by Taweel et al. (2005) it was found that feeding perennial ryegrass diets containing from 149 to 181 g of WSC kg⁻¹ DM resulted in a mean pH value of 5.8. The observed value was well below the value considered optimal for cellulyolysis (0.7, Van Soest, 1994) and also below the value that is considered to be inhibitory for cellulytic microorganisms (6.2, Orskov, 1992; Van Soest 1994). Also, the supplementation of GS with maize silage (GS-MS) did not improve the EMPS when compared to feeding of GS alone (Table 8). The observation is not in accordance with the review by Givens and Rulquin (2004) reporting that MS at inclusion rate in the range from 0.075 to 0.600 supports greater EMPS than GS-based diets (48.4 vs. 39.1 g MN per kg OMADRI).

It was expected that supplementation of GS with MS, FB or M would enhance the EMPS through improvement of the degree of synchrony between the supply of fermentable energy and degradable protein in the rumen as reported by Sinclair et al. (1993, 1995). Synchronous release of the above mentioned diet components is expected to provide specific N compounds (peptides, amino acids) required by rumen microbes that cannot be supplied by the rumino-hepatic cycle or supplementation of the diets with urea. It was observed that in the present experiment the I₅ of GS was not improved when it was supplemented with MS or FB while in the case of supplementation with M the improvement was small (Table 6). The observation can be attributed to the fact that a) the urea which was also added to diets to make them isonitrogenous reduced the I₅ values of supplemented diets and b) GS per se was already characterised by relatively high I₅ which was considerably higher than reported by Verbić et al. (1999) (0.75 vs. 0.22 to 0.33). It seems that, in the present experiment, the synchrony of CP and OM
degradation in the rumen can not be consid- ered as an important factor affecting the EMPS. A similar observation was also reported by Kaswari et al. (2007).

Metabolizable protein and nitrogen balance

The concentrations of metabolizable protein in the diets were roughly assessed on the basis of the results on CP concentrations in the diet (Table 2), dietary CP degradabilities in the rumen (Table 5), true CP digestibilities in the total tract (Table 7) and MPS in the rumen (Table 8). The results are given in Table 9. Supplementation of GS with MS, FB or M resulted in a decrease of metabolizable protein concentration (from 5% in FB to 11% in M supplemented diet). When compared to GS, a decrease of metabolizable protein concentration in GS-MS was mainly due to a decrease in the supply of postruminally digested dietary protein while GS-FB and in GS-M both, a decrease in the supply of postruminally digested dietary protein as well as a decrease in the supply of digestible true microbial protein, were observed. In absolute terms the metabolizable protein concentrations in diets from the present experiment are higher than those obtained for maize silage (Verbič et al., 2005) and considerably higher than for GS values reported by Verbič et al. (1999).

There was a tendency towards higher N retention in GS and GS-MS when compared to GS-FB and GS-M (Table 10). However, the differences between GS and other diets were not statistically significant (P>0.1) and not related to metabolizable protein concentrations in the diets. It can be supposed that N retention may be affected by metabolizable protein supply only in cases in which the latter is lower than the requirements. The metabolizable proteins were supplied in excess (101, 94, 97, 89 g per day) of the estimated requirements (91, 93, 96 and 91 g per day in GS, GS-MS, GS-FB and GS-M, respectively) except in the GS-M diet. The above mentioned requirements were estimated on the basis of average animal weights, (50.6, 51.1, 50.8 and 50.7 kg) and daily weight gains (143, 153, 163 and 145 g) in GS, GS-MS, GS-FB and GS-M, respectively) using AFRC (1993) recommendations.

Conclusions

The results suggest that highly wilted GS prepared under favourable wilting conditions and containing high concentrations ofWSC supports high EMPS in the rumen. It seems that, in such silages, there is no room for an improvement in the EMPS by supplementation of the diets with either starch or WSC rich feeds. Highly wilted silage is also characterised by a relatively well synchronised N and OM degradation in the rumen which can not be markedly improved by diet supplementation. Therefore, synchrony of OM and CP degradation in the rumen can not be considered as important factors affecting EMPS in grass silages with high DM and WSC concentration. Supplementation of highly wilted GS silages with feeds rich in starch or WSC may lead to impaired metabolizable protein supply when urea is used as a source of rumen degradable N.

Table 9. Digestion of protein along the digestive tract of sheep in grass silage-based diets (in g kg⁻¹ DMI).

| Parameter                               | GS   | GS-MS  | GS-FB  | GS-M  |
|-----------------------------------------|------|--------|--------|-------|
| Dietary protein intake                  | 173  | 172    | 174    | 176   |
| Digested in the rumen                   | 131  | 137    | 135    | 143   |
| Digested postruminally                  | 34   | 27     | 31     | 25    |
| Digested in the total tract             | 165  | 164    | 166    | 168   |
| Digestible true microbial protein       | 64   | 64     | 62     | 61    |
| Metabolizable protein                   | 98   | 90     | 93     | 87    |

Table 10. Nitrogen (N) excretion and balance in grass silage-based diets (g kg⁻¹ N intake).

| Parameter                               | GS   | GS-MS  | GS-FB  | GS-M  | SEE  | Significance |
|-----------------------------------------|------|--------|--------|-------|------|--------------|
| N excretion in faeces                   | 282a | 240b   | 283bc  | 271bc | 20   | P<0.05       |
| N excretion in urine                    | 611a | 644b   | 637bc  | 630bc | 33   | P<0.05       |
| N retention                             | 107  | 116    | 100    | 98    | 26   | ns           |

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