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# Effects of forage supplements on milk production and chemical properties, *in vivo* digestibility, rumen fermentation and N excretion in dairy cows offered red clover silage and corn silage or dry ground corn

G. Salcedo[1]†, L. Martínez-Suller[2], H. Arriaga[3], P. Merino[3]
[1]*Instituto de Educación Secundaria "La Granja", Department of Agriculture Technology, Heras 39792 (Cantabria), Spain*
[2]*Water and Environmental Science and Technology Department, Cantabria University, Spain*
[3]*NEIKER-Tecnalia, Ecotechnologies Department, Derio 48160, Spain*

**This study concerned the effects of partial substitution of clover silage with high starch forages on milk production and chemical composition, *in vivo* digestibility, rumen fermentation pattern and nitrogen excretion of dairy cows. Sixteen dairy cows were separated into two groups and were assigned to treatments in a two-period crossover design. Two forage supplements were used: corn silage (CS) and dry ground corn (DG). All animals received 4.5 kg of concentrate dry matter per day. Results showed no significant difference between the forage supplements for milk production, while significant differences ($P<0.01$) were observed for milk fat, milk protein and nitrogen utilisation efficiency (42 v. 4.0 g/kg, 3.5 v. 3.3 g/kg and 222 v. 188 g/kg, respectively, for DG and CS). Faecal N excretion did not differ between forage supplements, but urinary N excretion was higher for CS ($P<0.05$). No significant differences were observed between treatments for rumen fluid pH or for rumen fluid concentrations of ammonium nitrogen or of acetic, propionic or butyric acids. Dry matter intake and the *in vivo* digestibility of dry matter, organic matter, acid detergent fibre and neutral detergent fibre were all higher for CS compared with DG.**

*Keywords:* clover silage; dairy cow; digestibility; forage supplement; intake

†Corresponding author: gregoriosalce@ono.com

## Introduction

High quality forages frequently have a significant concentration of non-protein N (NPN) (Muck, 1987). Non-protein N is composed of oligopeptides, free amino acids, ammonium compounds and others small molecules that contribute directly to rumen ammonia concentration. Albrecht and Muck (1991) and Papadopoulos and McKersie (1983) observed NPN concentrations in red clover silage (RCS) that were around 30 to 40% lower than in alfalfa silage, which has an NPN concentration of 540 g/kg N (Broderick and Albrecht, 1994). A polyphenol-oxidase enzymatic system in red clover changes phenols to quinones (Jones, Muck and Hatfield, 1995), which have an immediate effect on proteases that degrade silage proteins thus reducing ammonia N ($NH_3$-N) synthesis.

In an experiment with dairy cows, Broderick, Walgenbach and Maignan (2001) reported a lower intake for cows on RCS than for cows offered alfalfa silage, possibly due to higher metabolic satiety attributed to higher dry matter (DM) digestibility (678 and 604 g/kg for red clover silage and alfalfa silage, respectively). Similar milk production, fat, protein, lactose and non-fat solids were obtained for both silage-based diets. When RCS was used in place of alfalfa silage, urea concentration in milk and rumen ammonium concentration decreased, while daily liveweight gain increased.

The present study complements previous research with dairy cows, fed with alfalfa or clover silages (Salcedo, 2004), in which N utilisation and milk quality and production were studied. Although nowadays dry alfalfa and corn silage (CS) are the forage supplements most used on commercial dairy farms in Cantabria (Spain), dry ground corn (DG) is becoming more popular due to advantages such as ease of handling, lower storage space requirement, etc. (Salcedo, 2001). The most important difference between CS and DG is that DG has not undergone fermentation, but has been subjected to increased temperature during the drying process, which promotes a reduction in the rumen degradability of its starch content.

The main objective of the current experiment was to compare the effects of a partial substitution of RCS with CS or DG on milk yield and composition, diet digestibility, N excretion and rumen metabolic profile of dairy cows.

## Materials and Methods

### *Animals and diets*

Sixteen multiparous Friesian cows (average (s.d.): parity 2.6 (0.8), body weight (BW) 604 (12) kg, days in milk 208 (22), milk yield 19.8 (3.74) kg/day, milk fat 38.9 (3.5) g/kg and milk protein 35.2 (2.2) (g/kg)) were selected from the experimental dairy herd of Instituto de Educación Secundaria "La Granja" (Cantabria, Spain) and assigned randomly to one of two experimental diets. One ruminally cannulated cow (10 cm internal diameter: Bar Diamond Inc., Parma, USA) was assigned to each dietary treatment. All animals were cared for in accordance with the guidelines of the European Directive 86/609/EEC of 24 November 1986 concerning the protection and welfare of animals used for experimental and other scientific purposes.

Diets were formulated to fit the nutritional requirements of animals. All forages (RCS, CS and DG) were offered *ad libitum* whereas both diets were supplemented with 4.5 kg DM of concentrate (fresh weight composition (g/kg): barley meal 824, soybean meal 141, bicalcium phosphate 11.4, sodium bicarbonate 19 and mineral mix 4) per day.

Red clover was cut, with a rotary mower-conditioner, at the pre-bud state and wilted for 48 h before baling. No preservative was used during the production of silage bales, which were allowed to ferment for at least 4 months before they were used. Corn for silage was harvested, at the 1/3 milk state, with a corn grinder and conserved in a silage clamp without preservative.

*Experimental design*

*Experiment 1:* The 16 dairy cows were assigned to a 2×2 cross-over experiment; two types of starchy forage supplement (CS or DG) × two periods. Each period was 30 days; 10 adaptation days and 20 test days. Individual body weight was recorded at the beginning and at the end of each period.

RCS was offered *ad libitum* daily at 0930 and immediately afterwards, CS or DG was offered separately and *ad libitum* as well. Animals had access to both forages (RCS and CS or DG) throughout the day. Concentrate was offered daily (0800 and 1530) on an individual animal basis using headlocks. Water and mineral salts were available *ad libitum.*

Daily intake of RCS, CS and DG was calculated on a group basis as the difference between offered and rejected silages. Feed samples were taken twice per week during the test period for the determination of chemical composition: pH, $NH_3$-N and volatile fatty acids (VFA).

Milk yield was recorded at each milking (0730 and 1630) and samples were retained from each milking, during the 20 test days, for laboratory analyses.

*Experiment 2:* At the end of Experiment 1 four of the 16 Friesian cows were allocated to metabolism stalls (2.5 m × 1.7 m) equipped with rubber beds and headlocks. Cows were offered the same experimental diets used in Experiment 1 during two 14-day experimental periods (10 adaptation days and 4 test days) using a cross-over design.

Cows were milked individually, twice per day, in the metabolism stalls using a portable milking machine. Immediately after milking, concentrate was offered to animals. Water and mineral salts were offered *ad libitum*. For each diet and period, all animals were observed over five 10 min periods to evaluate the time taken to masticate one rumen bolus. Quantity of forage offered (RCS, CS and DG) to animals was calculated according to the results obtained in Experiment 1.

Faeces were collected and weighed daily and a subsample (250 g) was retained for laboratory analysis. Urine was collected through a Foley catheter and stored in 25 L containers with 1 L of dilute (1 M) $H_2SO_4$ solution to avoid ammonia loss. Blood samples were collected after the morning milking during the 4-day test period to determine glucose concentration.

Samples of rumen liquid were obtained from the two fistulated cows on each of the last 3 days of each sampling period and stored in sterile hermetically sealed containers without preservative. The post-prandial rumen fluid was extracted after 0 (0745), 2, 4, 7.5, 8.5 and 10.5 h.

*Analytical procedures*

*Feed:* Forage pH was determined using a Crison BasiC20 pH-meter; DM concentration of forage and concentrate were determined by drying at 60 °C for 48 h. Ash was determined after igniting the samples at 550 °C. The concentration of $NH_3$-N was determined by distillation with magnesium oxide (Kjeltec 1002, Tecator), and crude protein (CP) was by Kjeldahl method (total N × 6.25). The soluble protein (N × 6.25) concentration of silages and concentrate was determined

by Kjeldhal after maceration of the fresh sample in water at 80 °C.

Acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin were determined on a dry sample of RCS according to Goering and Van Soest (1970). All samples were split to allow determination of the N concentration of the fibre residue (Van Soest, 1965). In the case of samples of CS and DG the NDF was determined using amylase according to Van Soest, Robertson and Lewis (1991).

Fat was extracted with petroleum ether at 40 to 60 °C with a Soxtec™ (AOAC, 1990). *In vitro* organic matter (OM) digestibility was determined by the NDF-cellulose enzymatic method (Riveros and Argamenteria, 1987). VFAs were determined using high-performance liquid chromatography (HPLC) (Shimadzu SPD-10 A fitted with a Shodex RS pack KG-811 column). Starch determination was conducted by endoamylase and exo-glucosidase incubation before glucose assay (Sigma no. 510-A; Sigma Chemical Co.), as described by Herrera-Saldana *et al.* (1990). Calcium and magnesium were analysed on an atomic absorption spectrophotometer. Potassium was analysed using a flame photometer and phosphorus determined by a colorimetric method, based on nitro-molibdo-vanadate, in the Laboratorio Agroalimentario (MAPA) of Santander (Spain).

During the two periods of Experiment 2, samples of RCS, CS and DG were introduced into the rumen of each cow to determine the degradability of crude protein and starch. Samples were weighed (3 g) in 13 cm × 7.7 cm nylon bags (pore size of 45 μm). Upon removing samples, bags were rinsed with clean cold water for three periods of 5 min and dried in an oven at 60 °C for 48 h. Protein and starch degradability of concentrate were obtained according to NRC (2001). Whole and split grain concentrations in CS and DG were calculated on 350 g (14 × 25 g subsamples) of each forage supplement by counting manually. Samples (150 g) of CS and DG were weighed and manually separated to characterize particle size according to three groups: >2 cm, 1 to 2 cm, and <1 cm.

*Milk:* CP (total N × 6.38), fat, lactose and non-fat solid concentrations were determined using a Milko-Scan 4000 device in the Interprofessional Dairy Laboratory of Santander (Cantabria, Spain). Urea concentration was determined according to L 155 12/07/1973 European Communities in the Animal Nutrition Laboratory of I.E.S. "La Granja" (Cantabria, Spain). Non-casein N (NCN) was determined by the Kjeldahl method after precipitation with 10% acetic acid plus 1 M sodium acetate (Casado, 1982). Casein N was estimated as the difference between total N and NCN.

*Rumen liquor:* The pH of rumen fluid was determined immediately after sample withdrawal using a pH meter (Crison BasiC20). Samples were then centrifuged and analyzed for $NH_3$-N by distillation with magnesium oxide (Kjeltec 1002, Tecator). An aliquot of rumen liquid was filtered, acidified with 5 mL of 6 M HCl per 100 mL of rumen liquid, and finally frozen for subsequent analysis of VFA by HPLC (Shimadzu SPD-10 AV fitted with a Shodex RS pack KG-811 column).

*Faeces, urine and blood:* The DM concentration of faeces was determined by drying 100 g in an oven at 60 °C for 48 h. Ash was determined from loss on ignition at 550°C and the organic matter was determined by difference. ADF and NDF were determined according to Goering and Van Soest (1970) and total N was determined by the Kjeldahl method on fresh samples. N concentration in urine

was determined by the Kjeldahl method, while blood glucose concentration was determined using a glucometer (GX) supplied by Bayer.

*Data calculation and analysis procedures*

The effective degradability of starch and protein were calculated as per Ørskov and McDonald (1979). The efficiency with which N intake was converted into N excreted in milk and the efficiency of metabolisable energy conversion into gross energy in milk (Tyrrell and Reid, 1965) were calculated as the ratio of output to input.

The *in vivo* digestibility was predicted from NDF and *in vitro* enzymatic digestibility using the equation of Argamentería *et al*. (1995) for RCS and the equation of Riveros and Argamenteria (1987) for CS and DG. Metabolisable energy (MJ/kg DM) was calculated as 0.016 × OMD (MAFF, 1984; OMD = organic matter digestibility (g/kg)).

Data for milk yield and composition, nutrient balance, chemical composition of faeces, urine and blood, excretion and digestibility were analysed using Proc GLM of SAS (1985). The model used was:

$$Y_{ijk} = \mu + D_i + P_j + C_k + \varepsilon_{ijk},$$

where Y = observation, μ = population mean, $D_i$ = diet (CS or DG), $P_j$ = period (1, 2), $C_k$ = cow effect (1…16 for Experiment 1, 1...4 for Experiment 2) and $\varepsilon_{ijk}$ = residual error.

The chemical composition of rumen fluid (Experiment 2) was analysed by repeated measures analysis over time according to the model:

$$Y_{ijkl} = \mu + D_i + P_j + C_k + T_l + D{\times}T_{il} + \varepsilon_{ijkl}$$

where Y = observation, μ = population mean, $D_i$ = diet (CS or DG), $P_j$ = period, (1, 2), $C_k$ = cow (1, 2), $T_l$ = time of sampling, (1…6) and $\varepsilon_{ijkl}$ = residual error.

## Results and Discussion

The chemical composition of the feeds is shown in Table 1. Ammonia concentration in red clover silage was significantly higher, while the concentrations of acetic acid and soluble N, and pH were below those given in INRA (1981), likely due to higher moisture concentration.

Crude protein, soluble protein, and non-fibrous carbohydrate (NFC; equals 1000 minus the concentrations (g/kg) of CP, NDF, ash and fat) concentrations were higher for CS than for DG, as was effective degradability of CP. In contrast, the concentrations of ADF, NDF, OM, starch, lignin and minerals were higher for DG. The higher starch concentration could be due to the lower maturity status of the CS, or because part of the starch was used during fermentation of CS. Andrae *et al*. (2001) and Salcedo (2002c) reported an increase in starch concentration and a corresponding decrease in the concentrations of ADF and NDF at the end of the maturity phase (even though for DG the temperature of the drying process would favour artificial lignin synthesis).

The high *in vivo* digestibility of OM obtained for CS and DG in this study are within the range reported by Barrière *et al*. (1993). The rumen degradability of starch was higher for CS ($P<0.05$), probably due to a higher water concentration in the grains.

About 62.5% particles were in the range 1 to 2 cm for both forages. A particle size greater than 2 cm was found in 26.0% and 22.8% of DG and CS, respectively, in agreement to Andrae *et al*. (2001).

**Table 1. Chemical composition, digestibility and physical attributes of feeds**

| Component | Feed[†] | | | | | | |
|---|---|---|---|---|---|---|---|
| | RCS | | CS | | DG | | Concentrate |
| Dry matter (DM) (g/kg) | 227 | (33.7)[‡] | 349 | (10.6) | 922 | (0.2) | 907 |
| Organic matter (g kg/DM) | 910 | (4.1) | 968 | (4.5) | 977 | (0.2) | 917 |
| Crude protein (CP) (g/kg DM) | 192 | (15.6) | 92.4 | (3.2) | 80.5 | (0.34) | 170 |
| Ether extract (g/kg DM) | 37 | (0.2) | 32.6 | (0.3) | 35.5 | (0.18) | 31 |
| Neutral detergent fibre (g/kg DM) | 388 | (28.3) | 465 | (22.7) | 516 | (5.3) | 19.2 |
| Acid detergent fibre (g/kg DM) | 257 | (8.2) | 253 | (14.4) | 285 | (3.8) | 73 |
| Metabolisable energy (MJ/kg DM) | 10.4 | (0.45) | 10.9 | (0.25) | 11.4 | (0.18) | 13.2 |
| Starch (g/kg DM) | 4.5 | (0.1) | 257 | (3.0) | 336 | (1.4) | 492 |
| Starch degradability parameters[§] | | | | | | | |
| a (g/kg starch) | – | – | 781[y] | (2.8) | 625[z] | (5.0) | – |
| b (g/kg starch) | – | – | 196[z] | (3.4) | 326[y] | (5.4) | – |
| c | – | – | 0.12[y] | (0.002) | 0.09[y] | (0.001) | – |
| eD (g/kg starch) | – | – | 913[y] | (4.0) | 820[z] | (2.8) | – |
| Crude protein degradability parameters[§] | | | | | | | |
| a (g/kg CP) | 512 | (28.4) | 362[y] | (4.2) | 317[z] | (4.0) | – |
| b (g/kg CP) | 325 | (25.5) | 417[z] | (8.9) | 451[y] | (4.1) | – |
| c | 0.09 | (0.001) | 0.081[y] | (0.009) | 0.052[z] | 0.001 | – |
| eD (g/kg CP) | 711 | (0.95) | 604[y] | (5.0) | 526[z] | (6.0) | – |
| Rumen undegraded protein (g/kg CP) | 288 | (0.95) | 396[z] | (5.3) | 473[y] | (6.0) | 24.6 |
| N tied to ADF (g/kg ADF) | 57.8 | 1.5 | – | – | – | – | – |
| N tied to (g/kg NDF) | 196 | 1.6 | – | – | – | – | – |
| Non-protein N (g/kg N) | 408 | (1.23) | – | – | – | – | – |
| $NH_3$-N (g/kg N) | 84.5 | (10.5) | 39.9 | (2.8) | – | – | – |
| Lignin (g/kg DM) | 89.2 | (0.9) | 21.8 | (0.8) | 24.1 | 0.5 | – |
| Ca (g/kg DM) | 15.1 | (0.6) | 2.5 | (0.05) | 2.8 | (0.02) | 7.3 |
| P (g/kg DM) | 2.9 | (0.05) | 2.6 | (0.05) | 3.2 | (0.016) | 4.2 |
| Mg (g/kg DM) | 4.4 | (0.05) | 2.9 | (0.05) | 3.1 | (0.04) | 1.7 |
| K (g/kg DM) | 30.6 | (0.1) | 1.7 | (0.1) | 1.8 | (0.13) | 6.9 |
| pH | 3.82 | 0.11 | 3.62 | 0.002 | – | – | – |
| Lactic acid (g/kg DM) | 83.3 | (0.72 | 57.6 | (1.45) | – | – | – |
| Acetic acid (g/kg DM) | 26.0 | (0.33) | 16.2 | (0.03) | – | – | – |
| NFC, g/kg (DM) | 293 | (12.5) | 378 | (5.4) | 345 | (4.1) | – |
| *In vitro* DM digestibility (g/kg) | 653 | (28.1) | 684 | (15.8) | 717 | (7.3) | |
| Particle size (g/kg DM): | | | | | | | |
| >2 cm | | | 22.8 | (2.15) | 26.2 | (1.44) | 1.44 |
| 1–2 cm | – | – | 63.9 | (1.79) | 61.2 | (1.51) | 1.51 |
| <1 cm | | | 13.2 | (1.61) | 12.5 | (2.1) | 2.1 |
| Whole grains (g/kg DM) | – | – | 300 | (25.7) | 345 | (11.9) | – |

[†] RCS = red clover silage, CS = corn silage, DG = dry ground corn.
[‡] Values within brackets are s.d.
[§] a = rapidly degradable fraction, b= slowly degradable fraction, c = rate (%) of degradation over time, eD = effective degradability assuming a proportional ruminal outflow rate of 0.06 per hour.
[yz] Significantly different ($P<0.05$) within a row.

*Nutrient intake*

Intakes of DM and digestible OM were significantly higher ($P<0.001$) with the diet containing CS compared to the GS diet (Table 2) and this was attributed to high digestibility of NDF, ADF, OM and DM observed (Experiment 2, Table 3). The lower time required to masticate a bolus (Table 3) could also account for the higher intake for the diet containing CS. No significant differences were observed for N intake (Table 3).

**Table 2. Nutrient intake for diet of red clover silage with supplement: Experiment 1**

| Component | Supplement | | s.e. |
|---|---|---|---|
| | Corn silage | Dry ground corn | |
| Total dry matter (DM) (kg/day) | 18.8 | 18.0 | 0.081 |
| Clover silage DM (kg/day) | 10.0 | 9.87 | 0.073 |
| Supplement DM (kg/day) | 4.34 | 4.00 | 0.12 |
| Metabolisable energy (ME) (MJ/day) | 212 | 205 | 0.83 |
| Crude protein (CP) (kg/day) | 3.09 | 2.95 | 0.013 |
| Rumen fermentable organic matter (kg/day) | 8.43 | 7.85 | 0.042 |
| Rumen degradable protein (RDP) (kg/day) | 2.18 | 2.07 | 0.0097 |
| Soluble CP (kg/day) | 1.28 | 1.22 | 0.0066 |
| RDP/ME (g/MJ) | 1.65 | 1.62 | 0.0044 |
| Neutral detergent fibre (kg/day) | 6.79 | 6.59 | 0.032 |
| Acid detergent fibre (kg/day) | 4.01 | 4.01 | 0.014 |
| Starch (kg/day) | 3.37 | 3.50 | 0.016 |
| Non-fibrous carbohydrate (kg/day) | 5.19 | 6.41 | 0.033 |
| Ca (g/day) | 192 | 200 | 1.17 |
| P (g/day) | 49.0 | 60.3 | 0.48 |
| Mg (g/day) | 50.8 | 60.8 | 0.49 |
| K (g/day) | 341 | 342 | 2.18 |
| Digestible organic matter[1] (kg/day) | 13.0 | 12.2 | 0.061 |

[1] Based on *in vivo* digestibilities determined with sheep fed at maintenance.

**Table 3. Nutrient balance estimates for diet of red clover silage with supplement: Experiment 2**

| Variable[1] | Forage supplement | | s.e. | P value |
|---|---|---|---|---|
| | Corn silage | Dry ground corn | | |
| Total dry matter (DM) intake ( kg/day) | 18.3 | 17.9 | 0.095 | <0.001 |
| Clover silage DM intake (kg/day) | 10.4 | 9.95 | 0.14 | <0.001 |
| Intake of supplement DM (kg/day) | 3.46 | 3.41 | 0.031 | 0.542 |
| Intake of N (g/day) | 493 | 505 | 4.34 | 0.169 |
| Output of fresh faeces (kg/day) | 38.2 | 36.4 | 0.54 | <0.001 |
| Output of faecal DM (kg/day) | 5.58 | 6.24 | 0.12 | <0.001 |
| Output of urine (L/day) | 22.3 | 21.1 | 0.26 | 0.017 |
| Output of N in milk (g/day) | 93.2 | 102.8 | 1.55 | <0.001 |
| Output of N in urine (g/day) | 182 | 177 | 1.26 | 0.026 |
| Output of N in faeces (g/day) | 201 | 200 | 3.35 | 0.959 |
| Faecal N/ N intake (g/g) | 0.408 | 0.398 | 0.0087 | 0.594 |
| Urinary N/N intake (g/g) | 0.370 | 0.351 | 0.0041 | 0.012 |
| NDF in faeces (g/kg DM) | 459 | 496 | 5.8 | <0.001 |
| ADF in faeces (g/kg DM) | 329 | 349 | 3.2 | <0.001 |
| Digestibility coefficients (g/kg) | | | | |
| DM | 696 | 676 | 5.0 | 0.045 |
| Organic matter | 744 | 729 | 3.9 | 0.041 |
| NDF | 600 | 558 | 7.7 | 0.002 |
| ADF | 526 | 486 | 9.6 | 0.032 |
| N | 592 | 602 | 8.7 | 0.594 |
| Corn grains in faeces (g/kg) | 25.8 | 39.0 | 1.8 | <0.001 |
| Glucose on blood (mg/L) | 519 | 544 | 4.0 | <0.001 |
| Mastication time per bolus (s) | 42.5 | 44.6 | 0.42 | 0.006 |
| Diet moisture (g/kg) | 697 | 659 | 5.7 | <0.001 |

[1] NDF = neutral detergent fibre, ADF = acid detergent fibre.

Hazard *et al*. (2001) observed a higher feed intake (3.0 and 3.1% of bodyweight for CS and DG, respectively) in an experiment with dairy cows offered clover silage and substituting clover silage with CS plus 3.4 kg of alfalfa hay, and 5.2 kg of concentrate.

Grant and Mertens (1992) suggested a negative relationship between ADF digestibility and starch concentration, which agrees with our data (r –0.64, $P<0.05$ for ADF; r –0.80, $P<0.01$ for NDF). Viersma *et al*. (1993) suggested that the reduced digestibility of plant material at maturity is another possible explanation for the lower ADF digestibility. However, fermentable OM intake was higher when CS was used (Table 2) compared with DG and this may be related to the higher rapidly degradable starch concentration of CS. This result is similar to that obtained by Salcedo (2004) and slightly lower than that shown by Broderick *et al*. (2002) with alfalfa silage.

NDF intake was higher with the CS diet, which is likely to be due to the higher intake of RCS with this diet. However, NDF concentration was similar between diets (accounted for 36.6% and 36.1% of total DM intake for DG and CS, respectively). These results are in agreement with Mertens (1992), who suggested that 35% of NDF in the total DM intake is the optimum to maximize DM intake. Equally the average value for NDF consumption per 100 kg bodyweight was 1.1 kg which is consistent with the findings of Mertens (1987).

Metabolizable energy intake (average 208 MJ per cow per day; Experiment 1) was similar to values in NRC (1989). The intakes of CP, soluble CP, RDP (rumen degradable protein) and RUP (rumen undegradable protein) were significantly ($P<0.001$) higher with the CS diet, probably reflecting the higher DM intake with this diet (Table 2). The intakes recorded are higher than NRC recommendations for CP and RDP, given the observed milk yield. This fact is probably due to high CP concentration in clover silage, which accounted for 63.5% of RDP intake. Even with this high CP consumption, metabolisable protein intake (predicted by CNCPS 5.0, Fox *et al*., 2003) was 1.954 and 1.883 g/day for DG and CS diets, respectively. These average values were 18.2 and 24.5% higher than NRC (2001) recommendations. Similarly, RDP per unit of metabolisable energy did not differ between diets but the average value (Table 2) was considerably higher than the ARC (1980) recommendation (1.3 g/MJ) for optimal microbial protein synthesis.

The intake of starch, NFC, Ca, P and Mg was higher in diets supplemented by DG ($P<0.05$) whereas K intake did not differ significantly between diets. Intake of P was just above the requirements estimated by NRC (1989), given the observed milk yield. On the contrary, Ca, Mg and K intakes exceeded the recommended values.

*Digestibility and N balance (Experiment 2)*

The DM intake was higher for cows on the CS diet (Table 3; $P<0.001$) due to the higher RCS intake ($P<0.001$), in accordance with results in Experiment 1. There were no significant differences in intake between the CS and DG supplements (Table 3). Faecal output was slightly lower with the CS diet (5.5 v. 6.2 kg DM per day) probably due to higher digestibility of DM, NDF and ADF ($P<0.05$, $P<0.01$ and $P<0.05$, respectively). In contrast, the highest urine volume was registered for the CS diet ($P<0.05$), probably due to the higher moisture concentration of this diet and not to a higher N intake (Table 3).

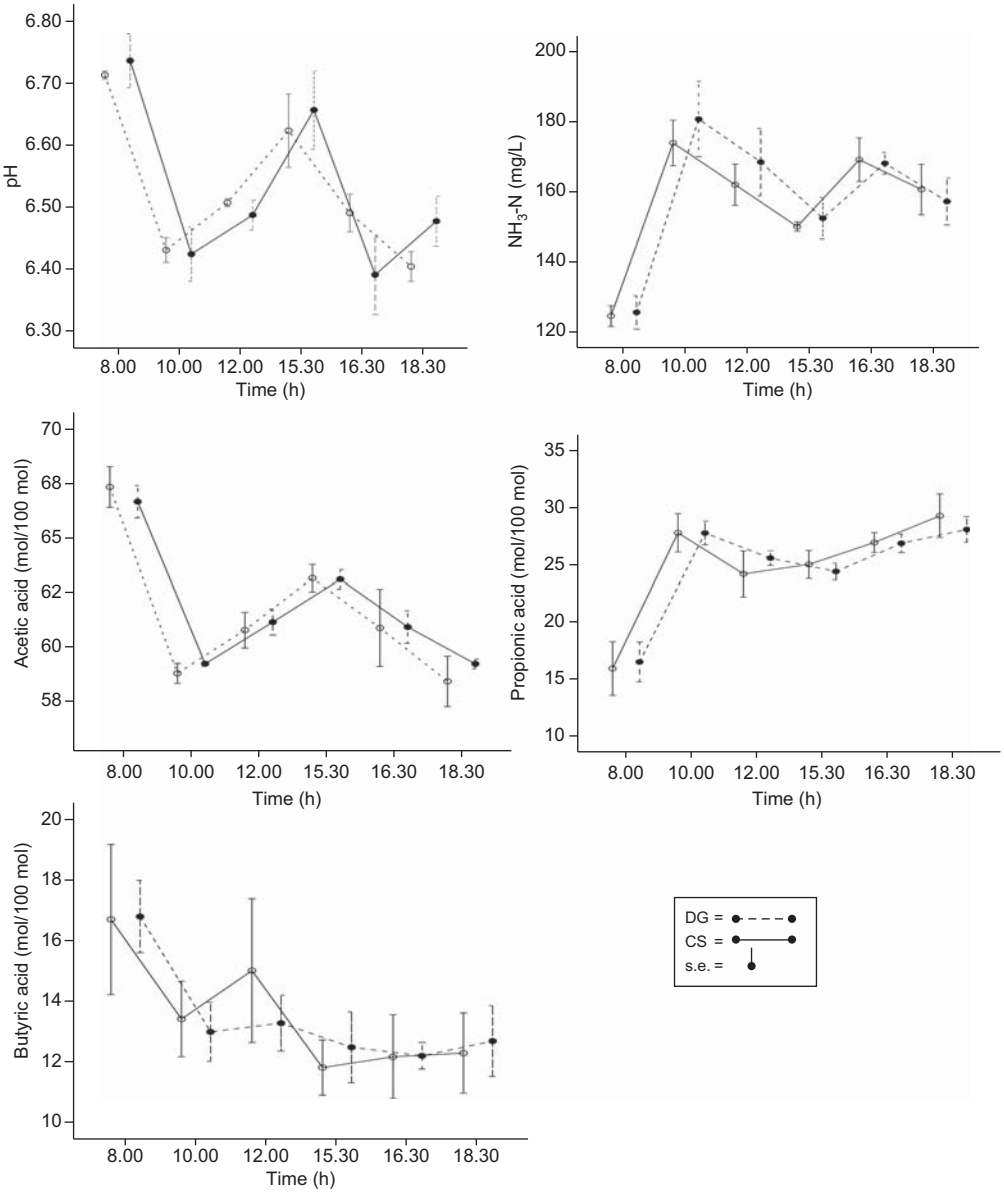


*Figure 1. Characteristics of rumen fluid for diets supplemented with either corn silage (CS) or dry ground corn (DG): pH, ammonium nitrogen ($NH_3$-N) concentration, and the proportions (relative to total volatile fatty acids) of acetic, propionic and butyric acids. (The values at each time point are offset to aid legibility.)*

For this reason, the higher N excretion in urine for cows on the CS diet ($P<0.01$) is probably attributed to the difference in urine output (1.2 L) between the diets. Furthermore, no significant difference was observed for N digestibility (Table 3).

*Rumen fermentation*

The rumen fermentation characteristics are shown in Figure 1. The pH of rumen fluid was similar between diets, with an average value of 6.5, due probably to the similar starch and NDF concentrations in the diets. For both forages, rumen pH decreased immediately following concentrate intake, showing the lowest value at the 1000 time point. This is probably due to the rapid fermentation of non-fibrous carbohydrates and is characteristic when concentrate is offered twice daily (Krause, Combs and Beauchemin, 2002).

As for pH, $NH_3$–N concentration was not affected by supplement type. However, this component showed significant differences ($P<0.001$) over time. The highest $NH_3$-N concentration was observed 2 h after concentrate intake and was slightly higher for DG supplement (Figure 1), probably due to lower starch degradation in the rumen (Philippeau and Michalet-Doreau, 1997). Andrae *et al.* (2001) observed increased ruminal digestibility of starch in corn silage when the grain was classified as in the milky-glass phase. The results from the present study are similar to those reported by Salcedo (2004), probably due to the high availability of soluble carbohydrate in the rumen. In any event, N-$NH_3$ concentrations were higher than the maximum concentration indicated by Satter and Styler (1974); 50 mg/L of rumen fluid.

No significant differences were observed between forages for the proportions of the various VFAs in rumen fluid, while significant differences were observed over time. For both diets, the highest acetic acid proportion occurred just before concentrate was offered, in both the morning and afternoon. The lowest acetic acid proportion was observed 2 h after concentrate intake, likely due to the quantity of forage in the rumen.

*Milk production and chemical composition*

Milk production did not differ significantly between treatments in Experiment 1 (Table 4). However, significant differences were observed for fat-corrected milk yield ($P<0.05$). Other researchers, such as Montgomery, Baxter and Bearden (1976), Lessard and Fisher (1980), Dulphy *et al*. (1984), Thomas, Aston and Daley (1985), Bando and Deoka (1990) and Chenais, Le Gall and Jullien (1993), have observed profitable effects for milk production when leguminous silage was included in the diet, probably due to a higher intake. However, Moran and Wamungai (1992) had to suspend (after 8 weeks) an experiment with diets based on CS (60%) and clover silage (40%) due to a sharp reduction in milk production. The nutritional deficiencies were caused by inadequately wilted clover silage, resulting in a large amount of effluent, with consequent losses of sugars.

The highest fat concentration was observed with the DG diet ($P<0.05$). Probably the due to the lower *in vivo* digestibility of NDF and/or the longer ($P<0.01$) time required to masticate a bolus with the DG diet (Table 3), in spite of equivalent acetic acid concentrations in rumen fluid between diets (Figure 1).

The highest milk protein concentration was observed with the DG diet ($P<0.001$), probably due to lower starch degradation in DG (Table 1) leading to a higher proportion of the starch being digested in the small intestine, as confirmed by the differences in the concentration of glucose in blood (Table 3). In a previous study (Salcedo, 2004), the highest protein concentration was observed for RCS conserved with DG. Another possible explanation for the higher CP concentration in

**Table 4. Mean values for effects of dietary supplement on milk production traits, milk composition and efficiency: Experiment 1**

| Variable | Supplement | | s.e. | P value |
|---|---|---|---|---|
| | Corn silage | Dry ground corn | | |
| Milk yield (kg/day) | 18.0 | 18.9 | 0.31 | 0.316 |
| Fat-corrected milk yield[a] (kg/day) | 17.9 | 19.5 | 0.32 | 0.017 |
| Fat yield (kg/day) | 0.71 | 0.79 | 0.014 | 0.006 |
| Protein yield (kg/day) | 0.59 | 0.67 | 0.010 | <0.001 |
| Non-fat solids yield (kg/day) | 1.62 | 1.74 | 0.029 | 0.035 |
| Gross energy (GE) yield in milk (MJ/day) | 56.3 | 60.9 | 1.01 | 0.022 |
| Milk composition | | | | |
| Protein (g/kg) | 33.3 | 35.3 | 0.22 | <0.001 |
| Fat (g/kg) | 40.0 | 42.0 | 0.43 | 0.025 |
| Casein (g/kg) | 26.3 | 27.8 | 0.17 | <0.001 |
| Lactose (g/kg) | 48.5 | 48.6 | 0.14 | 0.675 |
| Non-fat solids (g/kg) | 90.1 | 91.5 | 0.22 | 0.002 |
| Urea (mg/L) | 149 | 140 | 0.48 | <0.001 |
| Output of N in milk/N intake (g/g) | 0.188 | 0.222 | 0.0036 | <0.001 |
| Output of GE in milk/ME[b] intake (MJ/MJ) | 0.266 | 0.298 | 0.0049 | <0.001 |
| Somatic cell count | 134 | 287 | 21.92 | <0.001 |
| Body-weight gain (g/day) | 88 | 133 | 0.47 | 0.263 |

[a] Fat concentration of 40 g/kg.
[b] Metabolisable energy.

milk with the diet DG could be improved energy utilisation, as confirmed by the higher concentrations of casein and urea in milk (P<0.001; Table 4).

*Nutrient utilisation*

The efficiency of ME use for milk production was higher for the DG diet (P<0.001; Table 4), probably due to the dehydration process that may have reduced the degradability of starch thereby furnishing more glucose to the intestine. This is consistent with increased glucose concentration in blood (P<0.001; Table 3), higher body-weight gain, lower milk urea concentration (P<0.001; Table 4) and higher milk protein concentration (P<0.001; Table 4).

Ortega and Mendoza (2003) observed that part of the starch could escape rumen fermentation for diets with a high starch concentration and thus increase available starch for absorption as glucose. However, the capacity of the small intestine of a ruminant to digest an elevated quantity of starch is disputed (Waldo, 1973; Croome, Bull and Taylor, 1992) due to low pancreatic amylase output (Coombe and Smith, 1974) and low glucose absorption (Ørskov, 1986; Kreikemeier *et al*., 1991; Tanigshu *et al*., 1995). In an experiment with grazing dairy cows supplemented with 4, 2 or 0 kg of DG plus 2.6 kg of concentrate, the highest glucose concentration (average values were 627, 613 and 593 mg/L, respectively) was found for cows on 4 kg probably due to a higher starch intake (Salcedo, 2004).

Ruminal fermentation of silage has negative effects on the synchronisation between available energy and protein in the rumen (Prates *et al.,* 1986) and could be an explanation for the lower efficiency observed for energy utilisation when CS was substituted by DG. In a similar experiment, Salcedo (2002a,b) observed higher efficiencies when grazing animals were supplemented with 2 kg of concentrate (0.264) than when these animals were offered grass silage as a substitute for

grazing (0.226). This finding is in agreement with Thomas (1982), who observed a lower utilisation of soluble carbohydrate for microbial development with silage than with fresh forage, probably due to the transformation of components that occurs during the ensilage process.

The results obtained for the efficiency of metabolisable energy utilisation for milk synthesis (Table 4) are slightly higher than the values of 0.264 and 0.275 reported by Salcedo (2001) for cows (172 days in milk) offered grass silage and 4 kg of concentrate plus 4 or 2 kg of DG. Nocek and Tamminga (1991) suggested that starch is used more efficiently when it is not digested in the rumen.

The efficiency of N utilisation for milk production was higher for DG than for CS ($P<0.001$). This is probably due to a more ideal rumen balance between protein and energy. Dewhurst *et al*. (2003) obtained similar efficiency values with dairy cows offered RCS supplemented with 4 or 8 kg of concentrate (0.188 and 0.197, respectively). The values for efficiency of N utilisation for milk production are higher than those reported by Salcedo (2004) in a similar experiment, probably due to the lower N intake in the present experiment (Table 4).

## Conclusions

The substitution of CS by DG in diets of dairy cows offered clover silage does not increase milk production. Milk protein concentration is higher when DG is used compared with CS; this significantly improves the conversion efficiency of feed N into N in milk.

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