Effect of replacing organic grass-clover silage from primary growth with regrowth on feed intake and milk yield of dairy cows

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ABSTRACT

Under Norwegian conditions diets based on primary growth silage (PG) typically increase milk yield compared to silage prepared from the regrowth (RG). Organic PG, dominated by immature grasses, are often high in energy and low in CP, whereas the opposite is the case for organic RG harvests, dominated by clover. Here, we tested the hypotheses that increasing proportions of RG will reduce the total supply of metabolizable energy, but increase the CP-intake, and that there is a dietary optimal mix of PG and RG to meet requirements for optimal milk production. Sixteen Norwegian Red cows were used in an experiment designed with four balanced 4 × 4 Latin squares with 21-days periods to evaluate the effect of incremental replacement of PG with RG on feed intake, nutrient digestion and milk production. Silages were prepared from PG and RG of an organically managed grassland. Treatments comprised silages fed *ad libitum* with RG replacing PG in ratios of 0, 0.33, 0.67 and 1 on DM basis. Additionally concentrate was offered with 8 kg for pluriparous and 7 kg for primiparous cows. The PG had higher contents metabolizable energy (ME), potentially degradable neutral detergent fiber (NDF) and water soluble carbohydrates, while RG contained more crude protein (CP) and indigestible NDF. The already mentioned characteristics led to higher intakes of DM, organic matter, NDF and ME, and lower intakes of CP and indigestible NDF with increasing proportions of PG in the diet. Milk yield tended to be higher when PG and RG were offered as a mixture than when fed alone. The milk fat concentration decreased linearly with increasing proportions of RG proportion, while protein concentration was unaffected by diet. This led to a similar production of energy corrected milk among cows fed diets containing PG while cows fed pure RG diet produced 0.9 kg less daily. Silage energy concentration and energy intake influenced milk production more than CP supply**.**

Key words: dairy cow, organic milk production, regrowth, silage, grass-clover

INTRODUCTION

Organic dairy production in Norway is becoming more intensive, where increased milk yield has been achieved from increasing amounts of concentrate feed (TINE Rådgiving 2012). High-grade protein plant sources have limited (e.g. rapeseed and pulses) or no (e.g., soybeans) cultivation potential in Norway. Import of particularly soybeans is controversial (Leiber 2014). Homegrown forages may be cheaper to produce but only a high quality forage will realize a profitable production. Grassland yield and productivity of organic dairy production depend largely on biological N-fixation by legumes (Steinshamn 2010). To obtain forage with high fiber digestibility and thereby high energy content, the spring growth has to be harvested when plants are at an early stage of phenological development. Although the growing season in most of Norway is short, such an early primary growth (PG) may constitute less than 50% of the total annual yield and be low in crude protein (CP) (Steinshamn and Thuen 2008). The following regrowth (RG) harvests are often low in NDF, but its digestibility and energy value are found to be inferior to that in PG (Huhtanen et al. 2007). This might have nutritional and production consequences.

Dairy cows, fed grass silage from RG, were found to yield less milk than those fed grass silage from PG, which was ascribed to lower dry matter (DM) intake of RG silage (Kuoppala et al. 2008; 2010). Bertilsson and Murphy (2003) found that cows fed pure red clover silage from RG had higher feed intake than those fed pure red clover silage from PG, but milk yield and composition were similar. Additionally they found higher NDF content, lower proportion of potentially degradable NDF (pdNDF) and a lower rate of degradation of NDF in red clover silage prepared from RG than from PG. Vanhatalo et al. (2009) found that DM intake of grass silage decreased, while intake of red clover silage increased with advancing maturity, when comparing different growth stages in the PG of grass and red clover silages. In organic grass-clover harvests, clover contributes to a significant amount of CP in the total crop. Due to slower spring growth rate compared to grass species, the clover proportion is often low in PG of organic grass-clover swards harvested at early grass maturity stages, while in RG harvests the clover proportion is often high (Steinshamn and Thuen 2008). Furthermore, a slow release of N from manure at low spring temperatures limits the CP content in grasses in organic fields (Steinshamn 2001). Therefore, organic PG at high latitudes in Scandinavia, dominated by immature grasses, are often high in energy and low in CP. The CP in conventional agriculture normally starts with high concentrations and sinks rapidly with increasing maturity (Vanhatalo et al. 2009). Because of the mentioned slow N-release in organic clover-dominated RG harvests, the CP concentration is higher than the PG. However, this difference in estimated energy and nutrient contents of PG and RG silages is recognized, but few feeding trials with dairy cows have been carried out in which PG and RG are compared (Khalili et al. 2005; Kuoppala et al. 2008; Halmemies-Beauchet-Filleau et al. 2014).

The object of this study was to see if there is a synergetic effect on DMI and milk production when fiber and CP in organic grass-clover silages from PG and RG are mixes, or if the milk production is better of when feeding one of the silages alone. Based on available knowledge, the hypotheses were that increasing dietary proportions of RG will reduce the total supply of metabolizable energy, but increase the N-intake, and that there is a dietary optimal mix of PG and RG to meet the requirements for the highest milk energy production.

MATERIALS AND METHODS

Experimental Design and Animals

An experiment consisting of four balanced 4 × 4 Latin squares was conducted, each with 4 Norwegian Red cows, 4 organic grass-clover silage diets, and four 21-day periods. Each 21-day period was split in a 9-day adaption period and a 12-day sampling period. The 16 cows entered the experiment in early stage of lactation with (mean ± standard deviation) 36 ± 17 days in milk and BW of 623± 78 kg. At the end of the study, the BW had increased to 643 ± 68 kg. One cow was excluded from the experiment in two periods due to indigestion. Two squares were performed in fall 2012 (one with pluriparous cows and one with primiparous cows) and two squares were performed in spring 2013 (both with pluriparous cows).

Grass-clover Silages

Both experimental silages were harvested from the same organically managed ley (Counc. of the Eur. Union 2007) in its second and third production year in Ås, Norway (59°40’19”N, 10°46’33”E) in 2012. This ley has been organically cultivated since 1993. Cattle slurry was applied at rates of 30 ton/ha on May 2 and 15 ton/ha on June 15 in accordance with the regulations of organic production in the European Union (Counc. of the Eur. Union 2007). The PG harvest was conducted at early booting stage of timothy (*Phleum pratense L.* cv. ‘Grindstad’) on June 7, and it consisted of timothy (41.9%), meadow fescue (*Festuca pratensis Huds*. cv. ‘Fure’, 25.3%), white clover (*Trifolium repens L.* cv. ‘Hebe’, 11.3%), smooth meadow grass (*Poa pratensis L.* cv. ‘Knut’, 8.1%), red clover (T*rifolium pratense L.* cv. ‘Bjursele’, 6.5%) and other legumes, grasses, herbs, and weeds (6.9%). The RG was harvested on July 26 when crop NDF was estimated to be similar to that of the PG. The RG consisted of white clover (39.3%), timothy (29.2%), meadow fescue (14.2%), couch grass (*Elytrigia repens*, 6.0%), smooth meadow grass (5.2%), red clover (1.4%), and other legumes, grasses and weeds (4.7%). The botanical composition was determined after walking the whole field in a diagonal grid directly before each harvest. Every tenth step a handful of sward was cut with a pair of scissors. The total sample was then manually sorted into botanical components. Accordingly, all species were separately dried at 105 ºC to constant weight (minimum 24 h) and botanical composition was expressed on DM basis.

Silage Production

The crops were cut with Kuhn FC 302 G (Kuhn S.A., Saverne, France) mower and wilted for 9-10 h during daytime or 20-21 h over-night in PG, and 9-12 h during daytime or 27-30 h over-night in RG. Thereafter crops were baled using Orkel GP 1260 (Orkel AS, Fannrem, Norway) fixed chamber roundbaler with 20 fixed knives and a theoretical 54 mm chop length. Ensil®Pluss silage additive (540 g/kg formic acid, 180 g/kg propionic acid; Felleskjøpet Agri SA, Lillestrøm, Norway) was applied to the crop at 5.1 and 5.2 L/ton for PG and RG, respectively. Immediately after baling, each bale was weighed, and a core sample was taken to produce composite samples for approximately eight bales. The bales were wrapped in 8 layers of 0.75 m wide and 0.025 mm thick white Trioplus 2000 (Trioplast, Smålandsstenar, Sweden) stretch plastic film using Tanco Autowrap 280 ARC (Tanco Autowrap Ltd., Co. Carlow, Ireland) wrapping machine. Yields of harvested herbage were 2.84 ton DM/ha in PG and 2.05 ton DM/ha in RG.

The weather was sunny with no precipitation during wilting of the PG, but humid and a few raindrops (0.1 mm) when mowing began in the RG. Thereafter weather cleared up. The mean daily temperature was 11.1ºC and total precipitation 77 mm in May and until PG harvest began on June 5. From PG until the RG harvest, the mean temperature was 14.5ºC, and total precipitation 157 mm.

Experimental Diets

Experimental treatments comprised diets with RG replacing PG in the DM ratio 0, 0.33, 0.67 and 1 (treatments D1, D2, D3, and D4, respectively). Cows were fed silage *ad libitum* allowing 10% refusals. Before feeding, the silage was chopped using a roundbale chopper (Serigstad RBK 1202, Serigstad Agri, Bryne, Norway) and further with an Epple Blasius 940 (Epple Maschinen GmbH, Wiesensteig, Germany) to a chop length of 3-4 cm. For each cow, the correct proportions of the grass-clover silages were weighed in based on daily DM-determinations. The cows were individually fed three times a day and feeds were divided into the correct weight for each meal. Finally, the mixed-diets were thoroughly mixed by hand to minimize feed selection.

A compound concentrate (Felleskjøpet Agri SA, Lillestrøm, Norway) was fed 8 kg/d (as fed basis) to pluriparous- and 7 kg/d to primiparous cows containing peas (26.8%), oats (16.8%), wheat (16.5%), barley

(15%), rapeseed cake (10%), molasses (5.5%), rapeseeds (5%), $CaCO_3$ (1.3%), $Ca(H_2PO_4)_2$ (0.96%), MgO (0.66%), NaCl (0.6%), Na_2SO_4 (0.63%) and a complementary micro-mineral and vitamin mix (0.25%). The amount of added micro minerals and vitamins from the mix in the concentrate (per kg) was Ca (2,95 g), Cu (15 mg), Zn (65 mg), Mn (20 mg), I (3.5 mg), Co (0.25 mg), Se (0.3 mg), Vitamin A (5000 IU), Vitamin D (2000 IU) and Vitamin E (40 mg).

Cows were housed in a tie-stall with continuous access to water and were fed at 0630, 1415 and 2200 h. Daily feed residues remained in the feed through until directly before the morning feeding. Collection and recording of refusals were manually weighed once daily. Milking was conducted *in situ* daily at 0700 and 1700 h. All experimental procedures were carried out in accordance to the laws and regulations controlling experiments with live animals in Norway, made by the Norwegian University of Life Sciences Animal Care and Use Committee and the Norwegian Animal Research Authority.

Sampling, Recordings and Chemical Analyses

Feed intake and milk yield data from day 10 to 21 in each period were used. Daily samples of 500 g from each of the two silages were collected and freezed on Monday to Friday to provide a pooled sample from each week. After thawing a portion of each pooled sample was oven-dried at 59ºC to constant weight (minimum 24 h) and weighed warm. These portions formed the basis for daily DM intakes (DMI) of silage after correction for volatiles (Norfor 2007b). Analyses of pH was conducted using a Thermo Orion 420A+ pH-meter with Orion 9107BN electrode (Thermo Scientific,Beverly, MA, USA). Other portions of the pooled samples were freeze-dried. After drying, samples were equilibrated to room humidity overnight and milled through a 1-mm screen (Retsch GmbH cutting mill, Haan, Germany). Dried samples were analyzed for DM, ash, fat, water soluble carbohydrates (WSC) and NDF as described by Randby et al. (2010). Lignin was analyzed with H_2SO_4 corrected for ash as described by Van Soest et al. (1991) modified according to AOAC (1984). Contents of acid detergent fiber (ADF), NDF-N and ADF-N were equally corrected for ash, Kjeldahl-N was analyzed at Kjeltec 2460 (Foss Electric, Hillerød, Denmark). Indigestible NDF (iNDF) was determined according to Norfor *in sacco* standard 070910 (NorFor 2007a) using Sefar Petex 07-11/5-cloth (Sefar AG, Heiden, Switzerland) and 288 h intraruminal incubation. Potentially degradable NDF was calculated as NDF – iNDF. Metabolizable protein content, expressed as amino acids absorbed in the small intestine (AAT) and protein balance in the rumen (PBV), was calculated on background of the evaluation system according to Madsen et al. (1995), based on a fixed value for ruminal efficient protein degradability in experimental silages of 0.80 (Spörndly 2003). The

AAT/PBV-system of Madsen et al. (1995) was chosen and considered adequate for this experiment. Minerals were analyzed with inductively coupled plasma atomic emission spectroscopy on IRIS Intrepid II XSP (Thermo Fisher Scientific, Waltham, MA, USA). Chemical composition of the concentrates were analyzed with the same methods as used for the grass-clover silages. Additionally DM was determined at 105ºC and starch was determined by an enzymatic method (α-amylase and amyloglucosidase) (Megazyme, Wicklow, Ireland) in samples milled through a 0.5 mm screen.

Aliqout milk samples were collected with fractional sampling milk meters (Tru-Test Industries Ltd, Auckland, New Zealand) weekly in six subsequent milkings on day 11 to 14 and 18 to 21. Milk samples were analyzed for fat, protein, lactose, urea and free fatty acids using an infrared spectrophotometer (MilkoScan 6000, Foss Electric, Hillerød, Denmark). Calculations of energy corrected milk yield (ECM) included the concentration of lactose and was done according to Volden (2011).

Digestibility Study and Feed Value Calculations

In vivo digestibility of silages and concentrate was determined at maintenance level using three wethers per feed. Both PG- and RG-silages were single fed at 880 g/d DM, while the concentrate was evaluated feeding 450 g concentrate and 450 g DM of RG-silage a day. The contents of metabolizable energy (ME) and net energy (NE_L) were calculated on the basis of Van Es (1978).

Statistical Analysis

Mean values of measurements from day 10-15 and 16-21 in each period were used for feed intake and milk production results. All data were analyzed using the MIXED procedures of SAS software (SAS Institute Inc 2011) with the following model:

$$Y_{ijkl} = \mu + c_i + D_j + P(S)_{kl} + S_l + e_{ijkl},$$

where μ is the overall mean, c is the random effect of cow (i = 1 through 16) and D (j = 1 through 4), P(S) (k and l = 1 through 4) and S (l = 1 through 4) are the fixed effects of diet, period within square and square, respectively. Sum of squares were divided into orthogonal contrasts to assess linear and quadratic effects of the diets. No cubic effects were observed and they are therefore not included.

Results were considered significant at $P < 0.05$, and *P*-values between 0.05 and 0.1 were considered trends, while $P > 0.1$ were considered non-significant.

RESULTS

Chemical Contents of Experimental Feeds

The DM content of the wilted grass-clover crops before ensiling were 36.6% and 31.3% in PG and RG, respectively. On DM basis, 1 kg PG consisted 934 g organic matter (OM), 470 g NDF and 107 g CP. These proportions changed in the RG to respectively 915, 447 and 138 g/kg DM.

Table 1 shows the chemical composition of the experimental silages and concentrate. Silages were restrictively fermented and of good quality with low NH_3-N values and no butyric acid. Contents of OM and NDF were slightly higher in the PG than in RG. However, iNDF in NDF was 50% higher and WSC concentration 50% lower in RG than PG, while the CP content was 19% higher in RG than in PG. The CP in RG had higher concentrations of neutral detergent indigestible N (NDIN) and marginally higher concentrations of acid detergent indigestible N (ADIN).

The PG had higher in vivo digestibility of DM, OM and NDF, and higher energy values and AAT value than RG (Table 2). The in vivo digestibility of CP was higher in RG than in PG.

Nutrient Intake

Daily dietary nutrient intake of dairy cows offered the four silage diets are presented in Table 3. There was a significant linear effect of treatment on intake of all dietary parameters except ADF. As such, intake of DM, OM, NDF, pdNDF, WSC, starch and AAT decreased, while CP, PBV, iNDF and fat increased when replacing PG with RG. Total DMI decreased with 5.5% (1.09 kg DM), when PG was totally replaced with RG. The PG had at the same time higher concentrations of OM in the DM, which made the differences in OM intake between diets bigger and resulted in a 6.7% reduction when replacing PG with RG. Although the total intake of N-fractions in RG was higher, there were at the same time higher concentrations of NDIN and ADIN.

Milk Production

There was a tendency (P=0.06) to quadratic response in daily milk yield to increasing proportion of RG in the diet, with the lowest yield in D4 and the highest in D3 (Table 4). However, daily ECM yield decreased linearly with increasing proportion of second cut silage in the diet. Cows fed the D4 diet yielded on average 0.9 kg ECM less than the average of the diets containing PG. A similar effect was seen in daily milk fat yield, while milk fat concentration showed a clear linear decline to increasing proportion of RG. Protein yield showed a quadratic response to dietary treatments, with similar yields in D1, D2 and D3 and a 20 g smaller daily yield in D4. The increasing quadratic effect with increasing proportions of RG in the diet was more pronounced on milk urea than on the protein yield.

Energy and Nitrogen Use Efficiency

The decreasing intake of ME with increasing proportions of RG and still with a high milk production increased the conversion rate of ME into ECM (Table 4). To assess the N efficiency of turning feed N into milk N, the milk N produced was divided with the feed N intake. The N efficiency decreased with increasing RG proportions (Table 4).

DISCUSSION

Silage Composition

The energy content in grass species like timothy decreases rapidly after shooting due to lignification of plant cell walls. In organically managed Scandinavian grasslands, the herbage CP content is generally lower than in conventional because of exclusive use of manure as fertilizer (Byström et al. 2002; Steinshamn and Thuen 2008). Early harvested organic PG are therefore usually high in energy but low in CP. The CP content increases in the RG, due to increasing proportions of clover (Steinshamn and Thuen 2008). This experiment compared the effects of replacing silage from early PG with its RG on feed intake and milk production. We hypothesized that dairy cows offered a mixture of PG and RG silage would perform better than feeding each silage alone.

We aimed for equal NDF contents in both experimental crops, but ended up with lower concentrations in the RG. The result was a consequence of two factors; there was twice as much clover in RG than in PG and clover species have lower NDF concentrations than grasses (Huhtanen et al. 2006). Especially in relatively mature stands, clover species contribute to raising CP concentrations in grass-clover crops (Vanhatalo et al. 2008). Therefore, in line with our expectations, RG had more CP but less energy due to significantly higher proportions of iNDF and thereby lower digestibility. This is normally the case between PG and RG, regardless of conventionally or organically harvested crops (Khalili et al. 2005; Kuoppala et al. 2008). Higher clover proportion with higher CP content in RG compared to PG were expected as other studies have shown an increased clover proportion from 18% to 35% in respectively PG and RG (Govasmark et al. 2005; Steinshamn and Thuen 2008).

Nutrient Intake and Digestibility

In experiments with pure grass silages, Khalili et al. (2005) and Kuoppala et al. (2008) showed declining DMI with decreasing amounts of PG, which supported the finding of the linear decline in DMI when replacing PG with RG. The higher clover content in RG probably prevented a bigger difference in DMI. Increasing maturity of clover species have shown smaller decreases in DMI than in grasses within PG (Vanhatalo et al. 2009), however it is not certain that a pure clover diet would increase DMI, but there is an obvious positive effect of mixing clover and grass on DMI (Bertilsson and Murphy 2003; Dewhurst et al. 2003; Steinshamn 2010). Huhtanen et al. (2007) reported that the best way to predict silage DMI, independent of growth stage and harvest, was to use digestible OM in silage (*D*-value) combined with NDF quality. The model cannot accurately predict the intake response of replacement of grass with clover. Clover is known to have higher passage rate and total digestibility than grasses (Kuoppala et al. 2009). Nonetheless, the linearly decreasing silage DMI from PG to RG in this experiment can still be supported, as the PG was higher in both *D*-value and pdNDF content. Regardless of diet, the intake of starch and free glucose ranged from 10.9 to 11.5% of the total DMI. At these levels, glucose and starch do no not reduce NDF digestion significantly, compared to levels exceeding 20% of total DMI (Khalili and Huhtanen 1991; Stensig et al. 1998).

Higher energy concentration in PG increased the energy intake differences between diets compared to the differences in DMI, up to 10%. The hypothesis on decreasing energy intake and increasing N-intake with increasing proportion RG was met. Diets containing PG also offered the rumen microbes more energy from carbohydrates, which probably gave a more effective protein synthesis (Madsen et al. 1995). Under the above conditions, increased microbial activity and protein synthesis could partly compensate for lower CP in PG compared to RG silage. Furthermore, the CP concentration in the concentrate was higher than in the forages and increased the total CP concentration in D1 to 131 g/kg DM, which likely provided a sufficient N supply to the rumen microbes for an effective protein synthesis (Schwab et al. 2005). On the other hand, the milk urea of 2.25 mMol/L in D1 is below 2.6 mMol/L, which is considered the lower limit for sufficient supply of rumen degradable protein (RDP) (Nousiainen 2004). When comparing these findings it is clear that D1 is on the lower border or below of what can be tolerated of CP in the diet for the microbial protein synthesis. On the contrary, the CP of the RG had higher concentrations of both NDIN and ADIN. Whilst these N fractions are predominantly associated with rumen undegradable protein (RUP), Acid detergent indigestible N is considered indigestible in a diet with grass-clover silage (Van Soest 1994; Licitra et al. 1996) whereas NDIN is loosely bound to fiber, and as such a valuable source of N for the animal (Van Soest 1994). The latter will most likely be a part of RUP, depending on passage rates through the rumen. This meant, as the NDIN had a bigger share of CP in the RG it might have probably increased the level of RUP. The diet with pure RG was lower in AAT but still within recommended levels for the measured yield level, as the PBV was far above recommended levels for the respective diet. Thereby, forage RDP that possibly could have supported a higher rumen protein synthesis was lost due to a shortage of rumen digestible carbohydrates. Requirements of AAT were met in all diets while the PBV was calculated to be above minimum requirements in all diets. Still diets with a major proportion of RG had a high PBV value, which resulted in a lower microbial protein synthesis than if the diet was denser in energy. That means that there could have been produced more protein of microbial origin if the energy concentration had been higher (Madsen et al. 1995).

Intake of macro- and micro minerals, except for Se, was all within recommended levels for lactating cows. Selenium was provided at around 50% of the recommended 0.35 mg/kg DM (National Research Council 2001). The concentrate offered about 90% of the Se, reflecting the common challenge in Norwegian soils with low concentrations of Se. The K:Mg-ratio was slightly above recommendations. A too high ratio will make an impact on the Mg absorption. In this case, it will not have a practical importance (Newton et al. 1972).

Milk Production and Energy Utilization

Primiparous cows constituted 25% of the participating cows in the present study. Isolated, they had about 10% lower milk yield than the pluriparous cows. However, primiparous and pluriparous cows showed throughout similar diet effects on milk contents and conversion rates. Calculations based on the Nordic feed evaluation system, Norfor (Volden 2011), suggest that cows in the current experiment required 128.6 MJ NE_L daily to be able to produce 29.1 kg ECM/day with a body weight of 640 kg. This was barely the case in D4. There were a 0.3 MJ NE_L deficit in D4, while D3 offered an energy balance and the other diets had a surplus of energy. A positive energy balance on most diets is in line with the BW increase measured in average for all cows during the course of the experiment.

Milk fat concentration from cows fed diets containing PG was higher than from cows fed RG diets, possibly due to a significant higher dietary proportion of pdNDF (Beauchemin 1991) and WSC (Huhtanen et al. 2003). In addition, the RG contained more clover. Clover, and especially red clover, is known to reduce milk fat concentrations compared to grass species (Vanhatalo et al. 2009; Steinshamn 2010). The total milk fat yield remained similar in all diets except D4, due to higher milk yields in D2 and D3.

Milk protein yield (MPY) showed a quadratic response favoring the mixed diets, however, with small differences between diets. That makes this finding less significant and shows that higher dietary CP contents will not necessarily increase MPY (Huhtanen and Hristov 2009). Because of the higher CP content in RG, the N utilization efficiency decreased with increasing levels of RG. This shows that clover had a poorer conversion rate than grasses due to higher CP levels (Dewhurst et al. 2003). Still, compared to other studies with conversion rates ranging from 0.25 to 0.33 (Kuoppala et al. 2008; Eriksson et al. 2012; Randby et al. 2012), all the conversion rates in the present study were good (0.30-0.32), due to the overall moderate CP intake.

The slightly lower milk yield observed when cows were offered D4 supports findings of other experiments with lower yields in diets with RG (Peoples and Gordon 1989; Khalili et al. 2005; Kuoppala et al. 2010). Reports by Randby (1992); Vanhatalo et al. (2009) and Steinshamn (2010) suggest that feeding dairy cows legume silages results in a higher silage intake and thereby a higher milk production than pure grass silage. Dewhurst et al. (2003) found that 50% clover did not differ from a pure clover diet on DMI and milk production. However, it was also reported that pure clover diets may decrease milk yield compared to mixed grass-clover silages (Halmemies-Beauchet-Filleau et al. 2014). Based on the mentioned findings the highest feed intake and milk production should have occurred in D4. However, the effect of growth, PG versus RG, with its effect on fiber content and quality and thereby energy value are obviously more important; thus decreasing feed intake and milk production in D4 compared to the diets containing PG. Kuoppala et al. (2008) showed a clear correlation of ME intake and ECM yield. It is obvious that higher intakes ME will increase the milk yield. On the other hand, the RG contributed to a better utilization of pdNDF into milk.

Organic milk production has limitation on the use of concentrate (Counc. of the Eur. Union 2007). Similar diets to those used in this experiment, could comprise less concentrate, which would imply even lower dietary CP concentrations in the total diet. Calculations suggest that there would be expectations of too low PBV in D1, if the concentrate was fed at less than 4 kg a daily (Madsen et al. 1995). This means that there would be too little RDP for the microbes to synthesize into microbial protein. On the other hand, the NE_L would be higher than the cow could utilize for milk synthesis and, as such, the excess of energy could be partitioned into body reserves. All three diets comprising RG came out with a positive PBV even in a diet theoretically without concentrate. This suggests that a diet based on a mix of PG and RG would be more optimal than the pure PG or RG diets, if concentrate levels were below 4 kg daily, or if the actual concentrate contained less CP than in the current study. With 4 kg concentrates, or less, the hypothesis on a dietary optimal mix of PG and RG would probably have been met, but with 8 kg concentrates, as in the current study, the hypothesis was rejected because pure PG was equally good as the mixed diets.

CONCLUSION

The protein supply was not a key factor for the milk yield response. Sufficient energy supply with the PG was more important. The overall observed nitrogen use efficiency was good but significantly better with PG in the diet. As long as there were at least 33% PG in the diet, the ECM did not decrease. Milk yield tended to be higher in mixed diets, but lower fat content compared to the diet with pure PG took the overall advantage away from the mixed diets. Still in the practical use for farmers, who have to use all available resources at the farm, the mixed diets will provide a higher milk production and better economy than feeding each harvest separate.

ACKNOWLEGDEMENTS

The project was funded by the Norwegian Agricultural Agreement Research Fund (Project number 207755 in The Research Council of Norway), the County Governors of Sør- and Nord-Trøndelag, the Sør- and Nord-Trøndelag County Authorities, TINE SA and the Norwegian Agricultural Extension Service. The authors have no financial or other conflict of interest in the manuscript. Further, the authors acknowledge Torstein Garmo for his help with botanical composition, Egil Prestløkken for conducting a digestibility experiment with our feeds and finally the always present and helpful staff at the experimental unit led by Dag Kristoffer Forberg.

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Table 1.
The chemical composition of organic grass-clover silages and concentrate offered dairy cows.

Item	Primary growth		Regrowth		Concentrate	
	Mean	SE [a]	Mean	SE [a]	Mean	SE [a]
Dry matter, g/kg	369	0.49	336	0.38	876	3.9
pH	4.4	0.01	4.3	0.01		
g/kg DM						
Organic matter	932	0.47	915	0.48	922	0.69
Crude protein	116	1.0	138	0.90	165	0.25
Water soluble carbohydrates	39	2.0	26	0.64	64	0.86
Starch [b]					372	2.18
NDF [c]	501	3.4	473	2.0	154	2.8
Indigestible NDF [c]	63	1.2	97	2.6	56	1.4
Potentially digestible NDF [c]	439	3.2	377	3.7	98	3.4
Non fiber carbohydrates	246	4.0	218	2.3	566	3.0
ADF [d]	294	2.1	324	1.1	87	1.2
Acid detergent lignin	39	2.6	38	0.52	33	3.4
Fat	28	0.55	33	0.66	54	0.63
NDIN [e]	1.98	0.04	3.38	0.07	1.99	0.04
ADIN [f]	0.92	0.04	1.12	0.06	0.64	0.04
Lactic acid	13.0	0.50	17.5	0.62		
Formic acid	3.71	0.18	3.83	0.20		
Acetic acid	2.55	0.09	3.34	0.08		
Propionic acid	0.84	0.04	0.92	0.05		
Burtyric acid	0.00	0.00	0.00	0.00		
Ethanol	4.58	0.34	1.16	0.08		
Ammonia N (*g/kg N*)	11.5	0.94	14.0	0.81		
Vitamins and Minerals						
Vitamin A, *IU/kg*					5000	
Vitamin D, *IU/kg*					2000	
Vitamin E, *mg/kg*					40	
Ca, *g/kg DM*	5	0.13	7	0.20	11	0.28
P , *g/kg DM*	3	0.05	3	0.05	7	0.10
K , *g/kg DM*	20	0.39	21	0.22	10	0.07
Mg, *g/kg DM*	2	0.04	2	0.05	7	0.22
Na, *g/kg DM*	1	0.07	2	0.10	5	0.12
Cu, *mg/kg DM*	12	0.94	12	1.00	24	0.44
Zn, *mg/kg DM*	28	1.78	30	1.91	130	1.70
Mn *mg/kg DM*	41	2.37	52	2.56	81	15.2
Se, *mg/kg DM*	0.02	0.007	0.01	0.002	0.45	0.054

[a] Standard error

[b] Starch inclusive glucose.

[c] Neutral detergent fiber.

[d] Acid detergent fiber exclusive of ash.

[e] Neutral detergent insoluble nitrogen.

[f] Acid detergent insoluble nitrogen.

Table 2.

Apparent digestibility of nutrients on wethers and calculated feed values of primary growth (PG), regrowth (RG) and concentrate.

Item	PG	RG	SEM [a]	*P*-value	Concentrate
Sheep digestibility					
Dry matter	0.72	0.68	0.01	0.01	0.79
Organic matter	0.75	0.71	0.00	0.01	0.83
Crude protein	0.61	0.65	0.01	0.03	0.76
NDF [b]	0.73	0.68	0.01	0.03	0.46
Fat	0.86	0.86	0.01	0.34	0.85
Feed values					
ME [c], *MJ/kg DM*	10.3	9.7	0.67	<0.01	11.6
NE_L [d], *MJ/kg DM*	6.02	5.77	0.31	<0.01	6.61
AAT [e], *g/kg DM*	72.0	67.8	0.20	<0.01	71.2
PBV [f], *g/kg DM*	-7.80	19.9	0.81	<0.01	36.3
D-value [g]	698	649	0.36	<0.01	728

[a] Standard error of the mean

[b] Neutral detergent fiber.

[c] Metabolizable Energy.

[d] NEL, calculated according to Van Es (1978).

[e] Amino acids absorbed in the intestine (Madsen et al., 1995).

[f] Protein balance in the rumen (Madsen et al., 1995).

[g] Digestible organic matter in dry matter.

Table 3.

Effect of diets with regrowth replacing primary growth in the DM ratio 0, 0.33, 0.67 and 1 (treatments D1, D2, D3, and D4, respectively) on daily basis feed intake with orthogonal contrasts

Item	Diet					Orthogonal contrasts	
	D1	D2	D3	D4	SEM	Linear	Quadratic
DM intake, kg/d							
Grass-clover silage	15.1	15.0	14.4	14.0	0.49	<0.01	0.16
Concentrate	6.8	6.8	6.8	6.8	0.01		
Total	21.9	21.8	21.2	20.7	0.49	<0.01	0.14
Intake, kg/d							
Organic matter	20.3	19.9	19.6	19.0	0.49	<0.01	0.55
NDF [a]	8.60	8.44	7.92	7.67	0.248	<0.01	0.58
Indigestible NDF [a]	1.32	1.46	1.56	1.71	0.044	<0.01	0.74
pdNDF [b]	7.28	6.99	6.36	5.96	0.209	<0.01	0.44
ADF [c]	5.02	5.13	5.03	5.12	0.154	0.45	0.84
Acid detergent lignin	0.80	0.81	0.76	0.76	0.022	0.02	0.81
Water soluble carbohydrates	1.04	1.03	0.98	0.96	0.020	<0.01	0.85
Non fiber carbohydrates	7.29	7.31	7.13	7.05	0.125	<0.01	0.29
Starch [d]	2.39	2.39	2.39	2.38	0.004	0.04	0.18
Fat	0.85	0.87	0.87	0.89	0.020	<0.01	0.97
Crude protein	2.86	2.95	2.96	3.04	0.064	<0.01	0.84
Intake, g/d							
NDIN [e]	57.0	62.1	65.5	71.8	1.68	<0.01	0.48
ADIN [f]	21.7	23.4	24.4	26.4	0.63	<0.01	0.74
Ammonia-N	3.14	3.67	3.89	4.37	0.158	<0.01	0.83
AAT [g]	1565	1543	1469	1428	36.1	<0.01	0.41
PBV [h]	129	252	384	528	7.3	<0.01	0.13
MJ ME/d [i]	239	235	224	217	5,1	<0.01	0.38
MJ NE_L/d [j]	141	139	132	128	3.0	<0.01	0.36
DMI/BW [k]	0.0346	0.0347	0.0337	0.0331	0.0010	<0.01	0.21

[a] Neutral detergent fiber.

[b] Potentially degradable NDF.

[c] Acid detergent fiber exclusive of ash.

[d] Starch inclusive of glucose.

[e] Neutral detergent insoluble nitrogen.

[f] Acid detergent insoluble nitrogen.

[g] Amino acids absorbed in the intestine (Madsen et al., 1995).

[h] Protein balance in the rumen (Madsen et al., 1995).

[i] NE_L, calculated according to Van Es (1978).

[j] ME, calculated according to Van Es (1978).

[k] DM intake, kg/kg BW.

Table 4.

Effect of diets with regrowth replacing primary growth in the DM ratio 0, 0.33, 0.67 and 1 (treatments D1, D2, D3, and D4, respectively) on milk production and efficiency measures with orthogonal contrasts.

	Diet				SEM [a]	Orthogonal contrasts	
Item	D1	D2	D3	D4		Linear	Quadratic
Milk *kg/d*	30.2	30.2	30.6	29.9	1.16	0.50	0.06
ECM [b] *kg/d*	30.0	29.9	29.9	29.1	1.20	<0.01	0.11
Milk composition							
Fat, *%*	4.07	4.02	3.93	3.88	0.143	<0.01	0.99
Protein, *%*	3.12	3.13	3.10	3.11	0.046	0.27	0.87
Lactose, *%*	4.79	4.74	4.79	4.79	0.049	0.76	0.36
Urea, *mmol/L*	2.25	2.67	3.01	3.70	0.091	<0.01	0.01
Free fatty acids, *mEq/L*	0.44	0.34	0.34	0.37	0.059	0.20	0.08
Yield of milk components, kg/d							
Fat	1.22	1.21	1.20	1.16	0.064	<0.01	0.19
Protein	0.936	0.941	0.942	0.922	0.0293	0.14	0.04
Lactose	1.44	1.43	1.46	1.43	0.047	0.71	0.39
Efficiency measures							
ECM/MJ ME	0.126	0.127	0.134	0.134	0.0034	<0.01	0.55
ECM/Dry matter intake	1.37	1.37	1.41	1.40	0.036	0.07	0.78
Milk N/Feed N [c]	0.320	0.312	0.313	0.298	0.0067	<0.01	0.30

[a] SEM = standard error of the means.

[b] ECM = Energy corrected milk, ME = Metabolizable energy.

[c] Proportion of nitrogen in total ingested feed converted into nitrogen in milk.