A comparison of organic grass-clover silages from primary growth and regrowth in dairy cows

En sammenligning av økologisk grass-kløver surfôr fra første- og andreslått til melkekyr

Philosophiae Doctor (PhD) Thesis

Sondre Stokke Naadland

Department of Animal- and Aquacultural Sciences

Faculty of Veterinary Medicine and Biosciences

Norwegian University of Life Sciences

Ås 2016

Avhandling nr 2016:24

ISSN 1894-6402

ISBN 978-82-575-1353-5

Contents

Acknowledgements

This study was part of the project “Nutrient supply and productivity in organic forage and milk production - improved forage production and utilization based on local resources” led by Norwegian Institute of Bioeconomy Research (NIBIO). The project was funded by the Norwegian Agricultural Agreement Research Fund, the County Governors of Sør- and Nord-Trøndelag, the Sør- and Nord-Trøndelag County Authorities, TINE SA, and the Norwegian Agricultural Extension Service.

Firstly, I would like to express my gratitude to my main-supervisor Dr. Åshild Taksdal Randby for her extreme enthusiasm and valuable guidance through this process. I am also very thankful for the vast knowledge in organic dairy production that my co-supervisor Dr. Håvard Steinshamn (NIBIO) has brought to me. He was also the project leader of the project. Thanks to Dr. Sophie Julie Krizsan (NJV-SLU) for conducting fiber analyses and your good propositions in preparation of the papers.

Thanks to Dr. Torstein Garmo for his help with determination of botanical composition in my silages and Dr. Egil Prestløkken for conducting a digestibility experiment with our feeds. I would also like to thank the always present and helpful staff at the Animal Experimental Unit led by Dag Kristoffer Forberg.

Thanks to all friends and colleagues at The Department of Animal and Aquacultual Sciences for a good and open working environment. A special thanks to Tonje Marie Storlien, Jon Kristian Sommerseth and David Galmeus for valuable discussions.

Thanks to my supportive parents and brother. Finally, but not least the biggest and sincere gratitude to Solveig and our two sons, Jakob and Lavrans, for your great support and inspiration.

Ås, January 2016

Sondre Stokke Naadland

Summary

The annual milk yield of Norwegian organic dairy cows increased by 18% in 2013 compared to 2007 levels. The increased milk yield in this period was achieved by increased levels of concentrate feed in the diet. Given that, at least 50% of the dry matter (DM) in the daily diets of organic dairy cows should consist of forage during the first three months of lactation and at least 60% during the remaining lactation, feeding of high-quality forages are foreseen to be the cornerstone for the future increases in milk yield. Organic primary growth (PG) silages are usually high in metabolizable energy (ME), and in contrast to conventionally produced PG silages, they are low in crude protein (CP). Lower concentrations of potentially degradable neutral detergent fiber (pdNDF) are known to decrease ME concentration in the regrowth (RG) compared to PG. However, the CP concentration of the organic RG is higher than that of the PG, reflecting an increased inclusion of legumes with higher levels of CP than grasses. The low legume inclusion in the PG silage reflects higher temperature requirements of legumes than grasses.

To the best of my knowledge, all previous studies on grass- and legume silages from PG and RG to dairy cows have focused on feeding strategies in conventional dairy systems. Research on organic forages has only dealt with grass-clover silages, with no particular focus on PG and RG. There seemed to be a need to study the combined impact of organic PG and RG silages on feed characteristics and dairy cow performances. Therefore, this study investigated the impact of organic PG and RG grass-clover silages on feed intake, fiber- and nitrogen (N) metabolism and milk production of dairy cows.

In this study, 16 cows, grouped in four Latin squares, were fed organic grass-clover PG and RG silages harvested in Ås, Norway in 2012. Four experimental diets where RG replaced PG in the dry matter ratios of 0 (diet 1), 0.33 (diet 2), 0.67 (diet 3) and 1 (diet 4) were fed. Additionally, cows were offered 8 kg concentrate daily. Results indicated that dry matter intake (DMI) was the highest in dairy cows fed with diet 1 (15.1 kg/d), linearly declining to 14.1 kg/d in diet 4. Because of the higher concentration of ME in the PG, the calculated differences in total ME intake were more substantial than the differences in DMI (239–217 MJ ME/d). The increased differences in ME intake may be attributed to the higher organic matter concentration and digestibility, and lower indigestible neutral detergent fiber (NDF) concentration in the PG than in the RG silage

Rumen digestion of NDF was favored when the mixed diets (diet 2 and 3) were fed. Low average rumen ammonia (NH_3) concentrations of 4.9 mmol/L confirmed the low CP content in diet 1. Rumen NH_3 concentrations in this study increased to 6.4 and 7.0 mmol/L in diet 2 and 3, which promoted a faster digestion rate of NDF. Feeding diet 1 resulted in a milk urea concentration of 2.3 mmol/L, indicating a diet with low rumen degradable protein concentration for a sufficient rumen microbial protein synthesis. In particular, diet 1 promoted a high omasal flow of pdNDF, which resulted in a higher volume of pdNDF fecal excretion than in the other three diets. Higher fecal excretion of pdNDF in diet 1 can be explained by the differences in plant anatomy. That is, most stem strata of grass species are lignified as opposed to high lignin concentration in one stratum in legumes. This makes a larger part of the grass stem slowly digestible compared to legumes. Overall, the low ruminal NH_3 concentration and the stem structure in grasses are the possible reasons for the increased fecal excretion of pdNDF with increasing proportions of PG.

No effect of the higher CP intake with increasing proportions of RG in the diet was observed in the daily omasal CP flow. However, urea concentrations in blood, milk and urine all increased with increasing proportions of RG in the diet. Neither the total amino acid (AA) nor any single AA differed between diets in the daily omasal flow. Two AA, histidine and methionine, may have been present in limited quantities in all diets. Omasal flows of histidine and methionine were below recommendations.

The energy corrected milk (ECM) production (29.9–30.0 kg/d) was similar in all PG containing diets. Feeding of diet 4 promoted a lower daily ECM yield (29.1 kg/d) and lower milk fat- and protein yields. Milk fat content was lower in diet 4 (3.9%) than that in diet 1 (4.1%), reflecting the pattern of rumen butyrate concentrations. Milk protein concentrations were similar in all diets, but daily milk protein yield was the highest in diet 2 and 3, and the lowest in diet 4, reflecting the poorer N conversion in diet 4 than in all other diets.

Cows fed all three diets containing PG produced similar milk yields, but diet 2 and 3 promoted a more efficient energy conversion and a similar N conversion of feed into milk compared to diet 1. The most important suggestion from this study is for farmers to avoid feeding pure RG silages. It is important to note that low CP concentration in PG or in the concentrate, or with a lower concentrate supplementation, dietary CP concentrations may become too low and reduce milk yields. Therefore, feeding of a mixture of organic PG and RG silages may be a secure strategy for maintaining high milk yields from dairy cows. Since the current study investigated the impact of two harvests of grass-clover silages, inclusion of a third harvest as the current practice in intensive organic dairy systems can be studied further. Further research may focus on feeding red clover and less concentrate, in contrast to the current study where the diet was dominated by white clover and relatively high concentrate levels.

Sammendrag

Økologiske melkekyr i Norge har økt sin årlige melkeytelse med 18% mellom 2007 og 2013. Økningen er blitt oppnådd ved hjelp av en større kraftfôrandel i rasjonen. Minst 50% av tørrstoffet i økologiske fôrrasjoner til melkekyr skal komme fra grovfôr i de første tre månedene av laktasjonen og minst 60% i resten av laktasjonen. Derfor bør økt melkeytelse i fremtiden baseres på grovfôr av god kvalitet. Økologisk surfôr fra førsteslått (PG) har vanligvis høye nivåer av energi, men i motsetning til en konvensjonelt produsert PG, er den lav i råprotein (CP). Lavere konsentrasjoner av fordøyelig fiber (NDF) reduserer energikonsentrasjonen i gjenveksten (RG) sammenlignet med PG. En større andel belgvekster i økologisk RG øker konsentrasjonen av CP i forhold til PG. Belgvekster inneholder mer CP enn gress. Den økte andelen av belgvekster i RG beskriver belgvekstenes høyere temperaturbehov i forhold til gressarter.

Så langt jeg vet, har alle tidligere forsøk med fôring av surfôr med gress og belgvekster fra PG og RG til melkekyr hatt fokus på fôringsstrategier i konvensjonelle melkebruk. Forskning på økologisk fôr har bare studert gress-kløversurfôr, uten fokus på PG og RG. Det syntes å være et behov for å studere den kombinerte effekten av økologisk surfôr fra PG og RG på fôregenskaper og ytelse hos melkekyrne. Derfor så denne studien på effekten av økologisk gress-kløversurfôr fra PG og RG på fôropptak, omsetning av fiber og nitrogen (N) og melkeproduksjon hos kyr.

I denne studien ble 16 kyr gruppert i fire latinske kvadrater og fôret med økologisk gress-kløversurfôr fra PG og RG. Fôret ble høstet på Ås i 2012. Fire forsøksrasjoner ble fôret der RG erstattet PG i tørrstoffandeler på 0,0 (rasjon 1), 0,33 (rasjon 2), 0,67 (rasjon 3) og 1,0 (rasjon 4). Kyrne ble tildelt 8 kg kraftfôr daglig. Resultatene viste at tørrstoffinntak (DMI) var høyest hos melkekyr fôret med rasjon 1 (15,1 kg/d), og avtok lineært til 14,1 kg/d i rasjon 4. På grunn av høyere energikonsentrasjon i PG ble de beregnede forskjellene i totalt energiopptak større enn forskjellene i DMI (239–217 MJ ME/d). Den økte forskjellen i energiopptaket kan tilskrives høyere konsentrasjon av organisk stoff og bedre fordøyelighet, og mindre ufordøyelig NDF i PG.

Vomfordøyeligheten av NDF var best når de blandede rasjonene (rasjon 2 og 3) ble fôret. Konsentrasjonene av ammoniakk (NH_3) i vomma ble målt. Rasjon 1 hadde en lav gjennomsnittlig vomkonsentrasjon av NH_3 på 4,9 mmol/L og bekreftet det lave

nivået av CP i rasjon 1. Vomkonsentrasjonene av NH_3 økte til 6.4 og 7.0 mmol/L i rasjon 2 og 3, som fremmet en raskere nedbrytningshastighet av NDF. Fôring av rasjon 1 resulterte i en melkeurea på 2.3 mmol/L, noe som indikerer en rasjon med for lite vomnedbrytbart CP i rasjonen for en tilstrekkelig mikrobiell proteinsyntese i vomma. Spesielt rasjon 1 fremmet en rask daglig passasje av potensielt nedbrybar NDF (pdNDF) til bladmagen, noe som ga større gjødselutskilling av pdNDF i forhold til de andre tre rasjonene. Økt gjødselutskilling av pdNDF skyldtes forskjeller i planteanatomien mellom belgvekster og gress. I gressfamilien blir de fleste stengelsjiktene lignifisert. Hos belgvekster er lignin konsentrert i ett sjikt. Det fører til at en større del av gresstengelen fordøyes sakte i forhold til hos belgvekster. De sannsynlige årsakene til økt pdNDF-utskilling med økende andeler av PG i rasjonen er den lave vomkonsentrasjonen av NH_3 og ligninstrukturen i gresstengelen.

Det økende opptaket av CP med økende rasjonsandeler av RG ga ingen økning av daglig passasje av CP til bladmagen. Likevel økte konsentrasjonene av urea i blod, melk og urin med økende rasjonsandeler av RG. Bladmagepassasjen var lik for alle rasjoner i både total aminosyremengde og i mengden av hver enkelt aminosyre. To aminosyrer, histidin og metionin, kan ha blitt fôret i begrensende mengder for melkeproduksjonen. Passasjen til bladmagen av både histidin og metionin var lavere enn anbefalingene.

Alle rasjoner som inneholdt PG ga omtrent lik ytelse av energikorrigert melk (29,9 til 30,0 kg/d). Rasjon 4 ga lavere daglig ytelse av energikorrigert melk (29,1 kg/d), melkefett og melkeprotein. Konsentrasjonen av melkefett avtok lineært fra rasjon 1 (4,1%) til rasjon 4 (3,9%), hvilket gjenspeilte smørsyrekonsentrasjonen i vomma. Det var ingen sikre konsentrasjonsforskjeller mellom rasjonene i melkeprotein, men daglig proteinytelse var høyest i rasjon 2 og 3, og lavest i rasjon 4, noe som gjenspeilte den dårligere N-utnyttelsen ved fôring av rasjon 4 i forhold til de andre rasjonene. Kyrne hadde lik melkeytelse da de tre rasjonene som inneholdt PG ble fôret, men rasjon 2 og 3 ga en mer effektiv energiutnyttelse og en tilsvarende N-utnyttelse av fôr til melk sammenlignet med rasjon 1.

Den beste anbefalingen fra denne studien er å unngå fôring RG-surfôr alene. Likevel kan en lav konsentrasjon av CP i PG eller i kraftfôret, eller med mindre kraftfôr i rasjonen gi en for lav totalkonsentrasjon av CP, og føre til redusert melkeytelse. Derfor kan fôring av en blanding av økologisk gress-kløversurfôr fra PG og RG være en sikker strategi for å opprettholde en høy melkeytelse hos melkekyrne. Siden denne studien bare undersøkte effekten av to slåtter med gress-kløversurfôr, kan det i fremtidig forskning være aktuelt å se nærmere på tredjeslåtten i tråd med praksis i intensiv økologisk melkeproduksjon. Videre forskning kan dessuten se på effekten av å bytte ut hvitkløver med rødkløver eller bruke lavere kraftfôrnivåer enn det som ble brukt i denne studien.

Abbreviations

AA	amino acid	NDHRS	Norwegian Dairy Health Recording System
CP	crude protein	NH_3	ammonia
DM	dry matter	NE_L	net energy for lactation
DMI	dry matter intake	OM	organic matter
ECM	energy corrected milk	PBV	protein balance in rumen
His	histidine	pdNDF	potentially degradable neutral detergent fiber
iNDF	indigestible neutral detergent fiber	PG	primary growth
Leu	leucine	RDP	rumen degradable protein
Lys	lysine	RG	regrowth
ME	metabolizable energy	RUP	rumen undegradable protein
Met	methionine	TNAN	total non-ammonia nitrogen
MJ	mega joule	WSC	water soluble carbohydrate
MNAN	microbial non-ammonia nitrogen		
N	nitrogen		
NAN	non-ammonia nitrogen		
NDF	neutral detergent fiber		

List of original papers

I. Naadland, S.S., Steinshamn, H., Randby, Å.T. 2015. Effect of replacing organic grass-clover silage from primary growth with regrowth on feed intake and milk yield of dairy cows. *Organic Agriculture. DOI: 10.1007/s13165-015-0144-0.*

II. Naadland, S.S., Steinshamn, H., Krizsan, S.J., Randby, Å.T. 2015. Effect of replacing organic grass-clover silage from primary growth with regrowth on fiber digestion in dairy cows. *Animal. Submitted.*

III. Naadland, S.S., Steinshamn, H., Krizsan, S.J., Randby, Å.T. 2015. Effect of replacing organic grass-clover silage from primary growth with regrowth on N digestion in dairy cows. *Animal Feed and Technology. Submitted.*

Paper I is reproduced with kind permission of the publisher

General introduction

Organic agriculture has evolved on the idea that everything taken from nature should be returned in some way to create an everlasting sustainable cycle. This idea makes the organic agriculture less intensive than the conventional agriculture. Several studies have indicated that organic agriculture leaves a smaller footprint in terms of greenhouse gases (Kassow *et al.*, 2010, Refsgaard *et al.*, 2012, Smith *et al.*, 2015). Ruminants are responsible for a considerable share of the agriculture related greenhouse gases. Lower emissions in organic ruminant husbandry might be related to more grazing and less energy-demanding harvesting processes compared to the conventional farms. Biodiversity is proposed to be better in organic agriculture than in conventional. However, Hole *et al.* (2005) found no differences between conventional multicultures and organic agricultures. However, industrial large-scale agriculture with a limited number of various crops were not beneficial for biodiversity.

Organic cows produce less milk than cows in conventional farming systems (Sato *et al.*, 2005). Recent Norwegian statistics have shown around 15% lower milk yields in organic versus conventional dairy cows (TINE Rådgiving, 2014a, Landbruksdirektoratet, 2015). The cows' health can be improved for that reason alone, as the immune system gets energy to avoid a suppressed state. Earlier studies from the 1990's mainly indicated a better health situation in organic dairy production except in a study showing an inferior reproductive health compared to conventional production (Reksen *et al.*, 1999, Hardeng and Edge, 2001). However, feeding and management has changed since then, and more recent studies questioned the better health of organic cows. Valle *et al.* (2007) found that disease recordings per cow in the Norwegian Dairy Health Recording System (NDHRS) from organic dairy farms were fewer than from conventional farms and a closer look indicated fewer treated cases in organic dairy farms. They meant that this was not a question of organic- versus conventional dairy production, but different management like drying of mammary quarters instead of medical treatment. Another study looking into the NDHRS from 2005 until 2007 did not show any health related differences between organic and conventional dairy production. Two findings are worth mentioning; organic cows had lower somatic cell count but a higher prevalence of dry mammary quarters (Garmo *et al.*, 2010). Organic dairy production has to meet strict regulations upon animal welfare. Calves must be provided with a minimum of three months of natural cow milk and the three first days after birth they must have access to a suckling cow (Council of the European

Union, 2007). Free access to milk in the suckling period is shown to increase body weight gain and provide higher milk yields in the first lactation (Johnsen *et al.*, 2015).

Organic dairy farming in Norway is changing from being extensive in small units to intensive operated larger units. Farmers have increased the annual milk yield per organic cow from 5890 kg in 2007 to 6960 kg in 2013, mostly by increased use of concentrate from 9050 to 11,540 MJ net energy lactation (NE_L) per cow and year, in the same period (TINE Rådgiving, 2014b). Additionally, improved genetics has increased milk yield (Storli *et al.*, 2014). The organic cows constituted 4.7% of the total Norwegian dairy cow population in 2014 and the share has declined the last couple of years (TINE Råvare, 2015), despite the national goal of 15% organic production in 2020 (Landbruks- og matdepartementet, 2011). Individual milk yield in organic farming has increased more than in conventional farming, which might eradicate some of the effect of a declining share of the organic cow population.

Concentrate feeds in Norway are mostly purchased with a significant proportion of imported ingredients. Especially protein dense crops have limited (e.g., rapeseed and pulses) or no (e.g., soybeans) cultivation potential in Norway, and imported feeds are often cheaper than the domestic produced feeds. This is a challenge as imported feeds are controversial. One of the basic ideas for organic agriculture is that feed for livestock should be primarily produced locally (Leiber, 2014). Additionally, at least 50% of the dry matter intake (DMI) in the daily diets of organic dairy cows should consist of forages during the first three months of lactation and at least 60% during the remaining lactation (Council of the European Union, 2008). Grassland covered 82% of the Norwegian organic managed area in 2014 (Landbruksdirektoratet, 2015), and the most pronounced challenges for the organic dairy farming is generally to produce large enough crops with a sufficient crude protein (CP) concentration. Improved forage quality is the most important topic to pursue in the intensifying organic dairy production. Challenges are different from conventional fields due to the restrictions on use of mineral fertilizer in organic farming (Council of the European Union, 2007). Fields are harvested multiple times each year and the organic crops have generally low CP concentrations especially in the primary growth (PG). In the regrowth (RG), the CP usually increase because of an increasing proportion of legumes. White clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.) are the most common grassland legumes in Norway. The clover species have a slow spring growth rate in the cold Norwegian climate. Increasing temperatures between PG and RG increase the share of legumes in forages (Steinshamn and Thuen, 2008, Alstrup *et al.*, 2016). Red clover is the preferred legume in forage production because of its high yields compared to white clover and easier and more persistent establishment compared to lucerne (*Medicago sativa*).

Digestibility of forages is primarily determined by stage of maturity at harvest, and digestibility is often better in PG diets than in RG diets. The digestibility determines to a large extent the DMI (Huhtanen *et al.*, 2007). 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 DMI of RG silage (Kuoppala *et al.*, 2008, 2010). Grasses seem to be more likely to decrease DMI with both increasing maturity and from PG to RG compared to legumes (Huhtanen *et al.*, 2007). Bertilsson and Murphy (2003) found that feeding pure red clover silage from RG to dairy cows promoted a higher DMI than red clover from PG. However, milk yields and composition were similar. Different legumes seem to give similar DMI (Dewhurst *et al.*, 2003a)

Legumes have a higher CP concentration than grasses and they promote an increased CP concentration in grass species in mixed leys (Gierus *et al.*, 2012). The composition of legumes does also vary depending on species. White clover has usually a higher CP concentration than red clover (Dewhurst *et al.*, 2003a, Kornfelt *et al.*, 2013). Solubility is used to fractionate CP into different groups. Red clover has more slowly degradable CP than grasses and a higher concentration of rumen undegradable protein (RUP), which potentially transfers more of the CP digestion to the intestine. Rumen microbes are responsible for the initial degradation of CP to peptides, amino acids (AA) and simple nitrogen (N) fractions like ammonia (NH_3) and urea. The N fractions are thereafter reconfigured into new AA and proteins. The different plant species have different conformations of AA in their CP. The digestion impact of the conformation has been subject to several studies. Even though the conformation is changed by the rumen microbiota, it is obvious that various single AA in different feeds can be subject to limit milk production in dairy cows depending on the dietary composition. In forage based diets to dairy cow, three different AA are found to be the first limiting AA. Grasses are found to provide limited quantities of His (Vanhatalo *et al.*, 1999, Korhonen *et al.*, 2000), while red clover may be short of Met (Vanhatalo *et al.*, 2009). In addition, Lys is proposed to limit production in red clover based diets (Brito *et al.*, 2007). Studies on AA in white clover are few or non-exciting.

The neutral detergent fiber (NDF) digestibility is different between PG and RG. Feed digestibility is largely derived by the amount of potentially degradable NDF (pdNDF). Digestibilities of PG are known to be better than in RG (Huhtanen *et al.*, 2007). Grasses contain more NDF than legumes and white clover has a lower NDF content than red clover (Dewhurst, 2013). However, the concentration of indigestible NDF (iNDF) in legumes is bigger than in grasses. Considering the higher iNDF concentration, legumes are more rapidly degraded in rumen than grasses (Vanhatalo *et al.*, 2009, Kammes and Allen, 2012). White clover more than red clover are shown to reduce rumen load when fed in a significant proportion of the diet, due to a faster particle breakdown compared to grass based diets (Dewhurst *et al.*, 2003b, Kuoppala *et al.*, 2009). The reduced rumen load can cause an increased DMI and milk yield. This is derived from the forage stem structure. Legumes have concentrated their indigestible lignin in the xylem and the rest stem tissue is almost completely digestible. (Wilson and Kennedy, 1996). Grasses on the other hand, has lignin distributed throughout all tissues except the phloem. Even with a lower lignin content than legumes, the lignin in grasses protects a larger amount of cell walls from digestion and slows the digestion down (Buxton and Russell, 1988). Therefore, legumes promotes bigger rumen pools of iNDF while grasses may increase rumen pools of pdNDF (Kuoppala *et al.*, 2004). Maturity will also increase the iNDF rumen pool in both legumes and grasses.

Early harvested organic PG in Scandinavia usually yields mostly grasses with low CP concentrations (Steinshamn and Thuen, 2008). The energy concentration is high, while the CP concentration is usually too low for a sufficient supply to dairy cows (Schwab *et al.*, 2005). The temperature during summer is higher than in the spring, which is beneficial for the growth of legumes and accordingly the legume proportion increases in the RG. Legumes are normally rich in CP and accordingly CP increase in the RG yield. The energy concentration decrease and the CP conformation changes from large shares of rumen degradable protein (RDP) in PG to increasing shares of RUP in RG. Pure organic grass diets for dairy production will be too low in CP and will have an insufficient protein balance in the rumen (PBV), meaning an excess of energy compared to CP, while RG with a higher legume proportion will most likely be higher in CP but with an insufficient energy concentration to sustain the rumen microbial activity. That may give the RG a too high PBV, which is opposite of the PG. On this background, it may be suitable to mix the PG and the RG to take advantage of their different properties.

Aims and hypotheses for the thesis

The thesis is a part of a larger research project entitled “Nutrient supply and productivity in organic forage and milk production - improved forage production and utilization based on local resources” led by Norwegian Institute of Bioeconomy Research (NIBIO). The primary objective of the project was to improve the basis for tactical and strategic choices in the production, use and utilization of grassland forage in organic milk production.

In the dairy cow study, we wanted to find the best mixture of organic PG and RG silages for during lactation. Silages were prepared from an early PG and the subsequent RG of an organically managed grass-clover ley. We investigated how silage from the different growths differed with respect to feed intake, rumen fermentation, cell wall digestion, nutrient digestion, rumen passage kinetics, and N metabolism including omasal canal AA flow and milk production in cows. With that goal, we conducted a feeding trial including totally 16 dairy cows in early lactation. They were offered four different diets consisting of PG grass-clover silages replaced by RG silage in the proportions 0, 0.33, 0.67 and 1 on dry matter (DM) basis. The number of cows was reduced to a half for the metabolic part of the experiment.

Hypotheses

- Increasing proportion of RG silage in the diet reduces the total supply of metabolizable energy, increases rumen accumulation of iNDF but increases passage rate and digestion rate of pdNDF, increases the intake of N, increases the flows of microbial non-ammonia N (MNAN) and dietary non-ammonia N to the intestine and increases the total flow of AA to the intestine but in a less balanced composition for milk production

- There is a dietary optimum in the ratio between PG and RG silage with respect to energy and protein supply and AA balance to high yielding dairy cows

Materials and methods

Grass-clover silages and experimental diets

An organically managed field located in Ås, Norway (59°40'19"N, 10°46'33"E) in its second and third production year was harvested twice to make experimental grass-clover silages from the PG and the first RG in 2012. The field has been organically cultivated since 1993. The PG was harvested 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 (cv. 'Hebe', 11.3%), smooth meadow grass (*Poa pratensis* L. cv. 'Knut', 8.1%), red clover (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%).

Experimental diets comprised diets with RG replacing PG in the DM ratio 0, 0.33, 0.67 and 1 (diet 1, diet 2, diet 3, and diet 4, respectively). Cows were fed silage *ad libitum* allowing 10% refusals. 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 (27%), oats (17%), wheat (17%), barley (15%), rapeseed cake (10%), molasses (6%), 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).

Experimental Design and Animals

Totally four 4 × 4 Latin squares each with four Norwegian red cows were conducted. In the production study all 16 cows participated, but in the metabolic studies of fiber and N digestion were conducted with two Latin squares. These eight cows were equipped with a rumen cannula (Bar Diamond Inc., Parma, ID, USA). The two last Latin squares consisted of non-cannulated cows including one square of primiparous cows. The cows entered the experiment in early lactation.

Each of the four periods lasted for 21 days consisting of 9 days of adaption and 12 days of sampling. Cows were housed in a tie-stall with continuous access to water and feed, and were fed three times daily at 0630, 1415 and 2200 h. Milking was conducted daily at 0700 and 1700 h.

Samples were collected from feeds, rumen, reticulum, feces, urine, milk and blood for the experimental studies. Methods and analyses are described in details in the attached papers to this thesis.

Brief summary of papers I-III

Paper I

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

The object of this study was to test if there is a synergistic effect on DMI and milk production of offering organic grass-clover silages as mixture of PG and RG, compared with feeding the two silages separately.

Main results

- Experimental grass-clover silages were typical representatives of Scandinavian PG and RG. The PG contained 6.02 MJ NE_L/kg DM and 115 g CP/kg DM. The RG contained 5.77 NE_L/kg DM and 138 g CP/kg DM.
- Forage DMI decreased from 15.1 to 14.0 kg/d with increasing RG proportions.
- Intake of NDF decreased from 8.60 to 7.67 kg/d with increasing RG proportions.
- Intakes of iNDF and CP increased from 1.50 to 1.93 kg/d and 2.86 to 3.04 kg/d, respectively, with increasing proportions of RG.
- Calculated energy intake decreased from 141.5 to 128.4 MJ NE_L/d with increasing RG proportions.
- Diets 1–3 had similar energy corrected milk (ECM) yield (29.9-30.1 kg/d) whereas diet 4 was lower at 29.1 kg/d.
- Daily fat concentrations and yields decreased linearly with increasing RG proportions.
- Daily milk protein yield had a quadratic effect with similar yield (936-942 g/d) for the diets 1–3 and lower yield (922 g/d) for diet 4.
- Conversion efficiency of metabolizable energy (ME) to ECM improved with increasing RG proportions.
- Conversion of feed N to milk N was impaired with increasing RG proportions.

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 N conversion was good but significantly better with PG in the diet. As long as there was at least 33 % PG in the diet, the ECM did not decrease. Milk yield tended to be higher in diet 2 and 3, but lower fat content compared to the diet 1 took the overall advantage away from the diet 2 and 3. Still, in the practical use for farmers, who have to use all available resources at the farm, mixed diets will provide a higher milk production and better economy than feeding each harvest separate.

Paper II

Effect of replacing organic grass-clover silage from primary growth with regrowth on fiber digestion of dairy cows

The objective of this study was to compare rumen fiber kinetics in lactating dairy cows fed diets based on PG and RG grass-clover silages produced from the same sward.

Main results

- Omasal flow of OM, NDF and pdNDF increased with increasing PG proportions.
- Omasal flow of iNDF and CP increased with increasing RG proportions.
- Rumen digestibilities of OM, NDF and pdNDF was highest in mixed diets.
- Total tract digestibilities of NDF, pdNDF and CP increased with increasing PG proportions.
- Rumen iNDF accumulated with increasing RG proportions, and rumen pdNDF accumulated with increasing PG proportions.
- Rumen passage rate of NDF and pdNDF was lowest for mixed diets, while the digestibility rate for pdNDF increased with increasing RG proportions.
- Fecal excretion of DM and NDF increased with increasing PG proportions and decreased excretion of iNDF and a decreasing tendency for fecal excretion of CP.
- Ruminal concentrations of NH_3-N, total volatile fatty acids and acetate increased with increasing RG proportions, while butyrate increased with increasing PG proportions.

Conclusion

Although rumen DM pool sizes were similar among diets, its composition differed: Increasing dietary RG proportion decreased pool sizes of NDF and pdNDF while pool sizes of iNDF and CP increased. A greater rumen digestion rate of NDF gave a more complete total tract NDF digestion, with lower excretion of pdNDF, with increasing RG proportion. The improved NDF digestion by cows offered diet 2 and 3 was the most probable reason for similar or higher milk fat- and protein yields compared with diet 1, where the highest net energy intake was calculated.

Paper III

Effect of replacing organic grass-clover silage from primary growth with regrowth on N digestion in dairy cows

To my knowledge, there are no metabolic studies comparing PG and RG from an organic field with emphasis on N-metabolism. The objective of this study was to compare N-metabolism in lactating dairy cows fed diets based on PG and RG grass-clover silages produced from the same field. Diets consisting of PG and RG were compared when fed alone or as mixtures, with emphasis on qualitative and quantitative AA supply to the intestine.

Main results

- Intakes of CP, neutral detergent indigestible N, acid detergent indigestible N, His, non-essential AA increased with increasing RG proportions.
- Omasal flow of N fractions and AA were similar among diets but the share of MNAN in total NAN (TNAN) increased with increasing PG proportions.
- True rumen CP digestibility showed no differences among diets apart from a numerically lower digestibility in diet 4.
- Total tract CP digestibility increased with increasing RG proportions.
- Urinary N excretion increased with increasing RG proportions and gave a poorer N utilization.
- Urea increased in blood and milk with increasing RG proportions.

Conclusion

Increasing dietary proportions of RG silage increased daily intakes of CP, total AA and some single AA, including His, but neither the total AA flow to the intestine nor the flow of any single AA differed between diets. Higher daily yields of milk and milk solids were observed for cows on diet 2 and 3 than on diet 1 and 4. A more complete NDF digestion caused by higher rumen NH_3 concentrations with diet 2 and 3 might have provided those cows more energy than diet 4, and therefore increased milk yield. Methionine seemed to be the first limiting AA in the grass-clover silages with His as a possible second limiting AA. Low concentrate concentrations of Leu probably related to rapeseed meal included in the concentrate mixture might also have been a potential limiting AA.

General discussion

Purpose of the study with the organic silages

This study was conducted to understand how organic grass-clover herbage might be offered to dairy cows to maximize utilization for milk production. Effects of PG versus RG has been studied earlier but only in pure stands of either grasses or legumes and in conventional systems (Khalili *et al.*, 2005, Kuoppala *et al.*, 2008, Halmemies-Beauchet-Filleau *et al.*, 2014). The pure grown stands give results that are easier to interpret. Different proportions of plant species and general properties of cuts may confound results from mixed grass-clover leys. Pure stands of grasses normally achieve a lower concentration of CP compared to grasses growing in mixed leys with legumes (Gierus *et al.*, 2012). In the present study, the legume proportions in the grass-clover silages increased from 18% in PG to 41% in RG, which is similar to other studies (Govasmark *et al.*, 2005, Steinshamn and Thuen, 2008, Alstrup *et al.*, 2016). The results of this study may be more complicated to interpret, but they are of applied relevance. Plant species are grown in mixtures in real farm situations and the proportions of grasses and legumes in the harvested crops in the current study is to be expected on farms (Steinshamn *et al.*, 2015).

The two experimental silages were typical organic Fennoscandian representatives. The PG showed high calculated ME concentrations and a low CP concentration. In the RG, calculated ME decreased and the CP concentration increased. These features are due to the exclusive use of manure fertilizer (Bystrom *et al.*, 2002, Steinshamn and Thuen, 2008, Steinshamn *et al.*, 2015). We aimed for equal NDF concentrations in both crops, but ended up with slightly lower concentrations in the RG because of a higher proportion of legumes. Grasses are higher in NDF compared to the dominating legumes of this study, white clover and red clover (Huhtanen *et al.*, 2006). Increasing iNDF concentrations and poorer digestibility in the RG caused a lower energy concentration (Khalili *et al.*, 2005, Kuoppala *et al.*, 2008). The increased iNDF arose by higher proportions of legumes and a generally higher iNDF concentration in RG-grasses (Nousiainen *et al.*, 2004, Kuoppala *et al.*, 2008). Still, the iNDF differences between the two grass-clover silages were relatively small. White clover, the most prominent species in RG, has a smaller increase of iNDF from PG to RG compared to red clover, and especially compared to grasses (Kornfelt *et al.*, 2013).

Feed intake

Dry matter intake of RG silages by dairy cows is generally lower than DMI of PG (Huhtanen *et al.*, 2007). Different studies with pure grass silages, as well as the present study have shown decreasing DMI with increasing proportions of RG (Khalili *et al.*, 2005, Kuoppala *et al.*, 2008). The increased inclusion of legumes in RG may have inhibited an even bigger drop in DMI. Several studies have shown beneficial effects in DMI with increasing proportions of legumes in grass-clover silages (Bertilsson and Murphy, 2003, Dewhurst *et al.*, 2003a, Steinshamn, 2010). The PG had a higher *D*-value and pdNDF concentration, which is shown to increase DMI (Huhtanen *et al.*, 2007).

Daily DMI increased less than daily ME intake with increasing PG proportions, due to the higher ME concentration in PG compared to the RG. Daily CP intake decreased less than the actual CP concentration difference between PG and RG with increasing proportions of PG in the diet. The PG offered the rumen microbes more digestible carbohydrates as substrate for the protein synthesis, which probably compensated a lower CP intake (Madsen *et al.*, 1995). Dietary CP concentration at 131 g/kg DM should be sufficient for an adequate microbial protein synthesis in rumen (Schwab *et al.*, 2005), but milk urea was below 2.6 mmol/L for diet 1. It is expected that such results indicate an insufficient supply of RDP (Nousiainen, 2004). The calculated intakes of AA to the small intestine (AAT) were sufficient in all diets and the PBV were above minimum requirements but diet 3 and 4 had PBV levels higher than recommended. This means that microbial protein synthesis in rumen could have been better if more soluble carbohydrates was provided (Madsen *et al.*, 1995).

Rumen fermentation

Rumen pH fell below six for a maximum of three hours between feedings and did likely not reduce NDF digestion significantly. On the other hand, transitory low pH may have been beneficial for an increased omasal non-ammonia N (NAN) flow (Calsamiglia *et al.*, 2002). The NDF digestion may be impaired by too low NH_3 concentrations in rumen and 4.1 mmol/L is suggested as a limit (Broderick *et al.*, 2010). All diets containing PG fell below 4.1 mmol/L for about five out of the eight hours between feedings. The daily average of NH_3 when feeding diet 1 was 4.9 mmol/L and close to the mentioned limit. Cows on the diet 1 had a rumen NH_3 concentration of 2.2 mmol/L before the morning feeding, which probably resulted in a reduced microbial growth in rumen during night hours. Diet 2 and 3 were at higher diurnal average NH_3

concentrations, which likely improved rumen NDF digestion in mixed diets compared to diet 1. Still, rumen microbes seem to some extent to adapt to the actual diet and similar rumen NH_3 concentrations are observed in other experiments with higher dietary CP concentrations (Sannes *et al.*, 2002, Kuoppala *et al.*, 2009). More rapid degradation of pdNDF of the legumes in the RG seemed to increase rumen acetate and decrease rumen butyrate. A poorer NDF digestion was expected in the RG because of a higher inclusion of slowly degradable compounds. On the other hand, a bigger proportion of legumes in the RG increased pdNDF digestion rate, in line with Kuoppala *et al.* (2009), (2010). The increasing rumen butyrate concentration with increasing proportions of PG was caused by the higher concentration of water soluble carbohydrates (WSC) in PG compared to the RG, in line with Khalili and Huhtanen (1991) and Oba (2011).

Digestion of NDF and flow rates

Pure clover silages are known to reduce the rumen DM pool, but a synergistic effect of grass and clover maintained similar DM pool in all diets in our study, similar to other studies comparing PG and RG (Moseley and Jones, 1984, Dewhurst *et al.*, 2003b, Kuoppala *et al.*, 2009). White clover has a higher rumen fermentation rate that gives a bigger small particle phase and rumen outflow rate compared to other legumes and grasses (Dewhurst *et al.*, 2003b). Legumes and grasses, in general, have different stem structures, which led to increased rumen accumulation of pdNDF with increasing proportions of PG and increased rumen accumulation of iNDF with increasing proportions of RG. The PG contained more grasses than the RG, which have lignin spread out in most strata of the stem tissue. Legumes have their lignin concentrated in the xylem stratum of the stem, which increase digestion with increasing proportions of RG (Wilson and Kennedy, 1996). Also lower pdNDF concentrations and intake in the RG contributed to an increasing pdNDF digestion rate with increasing proportions of RG, in line with Weisbjerg *et al.* (2008). The presented results explain the improved NDF digestion of the RG despite higher concentrations of indigestible feed fractions in line with Kuoppala *et al.* (2009) and Halmemies-Beauchet-Filleau *et al.* (2013).

Diet 2 and 3 had higher rumen NDF digestibilities compared to diet 1 and 4, which can be related to sufficient rumen NH_3 concentrations that promoted a higher microbial activity in rumen. Diet 4 had the highest rumen concentration of NH_3, and the from that point of view the best prerequisite for a good NDF digestibility, but ruminal accumulation of iNDF may have reduced rumen NDF digestibility. Anyway, the higher rumen passage rate with increasing proportions of RG resulted in a linearly improving total tract NDF digestibility, in line with Kuoppala *et al.* (2009). Even though the proportion of digested NDF was smaller with increasing proportions of PG, the digested quantity of pdNDF was still bigger. The majority of the NDF is digested in rumen and the omasal flow of pdNDF increased with increasing proportions of PG. An increasing quantity of pdNDF was recovered in feces with increasing dietary proportions of PG. The higher fecal excretion of pdNDF indicates that there were a potential for increased milk yield. The low rumen NH_3 concentration in diet 1 was the probable reason for a slightly depressed milk yield compared to the mixed diets. Still quantitatively, diet 1 promoted the highest pdNDF digestion of all diets.

Supply of nitrogen

Similar to Vanhatalo *et al.* (2009), lower N intakes with PG in the diet increased the rumen outflow rate of N due to more accessible rumen soluble carbohydrates and accordingly a more intensive microbial protein synthesis. This was shown in the omasal flow of NAN. Although the TNAN was similar in all diets, there were an increasing share of MNAN in TNAN with increasing proportions of PG as in previous studies (Merry *et al.*, 2006, Vanhatalo *et al.*, 2009, Halmemies-Beauchet-Filleau *et al.*, 2014). Concentrations of urea in blood, urine and milk reflected the rumen NH_3 concentrations. Overall, those concentrations were in the lower reference area reflecting the similar low dietary CP concentrations. Even if all dietary CP concentrations were below 16.5% in DM, the urea concentrations increased with increasing proportions of RG. Urea concentrations are not expected to increase with the mentioned dietary CP concentrations unless the supply of ME is insufficient (Castillo *et al.*, 2001, Broderick, 2003, Colmenero and Broderick, 2006).

In silages based on grass and red clover, respectively, His and Met are found to be the most limiting AA on milk production (Ahvenjärvi *et al.*, 1999, Korhonen *et al.*, 2000, Vanhatalo *et al.*, 2009). Our study supports those findings as the grass dominated PG was lower in His, while the more clover dominated RG was lower in Met. However, the individual concentrations of both AA in both silages seemed to be below the ideal 2.2% of CP (Lee *et al.*, 2012). Concentrations of His were closer to the ideal concentration than what Met was. For a daily production of 1 kg milk protein Vyas and Erdman (2009) predicted a intake requirement of 40 g/d Met and 130 g/d Lys. Intakes of Met were close to 40 g/d in all diets, while Lys was offered in remarkably higher quantities. This suggests that Met was more limiting than Lys. Differences among AA on the level of intake were obliterated in the omasal flow.

Rapeseed constituted 15% of the concentrate. In rapeseed meal, Leu is recognized to be the first limiting AA (Boisen *et al.*, 2000). All diets in our study had contents of Leu slightly below recommendations (National Research Council, 2001). Rapeseed is found to be a useful protein source that can be grown in temperate climates (Huhtanen *et al.*, 2011). For Norwegian organic dairy production, this may be of importance, as at least 60% of the feedstuff must be locally produced (Council of the European Union, 2007). Rapeseed seems to be the best homegrown protein source for dairy cows (Khalili *et al.*, 2002).

It was expected that the omasal flow of total AA should increase with increasing proportions of RG, but the omasal flow of AA were similar between all diets. Additionally, it was expected that the milk protein synthesis should be depressed with increasing proportions of RG. This study could not support that hypothesis.

Milk production

The primiparous cows had approximately 10% lower milk yield than the pluriparous cows. However, milk contents and conversion rates from feed to milk of energy and N were similar regardless of parity. In the milk production results, we concluded slightly different between paper I (all 16 cows) and papers II and III (eight cannulated cows).

In line with earlier experiments, diet 4 promoted a lower milk yield than diets containing PG (Peoples and Gordon, 1989, Khalili *et al.*, 2005, Kuoppala *et al.*, 2008). Inclusion of legumes in grass silages is generally reported to increase DMI and milk yield (Randby, 1992, Vanhatalo *et al.*, 2009, Steinshamn, 2010). Already at a 50% inclusion of legumes, the full effect may be obtained (Dewhurst *et al.*, 2003a). In the present study, growth (PG vs. RG) and NDF properties seemed to be more important for DMI and milk yield than the inclusion of legumes. Calculations using the Norfor system (Volden, 2011) showed that there were an energy deficit in diet 4 and an exact energy balance in diet 3. We did not observe a clear advantage of diet 2 and 3 compared to diet 1 in paper I. All three diets containing PG had similar ECM yields with 16 participating cows in the study. Diet 4 gave a lower ECM yield. This changed slightly in paper II and III, where the diet 2 gave the numerically highest production. The more efficient NDF digestion in diet 2 and 3 compared to diet 1 supports the increased ECM yields. The PG alone seemed to have a too low CP concentration, which resulted in sub-optimal rumen NH_3 concentrations and reduced NDF digestion. As we discussed in paper I, a reasonable high share of concentrate in the diet probably saved diet 1 from providing a lower milk yield. The concentrate increased the CP concentration in the total diet.

The milk fat synthesis in the mammary gland is primed by butyrate Therefore, increasing rumen butyrate concentrations normally increase milk fat yield (Van Soest, 1994). Increasing grass maturity and legume proportion decrease rumen butyrate concentrations (Vanhatalo *et al.*, 2009, Steinshamn, 2010, Dewhurst, 2013), which can explain the decreasing milk fat concentration with increasing proportions of RG. The higher intakes of pdNDF (Beauchemin, 1991) and WSC (Huhtanen *et al.*, 2003) with diets containing PG promoted an increased milk fat synthesis. Due to a marginally higher milk yield with diet 2 and 3 compared to diet 1, daily milk fat yield with the three PG containing diets was similar.

Similar to the findings by Huhtanen and Hristov (2009), the higher dietary CP intake with RG did not promote a higher milk protein yield. The higher CP concentrations in the RG with more legumes promoted lower N conversion rates than the PG (Dewhurst *et al.*, 2003a). Still, due to generally low dietary CP concentrations the N conversion rates were good (Kuoppala *et al.*, 2008, Eriksson *et al.*, 2012, Randby *et al.*, 2012). Even in milk protein, the pure RG diet gave a lower production than the PG containing diets.

Conclusions

Judged by milk production results, energy intake was more important than CP intake for milk yield response. Low CP concentrations in PG promoted the best N conversion in the experiment, but overall the N conversion was good. Apparently, due to increased ruminal NH_3-concentration, the two mixed diets promoted a better NDF digestion than the two pure diets, which further elicited a higher production of milk solids. Higher N intake with increasing proportions of RG did not improve N digestion and metabolism. Neither omasal flow of total AA nor any single AA differed between diets, which is in line with a similar milk protein yield among diets. Of the single AA, one or two AA seemed to limit milk production in all diets; Met and His. Rapeseed from the concentrate may have reduced the dietary Leu concentrations around recommended level as well.

Similar yields of ECM were maintained as long as at least 33% of the forage diet consisted of PG. In total, the PG dominated mixed diet promoted the highest ECM, due to highest milk yield, daily milk fat yield and milk protein yield.

Recommendations

Farmers' advice

Often, feeding organic PG give a higher milk yield than feeding organic RG. If possible, the two cuts should be fed simultaneously. This will likely provide a better NDF digestion through a sufficient N and carbohydrate concentration in rumen and thereby increase the production of milk solids. Offering pure PG in combination with less concentrate than in the present study or, feeding a concentrate with a lower CP concentration may cause a bigger reduction in milk yield than presently observed.

A silage mixture with up to 67% RG in the diet did not seem to influence milk production negatively. In this way, it should be easy to utilize the entire organic harvest from both the PG and the RG. The most obvious recommendation from this study is to avoid feeding the RG alone.

Future research

The experimental silages used in this study were typical organic silages in Fennoscandia. These silages were botanically heterogeneous, which compared to botanically homogenous silages may give the silages different properties in the bovine digestive tract. The herbage harvested in this study had a big share of white clover. White clover has somewhat different properties compared to red clover, which is more common in grass-clover silages. Digestion of red clover may differ from white clover in terms of more rumen undegradable compounds. Therefore, it could be useful to repeat a similar experiment with more red clover rather than white clover, or red clover as the only legume. Inclusion of the third harvest could also be interesting, as it is increasingly common to use an intensive management system in organic milk production.

Feeding diets with less concentrate is also an option for further research, especially to challenge the limitation on N supply of the organic PG.

References

Ahvenjärvi S, Vanhatalo A, Huhtanen P and Varvikko T 1999. Effects of supplementation of a grass silage and barley diet with urea, rapeseed meal and heat-moisture-treated rapeseed cake on omasal digesta flow and milk production in lactating dairy cows. Acta Agriculturae Scandinavica Section a-Animal Science 49, 179-189.

Alstrup L, Søegaard K and Weisbjerg M 2016. Effects of maturity and harvest season of grass-clover silage and of forage-to-concentrate ratio on milk production of dairy cows. Journal of Dairy Science 99, 328-340.

Beauchemin K 1991. Effects of dietary neutral detergent fiber concentration and alfalfa hay quality on chewing, rumen function, and milk production of dairy cows. Journal of Dairy Science 74, 3140-3151.

Bertilsson J and Murphy M 2003. Effects of feeding clover silages on feed intake, milk production and digestion in dairy cows. Grass and Forage Science 58, 309-322.

Boisen S, Hvelplund T and Weisbjerg MR 2000. Ideal amino acid profiles as a basis for feed protein evaluation. Livestock Production Science 64, 239-251.

Brito A, Broderick G, Colmenero JO and Reynal S 2007. Effects of feeding formate-treated alfalfa silage or red clover silage on omasal nutrient flow and microbial protein synthesis in lactating dairy cows. Journal of Dairy Science 90, 1392-1404.

Broderick GA 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. Journal of Dairy Science 86, 1370-1381.

Broderick GA, Huhtanen P, Ahvenjärvi S, Reynal SM and Shingfield KJ 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle--a meta-analysis. Journal of Dairy Science 93, 3216-3230.

Buxton DR and Russell JR 1988. Lignin Constituents and Cell-Wall Digestibility of Grass and Legume Stems. Crop Science 28, 553-558.

Byström S, Jonsson S and Martinsson K 2002. Organic versus conventional dairy farming–studies from the Öjebyn Project. In Proceedings of the UK Organic Research Conference, 179-184.

Calsamiglia S, Ferret A and Devant M 2002. Effects of pH and pH fluctuations on microbial fermentation and nutrient flow from a dual-flow continuous culture system. Journal of Dairy Science 85, 574-579.

Castillo A, Kebreab E, Beever D, Barbi J, Sutton J, Kirby H and France J 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. Journal of Animal Science 79, 247-253.

Colmenero JJO and Broderick GA 2006. Effect of Dietary Crude Protein Concentration on Milk Production and Nitrogen Utilization in Lactating Dairy Cows. Journal of Dairy Science 89, 1704-1712.

Council of the European Union 2007. Counc. of the Eur. Union (2007) Council Regulation (EC) No. 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. Official Journal of the European Communities 189, 20.7.2007, 1-23.

Council of the European Union 2008. Commission regulation No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. Official Journal of the European Union. L250: 1-84.

Dewhurst R 2013. Milk production from silage: comparison of grass, legume and maize silages and their mixtures. Agricultural and Food Science 22, 57-69.

Dewhurst R, Fisher W, Tweed J and Wilkins R 2003a. Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. Journal of Dairy Science 86, 2598-2611.

Dewhurst R, Evans R, Scollan N, Moorby J, Merry R and Wilkins R 2003b. Comparison of grass and legume silages for milk production. 2. In vivo and in sacco evaluations of rumen function. Journal of Dairy Science 86, 2612-2621.

Eriksson T, Norell L and Nilsdotter-Linde N 2012. Nitrogen metabolism and milk production in dairy cows fed semi-restricted amounts of ryegrass–legume silage with birdsfoot trefoil (*Lotus corniculatus* L.) or white clover (*Trifolium repens* L.). Grass and Forage Science 67, 546-558.

Garmo RT, Waage S, Sviland S, Henriksen BI, Østerås O and Reksen O 2010. Reproductive Performance, Udder Health, and Antibiotic Resistance in Mastitis Bacteria isolated from

Norwegian Red cows in Conventional and Organic Farming. Acta Veterinaria Scandinavica 52, 1-13.

Gierus M, Kleen J, Loges R and Taube F 2012. Forage legume species determine the nutritional quality of binary mixtures with perennial ryegrass in the first production year. Animal Feed Science and Technology 172, 150-161.

Govasmark E, Steen A, Bakken A, Steinshamn H, Strøm T and Hansen S 2005. Factors affecting the concentration of Zn, Fe and Mn in herbage from organic farms and in relation to dietary requirements of ruminants. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 55, 131-142.

Halmemies-Beauchet-Filleau A, Vanhatalo A, Toivonen V, Heikkilä T, Lee MRF and Shingfield KJ 2013. Effect of replacing grass silage with red clover silage on ruminal lipid metabolism in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. Journal of Dairy Science 96, 5882-5900.

Halmemies-Beauchet-Filleau A, Vanhatalo A, Toivonen V, Heikkilä T, Lee MRF and Shingfield KJ 2014. Effect of replacing grass silage with red clover silage on nutrient digestion, nitrogen metabolism, and milk fat composition in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. Journal of Dairy Science 97, 3761-3776.

Hardeng F and Edge VL 2001. Mastitis, Ketosis, and Milk Fever in 31 Organic and 93 Conventional Norwegian Dairy Herds. Journal of Dairy Science 84, 2673-2679.

Hole DG, Perkins AJ, Wilson JD, Alexander IH, Grice PV and Evans AD 2005. Does organic farming benefit biodiversity? Biological Conservation 122, 113-130.

Huhtanen P and Hristov AN 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. Journal of Dairy Science 92, 3222-3232.

Huhtanen P, Nousiainen J and Rinne M 2006. Recent developments in forage evaluation with special reference to practical applications. Agricultural and Food Science 15, 293-323.

Huhtanen P, Rinne M and Nousiainen J 2007. Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. International Journal of Animal Biosciences 1, 758-770.

Huhtanen P, Hetta M and Swensson C 2011. Evaluation of canola meal as a protein supplement for dairy cows: A review and a meta-analysis. Canadian Journal of Animal Science 91, 529-543.

Huhtanen P, Nousiainen JI, Khalili H, Jaakkola S and Heikkilä T 2003. Relationships between silage fermentation characteristics and milk production parameters: analyses of literature data. Livestock Production Science 81, 57-73.

Johnsen JF, Zipp KA, Kälber T, Passillé AMd, Knierim U, Barth K and Mejdell CM 2015. Is rearing calves with the dam a feasible option for dairy farms? – Current and future research. Applied Animal Behaviour Science.

Kammes K and Allen M 2012. Rates of particle size reduction and passage are faster for legume compared with cool-season grass, resulting in lower rumen fill and less effective fiber. Journal of Dairy Science 95, 3288-3297.

Kassow A, Rahmann G, Blank B, Paulsen HM and Aulrich K 2010. Studies on greenhouse gas emissions in organic and conventional dairy farms. Ressortforschung für den Ökologischen Landbau, 65-76.

Khalili H and Huhtanen P 1991. Sucrose supplements in cattle given grass silage-based diet. 1. Digestion of organic matter and nitrogen. Animal Feed Science and Technology 33, 247-261.

Khalili H, Kuusela E, Suvitie M and Huhtanen P 2002. Effect of protein and energy supplements on milk production in organic farming. Animal Feed Science and Technology 98, 103-119.

Khalili H, Sairanen A, Nousiainen J and Huhtanen P 2005. Effects of silage made from primary or regrowth grass and protein supplementation on dairy cow performance. Livestock Production Science 96, 269-278.

Korhonen M, Vanhatalo A, Varvikko T and Huhtanen P 2000. Responses to graded postruminal doses of histidine in dairy cows fed grass silage diets. Journal of Dairy Science 83, 2596-2608.

Kornfelt LF, Nørgaard P and Weisbjerg MR 2013. Effect of harvest time of red and white clover silage on chewing activity and particle size distribution in boli, rumen content and faeces in cows. animal 7, 909-919.

Kuoppala K, Rinne M, Nousiainen J and Huhtanen P 2008. The effect of cutting time of grass silage in primary growth and regrowth and the interactions between silage quality and concentrate level on milk production of dairy cows. Livestock Science 116, 171-182.

Kuoppala K, Ahvenjärvi S, Rinne M and Vanhatalo A 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 2. Dry matter intake and cell wall digestion kinetics. Journal of Dairy Science 92, 5634-5644.

Kuoppala K, Rinne M, Ahvenjärvi S, Nousiainen J and Huhtanen P 2004. Digestion kinetics of NDF in dairy cows fed silages from primary growth and regrowth of grass. Journal of Animal and Feed Sciences 13, 127-130.

Kuoppala K, Rinne M, Ahvenjärvi S, Nousiainen J and Huhtanen P 2010. The effect of harvesting strategy of grass silage on digestion and nutrient supply in dairy cows. Journal of Dairy Science 93, 3253-3263.

Landbruks- og matdepartementet 2011. Melding til Stortinget 9 (2011-2012) Velkommen til bords. In (Ed. Fagbokforlaget), https://www.regjeringen.no/no/dokumenter/meld-st-9-20112012/id664980/?ch=1&q=.

Landbruksdirektoratet 2015. Produksjon og omsetning av økologiske landbruksvarer. Rapport for 2014. In (Ed. TT Ha), p. 98. https://www.slf.dep.no/no/produksjon-og-marked/kjott-og-egg/marked-og-pris/_attachment/45676?_ts=14c992a5698&download=true.

Lee C, Hristov A, Cassidy T, Heyler K, Lapierre H, Varga G, de Veth M, Patton R and Parys C 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. Journal of Dairy Science 95, 6042-6056.

Leiber F 2014. Resigning protein concentrates in dairy cattle nutrition: a problem or a chance? Organic Agriculture. 4, 269-273.

Madsen J, Hvelplund T, Weisbjerg MR, Bertilsson J, Olsson I, Spörndly R, Harstad OM, Volden H, Tuori M, Varvikko T, Huhtanen P and Olafsson BL 1995. The AAT/PBV protein evaluation system for ruminants - a revision. Norwegian Journal of Agricultural Sciences Supplement, 3-37.

Merry RJ, Lee MR, Davies DR, Dewhurst RJ, Moorby JM, Scollan ND and Theodorou MK 2006. Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. 1. In vitro and in vivo studies of nitrogen utilization. Journal of Animal Science 84, 3049-3060.

Moseley G and Jones J 1984. The physical digestion of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) in the foregut of sheep. British Journal of Nutrition 52, 381-390.

National Research Council 2001. Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001. The National Academies Press, Washington, DC.

Nousiainen J 2004. Development of tools for the nutritional management of dairy cows on silage-based diets. PhD thesis. University of Helsinki.

Nousiainen J, Ahvenjärvi S, Rinne M, Hellämäki M and Huhtanen P 2004. Prediction of indigestible cell wall fraction of grass silage by near infrared reflectance spectroscopy. Animal Feed Science and Technology 115, 295-311.

Oba M 2011. Review: Effects of feeding sugars on productivity of lactating dairy cows. Canadian Journal of Animal Science 91, 37-46.

Peoples A and Gordon F 1989. The influence of wilting and season of silage harvest and the fat and protein concentration of the supplement on milk production and food utilization by lactating cattle. Animal Production 48, 305-317.

Randby Å, Weisbjerg M, Nørgaard P and Heringstad B 2012. Early lactation feed intake and milk yield responses of dairy cows offered grass silages harvested at early maturity stages. Journal of Dairy Science 95, 304-317.

Randby ÅT 1992. Grass-clover silage for dairy cows. In 14th General Meeting of European Grassland Federation, Lahti, Finland, 272-275.

Refsgaard K, Bergsdal H, Berglann H and Pettersen J 2012. Greenhouse gas emissions from life cycle assessment of Norwegian food production systems. Acta Agriculturae Scandinavica, Section A — Animal Science 62, 336-346.

Reksen O, Tverdal A and Ropstad E 1999. A Comparative Study of Reproductive Performance in Organic and Conventional Dairy Husbandry. Journal of Dairy Science 82, 2605-2610.

Sannes R, Messman M and Vagnoni D 2002. Form of rumen-degradable carbohydrate and nitrogen on microbial protein synthesis and protein efficiency of dairy cows. Journal of Dairy Science 85, 900-908.

Sato K, Bartlett P, Erskine R and Kaneene J 2005. A comparison of production and management between Wisconsin organic and conventional dairy herds. Livestock Production Science 93, 105-115.

Schwab C, Huhtanen P, Hunt C and Hvelplund T 2005. Nitrogen Requirements of Cattle. In Nitrogen and Phosphorus Nutrition of Cattle: Reducing the Environmental Impact of Cattle Operations (eds. E Pfeffer and AN Hristov), pp. 13-70, CABI Publishing, Cambrigde, MA, USA.

Smith LG, Williams AG and Pearce BD 2015. The energy efficiency of organic agriculture: A review. Renewable Agriculture and Food Systems 30, 280-301.

Steinshamn H 2010. Effect of forage legumes on feed intake, milk production and milk quality–a review. Animal Science Papers and Reports 28, 195-206.

Steinshamn H and Thuen E 2008. White or red clover-grass silage in organic dairy milk production: Grassland productivity and milk production responses with different levels of concentrate. Livestock Science 119, 202-215.

Steinshamn H, Adler S, Frøseth R, Lunnan T, Torp T and Bakken A 2015. Yield and herbage quality from organic grass clover leys—a meta-analysis of Norwegian field trials. Organic Agriculture, 1-16.

Storli KS, Heringstad B and Salte R 2014. Effect of dams' parity and age on daughters' milk yield in Norwegian Red cows. Journal of Dairy Science 97, 6242-6249.

TINE Rådgiving 2013. TINE SA (The Norwegian Dairy Association), Ås, Norway. In, https://medlem.tine.no/cms/aktuelt/nyheter/statistikk/faglig-rapport.

TINE Rådgiving 2014. TINE SA (The Norwegian Dairy Association), Statistikksamling 2013. In (Ed. T Roalkvam), p. 69. TINE Rådgiving og Medlem, https://medlem.tine.no/cms/aktuelt/nyheter/statistikk/statistikksamling.

TINE Råvare 2015. TINE SA (The Norwegian Dairy Association), Melkeleveransene i juni 2015. In, https://medlem.tine.no/cms/aktuelt/nyheter/statistikk/stor-melkeproduksjon-f%C3%B8rste-halv%C3%A5r.

Valle PS, Lien G, Flaten O, Koesling M and Ebbesvik M 2007. Herd health and health management in organic versus conventional dairy herds in Norway. Livestock Science 112, 123-132.

Van Soest PJ 1994. Nutritional ecology of the ruminant. Comstock Publishing Associates, Ithaca, N.Y.

Vanhatalo A, Huhtanen P, Toivonen V and Varvikko T 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. Journal of Dairy Science 82, 2674-2685.

Vanhatalo A, Kuoppala K, Ahvenjärvi S and Rinne M 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. Journal of Dairy Science 92, 5620-5633.

Volden H 2011. NorFor Nordic feed evaluation system. Wageningen Academic Publishers, Wageningen, The Netherlands.

Vyas D and Erdman RA 2009. Meta-analysis of milk protein yield responses to lysine and methionine supplementation. Journal of Dairy Science 92, 5011-5018.

Weisbjerg M, Søegaard K, Hopkins A, Gustafsson T, Bertilsson J, Dalin G, Nilsdotter-Linde N and Spörndly E 2008. Feeding value of legumes and grasses at different harvest times. In Biodiversity and animal feed: future challenges for grassland production. Proceedings of the 22nd General Meeting of the European Grassland Federation, Uppsala, Sweden, 9-12 June 2008., pp. 513-515.

Wilson J and Kennedy P 1996. Plant and animal constraints to voluntary feed intake associated with fibre characteristics and particle breakdown and passage in ruminants. Crop and Pasture Science 47, 199-225.

Paper I

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

Sondre Stokke Naadland[1], Håvard Steinshamn[2] and Åshild Taksdal Randby[1]

(1) *Department of Animal and Aquacultural Sciences, Norwegian University of Life Science, Norway, 1432 Ås, Norway*

(2) *NIBIO, Norwegian Institute of Bioeconomy Research, 6630 Tingvoll, Norway*

Published in Organic Agriculture

Org. Agr.
DOI 10.1007/s13165-015-0144-0

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

Sondre Stokke Naadland · Håvard Steinshamn · Åshild Taksdal Randby

Received: 17 August 2015 / Accepted: 8 December 2015

Abstract Under Norwegian conditions, diets based on primary growth (PG) silage typically increase milk yield compared to silage prepared from the regrowth (RG). Organic PG, dominated by immature grasses, is often high in energy and low in crude protein (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-day 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 dry matter (DM) basis. Additionally, concentrate was offered with 8 kg for pluriparous and 7 kg for primiparous cows. The PG had higher content metabolizable energy (ME), potentially degradable neutral detergent fiber (NDF), and water-soluble carbohydrates, while RG contained more 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.

Keywords Dairy cow · Organic milk production · Regrowth · Silage · Grass-clover

S. S. Naadland (✉) · Å. T. Randby
Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway
e-mail: sondre.naadland@nmbu.no

H. Steinshamn
Norwegian Institute of Bioeconomy Research (NIBIO), 6630 Tingvoll, Norway

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

Published online: 16 December 2015

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, is 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 mixed 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 four Norwegian Red cows, four 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 body weight (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 years 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 t/ha on May 2 and 15 t/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 (*Trifolium 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 °C to constant weight (minimum 24 h) and botanical composition was expressed on a DM basis.

Silage production

The crops were cut with Kuhn FC 302G (Kuhn S.A., Saverne, France) mower and wilted for 9–10 h during daytime or 20–21 h overnight in PG and 9–12 h during daytime or 27–30 h overnight in RG. Thereafter, crops were baled using Orkel GP 1260 (Orkel AS, Fannrem, Norway) fixed chamber round baler 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/t 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 eight 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 t DM/ha in PG and 2.05 t 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 11.1 °C 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 14.5 °C and total precipitation 157 mm.

Experimental diets

Experimental treatments comprised diets with RG replacing PG in the DM ratios 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 round baler 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 4–5 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/day (as fed basis) to pluriparous- and 7 kg/day 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 59 °C 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 were 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, and 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 compositions of the concentrates were analyzed with the same methods as used for the grass-clover silages. Additionally, DM was determined at 105 °C and starch was determined by an enzymatic method (α-amylase and amyloglucosidase) (Megazyme, Wicklow, Ireland) in samples milled through a 0.5-mm screen.

Aliquot 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 (ECM) yield included the concentration of lactose and were 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/day 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 to 15 and 16 to 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 was 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 $P > 0.1$ was considered non-significant.

Results

Chemical contents of experimental feeds

The DM contents of the wilted grass-clover crops before ensiling were 36.6 and 31.3 % in PG and RG, respectively. On a DM basis, 1-kg PG consisted of 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

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

Item	Primary growth		Regrowth		Concentrate	
	Mean	SE	Mean	SE	Mean	SE
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					372	2.18
NDF	501	3.4	473	2.0	154	2.8
Indigestible NDF	63	1.2	97	2.6	56	1.4
Potentially digestible NDF	439	3.2	377	3.7	98	3.4
Non fiber carbohydrates	246	4.0	218	2.3	566	3.0
ADF	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	1.98	0.04	3.38	0.07	1.99	0.04
ADIN	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		
Butyric 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

SE standard error, *Starch* starch inclusive glucose, *NDF* neutral detergent fiber, *ADF* acid detergent fiber exclusive of ash, *NDIN* neutral detergent insoluble nitrogen, *ADIN* acid detergent insoluble nitrogen

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.

Table 2 Apparent digestibility of nutrients on wethers and calculated feed values of primary growth (PG), regrowth (RG), and concentrate

Item	PG	RG	SEM	*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	0.73	0.68	0.01	0.03	0.46
Fat	0.86	0.86	0.01	0.34	0.85
Feed values					
ME (MJ/kg DM)	10.3	9.7	0.67	<0.01	11.6
NE_L (MJ/kg DM)	6.02	5.77	0.31	<0.01	6.61
AAT (g/kg DM)	72.0	67.8	0.20	<0.01	71.2
PBV (g/kg DM)	−7.80	19.9	0.81	<0.01	36.3
D value	698	649	0.36	<0.01	728

SEM standard error of the mean, *NDF* neutral detergent fiber, *ME* metabolizable energy, NE_L NEL, calculated according to Van Es (1978), *AAT* amino acids absorbed in the intestine (Madsen et al. 1995), *PBV* protein balance in the rumen (Madsen et al. 1995), *D value* digestible organic matter in dry matter

Nutrient intake

Daily dietary nutrient intake of dairy cows offered the four silage diets is 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 is 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.

Table 3 Effect of diets with regrowth replacing primary growth in the DM ratios 0, 0.33, 0.67, and 1 (treatments D1, D2, D3, and D4, respectively) on daily basis feed intake with orthogonal contrasts

Item	Diet				SEM	Orthogonal contrasts	
	D1	D2	D3	D4		Linear	Quadratic
DM intake (kg/day)							
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/day)							
Organic matter	20.3	19.9	19.6	19.0	0.49	<0.01	0.55
NDF	8.60	8.44	7.92	7.67	0.248	<0.01	0.58
Indigestible NDF	1.32	1.46	1.56	1.71	0.044	<0.01	0.74
pdNDF	7.28	6.99	6.36	5.96	0.209	<0.01	0.44
ADF	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	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/day)							
NDIN	57.0	62.1	65.5	71.8	1.68	<0.01	0.48
ADIN	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	1565	1543	1469	1428	36.1	<0.01	0.41
PBV	129	252	384	528	7.3	<0.01	0.13
MJ ME/day	239	235	224	217	5,1	<0.01	0.38
MJ NE_L/day	141	139	132	128	3.0	<0.01	0.36
DMI/BW	0.0346	0.0347	0.0337	0.0331	0.0010	<0.01	0.21

SEM standard error of the mean, *NDF* neutral detergent fiber, *pdNDF* potentially degradable NDF, *ADF* acid detergent fiber exclusive of ash, *Starch* starch inclusive of glucose, *NDIN* neutral detergent insoluble nitrogen, *ADIN* acid detergent insoluble nitrogen, *AAT* amino acids absorbed in the intestine (Madsen et al. 1995), *PBV* protein balance in the rumen (Madsen et al. 1995), *MJ NE_L* NE_L, calculated according to Van Es (1978), *MJ ME/day* ME, calculated according to Van Es (1978), *DMI/BW* DM intake, kg/kg BW

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 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 was expected, as other studies have shown an increased clover proportion from 18to 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

Table 4 Effect of diets with regrowth replacing primary growth in the DM ratios 0, 0.33, 0.67, and 1 (treatments D1, D2, D3, and D4, respectively) on milk production and efficiency measures with orthogonal contrasts

Item	Diet				SEM	Orthogonal contrasts	
	D1	D2	D3	D4		Linear	Quadratic
Milk (kg/day)	30.2	30.2	30.6	29.9	1.16	0.50	0.06
ECM (kg/day)	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/day)							
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	0.320	0.312	0.313	0.298	0.0067	<0.01	0.30

SEM standard error of the means, *ECM* energy-corrected milk, *ME* metabolizable energy, *Milk N/feed N* proportion of nitrogen in total ingested feed converted into nitrogen in milk

prevented a bigger difference in DMI. Increasing maturity of clover species has 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), ADIN 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 that, 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 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 BW 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 ÅT (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 of 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 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 was 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.

Acknowledgments 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.

References

AOAC (1984) AOAC official method 7.074 fiber (acid detergent) and lignin in animal feed. In: Williams S (ed) Official methods of analysis of AOAC, 14th edn. Assoc. of Off. Anal. Chemists, Arlington, pp 162–163

Beauchemin K (1991) Effects of dietary neutral detergent fiber concentration and alfalfa hay quality on chewing, rumen function, and milk production of dairy cows. J Dairy Sci 74:3140–3151

Bertilsson J, Murphy M (2003) Effects of feeding clover silages on feed intake, milk production and digestion in dairy cows. Grass Forage Sci 58:309–322. doi:10.1046/j.1365-2494.2003.00383.x

Byström S, Jonsson S, Martinsson K (2002) Organic versus conventional dairy farming—studies from the Öjebyn project. The UK Organic Research Conference, Aberystwyth, pp 179–184

Counc. of the Eur. Union (2007) Council Regulation (EC) No. 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. Off J Eur Communities 189:1–23

Dewhurst R, Fisher W, Tweed J, Wilkins R (2003) Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. J Dairy Sci 86: 2598–2611

Eriksson T, Norell L, Nilsdotter-Linde N (2012) Nitrogen metabolism and milk production in dairy cows fed semi-restricted amounts of ryegrass–legume silage with birdsfoot trefoil (*Lotus corniculatus* L.) or white clover (*Trifolium repens* L.). Grass Forage Sci 67:546–558. doi:10.1111/j.1365-2494.2012.00882.x

Govasmark E, Steen A, Bakken A, Steinshamn H, Strøm T, Hansen S (2005) Factors affecting the concentration of Zn, Fe and Mn in herbage from organic farms and in relation to dietary requirements of ruminants. Acta Agri Scand, B—Soil Plant Sci 55:131–142. doi:10.1080/09064710510008586

Halmemies-Beauchet-Filleau A, Vanhatalo A, Toivonen V, Heikkilä T, Lee MRF, Shingfield KJ (2014) Effect of replacing grass silage with red clover silage on nutrient digestion, nitrogen metabolism, and milk fat composition in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. J Dairy Sci 97:3761–3776. doi:10.3168/jds.2013-7358

Huhtanen P, Hristov AN (2009) A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. J Dairy Sci 92: 3222–3232. doi:10.3168/jds.2008-1352

Huhtanen P, Nousiainen JI, Khalili H, Jaakkola S, Heikkilä T (2003) Relationships between silage fermentation

characteristics and milk production parameters: analyses of literature data. Livest Prod Sci 81:57–73
Huhtanen P, Nousiainen J, Rinne M et al (2006) Recent developments in forage evaluation with special reference to practical applications. Agri Food Sci 15:31
Huhtanen P, Rinne M, Nousiainen J (2007) Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. Animal 1:13
SAS Institute Inc (2011) SAS/STAT Software, Version 9.3. doi: citeulike-article-id:11105873
Khalili H, Huhtanen P (1991) Sucrose supplements in cattle given grass silage-based diet. 2. Digestion of cell wall carbohydrates. Anim Feed Sci Tech 33:263–273. doi:10.1016/0377-8401(91)90065-Z
Khalili H, Sairanen A, Nousiainen J, Huhtanen P (2005) Effects of silage made from primary or regrowth grass and protein supplementation on dairy cow performance. Livest Prod Sci 96:269–278
Kuoppala K, Rinne M, Nousiainen J, Huhtanen P (2008) The effect of cutting time of grass silage in primary growth and regrowth and the interactions between silage quality and concentrate level on milk production of dairy cows. Livest Sci 116:171–182. doi:10.1016/j.livsci.2007.10.002
Kuoppala K, Ahvenjarvi S, Rinne M, Vanhatalo A (2009) Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 2. Dry matter intake and cell wall digestion kinetics. J Dairy Sci 92:5634–5644. doi:10.3168/jds.2009-2250
Kuoppala K, Rinne M, Ahvenjarvi S, Nousiainen J, Huhtanen P (2010) The effect of harvesting strategy of grass silage on digestion and nutrient supply in dairy cows. J Dairy Sci 93: 3253–3263. doi:10.3168/jds.2009-3013
Leiber F (2014) Resigning protein concentrates in dairy cattle nutrition: a problem or a chance? Org Agri 4:269–273
Licitra G, Hernandez TM, VanSoest PJ (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim Feed Sci Tech 57:347–358. doi:10.1016/0377-8401(95)00837-3
Madsen J, Hvelplund T, Weisbjerg MR et al. (1995) The AAT/PBV protein evaluation system for ruminants—a revision. Nor J Agri Sci Suppl 35
National Research Council (2001) Nutrient requirements of dairy cattle: seventh revised edition, 2001. The Natl. Acad Press, Washington, DC
Newton G, Fontenot J, Tucker R, Polan C (1972) Effects of high dietary potassium intake on the metabolism of magnesium by sheep. J Anim Sci 35:440–445
NorFor (2007a) Norfor in sacco standard, Nordic feed evaluation system. Accessed 21.11 2014
Norfor (2007b) Norfor method for determination of dry matter vol 2015, 21 September 2007 edn
Nousiainen J (2004) Development of tools for the nutritional management of dairy cows on silage-based diets. PhD Thesis. Univ. of Helsinki
Peoples A, Gordon F (1989) The influence of wilting and season of silage harvest and the fat and protein concentration of the supplement on milk production and food utilization by lactating cattle. Anim Prod 48:305–317
TINE Rådgiving (2012) TINE SA (The Nor. Dairy Ass.), Ås, Norway
Randby ÅT (1992) Grass-clover silage for dairy cows. In: 14th Gen. Meet. of Eur. Grassl. Fed., Lahti, Finland, 8–11 June 1992 1992. pp 272–275
Randby Å, Nørgaard P, Weisbjerg M (2010) Effect of increasing plant maturity in timothy-dominated grass silage on the performance of growing/finishing. Norwegian Red Bulls Grass Forage Sci 65:273–286
Randby Å, Weisbjerg M, Nørgaard P, Heringstad B (2012) Early lactation feed intake and milk yield responses of dairy cows offered grass silages harvested at early maturity stages. J Dairy Sci 95:14
Schwab C, Huhtanen P, Hunt C, Hvelplund T (2005) Nitrogen requirements of cattle. In: Pfeffer E, Hristov AN (eds) Nitrogen and phosphorus nutrition of cattle: reducing the environmental impact of cattle operations, vol 1. CABI Publ, Cambridge, pp 13–70
Spörndly R (2003) Fodertabeller för idisslare. Rep. 257. Dep. of Anim. Nutr. Manag. Swedish Univ. Agric. Sci, Uppsala
Steinshamn H (2001) Effects of cattle slurry on the growth potential and clover proportion of organically managed grass-clover leys. Acta Agri Scand, B - Soil Plant Sci 51:12. doi: 10.1080/09064710127615
Steinshamn H (2010) Effect of forage legumes on feed intake, milk production and milk quality—a review Anim Sci Pap and Rep 28:12
Steinshamn H, Thuen E (2008) White or red clover-grass silage in organic dairy milk production: grassland productivity and milk production responses with different levels of concentrate. Livest Sci 119:14. doi:10.1016/j.livsci.2008.04.004
Stensig T, Weisbjerg MR, Hvelplund T (1998) Digestion and passage kinetics of fibre in dairy cows as affected by the proportion of wheat starch or sucrose in the diet. Acta Agri Scand, A - Anim Sci 48:12. doi:10.1080/09064709809362413
Van Es AJH (1978) Feed evaluation for ruminants. I. The systems in use from May 1977-onwards in the Netherlands. Livest Prod Sci 5:15. doi:10.1016/0301-6226(78)90029-5
Van Soest PJ (1994) Nutritional ecology of the ruminant, 2nd edn. Comstock Publ. Assoc, Ithaca
Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:15. doi:10.3168/jds.S0022-0302(91)78551-2
Vanhatalo A (2008) Effects of harvest time of red clover silage on milk production and composition. In: biodivers. and anim. feed: future chall. for grassl. prod. Proceedings of the 22nd Gen. Meet. of the Eur. Grassl. Fed. Swedish Univ. of Agri. Sci, Uppsala, pp 391–393
Vanhatalo A, Kuoppala K, Ahvenjärvi S, Rinne M (2009) Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. J Dairy Sci 92:14. doi:10.3168/jds.2009-2249
Volden H (2011) NorFor Nordic feed evaluation system Norfor—the Nordic feed evaluation system, vol 130. Wageningen Acad. Publ, Wageningen. doi:10.3920/978-90-8686-718-9

Paper II

Effect of replacing organic grass-clover silage from primary growth with regrowth on fiber digestion in dairy cows

S.S. Naadland[1], H. Steinshamn[2], S.J. Krizsan[3] and Å.T. Randby[1]

[1] *Department of Animal and Aquacultural Sciences, Norwegian University of Life Science, 1432 Ås, Norway*

[2] *NIBIO, Norwegian Institute of Bioeconomy Research, Department of Grassland and Forage, 6630 Tingvoll, Norway*

[3] *Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, 901 83 Umeå, Sweden*

Submitted to Animal

Effect of replacing organic grass-clover silage from primary growth with regrowth on fiber digestion in dairy cows

S.S. Naadland[1], H. Steinshamn[2], S.J. Krizsan[3] and Å.T. Randby[1]

[1] *Department of Animal and Aquacultural Sciences, Norwegian University of Life Science, 1432 Ås, Norway*

[2] *NIBIO, Norwegian Institute of Bioeconomy Research, Department of Grassland and Forage, 6630 Tingvoll, Norway*

[3] *Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, 901 83 Umeå, Sweden*

Corresponding author: Sondre Stokke Naadland. E-mail: sondre.naadland@nmbu.no

Short title: Fiber digestibility of silages in dairy cows

Abstract

There are differences in grass-clover proportions and chemical compositions between herbage from primary growth (PG) and regrowth (RG) in organic grass-clover leys. Mixing silages made from PG and RG may provide a more optimal diet to dairy cows than when fed separately. We tested the hypotheses that increasing dietary proportions of grass-clover silage made from RG compared with PG would increase digestion rate of potentially degradable NDF (pdNDF), and increase ruminal accumulation of indigestible NDF (iNDF). Eight rumen cannulated Norwegian Red cows were used in two replicated 4 × 4 Latin squares with 21-days periods. Organic

PG and RG silages were fed *ad libitum* in treatments with RG replacing PG in ratios of 0, 0.33, 0.67 and 1 on DM basis in addition to 8 kg concentrate. We evaluated the effect of the four diets on rumen- and total tract fiber digestibility. Increasing proportions of RG decreased silage intake by 7%. Omasal flow of pdNDF decreased whereas iNDF flow increased with increasing proportions of RG. Increasing proportions of RG decreased rumen pool sizes of NDF and pdNDF, while pool sizes of iNDF and CP increased. Increasing proportions of RG increased digestion rate of NDF, which resulted in greater total tract digestion of NDF. Pure PG diet had the highest calculated energy intake, but the improved rumen digestion of NDF by cows offered 0.33 and 0.67 of RG led to similar milk fat- and protein yields among the three diets with PG.

Keywords: dairy cows, digestibility, grass-clover, organic, regrowth

Implications

The diet based on grass-clover silage made from the primary growth provided most feed energy. However, feeding a moderate inclusion of silage made from the regrowth herbage increased rumen ammonia and improved digestion of fiber, which resulted in similar yields of milk, and milk fat and protein.

Introduction

Grassland legumes are important in organic livestock production because of their ability to fix airborne N_2 and high productivity without N fertilization. The clover species, white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.), are the most common legumes in Fennoscandinavia. Clovers have slower spring growth rates than grasses, and their proportion generally increases from PG to RG in organic grass-clover leys (Steinshamn and Thuen, 2008, Eriksen *et al*., 2012). Fiber properties are different in primary growth (PG) and regrowth (RG) as well as between grasses and legumes (Kuoppala *et al.*, 2009, 2010). Knowledge on the differences in fiber properties between species and cuts is important for dietary ration planning in ruminant production.

The concentrations of NDF and indigestible NDF (iNDF) increase with advancing maturity in grasses and legumes (Kuoppala *et al.*, 2009, Bayat *et al.*, 2011), but to a lesser extent in clover compared to grasses (Bertilsson and Murphy, 2003, Dewhurst, 2013). Pure grass silages from PG has normally higher concentrations of NDF and iNDF in NDF, and lower digestibility and energy concentration compared to RG (Khalili *et al.*, 2005, Kuoppala *et al.*, 2008). Legumes contains less NDF, higher iNDF proportion in NDF and the rumen degradation rate (k_d) of potentially degradable NDF (pdNDF) is faster compared to grasses (Kuoppala *et al.*, 2009, Kammes and Allen, 2012). Increasing proportions of clover with lower NDF concentrations in grass-clover silage is expected to increase dry matter intake (DMI), and thereby milk production, as suggested by Bertilsson and Murphy (2003) and Dewhurst *et al.* (2003a). Previous reports have shown faster particle breakdown and reduced rumen load in legumes compared to grasses (Bertilsson and Murphy, 2003, Dewhurst *et al.*, 2003b, Kuoppala *et al.*, 2009). However, diets with increasing proportions of legumes may accumulate iNDF in rumen due to the lower digestibility of RG compared to PG of grass-clover leys (Kuoppala *et al.*, 2004). Different properties of PG and RG as well as effects on intake and milk production by dairy cows are relatively well established for silages made of pure stands of grass and clover leys. However, few feeding trials with dairy cows have been carried out to investigate the effects of different proportions of silages made from PG and RG of mixed grass and clover leys on fiber digestion and metabolism in dairy cows. The objective of the present study was to compare rumen fiber kinetics in lactating dairy cows fed diets based on PG and RG grass-clover silages produced from the same sward. We hypothesized that diets based on grass-clover silage from PG fed to lactating dairy cows would provide more feed energy than diets with increasing proportions of grass-clover silage made from RG. Grass-clover silage made of RG will restrict intake and milk production due to increased rumen accumulation of iNDF.

Materials and methods

Experimental design and animals

Laws and regulations controlling experiments with live animals by Norwegian University of Life Sciences Animal Care and Use Committee and the Norwegian Animal Research Authority were implemented in the experiment. An experiment consisting of two replicated 4 x 4 Latin squares, each with 4 Norwegian Red cows, and four 21-day periods consisting of 9 days of adaption and 12 days of sampling, was conducted in fall 2012 and in spring 2013. The experimental treatments were four diets made of organic grass-clover silage from PG and RG harvested from the same field. Cows were equipped with rumen cannulae (Bar Diamond Inc., Parma, ID, USA) and entered the experiment at (mean ± SD) 56 ± 19 days in milk and BW

622 ± 83 kg. One cow was excluded from the experiment in two periods due to indigestion. Cows were housed in a tie-stall with continuous access to water and feed, and were fed three times daily at 0630, 1415 and 2200 h. Milking was conducted daily at 0700 and 1700 h.

Grass-clover silages and experimental diets

One PG and one RG silage were prepared from organically managed leys in Ås, Norway (59°40'N, 10°46'E) in 2012 (Council of the European Union, 2007). The ley mainly consisted of grass species like timothy (*Phleum pratense L.* cv. 'Grindstad') and meadow fescue (*Festuca pratensis Huds.* cv. 'Fure') together with white clover ('Hebe') and red clover ('Bjursele'). The PG and the RG contained respectively 11.3% and 39.3% white clover and 6.5% and 1.4% red clover. A detailed description of silage production and grass-clover silages was reported in Naadland *et al*. (2015, submitted results). Experimental treatments comprised diets with replacement of PG with RG silage in the proportions 0, 0.33, 0.67 and 1 (treatments D1, D2, D3, and D4, respectively) on DM basis. Cows were offered silage *ad libitum* allowing daily refusals of 10%. Silages were chopped to a median chop length of 3-4 cm, and before feeding the silage mixtures, they were hand mixed to minimize selection. Cows were additionally fed 8 kg (as fed basis) daily of a concentrate mixture containing peas (26.8%), oats (16.8%), wheat (16.5%), barley (15.0%), rapeseed cake (10.0%), molasses (5.5%), rapeseeds (5.0%) and a vitamins and mineral mixture (4.4%).

Sampling, recordings and chemical analyses

Daily samples of 1 kg from PG and RG silages were collected Monday to Friday for a pooled sample from each week in each period. The triple marker method demonstrated nutrient outflow from rumen and rumen digestibility (France and Siddons, 1986). On day 4 at 0800 h in each period a priming dose of 2,80 g Cr (Cr-EDTA) and 2,46 g Yb (Yb-acetate) was poured into rumen. Directly followed by a continuous infusion of 2,80 g Cr/d and 2,46 g Yb/d ending on day 14 at 1500 h. Infusions of flow markers were administered using a peristaltic pump (Cenco Instruments MIJ N.V., Breda, the Netherlands) with individual lines directly into the rumen of each cow. The third marker was internal iNDF supporting the two other markers to differentiate between fluid phase (FP), small particles (SP) and large particles (LP). The easier conductible sampling at the omasal orifice in reticulum was preferred over sampling in omasum (Krizsan *et al.*, 2010). Reticular spot samples were collected using a 250 mL Erlenmeyer flask with a rubber stopper to sample a totally 1200 mL. Samples were used to determine digesta flow to the omasal canal and collected 3 times daily on day 12 at 0900, 1330 and 1800 h, on day 13 at 0730, 1200 and 1630 h and on day 14 at 0600, 1030 and 1500 h. Samples of 600 g from each time point were pooled to a total of 5400 ml from each period. Pooled samples were frozen at -20ºC in the same container directly after sampling. After thawing , they were filtered and centrifuged at 1,000 × *g* for 10 min at 5ºC to separate the digesta into LP, SP and FP as described by Krizsan *et al.* (2010). Total collection of feces and urine to measure total digestibility was conducted from day 10 to 12. Urine was separated from the feces using a funnel device, bonded around to vulva, leading urine in a hose ending into a container. To prevent NH_3 volatilization the container was daily added 1.5 L with 10% H_2SO_4 solution. To assess ruminal fermentation, liquid samples of 250 ml were collected on day 17 at 0600, 0730, 0900, 1030, 1200, 1330, 1500 and 1630 h. Directly after sampling pH was measured. From each sampling, 9.5 mL ruminal liquid was filled in a 15 mL test tube with 0.5 mL formic acid for NH_3 analysis. A 50 mL test tube was added 2 mL formic acid and from each sampling 5 mL ruminal fluid for volatile fatty acids (VFA) analyses. Samples were kept at 4ºC until analyses. Rumen evacuations were conducted on day 19 and 21 at 0600 and 0930 h, at expected maximum and minimum rumen fill, respectively. From each Latin square, two cows were evacuated at 0600 h and two other cows at 0930 h on day 19. On day 21, cows and times were changed. Organic matter (OM), DM, CP, NDF and iNDF were analyzed.

Aliquot 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 and milk urea with infrared spectrophotometer (MilkoScan 6000, Foss Electric, Hillerød, Denmark). Blood samples were collected on day 18 at 0600, 0900 and 1200 h from the coccygeal vessels, which were considered similar to arterial blood entering the mammary gland. Blood collection tubes (Vacuette®, Greiner Bio-One, Frickenhausen, Germany) containing Li-heparin was used for glucose, non-esterified fatty acids and beta-hydroxybutyric acid (BHBA) analyses. Additionally serum tubes were used for urea analyses. Li-heparin tubes were immediately cooled and centrifuged (3000 × *g* for 10 min.). Serum tubes were stored at room temperature to coagulate for 2 h and centrifuged (3000 × *g* for 10 min.). All samples were pooled across sampling times to provide one sample per cow per period. Chemical analyses of feeds are described in detail in Naadland *et al*. (2015, submitted results). The same methods were used to assess chemical composition of digesta and fecal samples. Rumen fluid was analyzed for VFA with gas chromatography in Finnigan Focus GC (Thermo Fisher Scientific, Waltham, MA, USA) and NH_3-N using the flow injection analyzer FIAstar 5010 (Tecator AB, Höganäs, Sweden). The markers Cr and Yb in reticular contents and feces were analyzed in an atomic absorption spectrophonometer (GBC SavantAA Ser. No A6990, GBC Scientific Equipment, Hampshire, IL, USA) as described by Njåstad *et al.* (2014).

Calculations and statistical analysis

Fecal recovery was used to correct marker concentrations as described by Krizsan *et al.* (2010). Flows of OM were corrected for VFA (Ahvenjarvi *et al.*, 2002) and microbial OM. Results of rumen evacuations were based on the mean of both evacuations in each period. These results provided the basis for calculations of fractional rates of intake (k_i), passage (k_p) and digestion (k_d):

$$k_i = 1/24 \times (\text{intake, kg/d})/(\text{rumen pool size, kg});$$

$$k_p = 1/24 \times (\text{omasal canal flow, kg/d}) / (\text{rumen pool size, kg});$$

$$k_d = k_i - k_p.$$

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

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

where μ is the overall mean, c is random effect of cow (i = 1 through 8) and D (j = 1 through 4), P(S) (k = 1 through 4) and S (l = 1 and 2) are the fixed effects of diet, period within square and square. Sum of squares were divided into orthogonal contrasts to assess linear and quadratic effects of the diets. The following model for repeated measures with the MIXED procedure of SAS was used to assess the effect of experimental diets on diurnal variation in rumen fermentation:

$$Y_{ijklm} = \mu + c_i + D_j + P(S)_{kl} + T_m + (PT)_{km} + (DP)_{jk} + e_{ijklm},$$

where T is fixed effect of time after morning feeding. Other letters have the same meaning as mentioned above. Results were considered significant at $P < 0.05$, and P-values between 0.05 and 0.1 were considered trends, while $P \geq 0.1$ were considered non-significant.

Results

Grass silages, feed intake and fiber kinetics

Both silages were well preserved, with restricted fermentation (low concentration of fermentation acids), no butyric acid and low concentration of NH_3 and low pH (Table 1). Intakes of DM, OM, pdNDF and water soluble carbohydrates (WSC) increased with increasing proportions of PG in the diet whereas intakes of iNDF and CP increased with increasing proportions of RG (Table 2). Flows of DM through the omasal orifice did not differ between diets, while the flow of OM tended ($P = 0.09$) to decrease linearly with increasing RG proportion (Table 2). There were linear and quadratic responses to increasing RG proportion in the diet on omasal flow of NDF and pdNDF, with clearly highest values observed for D1, and lowest values for D2 and D4, respectively. The flow of iNDF increased linearly with increasing proportion of RG in the diet. There was a quadratic response to diet on rumen true OM, NDF and pdNDF digestibilities with the highest values observed for the mixed diets, D3 and D2, respectively (Table 2). Total tract digestibility of NDF tended to ($P = 0.06$) and that of pdNDF increased linearly with increasing RG proportion.

Silage type had no effect on rumen pool sizes of DM and OM (Table 3). Pool size of NDF tended to decrease (P = 0.05) while pdNDF decreased with increasing proportions of RG. On the other hand, pool sizes of iNDF and CP increased (P < 0.001) with increasing proportions of RG. There was a quadratic response of diet on k_p of pdNDF, with the lowest rate in D2 and similar and high rates in D1 and D4. A similar response (P = 0.07) to diet was observed for NDF. The k_d of pdNDF increased linearly with increasing proportions of RG. The amount of excreted feces DM reflected the DMI, and decreased with increasing RG proportion (Table 4). Fecal excretions of NDF were lowest, and iNDF highest, in D4. The excretion of N through feces tended (P = 0.07) to decrease with increasing RG proportion whereas N excretion through urine notably increased. The total N excretion through feces and urine was highest for D4, both measured as daily amount and as a proportion of ingested N.

Rumen fermentation

Diurnal pattern of rumen pH were similar among diets, with the highest values before the morning feeding (average value 6.35) and the lowest values 4.5 h after the morning feeding (average value 5.95) (Not presented). The rumen NH_3 concentrations increased linearly with increasing proportions of RG in the diet (Table 5). The dietary effect on ruminal NH_3 diminished around and after the afternoon feeding (Figure 1). The total VFA concentrations increased linearly with increasing proportions of RG in the diet (Table 5). Acetic acid was the main contributor to that result, as there were significantly higher concentrations in D4 than in all other diets. Butyrate and valerate decreased significantly with increasing RG proportion.

Milk production and blood metabolites

The highest daily milk yield and milk fat yield were observed for D2 (Table 6), and the same tendency was found for milk protein yield. This gave an overall significant quadratic effect for daily energy corrected milk yield, with the highest yield for D2 and the lowest yield for D4. Energy corrected milk yields of the three diets containing PG were similar. There were few detectable differences between diets on the measured blood parameters. Blood urea increased with increasing proportions of RG in the diet. At the same time, BHBA showed numerically decreasing plasma concentrations with increasing proportions of RG (Table 6).

Discussion

Properties of the two experimental silages

The purpose of the present study was to compare the effects of replacing organic grass-clover silage from PG with the first RG from the same field on rumen fiber kinetics. Other studies have mainly focused on pure stands of legumes and grasses (Dewhurst *et al.*, 2003a, Halmemies-Beauchet-Filleau *et al.*, 2013). The interpretation of results are simpler when plant species are grown and fed individually, as the effect of cut may be confounded with species effect when species are mixed and their relative proportion changes between cuts. However, species in pure stands may give the herbage different properties than when grown in mixtures. Especially grasses increases their CP concentrations when growing with legumes (Gierus *et al.*, 2012). The total clover content in the leys increased from 18 to 41% from PG to RG in the present study, which realistically is achieved in mixed grass-clover leys (Steinshamn and Thuen, 2008, Alstrup *et al.*, 2015). Therefore, the results from the present study has applied relevance. The differences in chemical composition between PG and RG were as expected, and can be seen as typical representatives of organic forages in Fennoscandinavia. Two reasons led to an increased iNDF concentration in the RG compared to PG: A significantly higher share of clover and a higher concentration of iNDF in the grasses (Nousiainen *et al.*, 2004, Kuoppala *et al.*, 2008). Grasses increase iNDF more than red clover from PG to RG (Bertilsson and Murphy, 2003), and red clover has shown a greater iNDF increase than white clover (Kornfelt *et al.*, 2013). Compared to the observation in the referred studies, the actual difference in iNDF between RG and PG silages was relatively small, probably because white clover was quantitatively the dominating legume in our study.

Rumen fermentation

The rumen pH did not fall below six for more than three hours between morning and afternoon feeding for any diet. That makes it unlikely that rumen pH inhibited fiber digestion (Calsamiglia *et al.*, 2002). Higher intakes of CP with increasing proportions of RG resulted in significantly higher NH_3-N concentrations in rumen even before morning feeding, which may have influenced fiber digestion. Fiber digestion is impaired by too low NH_3-N concentrations, and it is suggested that NH_3-N concentrations should not fall below 7 mg/dL (Broderick *et al.*, 2010). All diets except the one with pure RG fell below 7 mg/dL for around five of the eight hours interfeeding, while the pure RG diet was in suboptimal NH_3-N concentrations around three hours interfeeding. At least theoretically, the rumen microbes seemed to be on the border of what they could tolerate for further growth when PG was offered as only forage. That diet should have an inferior microbial reproduction before morning feeding compared to the other diets (Broderick *et al.*, 2010). However, the rumen microbes seem to adapt to the diet as other experiments including diets with far greater dietary CP levels ended up with similar ruminal NH_3-N concentrations as shown here (Sannes *et al.*, 2001, Kuoppala *et al.*, 2009). Rumen acetate is mainly derived from fermentation of fiber and molar acetate proportion increases with dietary NDF concentrations (Vanhatalo *et al.*, 2009). The observed increased rumen molar proportions of acetate and decreased rumen butyrate with increasing RG proportion were likely caused by more rapid digestion of pdNDF. Normally there would be expected lower NDF digestibility in RG, but the increasing proportion of clover promoted a faster digestion (Kuoppala *et al.*, 2009, 2010). Lower WSC concentration in RG than in PG silage might be the reason for the decreasing ruminal butyrate concentrations with increasing dietary RG proportion (Khalili and Huhtanen, 1991, Oba, 2011). The dietary effect on rumen butyrate concentrations were also reflected in the numerical differences in venous BHBA concentrations. Higher rumen butyrate concentrations in cows receiving PG diets may have contributed to the linearly increased milk fat production with increasing proportions of PG (Van Soest, 1994). Silage from increasing maturity of grasses and fed to dairy cows has also resulted in increased concentrations of rumen acetate and decreased concentrations of butyrate (Vanhatalo *et al.*, 2009), as in the present experiment with different cuts. Feeding legumes also results in higher rumen concentrations of both total VFA and acetate to butyrate ratio than grasses (Bertilsson and Murphy, 2003, Dewhurst *et al.*, 2003b, Vanhatalo *et al.*, 2009). Those previous experiments focused on plant species and maturity but not the effect of different cuts from mixed grass-clover, as in the present study. Rumen total VFA concentrations from PG and RG in pure grass silage are found to differ very little (Kuoppala *et al.*, 2010).

Digestion of NDF and flow rates

Rumen pool sizes in dairy cows are found to decrease when silage contain more than 50% legumes in the silage (Dewhurst *et al.*, 2003b). Therefore, with less than 50% legumes in the present study, similar quantities of rumen contents and DM pool size were expected. A smaller rumen DM pool would have been expected with a pure clover forage, but there is likely a synergistic effect of grass-clover silages to maintain a greater DM pool (Moseley and Jones, 1984, Dewhurst *et al.*, 2003b, Kuoppala *et al.*, 2009). Differences in NDF quality in rumen may explain the tendencies seen in OM digestibility, in other words an apparent positive synergistic effect of PG and RG mixes compared to pure diets on rumen digestibility of OM. Rumen accumulation of pdNDF with increasing proportions of PG was observed due to a proportional slower omasal canal flow compared to feed intake. Grasses dominated in the PG and the even distribution of lignin in the grass tissue makes the rate of cell wall digestion slower than in legumes (Wilson and Kennedy, 1996). The digestibility of pdNDF increased with increasing proportions of RG in both rumen and total tract. The mixed diets had slower k_p compared to the two pure diets. However, the k_d of pdNDF increased linearly with increasing proportions of RG with more legumes, which was probably due to lower NDF concentrations in the forage (Weisbjerg and Søegaard, 2008). At the same time, iNDF seemed to accumulate in rumen with increasing proportions of RG due to the rigid lignification in xylem of legumes. Other strata of the stem that contained no or very little iNDF were easily digested. This might be more obvious when the leaf to stem ratio declines with more advanced maturity (Wilson and Kennedy, 1996). Findings are in line with others (Kuoppala *et al.*, 2009, Halmemies-Beauchet-Filleau *et al.*, 2013). White clover has a higher fermentation rate in rumen compared to other grasses and legumes, which gives a higher small particle fraction and higher outflow rate (Dewhurst *et al.*, 2003b). These characteristics can explain the higher digestion rates of RG in spite of higher indigestible concentrations compared to PG. Decreasing DMI with increasing proportions of RG was probably caused by decreasing digestibility, more than the share of clover (Huhtanen *et al.*, 2007).

The higher rumen NDF digestibility in the mixed than the pure diets suggests greater microbial activity in the mixed diets. On the other hand, there were linearly increasing total VFA concentrations in rumen with increasing proportions of RG, suggesting that more VFA would be intermediary metabolized. Important are the ratios among different VFA. Butyrate is the primer of the milk fat synthesis (Van Soest, 1994). Especially immature grasses are beneficial for increased milk fat synthesis compared to grasses with advanced maturity and clover (Vanhatalo *et al.*, 2009). The pure RG diet may have suffered of an increasing accumulation of iNDF in rumen whereas the accumulation diminished with a 0.33 inclusion of PG. Increasing proportions of RG gave increasing total tract digestibility of NDF and pdNDF, probably caused by the increasing clover proportion (Kuoppala *et al.*, 2009). Bigger quantities of NDF was digested with increasing proportions of PG, but the proportion of digested NDF was greater with increasing proportions of RG. The increasing excretion of pdNDF with increasing proportions of PG suggests a potential for higher milk production. Increasing dietary proportions of PG gave a higher omasal pdNDF flow and the lowest rumen digestibility. Little pdNDF is digested in the intestine, which indicates a correlation between total digestibility and rumen digestibility (Kuoppala *et al.*, 2004, 2009). The consequence was that diets containing increasing proportions of PG lost more pdNDF through feces. Still, pure RG promoted the lowest milk production. That is explained by the bigger quantity of pdNDF digested due to higher dietary intake with increasing proportions of PG. If pure PG had been fed restrictively, the digestion of pdNDF would probably have improved, but milk yield would have dropped. Dietary effects on ECM were small within diets containing PG, but overall the mixed diets were preferable. They were similar in daily milk fat yield with the pure PG diet. In line with the hypothesis, increasing dietary proportions of organic RG increased digestion rate of pdNDF, assumingly due to its significant clover proportion. The PG offered a higher feed energy concentration and consumption compared with the RG, largely caused by a lower iNDF concentration. This resulted in higher daily production of milk solids. The hypothesis on increasing k_p of pdNDF with increasing dietary RG proportion was not confirmed. The k_p of pdNDF was lowest for the mixed diets, and contributed to highest rumen NDF digestion, which further may have contributed to similar daily milk solid production with the mixed diets as with pure PG, in spite of slightly lower daily OM intake.

Conclusion

Although rumen DM pool sizes were similar among diets, its composition differed: Increasing dietary RG proportion decreased pool sizes of NDF and pdNDF while pool sizes of iNDF and CP increased. A greater digestion rate of NDF gave a more complete total tract fiber digestion, with lower excretion of pdNDF, with increasing RG proportion. The improved NDF digestion by cows offered 0.33 or 0.66 of RG was the most probable reason for similar or higher milk fat- and protein yields compared with pure PG diet, where the highest net energy intake was calculated.

Acknowledgements

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 and the always helpful staff at the experimental unit led by Dag Kristoffer Forberg.

References

Ahvenjarvi S, Vanhatalo A and Huhtanen P 2002. Supplementing barley or rapeseed meal to dairy cows fed grass-red clover silage: I. Rumen degradability and microbial flow. Journal of Animal Science 80, 2176-2187.

Alstrup L, Søegaard K and Weisbjerg MR 2015. Effects of maturity and harvest season of grass-clover silage and of forage-to-concentrate ratio on milk production of dairy cows. Journal of Dairy Science, doi:10.3168/jds.2015-9802, Published online by Elsevier 21 October 2015.

Bayat AR, Rinne M, Kuoppala K, Ahvenjärvi S and Huhtanen P 2011. Ruminal large and small particle kinetics in dairy cows fed primary growth and regrowth grass silages harvested at two stages of growth. Animal Feed Science and Technology 165, 51-60.

Bertilsson J and Murphy M 2003. Effects of feeding clover silages on feed intake, milk production and digestion in dairy cows. Grass and Forage Science 58, 309-322.

Broderick GA, Huhtanen P, Ahvenjarvi S, Reynal SM and Shingfield KJ 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle-a meta-analysis. Journal of Dairy Science 93, 3216-3230.

Calsamiglia S, Ferret A and Devant M 2002. Effects of pH and pH Fluctuations on Microbial Fermentation and Nutrient Flow from a Dual-Flow Continuous Culture System. Journal of Dairy Science 85, 574-579.

Counc. of the Eur. Union (2007) Council Regulation (EC) No. 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. Official Journal European Communities 189, 20.7.2007, 1-23.

Dewhurst R 2013. Milk production from silage: comparison of grass, legume and maize silages and their mixtures. Agricultural and Food Science 22, 57-69.

Dewhurst RJ, Fisher WJ, Tweed JKS and Wilkins RJ 2003a. Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. Journal of Dairy Science 86, 2598-2611.

Dewhurst RJ, Evans RT, Scollan ND, Moorby JM, Merry RJ and Wilkins RJ 2003b. Comparison of grass and legume silages for milk production. 2. *In vivo* and *in sacco* evaluations of rumen function. Journal of Dairy Science 86, 2612-2621.

France J and Siddons RC 1986. Determination of digesta flow by continuous market infusion. Journal of Theoretical Biology 121, 105-119.

Gierus M, Kleen J, Loges R and Taube F 2012. Forage legume species determine the nutritional quality of binary mixtures with perennial ryegrass in the first production year. Animal Feed Science and Technology 172, 150-161.

Halmemies-Beauchet-Filleau A, Vanhatalo A, Toivonen V, Heikkilä T, Lee MRF and Shingfield KJ 2013. Effect of replacing grass silage with red clover silage on ruminal lipid metabolism in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. Journal of Dairy Science 96, 5882-5900.

Huhtanen P, Rinne M and Nousiainen J 2007. Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. Animal 1, 758-770.

Kammes KL and Allen MS 2012. Rates of particle size reduction and passage are faster for legume compared with cool-season grass, resulting in lower rumen fill and less effective fiber. Journal of Dairy Science 95, 3288-3297.

Khalili H and Huhtanen P 1991. Sucrose supplements in cattle given grass silage-based diet. 1. Digestion of organic matter and nitrogen. Animal Feed Science and Technology 33, 247-261.

Khalili H, Sairanen A, Nousiainen J and Huhtanen P 2005. Effects of silage made from primary or regrowth grass and protein supplementation on dairy cow performance. Livestock Production Science 96, 269-278.

Kornfelt LF, Nørgaard P and Weisbjerg MR 2013. Effect of harvest time of red and white clover silage on chewing activity and particle size distribution in boli, rumen content and faeces in cows. Animal 7, 909-919.

Krizsan SJ, Ahvenjärvi S, Volden H and Broderick GA 2010. Estimation of rumen outflow in dairy cows fed grass silage-based diets by use of reticular sampling as an alternative to sampling from the omasal canal. Journal of Dairy Science 93, 1138-1147.

Kuoppala K, Rinne M, Nousiainen J and Huhtanen P 2008. The effect of cutting time of grass silage in primary growth and regrowth and the interactions between silage quality and concentrate level on milk production of dairy cows. Livestock Science 116, 171-182.

Kuoppala K, Ahvenjarvi S, Rinne M and Vanhatalo A 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 2. Dry matter intake and cell wall digestion kinetics. Journal of Dairy Science 92, 5634-5644.

Kuoppala K, Rinne M, Ahvenjarvi S, Nousiainen J and Huhtanen P 2004. Digestion kinetics of NDF in dairy cows fed silages from primary growth and regrowth of grass. Journal of Animal and Feed Sciences 13, 127-130.

Kuoppala K, Rinne M, Ahvenjarvi S, Nousiainen J and Huhtanen P 2010. The effect of harvesting strategy of grass silage on digestion and nutrient supply in dairy cows. Journal of Dairy Science 93, 3253-3263.

Moseley G and Jones JR 1984. The physical digestion of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) in the foregut of sheep. British Journal of Nutrition 52, 381-390.

Njåstad KM, Adler SA, Hansen-Møller J, Thuen E, Gustavsson A-M and Steinshamn H 2014. Gastrointestinal metabolism of phytoestrogens in lactating dairy cows fed silages with different botanical composition. Journal of Dairy Science 97, 7735-7750.

Nousiainen J, Ahvenjärvi S, Rinne M, Hellämäki M and Huhtanen P 2004. Prediction of indigestible cell wall fraction of grass silage by near infrared reflectance spectroscopy. Animal Feed Science and Technology 115, 295-311.

Oba M 2011. Review: Effects of feeding sugars on productivity of lactating dairy cows. Canadian Journal of Animal Science 91, 37-46.

Sannes RA, Messman MA and Vagnoni DB 2001. Form of Rumen-Degradable Carbohydrate and Nitrogen on Microbial Protein Synthesis and Protein Efficiency of Dairy Cows. Journal of Dairy Science 85, 900-908.

Steinshamn H and Thuen E 2008. White or red clover-grass silage in organic dairy milk production: Grassland productivity and milk production responses with different levels of concentrate. Livestock Science 119, 202-215.

Van Es AJH 1978. Feed evaluation for ruminants. I. The systems in use from May 1977-onwards in The Netherlands. Livestock Production Science 5, 331-345.

Van Soest PJ 1994. Nutritional ecology of the ruminant. Comstock Publishing Associates, Ithaca, N.Y, USA.

Vanhatalo A, Kuoppala K, Ahvenjärvi S and Rinne M 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. Journal of Dairy Science 92, 5620-5633.

Weisbjerg MR and Søegaard K 2008. Feeding value of legumes and grasses at different harvest times. In in Proceedings to 22nd General Meeting of the European Grassland Federation, Uppsala, Sweden, 9-12 June 2008., Uppsala, Sweden, pp. 513-515.

Wilson JR and Kennedy PM 1996. Plant and animal constraints to voluntary feed intake associated with fibre characteristics and particle breakdown and passage in ruminants. Australian Journal of Agricultural Research 47, 199-225.

Table 1 The chemical composition of organic grass-clover silages (n = 16) and concentrate (n = 4). Silages were used in diets to dairy cows with regrowth replacing primary growth in the DM ratio 0, 0.33, 0.67 and 1 (treatments D1, D2, D3, and D4, respectively)

	Primary growth		Regrowth		Concentrate	
Item	Mean	SE	Mean	SE	Mean	SE
Dry matter, g/kg	369	0.5	336	0.4	876	3.9
pH	4.43	0.012	4.31	0.010		
g/kg dry matter						
Organic matter	932	0.5	915	0.5	922	0.7
CP	116	1.0	138	0.9	165	0.3
Water soluble carbohydrates	39	2.0	26	0.6	64	0.9
NDF	501	3.4	473	2.0	154	2.8
iNDF [1]	63	1.2	97	2.6	56	1.4
pdNDF [2]	439	3.2	377	3.7	98	3.4
ADL	39	2.6	38	0.5	33	3.4

[1] Indigestible NDF.

[2] Potentially degradable NDF.

Table 2 Effect of replacing organic grass-clover silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (treatments D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on feed intake, omasal flow and digestibilities (n = 16)

	Diet					Orthogonal contrasts	
Item	D1	D2	D3	D4	SEM	Linear	Quadratic
Dry matter intake, kg/d							
Grass-clover silage	15.1	14.9	14.4	14.1	0.70	<0.01	0.55
Total	22.1	21.9	21.4	21.0	0.70	<0.01	0.56
Intake [1]							
OM [2]	20.5	20.3	19.7	19.3	0.64	<0.01	0.51
NDF	8.64	8.40	7.97	7.72	0.382	<0.01	1.00
iNDF [3]	1.33	1.46	1.58	1.73	0.060	<0.01	0.72
pdNDF [4]	7.31	6.94	6.39	5.99	0.328	<0.01	0.90
Water soluble carbohydrates	1.06	1.05	1.00	0.99	0.030	0.02	0.92
CP	2.90	2.97	3.00	3.08	0.097	<0.01	0.77
MJ NEL/d [5]	147	143	137	132	4.6	<0.01	0.15
Omasal canal flow, kg/d							
OM	11.4	11.1	10.2	10.7	0.52	0.09	0.29
NDF	3.61	3.07	3.13	3.20	0.161	0.05	0.03
iNDF	1.15	1.26	1.33	1.47	0.047	<0.01	0.63
pdNDF	2.46	1.81	1.80	1.72	0.140	<0.01	0.02
CP	3.14	3.18	2.97	3.29	0.183	0.73	0.34
Digestibility in rumen, %							
OM, true	62.4	64.5	66.7	63.2	1.25	0.40	0.04
NDF	57.8	64.2	60.8	58.9	2.09	0.99	0.02
pdNDF	65.9	74.3	71.7	71.4	2.25	0.07	0.02
CP, true	64.6	63.8	65.8	60.7	2.51	0.36	0.35
Digestibility in total tract, %							
OM	74.1	75.5	75.7	75.4	0.58	0.11	0.15
NDF	63.7	65.8	65.9	66.8	1.18	0.06	0.54
pdNDF	72.9	76.3	78.0	81.5	1.18	<0.01	0.99
CP	68.5	71.0	72.2	73.2	0.61	<0.01	0.22
NDF digestibility, rumen/total	0.905	0.980	0.923	0.888	0.0279	0.35	0.05

[1] kg/d unless else is stated.

[2] Organic matter

[3] Indigestible NDF.

[4] Potentially degradable NDF.

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

Table 3 Effect of replacing organic grass-clover silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (treatments D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on rumen pool size, passage- and digestion kinetics (n=8)

	Diet					Orthogonal contrasts	
Item	D1	D2	D3	D4	SEM	Linear	Quadratic
Rumen content, kg	87.6	87.6	85.2	89.0	3.51	0.81	0.28
Rumen contents, kg							
DM [1]	10.92	11.00	10.67	11.03	0.465	1.00	0.58
OM [2]	10.02	10.07	9.74	10.01	0.434	0.73	0.64
CP	1.67	1.77	1.83	1.96	0.081	<0.01	0.64
NDF	6.53	6.44	6.00	6.07	0.285	0.05	0.69
pdNDF [3]	4.94	4.53	3.96	3.57	0.228	<0.01	0.98
iNDF [4]	1.60	1.91	2.04	2.50	0.116	<0.01	0.17
% / h							
DM, kp [5]	5.81	5.64	5.40	5.28	0.335	0.11	0.92
DM, kd [6]	2.74	2.87	3.14	2.84	0.222	0.53	0.30
OM, kp	6.34	6.16	5.92	5.82	0.366	0.15	0.89
OM, kd	2.33	2.44	2.72	2.39	0.245	0.64	0.33
NDF, kp	2.36	1.99	2.20	2.19	0.138	0.46	0.07
NDF, kd	3.24	3.59	3.38	3.32	0.235	0.97	0.31
pdNDF, kp	2.16	1.69	1.91	2.09	0.176	0.98	0.04
pdNDF, kd	4.19	4.89	4.86	5.23	0.348	0.04	0.61

[1] Dry matter

[2] Organic matter

[3] Potentially degradable NDF.

[4] Indigestible NDF.

[5] Rate of passage.

[4] Rate of digestion.

Table 4 Effect of replacing organic grass-clover silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (treatments D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on fecal and urinary excretion (n=8)

	Diet					Orthogonal contrasts	
Item	D1	D2	D3	D4	SEM	Linear	Quadratic
Feces, kg/d							
Dry matter	5.86	5.66	5.41	5.42	0.169	<0.01	0.25
CP	0.95	0.92	0.91	0.91	0.029	0.07	0.28
NDF	3.12	2.94	2.73	2.57	0.094	<0.01	0.84
iNDF[1]	1.15	1.26	1.33	1.48	0.047	<0.01	0.62
Urine, g/d							
N	87.5	98.8	106.1	125.9	3.62	<0.01	0.18

[1] Indigestible NDF.

Table 5 Effect of replacing organic grass-clover silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (treatments D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on rumen fermentation (n=8)

	Diet					*P*-value[1]
Item	D1	D2	D3	D4	SEM	Linear
pH	6.15	6.07	6.12	6.10	0.043	0.42
NH_3-N mg/dL	6.86	8.93	9.76	11.81	0.728	<0.01
Total VFA[2], mmol/L	117	122	123	126	4.3	0.01
In total VFA, mmol/mol						
Acetate	642	649	650	660	5.1	<0.01
Propionate	201	200	205	196	5.0	0.52
Butyrate	124	117	113	113	5.9	<0.01
Isobutyrate	5.86	5.92	5.85	6.01	0.371	0.71
Valerate	16.1	15.2	14.6	13.7	0.46	<0.01
Isovalerate	11.4	11.4	11.1	10.6	0.91	0.33
(Acetate + Butyrate)/Propionate	3.86	3.88	3.77	4.01	0.115	0.43

[1] Probability of significant effect of linear response to diet. The quadratic response to diet was not significant for any trait ($P \geq 0.25$).

[2] Volatile fatty acids.

Table 6 Effect of replacing organic grass-clover silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (treatments D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on milk production (n = 16) and blood metabolites from a coccygial blood vessel (n=8)

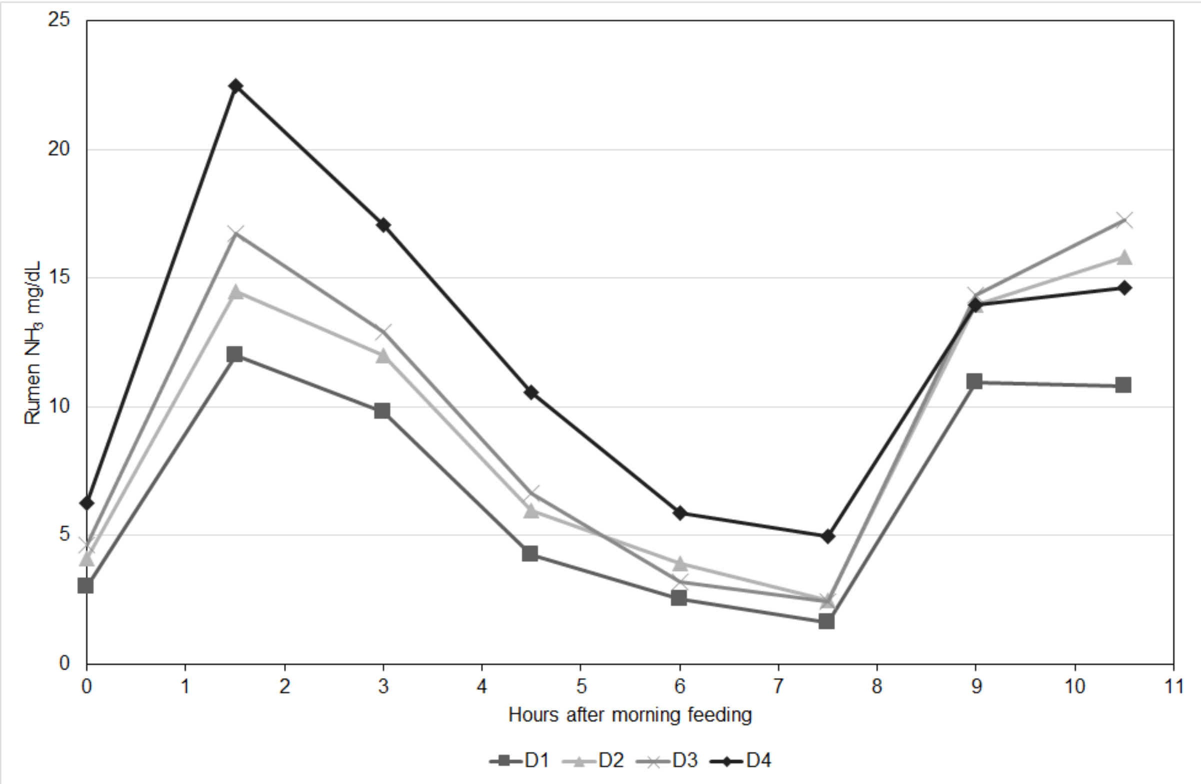
	Diet					Orthogonal contrasts	
Item	D1	D2	D3	D4	SEM	Linear	Quadratic
Milk kg/d	30.5	30.9	30.8	29.9	1.53	0.14	0.05
Energy corrected milk kg/d	30.6	31.0	30.4	29.3	1.97	<0.01	0.03
Yield of milk components, kg/d							
Fat	1.248	1.286	1.228	1.175	0.113	<0.01	0.04
Protein	0.959	0.978	0.964	0.940	0.039	0.10	0.02
Blood concentrations, mmol/L							
NEFA [1]	0.19	0.18	0.20	0.20	0.013	0.31	0.68
BHBA [2]	1.11	1.06	1.04	0.98	0.109	0.14	0.94
Glucose	3.18	3.19	3.23	3.18	0.093	0.88	0.62
Urea	1.85	2.47	2.81	3.65	0.224	<0.01	0.59

[1] Non esterified fatty acids.

[2] Betahydroxybutyric acid.

Figure captions

Figure 1 Effect of replacing organic grass-clover silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (treatments D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on diurnal variations of rumen NH_3-N concentrations (n=8).



Paper III

Effect of replacing organic grass-clover silage from primary growth with regrowth on N digestion in dairy cows

S.S. Naadland[a], H. Steinshamn[b], S. J. Krizsan[c], Å.T. Randby[a]

[1] *Department of Animal and Aquacultural Sciences, Norwegian University of Life Science, Norway, 1432 Ås, Norway*

[2] *NIBIO, Norwegian Institute of Bioeconomy Research, Department of Grassland and Forage, 6630 Tingvoll, Norway*

[3] *Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, 901 83 Umeå, Sweden*

Submitted to Animal Feed Science and Technology

Effect of replacing organic grass-clover silage from primary growth with regrowth on N digestion in dairy cows

S.S. Naadland[a], H. Steinshamn[b], S. J. Krizsan[c], Å.T. Randby[a]

[1] *Department of Animal and Aquacultural Sciences, Norwegian University of Life Science, Norway, 1432 Ås, Norway*

[2] *NIBIO, Norwegian Institute of Bioeconomy Research, Department of Grassland and Forage, 6630 Tingvoll, Norway*

[3] *Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, 901 83 Umeå, Sweden*

[*] Corresponding author: sondre.naadland@nmbu.no

Highlights

- Cows had higher N-intake from organic regrowth silage than from primary growth.
- A dietary mix of 0.67 primary growth and 0.33 regrowth maximized milk production.
- The flow of amino acids to the intestine did not differ between diets.
- Energy intake determined milk yield more than nitrogen intake.
- Methionine and histidine were probably the most limiting amino acids.

Abbreviations

AA, amino acid; AAT, amino acids to the intestine; BW, body weight; CP, crude protein; DM, dry matter; DMI, dry matter intake; EAA, essential amino acid; ECM, energy corrected milk; FP, fluid phase; LP, large particle phase; PBV, protein balance in rumen; PG, primary growth; RG, regrowth; SP, small particle phase.

Abstract

Clover, proportions and chemical composition of herbage differ between primary growth (PG) and regrowth (RG) in organic managed grass-clover fields. The difference in feed characteristics demand different supplementary feeding strategies to achieve high milk production by dairy cows, and a mix of silages made from PG and RG may provide a more optimal diet than fed separately. The RG normally offers more N in the diet than the PG because of a higher proportion of clover. Additionally, grasses and clovers have different amino acid (AA) profiles. Histidine seems to be a marginal AA in grasses, while methionine seems to be a marginal AA in clovers. Eight rumen cannulated Norwegian Red cows were used in two replicated 4 × 4 Latin squares with 21-days periods. Organic PG and RG silages were fed ad libitum in treatments with RG replacing PG in ratios of 0, 0.33, 0.67 and 1 on DM basis in addition to 8 kg/d of concentrate. Changing RG proportions from 0 to 1 increased N intake by 6% and rumen NH_3-concentrations from 4.9 to 8.4 mmol/L, but did not promote a better protein supply. Neither total ruminal outflow of AA nor the AA profile in the small intestine differed between dietary treatments. Methionine and histidine were probably the most limiting AA for a higher milk production. Limitations by histidine seemed more related to PG, while limitation by methionine seemed more related to RG.

Keywords: dairy cows, nitrogen, grass-clover, organic milk production, regrowth

1. Introduction

Organic agriculture depend on legumes and their ability to fix atmospheric N due to restrictions on the use of mineral fertilizers (Counc of the Eur Union, 2007). Grassland legumes used in Fennoscandinavia have a higher optimal growth temperature than their companion grasses, and due to low spring temperatures the legume proportion of the total yield from mixed leys is usually lower in the primary growth (PG) than in the regrowth (RG) (Steinshamn and Thuen, 2008; Eriksen et al., 2012). The organic PG has accordingly a relatively low N concentration due to a higher proportion of grasses, while the RG contains more legumes and has a higher N concentration than the corresponding PG (Steinshamn et al., 2015). It is desired to obtain a diet providing a high quantity of amino acids absorbed in the intestine (AAT), and a positive protein balance in the rumen (PBV), which depends on the amount of rumen digestible carbohydrates and N. Positive PBV-values describes sufficient amount of carbohydrates for the rumen microbial protein synthesis. Low N concentrations and high concentrations of rumen digestible carbohydrates in PG might initiate a negative PBV value, whereas PBV usually increases in a legume-rich RG. In mixtures with grasses, legumes usually promote an increased dry matter intake (DMI) and a correspondingly increased milk production compared to grasses alone (Dewhurst et al., 2003; Vanhatalo et al., 2009).

The grass protein has a greater share of rumen degradable protein (RDP) compared to legume protein, which potentially increase microbial protein synthesis (Halmemies-Beauchet-Filleau et al., 2014). Addition of the limiting essential AA (EAA) to an unbalanced forage AA-profile might increase milk production (Korhonen et al., 2000; Vanhatalo et al., 2009; Lee et al., 2012). Red clover (*Trifolium pratense* L.) dominated diets are probably primarily limited by Met (Vanhatalo et al., 2009). Levels of Met can be assumed similar in red clover and white clover (Reverter et al., 1999). Studies with grass based diets have shown

His to be the most limiting AA (Vanhatalo et al., 1999; Korhonen et al., 2000). Omasal flow of Met and His should each constitute 2.5% of total omasal crude protein (CP) flow (National Research Council, 2001; Lee et al., 2012). Lys is recommended at 7.2% of CP in omasal flow and in a 3:1 relationship to Met (National Research Council, 2001). However, restricted dietary Lys or a general negative PBV in early lactation is not expected to limit milk yield due to body tissue mobilization (Doepel et al., 2002; Mjoun et al., 2010).

To our knowledge, no previous studies have tested organic grass-clover silages made from PG and RG in the diets to lactating dairy cows with primary focus on the N metabolism. The objective of this study was to compare N metabolism with emphasis on qualitative as well as quantitative AA supply to the small intestine in lactating dairy cows fed diets based on PG and RG from grass-clover silages produced from the same field. We tested the hypotheses that increasing dietary RG proportions would increase AA flow to the small intestine, and that milk production from the RG with a large legume proportion is limited by a less balanced AA profile compared to PG.

2. Materials and methods

Laws and regulations controlling experiments with live animals by Norwegian University of Life Sciences Animal Care and Use Committee and the Norwegian Animal Research Authority were implemented in the experiment.

2.1. Experimental design and animals

An experiment consisting of two replicated 4 x 4 Latin squares, each with 4 Norwegian Red cows, and four 21-day periods consisting of 9 days of adaption and 12 days of sampling, was conducted in fall 2012 and spring 2013. Experimental treatments were four diets made of organic grass-clover silage from PG and RG harvested from the same field. Cows were equipped with rumen cannulae (Bar Diamond Inc., Parma, ID, USA) and entered the experiment at (mean ± SD) 56 ± 19 days *post partum* and BW 622 ± 83 kg. Indigestion excluded one cow from two experimental periods. Cows were housed in a tie-stall with continuous access to water and feed, and fed three times daily at 0630, 1415 and 2200 h. Milking was conducted daily at 0700 and 1700 h.

2.2. Grass-clover silages and experimental diets

The PG and RG silages were prepared from organically managed fields in Ås, Norway (59°40'N, 10°46'E) in 2012 (Council of the European Union, 2007). The ley consisted mainly of the grass species timothy (*Phleum pratense* L. cv. 'Grindstad') and meadow fescue (*Festuca pratensis* Huds. cv. 'Fure') and grassland legumes white clover (*Trifolium repens* L. cv. 'Hebe') and red clover ('Bjursele'). The PG and the RG contained 11.3% and 39.3% white clover and 6.5% and 1.4% red clover, respectively. Naadland et al. (2015) reported a detailed description of silage production and grass-clover silages. Experimental treatments comprised diets with replacement of PG and RG silage in the proportions 0, 0.33, 0.67 and 1 (treatments D1, D2, D3 and D4, respectively) on DM basis. Silages was offered *ad libitum* allowing 10% refusals daily. Silages were chopped to a median length of 4-5 cm and hand mixed before feeding to minimize selection. Cows were additionally fed 8 kg (as fed basis) daily of a concentrate mixture containing peas (26.8%), oats (16.8%), wheat (16.5%), barley (15.0%), rapeseed cake (10.0%), molasses (5.5%), rapeseed seeds (5.0%) and a vitamins and mineral mixture (4.4%; Natura Minovit Drøv, Felleskjøpet Agri BA, Lillestrøm, Norway) .

2.3. Sampling, recordings and chemical analyses

Daily samples of 1 kg from PG and RG silages were collected Monday to Friday for a pooled sample from each week in each period. Milk samples collected in six subsequent milkings weekly on day 11 to 14 as well as on day 18 to 21 were analyzed for fat, protein, lactose and urea with infrared spectrophotometer (MilkoScan 6000, Foss Electric, Hillerød, Denmark). Triple marker method demonstrated nutrient flow from rumen and rumen digestibility (France and Siddons, 1986). On day 4 at 0800 h in each period a priming dose of 2,80 g Cr (Cr-EDTA) and 2,46 g Yb (Yb-acetate) was poured into rumen, directly followed by a continuous infusion of 2,80 g Cr/d and 2,46 g Yb/d ending on day 14 at 1500 h. Infusions of flow markers were administered using a peristaltic pump (Cenco Instruments MIJ N.V., Breda, the Netherlands) with individual lines directly into the rumen of each cow. The third marker was indigestible NDF supporting Yb and Cr to differentiate digesta into a large particle (LP), small particle (SP) and fluid phase (FP), respectively. Additionally, an aqueous solution with 10% atom excess $(^{15}NH_4)_2SO_4$ (Sigma Aldrich (Isotec), Miamisburg, OH, USA) providing 200 mg/d of ^{15}N was infused from day 10 at 0600 h until day 14 at 1500 h. The easier conductible sampling at omasal orifice in reticulum was preferred over sampling in omasum (Krizsan et al., 2010). Reticular spot samples were collected using a 250 mL wide necked Erlenmeyer flask with a rubber stopper to sample 1200 mL. Samples were used to determine digesta flow to the omasal canal and collected 3 times daily on day 12 at 0900, 1330 and 1800 h, on day 13 at 0730, 1200 and 1630 h and on day 14 at 0600, 1030 and 1500 h. Samples of 600 g of each time point were pooled to a total of 5400 ml from each period. Pooled samples were frozen at -20ºC in the same container directly after sampling. After thawing the pooled samples were filtered and centrifuged at 1,000 × *g* for 10 minutes at 5ºC to separate the digesta into LP, SP and FP with the method described by Krizsan et al. (2010). Microbial mass was separated out of a 250 g sample directly after each sampling time as described by Ahvenjarvi et al. (2000). The native rumen ^{15}N-content was measured in a rumen content sample on day six.

Rumen evacuations were conducted on day 19 and 21 at 0600 and 0930 h, at expected maximum and minimum rumen fill, respectively. From each Latin square, two cows were evacuated at 0600 h and two cows at 0930 h on day 19. On day 21, cows and times were changed. Organic matter (OM), DM, N, NDFom and iNDF were analyzed. To assess ruminal fermentation, liquid samples of 250 ml were collected on day 17 at 0600, 0730, 0900, 1030, 1200, 1330, 1500 and 1630 h. From each sampling, 9.5 mL ruminal liquid was filled in a 15 mL test tube with 0.5 mL formic acid for NH_3 analysis and kept at 4ºC until analyses. Total collection of feces and urine to measure total digestibility was conducted from day 10 to 12. Urine was separated from feces using a funnel device, bonded around vulva, leading urine in a hose ending into a container. To prevent NH_3 volatilization the container was daily added 1.5 L with 10% H_2SO_4 solution.

Blood samples were collected on day 18 at 0600, 0900 and 1200 h from the coccygeal vessels, which were considered similar to arterial blood entering the mammary gland. It was used blood collection tubes (Vacuette®, Greiner Bio-One) containing Li-heparin for amino acids (AA) and BHBA analyses. Additionally a serum tube was used for urea analyses. Li-heparin tubes were immediately cooled and centrifuged (3000 × *g* for 10 min.). Serum-tubes were stored for 2 h at room temperature to coagulate and centrifuged (3000 × *g* for 10 min.). Plasma and serum were pooled across sampling times to provide one sample per cow per period.

Chemical analyses of feeds are described in detail in our previous paper (Naadland et al., 2015) The same analyses as used for feed were used on digesta and fecal samples. Rumen fluid was analyzed for NH_3-N using flow injection analyzer FIAstar 5010 (Tecator AB, Höganäs, Sweden). The markers Cr and Yb in reticular contents and feces were analyzed in an atomic absorption spectrophotometer (GBC SavantAA Ser. No A6990, GBC Scientific Equipment, Hampshire, IL), as described by Njåstad et al. (2014). The ^{15}N isotope was analyzed in reconstituted reticular samples, microbial samples and ruminal background samples. Each sample contained 100 µg of N, and they were weighed into tin capsules (PDZ Europa, Cheshire, UK). Additionally, 50 µL of KCO_3 solution (10 g/L) was pipetted onto each sample. Samples were dried at 60°C overnight to remove NH_3 residues. The enrichment of ^{15}N in the samples was analyzed in duplicate using PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples for individual AA analyses were freeze dried and ground to 0.5 mm before analyzing. The free AA were extracted with diluted HCl. Co-extracted N macromolecules were precipitated with sulfosalicylic acid and removed by filtration. The filtered solution was adjusted to pH 2.20. The AA were separated by ion chromatography and determined by ninhydrin reaction with photometric detection at 570 nm (Biochrom 30 Amino Acid Analyzer, Biochrom Ltd., Cambridge, UK).

2.4. Calculations and statistical analysis

Fecal recovery was used to correct the marker concentrations as described by Krizsan et al. (2010). The flow of OM was corrected for volatile fatty acids (Ahvenjarvi et al., 2002) and microbial OM.

The results of the rumen evacuations offered the basis of calculations for fractional rates of intake (k_i), passage (k_p) and digestion (k_d):

$$k_i = 1/24 \times (\text{intake, kg/d})/(\text{rumen pool size, kg});$$

$$k_p = 1/24 \times (\text{omasal canal flow, kg/d}) / (\text{rumen pool size, kg});$$

$$k_d = k_i - k_p.$$

Mean values of measurements from day 10-21 in each period were used for both feed intake and milk production results. All data were analyzed using the MIXED procedures of SAS software (SAS Institute Inc., 2012) 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 8) and D (j = 1 through 4), P(S) (k = 1 through 4) and S (l = 1 and 2) are the fixed effects of diet, period within square and square. Period was calculated as a repeated week value for feed intake and milk production. Sum of squares were divided into orthogonal contrasts to assess linear and quadratic effects of the diets.

3. Results

3.1. Silage quality, feed intake and milk production

Chemical composition of the two grass-clover silages is given in Table 1. It shows higher concentrations of OM, water soluble carbohydrates and NDFom in the PG and higher concentrations of N containing compounds in the RG. The PG had higher concentration of Met but lower concentration of His compared to the RG. Both silages were well preserved, with restricted fermentation (low concentration of fermentation acids and no butyric acid (not presented)) and low concentrations of NH_3-N and pH. Intakes of DM and OM were higher with increasing proportions of PG in the diet whereas intakes of total N, some AA (Asp, Cys, Glu, His, Phe, Ser, Thr and Tyr) and total non-essential AA (NEAA) increased with increasing proportions of RG (Table 2). The highest daily milk-, milk fat-, and milk protein yield were observed in D2 (Table 3). Accordingly, it was a quadratic effect of diet on energy corrected milk yield (ECM), with the lowest yield in D4. Milk urea concentrations increased with increasing proportions of RG.

3.2. Nitrogen metabolism, AA profile and blood metabolites

The omasal OM flow tended (P = 0.09) to decrease linearly with increasing RG proportions (Table 4). The share of microbial NAN in total NAN flowing into the omasum increased (P = 0.01) with increasing proportions of PG. There was no effect of diet on omasal flow of individual AA or total AA (Table 5). The ruminal NH_3 concentration increased linearly with increasing proportions of RG (Table 6). The N excretion through feces tended

(P = 0.07) to decrease whereas urinal N excretion increased (P < 0.01), with increasing RG proportions. Total N excretion through feces and urine was highest for D4, measured as daily amount and as a proportion of ingested N (Table 6). Blood urea increased with increasing RG proportions (Table 7). Increasing PG proportions tended (P=0.07) to increase blood concentrations of Leu while Glu tended (P = 0.07) to be lower when mixed diets were fed.

4. Discussion

4.1. Feed intake and milk production

The purpose of the present study was to compare the effects of PG and RG, prepared from the same organic grass-clover field, on N-metabolism of lactating dairy cows. Earlier studies have compared pure diets of grasses or legumes from the same cut or as mixtures of cuts (Bertilsson and Murphy, 2003; Dewhurst et al., 2003; Halmemies-Beauchet-Filleau et al., 2014). However, pure stands of grasses and legumes may have different chemical properties than when cultivated in mixtures. For instance, grasses are shown to have higher CP concentrations when grown in mixed leys with legumes and particularly with white clover (Gierus et al., 2012). The clover content increased from 18% in PG to 41% in RG, which is comparable to other studies (Steinshamn and Thuen, 2008; Steinshamn et al., 2015; Alstrup et al., 2016). Thus, the present results have applied relevance when using mixed silages before pure silages when testing the effects of cut on feed N metabolism in dairy cows.

Silages were typical representatives of Fennoscandinavian organic silages with increasing CP concentration and decreasing ME concentration from PG to RG (Steinshamn and Thuen, 2008). The decreasing DMI with increasing RG proportions in line with studies on grass silages (Khalili et al., 2005; Kuoppala et al., 2008). The RG silage has usually a poorer digestibility than PG (Huhtanen et al., 2007), while feeding legumes generally increase DMI relative to grass (Dewhurst et al., 2003; Moorby et al., 2009). In the present study, the effect of legume was confounded with the effect of growth period, and the effect of growth period on DMI has likely been stronger than the effect of legume proportion.

Concentrate increased dietary CP concentrations in all diets. Still, CP concentrations in diets were below 16.5%. Calculated N-efficiency does usually not decrease significantly with increasing dietary CP concentrations below this level (Castillo et al., 2001; Colmenero and Broderick, 2006). In the current experiment, highest milk production and lowest excretion of non-protein N in urine and milk was found on the pure PG diet with lowest CP content. Moreover, highest energy utilization was observed on the RG dominated diets with highest CP concentration. Together, this suggests that the dietary energy concentration was too low in the pure RG diet for an optimal rumen microbial N synthesis. The PBV was above recommended levels (Madsen et al., 1995). Legumes contains more RUP than grasses, which might offer insufficient N substrate for rumen microbial protein synthesis and a less ideal AA profile to the intestine (Vanhatalo et al., 2009).

4.2. Total N supply

Increasing proportions of PG and decreasing N intake increased the rumen N outflow rate (k_p) in line with Vanhatalo et al. (2009). This was likely due to an improved microbial protein synthesis caused by more rumen digestible feed energy.

Origin of CP in omasal flow differed between diets. Similar to previous studies, the PG promoted a higher share of microbial NAN in total NAN compared to RG (Merry et al., 2006; Vanhatalo et al., 2009; Halmemies-Beauchet-Filleau et al., 2014). The larger RUP concentrations in legumes can explain this. However, no dietary effect was found in omasal flow of total NAN, which confirmed a proportionally greater microbial activity with greater intakes of ME and increasing proportions of PG in line with Halmemies-Beauchet-Filleau et al. (2014).

Milk protein was produced in similar quantities in diets containing PG, while milk protein production was slightly lower in the pure RG diet. The surplus N was converted into urea, displayed as increasing blood and milk concentrations with increasing RG proportions. Increasing RG proportions was related to higher NH_3 concentrations in rumen, and underpins that energy supply limited microbial protein synthesis. The low rumen NH_3 concentrations in the pure PG diet appeared to limit the NDF digestion (Broderick et al., 2010). Higher NH_3 concentrations in the two mixed diets improved NDF digestibility, relatively to pure PG, in the present study. Urea concentrations in milk and blood were in the lower reference range (Kraft, 2005), in line with the low to moderate dietary N levels. Dietary CP concentrations were below 16.5%, and increasing levels of urea are not expected with sufficient quantities of ME (Castillo et al., 2001; Broderick, 2003; Colmenero and Broderick, 2006).

4.3. AA profile

His has been recognized as the first limiting AA in grass silages (Vanhatalo et al., 1999; Korhonen et al., 2000), and Met has been proposed to be the first limiting AA in red clover (Vanhatalo et al., 2009). In the present study, the concentration of His increased from the PG to the RG and Met decreased from the PG and the RG, and concentrations were similar to silages from other studies (Vanhatalo et al., 2009; Halmemies-Beauchet-Filleau et al., 2014). Lee et al. (2012) found that the ideal concentration of Met and His should be at 2.2% of MP. In the current study, His concentrations were slightly lower in both silages. However, the His concentrations were greater than the Met concentrations. Vyas and Erdman (2009) predicted that a 40 g/d intake of Met and 130 g/d of Lys would be sufficient for a 1000 g of daily milk protein yield, which is comparable to the present study. Intakes of Lys were higher than 130 g/d while Met were around 40 g/d, making Met possibly more limiting than Lys. The increasing intakes of His with increasing proportions of RG may confirm a possible limitation in grass silages (Vanhatalo et al., 1999; Korhonen et al., 2000). All the observed differences in intakes of AA disappeared when the digesta entered the intestine. Increasing RG proportions offered a greater total AA intake but all diets provided similar quantities of AA to the intestine due to greater microbial protein synthesis in rumen and lower N-intake in diets with increasing PG proportions.

Leu is proposed to be the first limiting AA in rapeseed meal (Boisen et al., 2000). Omasal flows of Leu in the present study were around 19% of EAA and slightly lower than recommendations (National Research Council, 2001). The concentrate contained 15% rapeseed meal and had a lower Leu concentration than both experimental silages. Ideally, animal feeding in organic farming should be based on local produced feedstuff, and rapeseed is a useful protein source that can be grown in temperate climates (Huhtanen et al., 2011). Rapeseed has shown a better production potential in diets based on organic grass-clover silages compared to peas in cold-temperate climate (Khalili et al., 2002).

We hypothesized that increasing dietary RG proportions would increase AA flow to the small intestine. The flows were similar for all diets and the hypothesis was rejected. In addition, the second hypothesis was rejected, as this study could not support that milk protein synthesis in the pure RG diet was limited by a less balanced AA profile compared to diets including PG.

5. Conclusion

Increasing dietary proportions of RG silage increased daily intakes of CP, total AA and some single AA, including His, but neither the total AA flow to the intestine nor the flow of any single AA differed between diets. Higher daily yields of milk and milk solids were observed for cows on the mixed diets than on the pure PG or RG diets. A more complete NDF digestion caused by higher rumen NH_3 concentrations with the mixed diets might have provided those cows with more energy than the pure PG diet, and therefore increased milk yield. Methionine seemed to be the first limiting AA in the grass-clover silages with His as a possible second limiting AA. Low concentrate Leu concentrations probably related to rapeseed meal included in the concentrate mixture might also have been a potential limiting AA.

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 and the always helpful staff at the experimental unit led by Dag Kristoffer Forberg.

References

Ahvenjarvi, S., Vanhatalo, A., Huhtanen, P., 2002. Supplementing barley or rapeseed meal to dairy cows fed grass-red clover silage: I. Rumen degradability and microbial flow. J Anim Sci 80, 2176-2187.

Ahvenjarvi, S., Vanhatalo, A., Huhtanen, P., Varvikko, T., 2000. Determination of reticulo-rumen and whole-stomach digestion in lactating cows by omasal canal or duodenal sampling. Brit J Nutr 83, 67-77.

Alstrup, L., Søegaard, K., Weisbjerg, M., 2016. Effects of maturity and harvest season of grass-clover silage and of forage-to-concentrate ratio on milk production of dairy cows. J of Dairy Sci 99, 328-340.

Bertilsson, J., Murphy, M., 2003. Effects of feeding clover silages on feed intake, milk production and digestion in dairy cows. Grass and Forage Sci 58, 309-322.

Boisen, S., Hvelplund, T., Weisbjerg, M.R., 2000. Ideal amino acid profiles as a basis for feed protein evaluation. Livest Prod Sci 64, 239-251.

Broderick, G.A., 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J of Dairy Sci 86, 1370-1381.

Broderick, G.A., Huhtanen, P., Ahvenjarvi, S., Reynal, S.M., Shingfield, K.J., 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle--a meta-analysis. J of Dairy Sci 93, 3216-3230.

Castillo, A., Kebreab, E., Beever, D., Barbi, J., Sutton, J., Kirby, H., France, J., 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. J of Animal Sci 79, 247-253.

Colmenero, J.J.O., Broderick, G.A., 2006. Effect of Dietary Crude Protein Concentration on Milk Production and Nitrogen Utilization in Lactating Dairy Cows1. J of Dairy Sci 89, 1704-1712.

Counc. of the Eur. Union, 2007. Council Regulation (EC) No. 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. Off. J. Eur. Communities 189, 20.7.2007, 1-23.

Dewhurst, R., Fisher, W., Tweed, J., Wilkins, R., 2003. Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. J of Dairy Sci 86, 2598-2611.

Doepel, L., Lapierre, H., Kennelly, J., 2002. Peripartum performance and metabolism of dairy cows in response to prepartum energy and protein intake. J of Dairy Sci 85, 2315-2334.

Eriksen, J., Askegaard, M., Søegaard, K., 2012. Complementary effects of red clover inclusion in ryegrass–white clover swards for grazing and cutting. Grass and Forage Sci 69, 241-250.

France, J., Siddons, R.C., 1986. Determination of digesta flow by continuous market infusion. J Theor Biol 121, 105-119.

Gierus, M., Kleen, J., Loges, R., Taube, F., 2012. Forage legume species determine the nutritional quality of binary mixtures with perennial ryegrass in the first production year. Anim Feed Sci Tech 172, 150-161.

Halmemies-Beauchet-Filleau, A., Vanhatalo, A., Toivonen, V., Heikkilä, T., Lee, M.R.F., Shingfield, K.J., 2014. Effect of replacing grass silage with red clover silage on nutrient digestion, nitrogen metabolism, and milk fat composition in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. J of Dairy Sci 97, 3761-3776.

Huhtanen, P., Hetta, M., Swensson, C., 2011. Evaluation of canola meal as a protein supplement for dairy cows: A review and a meta-analysis. Canadian J of Anim Sci 91, 529-543.

Huhtanen, P., Rinne, M., Nousiainen, J., 2007. Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. International J of Anim Biosci 1, 758-770.

Khalili, H., Kuusela, E., Suvitie, M., Huhtanen, P., 2002. Effect of protein and energy supplements on milk production in organic farming. Anim Feed Sci Tech 98, 103-119.

Khalili, H., Sairanen, A., Nousiainen, J., Huhtanen, P., 2005. Effects of silage made from primary or regrowth grass and protein supplementation on dairy cow performance. Livest Prod Sci 96, 269-278.

Korhonen, M., Vanhatalo, A., Varvikko, T., Huhtanen, P., 2000. Responses to graded postruminal doses of histidine in dairy cows fed grass silage diets. J of Dairy Sci 83, 2596-2608.

Kraft, W., 2005. Klinische Labordiagnostik in der Tiermedizin. Schattauer Verlag, Stuttgart.

Krizsan, S.J., Ahvenjärvi, S., Volden, H., Broderick, G.A., 2010. Estimation of rumen outflow in dairy cows fed grass silage-based diets by use of reticular sampling as an alternative to sampling from the omasal canal. J of Dairy Sci 93, 1138-1147.

Kuoppala, K., Rinne, M., Nousiainen, J., Huhtanen, P., 2008. The effect of cutting time of grass silage in primary growth and regrowth and the interactions between silage quality and concentrate level on milk production of dairy cows. Livest Sci 116, 171-182.

Lee, C., Hristov, A., Cassidy, T., Heyler, K., Lapierre, H., Varga, G., de Veth, M., Patton, R., Parys, C., 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. J of Dairy Sci 95, 6042-6056.

Madsen, J., Hvelplund, T., Weisbjerg, M.R., Bertilsson, J., Olsson, I., Spörndly, R., Harstad, O.M., Volden, H., Tuori, M., Varvikko, T., Huhtanen, P., Olafsson, B.L., 1995. The AAT/PBV protein evaluation system for ruminants - a revision. Norw J of Agricultural Sci Suppl, 3-37.

Merry, R.J., Lee, M.R., Davies, D.R., Dewhurst, R.J., Moorby, J.M., Scollan, N.D., Theodorou, M.K., 2006. Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. 1. In vitro and in vivo studies of nitrogen utilization. J of Anim Sci 84, 3049-3060.

Mjoun, K., Kalscheur, K.F., Hippen, A.R., Schingoethe, D.J., 2010. Performance and amino acid utilization of early lactation dairy cows fed regular or reduced-fat dried distillers grains with solubles. J of Dairy Sci 93, 3176-3191.

Moorby, J., Lee, M.R., Davies, D., Kim, E.J., Nute, G., Ellis, N., Scollan, N.D., 2009. Assessment of dietary ratios of red clover and grass silages on milk production and milk quality in dairy cows. J of Dairy Sci 92, 1148-1160.

Naadland, S.S., Steinshamn, H., Randby, Å.T., 2015. Effect of replacing organic grass-clover silage from primary growth with regrowth on feed intake and milk yield of dairy cows, Org Agr, Springer, http://link.springer.com/article/10.1007/s13165-015-0144-0, pp. 1-11.

National Research Council, 2001. Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001. The Natl Acad Press, Washington, DC.

Njåstad, K.M., Adler, S.A., Hansen-Møller, J., Thuen, E., Gustavsson, A.-M., Steinshamn, H., 2014. Gastrointestinal metabolism of phytoestrogens in lactating dairy cows fed silages with different botanical composition. J of Dairy Sci 97, 7735-7750.

Reverter, M., Lundh, T., Lindberg, J.E., 1999. Ileal amino acid digestibilities in pigs of barley-based diets with inclusion of lucerne (Medicago sativa), white clover (Trifolium repens), red clover (Trifolium pratense) or perennial ryegrass (Lolium perenne). Brit J Nutr 82, 139-147.

SAS Institute Inc., 2012. SAS/STAT® User's Guide. Cary, NC: SAS Institute Inc. .

Steinshamn, H., Adler, S., Frøseth, R., Lunnan, T., Torp, T., Bakken, A., 2015. Yield and herbage quality from organic grass clover leys—a meta-analysis of Norwegian field trials. Org Agr, 1-16.

Steinshamn, H., Thuen, E., 2008. White or red clover-grass silage in organic dairy milk production: Grassland productivity and milk production responses with different levels of concentrate. Livest Sci 119, 202-215.

Vanhatalo, A., Huhtanen, P., Toivonen, V., Varvikko, T., 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. J of Dairy Sci 82, 2674-2685.

Vanhatalo, A., Kuoppala, K., Ahvenjärvi, S., Rinne, M., 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. J of Dairy Sci 92, 5620-5633.

Vyas, D., Erdman, R.A., 2009. Meta-analysis of milk protein yield responses to lysine and methionine supplementation. J of Dairy Sci 92, 5011-5018.

Table 1

The chemical composition of organic grass-clover silages (n = 16) and concentrate (n = 4) offered dairy cows

	Primary growth		Regrowth		Concentrate	
Item	Mean	SE	Mean	SE	Mean	SE
Dry matter, g/kg	369	0.5	336	0.4	876	3.9
pH	4.43	0.012	4.31	0.010		
g/kg dry matter						
Organic matter	932	0.47	915	0.48	922	0.69
CP	116	1.00	138	0.90	165	0.25
NH_3	0.212	0.0269	0.309	0.0269		
Water soluble carbohydrates	39.3	1.99	26.0	0.64	63.6	0.86
NDF	501	3.4	473	2.0	154	2.8
ADL	39.0	2.61	37.5	0.52	33.0	3.41
AA g/100 g CP						
Cys	0.83	0.019	0.82	0.013	1.78	0.123
Met	1.49	0.035	1.35	0.039	1.18	0.106
Asp	9.14	0.169	10.30	0.206	8.43	0.813
Thr	4.49	0.117	4.51	0.199	3.33	0.258
Ser	4.12	0.109	4.19	0.183	3.71	0.315
Glu	10.19	0.171	10.13	0.270	17.10	1.350
Pro	4.90	0.089	4.75	0.083	4.95	0.354
Gly	4.77	0.111	4.70	0.148	3.47	0.264
Ala	6.42	0.159	6.03	0.174	3.43	0.369
Val	5.83	0.158	5.54	0.223	3.82	0.297
Ile	4.80	0.139	4.61	0.199	3.42	0.314
Leu	8.31	0.218	7.95	0.276	5.98	0.520
Tyr	2.91	0.087	2.46	0.105	2.15	0.198
Phe	5.27	0.125	5.25	0.164	3.94	0.343
His	1.83	0.031	2.00	0.069	2.20	0.185
Lys	5.27	0.074	5.05	0.165	5.19	0.449
Arg	3.65	0.062	3.43	0.083	5.68	0.464
BCAA [a]	18.9	0.51	18.1	0.70	13.2	1.13
NEAA [b]	43.3	0.86	43.4	1.06	45.0	3.63
EAA [c]	40.9	0.95	39.7	1.40	34.7	2.92

[a] Branched-chain amino acids (Val, Ile and Leu).
[b] Non-essential amino acids (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr).
[c] Essential amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).

Table 2
Effect of replacing silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (Diet D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on feed intake (n = 8)

	Diet					Orthogonal contrasts	
Item	D1	D2	D3	D4	SEM	Linear	Quadratic
DM intake, kg/d							
Grass-clover silage	15.1	14.9	14.4	14.1	0.70	<0.01	0.55
Total	22.1	21.9	21.4	21.0	0.70	<0.01	0.56
Intake							
Organic matter, kg/d	20.5	20.3	19.7	19.3	0.64	<0.01	0.51
NDF, kg/d	8.64	8.40	7.96	7.72	0.382	<0.01	1.00
Water soluble carbohydrates, g/d	1057	1048	1001	987	30.2	0.02	0.92
N, g,d	464	475	480	492	15.5	<0.01	0.79
AAT [a], g/d	1584	1549	1484	1439	55.5	<0.01	0.76
PBV [b], g/d	139	257	392	541	10.6	<0.01	0.15
MJ ME/d [c]	239	235	224	217	5.1	<0.01	0.38
Intake g/d							
Cys	35.1	35.5	35.7	36.1	0.87	0.05	0.96
Met	39.6	39.6	39.2	38.9	1.57	0.32	0.76
Asp	257.0	269.2	278.8	292.7	10.09	<0.01	0.82
Thr	117.0	119.5	120.7	122.6	4.93	0.03	0.84
Ser	114.9	117.7	119.1	121.3	4.53	0.01	0.89
Glu	375.5	381.3	383.2	388.7	10.61	0.03	0.97
Pro	142.9	144.7	145.3	146.9	4.95	0.17	0.98
Gly	123.4	125.6	126.4	128.1	5.10	0.08	0.88
Ala	151.9	153.1	152.3	152.4	6.67	0.94	0.78
Val	146.0	147.6	147.1	147.6	6.14	0.65	0.77
Ile	123.4	125.0	124.9	125.5	5.14	0.43	0.77
Leu	214.4	216.8	216.7	217.6	8.80	0.47	0.80
Tyr	75.7	74.6	72.6	70.3	3.00	<0.01	0.54
Phe	137.6	140.4	141.6	144.0	5.64	0.04	0.92
His	57.4	59.5	61.0	63.3	5.03	<0.01	0.89
Lys	152.1	153.9	153.7	154.9	5.49	0.38	0.88
Arg	129.5	130.3	129.9	130.3	3.72	0.73	0.87
BCAA [f]	484	489	489	491	20.1	0.51	0.78
NEAA [g]	1277	1302	1313	1336	45.7	0.02	0.95
EAA [h]	1117	1133	1135	1144	43.4	0.21	0.84
Total AA	2393	2434	2448	2481	89.0	0.06	0.90

[a] Amino acid to the intestine.
[b] Protein balance in rumen.
[c] ME, calculated according to Van Es (1978).
[d] Neutral detergent indigestible nitrogen.
[e] Acid detergent indigestible nitrogen.
[f] Branched-chain amino acids (Val, Ile and Leu).
[g] Non-essential amino acids (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr).
[h] Essential amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).

Table 3.

Effect of replacing silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (Diet D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on milk production (n = 8)

	Diet					Orthogonal contrasts	
Item	D1	D2	D3	D4	SEM	Linear	Quadratic
Milk, kg/d	30.5	30.9	30.8	29.9	1.53	0.14	0.05
ECM [a], kg/d	30.6	31.0	30.4	29.3	1.97	0.01	0.03
Milk composition							
Fat, %	4.07	4.02	3.93	3.88	0.143	<0.01	0.99
Protein, %	3.15	3.19	3.15	3.16	0.081	0.79	0.42
Lactose, %	4.79	4.74	4.79	4.79	0.049	0.76	0.36
Urea, mmol/L	2.23	2.50	2.92	3.57	0.155	<0.01	0.02
Yield of milk components, g/d							
Fat	1248	1286	1228	1175	113	0.01	0.04
Protein	959	978	964	940	39.4	0.10	0.02
Lactose	1445	1430	1455	1409	66.9	0.71	0.39
ECM/MJ ME [b]	0.126	0.127	0.134	0.134	0.0034	<0.01	0.55
Milk N/Feed N	0.324	0.324	0.317	0.300	0.0099	<0.01	0.09

[a] Energy corrected milk.

[b] Metabolizable energy.

Table 4.

Effect of replacing silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (Diet D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on daily omasal flow and digestibilities (n = 8)

	Diet					Orthogonal contrasts	
Item	D1	D2	D3	D4	SEM	Linear	Quadratic
Omasal canal flow, g/d							
OM [a]	11373	11052	10152	10651	524	0.09	0.29
MNAN [b]	339	335	310	333	17.9	0.49	0.37
DNAN [c]	203	215	195	242	17.2	0.12	0.22
TNAN [d]	541	549	506	573	32.0	0.62	0.22
CP	3142	3183	2971	3287	183	0.73	0.34
MNAN/TNAN % [e]	63.0	61.1	61.3	57.7	1.40	0.01	0.51
AA/CP % [f]	86.7	85.4	86.3	86.4	0.74	0.79	0.61
Digestibility in rumen, %							
OM, true	62.4	64.5	66.7	63.2	1.25	0.40	0.04
NDF	57.8	64.2	60.8	58.9	2.09	0.99	0.02
CP, true	64.6	63.8	65.8	60.7	2.51	0.36	0.35
Digestibility in total tract, %							
OM, apparent	74.1	75.5	75.7	75.4	0.58	0.11	0.15
NDF	63.7	65.8	65.9	66.8	1.18	0.06	0.54
CP	68.5	71.0	72.2	73.2	0.61	0.00	0.22

[a] Organic matter

[b] Microbial non-ammonia nitrogen.

[c] Dietary non-ammonia nitrogen.

[d] Total non-ammonia nitrogen.

[e] Percentage of MNAN in TNAN.

[f] Amino acids in total CP.

Table 5

Effect of replacing silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (Diet D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on omasal flow of amino acids (n = 8)

	Diet					Orthogonal contrasts	
Item	D1	D2	D3	D4	SEM	Linear	Quadratic
Omasal canal flow, g/d							
Cys	39.9	39.8	37.5	41.8	2.31	0.67	0.24
Met	57.8	57.5	53.8	60.0	3.29	0.84	0.26
Asp	331	335	310	350	21.1	0.66	0.28
Thr	146	147	136	153	8.7	0.78	0.27
Ser	121	122	114	126	7.2	0.78	0.36
Gln	426	425	394	437	25.1	0.99	0.31
Pro	119	116	111	124	7.2	0.73	0.24
Gly	134	135	127	141	7.4	0.65	0.34
Ala	170	170	155	171	8.7	0.77	0.31
Val	165	166	154	171	9.2	0.85	0.31
Ile	169	170	157	177	10.9	0.77	0.29
Leu	232	234	219	244	14.0	0.68	0.31
Tyr	90.2	89.4	87.6	95.5	6.75	0.60	0.47
Phe	154	155	146	164	9.8	0.57	0.26
His	52.2	52.8	49.9	55.5	3.05	0.53	0.33
Lys	182	189	173	198	13.9	0.50	0.38
Arg	140	141	137	148	9.0	0.56	0.49
BCAA [a]	566	570	530	592	34.0	0.75	0.30
EAA [b]	1298	1312	1225	1370	81.0	0.66	0.31
NEAA [c]	1431	1433	1337	1485	84.4	0.83	0.30
Total amino acids	2730	2745	2562	2855	165.2	0.75	0.31

[a] Branched-chain amino acids (Val, Ile and Leu).
[b] Non-essential amino acids (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr).
[c] Essential amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).

Table 6

Effect of replacing silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (Diet D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on rumen pool size, passage, digestion kinetics and excretion (n=8)

	Diet					Orthogonal contrasts	
Item	D1	D2	D3	D4	SEM	Linear	Quadratic
Rumen content, kg	87.6	87.6	85.2	89.0	3.51	0.81	0.28
Rumen contents, kg							
Dry matter	10.9	11.0	10.7	11.0	0.46	1.00	0.58
Organic matter	10.0	10.1	9.7	10.0	0.43	0.73	0.64
N	0.268	0.284	0.292	0.313	0.0129	<0.01	0.64
NH_3-N, mmol/L	4.90	6.37	6.97	8.43	0.520	<0.01	0.99
% / h							
Organic matter, k_p [a]	6.34	6.16	5.92	5.82	0.366	0.15	0.89
Organic matter, k_d [b]	2.33	2.44	2.72	2.39	0.245	0.64	0.33
NDF, kp	2.36	1.99	2.20	2.19	0.14	0.46	0.07
NDF, kd	3.24	3.59	3.38	3.32	0.24	0.97	0.31
N, k_p	7.88	7.47	6.82	6.70	0.452	0.02	0.68
N, k_d	-0.52	-0.31	0.14	0.05	0.327	0.11	0.60
Feces							
Dry matter, kg/d	5.86	5.66	5.41	5.42	0.169	<0.01	0.25
N, g/d	152.4	146.8	145.0	145.3	4.60	0.07	0.28
Urine N, g/d	87.5	98.8	106.1	125.9	3.62	<0.01	0.18
N in feces and urine, g/d	239.9	245.0	251.1	270.7	7.41	<0.01	0.15
N balance g/d [c]	71.5	85.4	80.2	78.0	8.00	0.67	0.29

[a] Rate of passage.
[b] Rate of digestion.
[c] N balance = N intake − (N in milk + N in feces + N in urine).

Table 7

Effect of replacing silages prepared from primary growth with regrowth in the DM ratio 0, 0.33, 0.67 and 1 (Diet D1, D2, D3, and D4, respectively) in the diet of lactating dairy cows on blood metabolites and amino acids from a coccygial blood vessel (n=8)

	Diet					Orthogonal contrasts	
Item	D1	D2	D3	D4	SEM	Linear	Quadratic
mMol/L							
BHBA [1]	1.11	1.06	1.04	0.98	0.109	0.14	0.94
Urea	1.85	2.47	2.81	3.65	0.224	<0.01	0.59
Total amino acids	3.81	3.34	2.51	3.53	0.166	0.38	0.15
μMol/L							
Cys	13.3	10.8	11.5	11.8	3.18	0.77	0.64
Met	29.7	28.8	33.3	28.4	2.50	0.97	0.42
Asp	11.1	10.0	10.6	11.2	1.02	0.77	0.30
Thr	140.8	133.3	142.8	120.5	12.54	0.36	0.55
Ser	131.9	123.1	137.0	132.2	9.41	0.72	0.83
Glu	76.7	61.7	73.4	76.9	4.91	0.57	0.07
Pro	104.7	90.2	103.5	103.5	8.19	0.79	0.38
Gly	427.5	371.1	413.9	447.7	31.07	0.45	0.15
Ala	338.7	296.6	299.8	290.3	19.42	0.11	0.40
Val	333.2	266.3	291.0	281.2	21.11	0.17	0.18
Ile	185.3	158.3	176.7	164.5	13.11	0.45	0.57
Leu	147.3	115.4	123.4	112.7	11.33	0.07	0.35
Tyr	57.6	50.2	55.3	51.2	4.08	0.44	0.68
Phe	53.5	46.9	52.8	50.0	3.32	0.75	0.57
His	41.2	28.7	28.1	30.7	7.18	0.32	0.29
Lys	131.5	105.5	110.8	112.8	9.43	0.23	0.15
Arg	98.4	75.3	84.0	80.6	7.84	0.17	0.18
Gln	267.9	241.5	280.2	267.0	18.16	0.65	0.71
Trp	50.0	42.9	55.8	52.2	4.53	0.33	0.70
BCAA [2]	666	540	591	558	43.4	0.17	0.29
EAA [3]	1310	1086	1194	1115	78.9	0.18	0.36
NEAA [4]	1515	1328	1468	1460	73.7	0.94	0.23
EAA % of TAA [5]	46.4	45.2	44.6	44.3	1.15	0.20	0.75
NEAA % of TAA	53.6	54.8	55.4	55.7	1.15	0.20	0.75

[1] Betahydroxy butyric acid.
[2] Branched-chain amino acids (Val, Ile and Leu).
[3] Non-essential amino acids (Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr).
[4] Essential amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).
[5] Total amino acid.