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

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ABSTRACT

Clover proportions, and thereby chemical composition of herbage, differ between primary growth (PG) and regrowth (RG) in organic managed grass-clover fields. The characteristics of PG and RG silages suggest different supplementary feeding strategies to sustain an efficient milk production in dairy cows. Silage made of the RG generally offers more crude protein (CP) in the diet than silage made of the PG because of an increasing proportion of clover later in the season. Additionally, grass and clover have different amino acid (AA) profiles. His has been suggested to be the first limiting AA in grass silage, while Met has been suggested to be the primarily limiting AA in red clover silage. 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 four diets with RG replacing PG silage in ratios of 0, 0.33, 0.67 and 1 on dry matter (DM) basis. Changing RG silage proportions from 0 to 1 increased daily CP intake from 2.90 to 3.08 kg 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. Met and His were probably the most limiting AA for a higher milk production. Limitations by His may be more related to diets based on PG, while production by cows fed diets based on more RG herbage were more likely limited by Met.

1. Introduction

Organic agriculture depends on legumes and their ability to fix atmospheric N_2 due to restrictions on the use of mineral fertilizers (Council of the European Union, 2007). The main forage for dairy cows in Fennoscandia is grass-clover silage prepared from temporary grassland, due to the relatively long winter. Grassland legumes used in Fennoscandia have a higher optimal growth temperature than their companion grasses. Due to low spring temperatures the herbage legume proportion in mixed leys is usually lower in the primary growth (PG), i.e. the spring growth after the winter dormancy,

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; iNDF, indigestible neutral detergent fiber; LP, large particle phase; ME, metabolizable energy; NAN, non ammonia nitrogen; NDF, neutral detergent fiber; NDFom, neutral detergent fiber expressed exclusive of residual ash; OM, organic matter; PBV, protein balance in the rumen; PG, primary growth; RDP, Rumen degradable fiber; RG, regrowth; RUP, rumen undegradable protein; SP, small particle phase.

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than in the regrowth (RG), the growth after a cut (Steinshamn and Thuen, 2008; Eriksen et al., 2012). The organic PG has a relatively lower N concentration due to the higher proportion of grass than compared with the corresponding legume-richer RG, as observed under both experimental and commercial farm conditions (Steinshamn et al., 2015; TINE Rådgivning, pers. commun.). 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 describe sufficient amounts 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). Thus, to high yielding cows, feeding a combination of silages prepared from PG and RG may provide a more optimal N supply than feeding the cuts separately.

The grass protein has a greater share of rumen degradable protein (RDP) compared to the legume protein, which potentially increase microbial protein synthesis (Halmemies-Beauchet-Filleau et al., 2014). Addition of the limiting essential amino acids (EAA) to an unbalanced forage amino acid (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), and 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 25 g/kg of total omasal crude protein (CP) flow (National Research Council, 2001 Lee et al., 2012). Lys is recommended at 72 g/kg of CP in omasal flow and in a 3:1 relationship to Met (National Research Council, 2001). However, restricted dietary Lys or a generally 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 (Norwegian Ministry of Agriculture and Food, 2010).

2.1. Experimental design and animals

An experiment consisting of two replicated 4×4 Latin squares, each with 4 Norwegian Red cows, and four 21-days 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 silages 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 $\pm$ SD) 56 ± 19 days in milk 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 feed was assigned in equal shares three times daily at 0630, 1415 and 2200 h. Milking was conducted twice 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 timothy (*Phleum pratense* L. cv. 'Grindstad') and meadow fescue (*Festuca pratensis* Huds. cv. 'Fure'), and the legumes white clover (*Trifolium repens* L. cv. 'Hebe') and red clover ('Bjursele'). The PG and the RG contained 113 g/kg and 393 g/kg white clover and 65 g/kg and 14 g/kg red clover, respectively. Naadland et al. (2015) have reported a detailed description of silage production and quality. 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 were chopped to a median length of 4.5 cm and hand mixed before feeding to minimize selection. Silages were offered *ad libitum* allowing 100 g refusals daily per kg silage fed. Cows were additionally fed 8 kg (on fresh basis) daily of a concentrate mixture containing peas (268 g/kg DM), oats (168 g/kg DM), wheat (165 g/kg DM), barley (150 g/kg DM), rapeseed cake (100 g/kg DM), molasses (55 g/kg DM), rapeseed seeds (50 g/kg DM) and a vitamins and mineral mixture (44 g/kg DM; Natura Minovit Drøv, Felleskjøpet Agri BA, Lillestrøm, Norway).