CH22 - Optimising ruminal function: the role of silage and concentrate in dairy cow nutrition to improve feed efficiency and reduce methane and nitrogen emissions

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Abstract

Ruminant farm animals contribute significantly to global greenhouse gas (GHG) emissions, but the emissions can be greatly reduced by nutrition. Sustainable dairy cow feeding strategy to mitigate GHG and N emissions should make the most of the unique ability of ruminants to convert local human-inedible biomass to high-quality dairy foods. In this chapter, we review the potential of silage plant species (grass, forage legumes, maize) and stage of maturity of silage crops as well as dietary forage to concentrate ratio to reduce environmental footprint of dairy cows in the temperate areas without compromising animal performance. Dairy cow performance is examined in terms of feed intake, milk yield, feed and N efficiency, and methane emission intensity. The role of concentrate composition (lipids, carbohydrates and protein) is also evaluated. As a case study, the potential of milled rapeseed to reduce environmental footprint of grass silage-based diet is evaluated in practical farm conditions.

Key words: dairy cow, forage species, starch, lipid, methane, nitrogen

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1 Introduction

Ruminant farm animals contribute significantly to global greenhouse gas (GHG) emissions including among others enteric methane (CH_4) and nitrous oxide (N_2O). Therefore, a lot of research efforts have been put on finding nutritional means to reduce GHG emissions especially from dairy production sector during recent years. There is a considerable diversity of potential dietary mitigation options under development including not only feeds, feeding management and nutrition but also rumen modifiers i.e. feeding specific substances that directly or indirectly inhibit methanogenesis or using biological control directed at reducing methanogens (Knapp et al., 2014). Some of the feed additives such as CH_4 inhibitor 3-nitrooxypropanol (3-NOP) are very promising not only in intensive dairy cow diets (Dijkstra et al., 2018; Van Gastelen et al., 2019), but also in all-forage cattle diets (Martinez-Fernandez et al., 2018). For now these applications are not available in practice. However, nutritional mitigation strategies based on altering forage and concentrate component of the diet are easily available and often more adaptable and applicable into practice than the available rumen modifiers. It has been estimated that potential of feeding and nutrition (including means such as e.g. improved forage quality, feeding grain and dietary lipids) to lower GHG emissions usually range from low to medium (10-30%) (Hristov et al., 2013; Knapp et al., 2014). However, Hristov et al. (2013) concluded that improving forage quality and the overall efficiency of dietary nutrient use is an effective way of decreasing CH_4 emission intensity in terms of CH_4 per unit of animal product.

Effectiveness of dietary strategies to mitigate GHG emissions be they forage or concentrate based strategies depends largely on their effects to rumen volatile fatty acid

(VFA) fermentation pattern. Any change in dietary composition in favour of propionate production reduces CH_4 owing to consuming reducing equivalents while diets in favour of acetate and butyrate formation generate H_2 for methanogenesis and thus increase CH_4 production as reviewed by Knapp et al. (2014). Also other fermentation processes such as rumen protein degradation and assimilation into microbial protein and biohydrogenation of fatty acids taking place in the rumen contribute to the balance of H_2. The former results in either a net consumption or net production of H_2, while the latter results in net consumption of H_2.

As forages are the main component of dairy cow diets local production of high-quality forage is of utmost importance to dairy farmers for maintaining and ensuring profitable milk production. Therefore, the choice of adopting GHG mitigating nutritional strategy appropriate at farm level depends a lot on geographical location of the farm, which largely determines climatic conditions and the forage plant species available for silage making. For instance, in hot regions silage crops are influenced by high temperatures negatively affecting crop yield and nutritive value while in cold regions a short and cool growing season may limit the use of crops such as maize sensitive to cool temperature as reviewed by Bernardes et al. (2018). This is often the case in Northern areas such as in Northern Europe, where grass species rather than use of maize predominate in silage production. According to Bernardes et al. (2018) climatic conditions affect all stages of silage production and utilization temperature being the most limiting factor. Silage production is dependent both on controllable factors e.g. plant species and stage of maturity, harvesting and ensiling methods and use of additives, and uncontrollable climate-related factors being thus vulnerable to considerable annual variation in silage fermentation quality and nutritive value.

Concentrate-based strategies include increasing proportion of concentrate in the diet and/or altering the type of carbohydrate (e.g. fibre vs. starch) or type of lipid (e.g. fatty acid profile) supplementation in the concentrate. Even though concentrate components such as grains and oilseeds in the diet are not as sensitive to annual changes in nutritive value relative to forages their contribution to dairy cow feed ration affects a lot on dry matter (DM) intake of forages and digestibility of dietary components e.g. fibre in the diet. According to Hristov et al. (2013) inclusion of concentrate feeds in the diet likely decreases CH_4 emission intensity particularly when inclusion is above 40% of dietary DM and rumen function is not impaired. On the hand, decreasing forage to concentrate ratio in dairy cow diets contradicts with endeavours at reducing use of human-edible components such as grains in the dairy cow feed rations. Sustainable dairy cow feeding strategy should make the most of the unique ability of ruminant animals to convert human-inedible biomass to high-quality animal-derived protein foods i.e. milk and meat.

In this chapter, we focus on reviewing recently accumulated literature from dairy cow physiological or milk production studies conducted on silage and/or concentrate supplemented diets including measurements on CH_4 production. We look at their dietary effects on the production parameters, feed efficiency (FE), nitrogen use efficiency (NUE) and CH_4 emission intensity in dairy cows in temperate zone conditions with emphasis in Europe and North America. We deal among others with the potential of plant species and stage of maturity of silage crops as well as dietary forage to concentrate ratio and concentrate composition to reduce environmental footprint of dairy cow production without compromising animal performance.

2 Role of silages

2.1 Silage plant species

Climatic conditions within the temperate area vary considerably and thus there is a large variation in forage species available for silage making. Major plant species include grasses, forage legumes and maize.

In northern areas, short and cool growing seasons with the rigours of a cold winter limit the choice of perennial grasses and legumes for silage making (Bernardes et al., 2018). The most widely used perennial forage species include timothy (*Phelum pratense*), perennial ryegrass (*Lolium perenne* L.) and various fescue species such as meadow fescue (*Festuca pratensis*) and tall fescue (*Festuca arundinacae* L.), red clover (*Trifolium pratense*) and lucerne (*Medicago sativa* L.) being the major forage legume species available for silage making (Wilkinson and Rinne, 2018; Bernardes et al., 2018). Though the production of lucerne is limited to southern parts of the zone e.g. in Scandinavia advancing climate change may enable its' production further northward in future (Järvenranta et al., 2016). Maize plant (*Zea mays* L.) is a tropical crop in origin, but as a valuable forage crop it is used wherever maize can grow, from temperate regions to the tropics. It is characterised with high quantities of low-cost starch per hectare coupled with a relatively high concentration of metabolizable energy, which makes this crop very attractive to farmers (Wilkinson and Rinne, 2018). Plant breeders have developed earlier maturing maize varieties, which can be used for whole-crop silage making in northern conditions. Thus, the area of maize cultivation for silage making is gradually expanding to the north the advancing climate change possibly contributing to it as well. The use of biodegradable film in cultivation also enable earlier sowing and harvesting of maize in the marginal areas of Northern Europe such as in Scandinavian countries.

Cool-season forage grasses are characterised with high digestibility attributed to low temperature and long day length, which delay lignification of cell walls (Huhtanen et al., 2013; Bernardes et al., 2018). As the decline in the rate of digestibility due to advancing maturity is slower with legumes than grasses mixing of these plant species for silage making is thus beneficial extending the optimal harvesting period of the herbages (Kuoppala, 2010). However, concentration of non-structural carbohydrates (NSC) including water soluble carbohydrates (WSC) and starch of these forages is often low and varies a lot depending on the climatic conditions. It affects ensiling potential and feed value of grass and legume forages and can be manipulated with harvesting time and ensiling methods such as use of additives (Vanhatalo and Jaakkola, 2016). There are also so called high-sugar ryegrass cultivars rich in WSC available for silage making (Moorby et al., 2006).

2.2 Grass silages

Of the grass silage management factors altering forage maturity stage at harvest has the greatest CH_4-mitigating potential while N fertilisation rate, use of additives or high-sugar ryegrass cultivars have no effect (Table 1). Feeding ryegrass-timothy (Warner et al., 2016; Warner et al., 2017), ryegrass (Brask et al., 2013a) or timothy (Pang et al., 2018) silage harvested at early relative to late stage of maturity in high-forage dairy cow diets has increased DM intake, energy-corrected milk yield (ECM) and FE considerably and decreased CH_4 emission intensity in terms of g CH_4 per kg ECM up to 20% (Warner et al., 2016; Warner et al., 2017). Moreover, reductions in CH_4 with improved silage quality were independent of the DM intake, and were smaller at late rather than early stage of lactation (Warner et al., 2017). They were neither attributable to acetate to propionate ratio, which was unchanged owing to the grass maturity (Brask et al., 2013a; Warner et al., 2016). However, the positive results were achieved at the expense of simultaneously decreasing NUE, which was remarkably high (-35%) when very early cut leafy stage grass silage was used (Warner et al., 2017) reflecting the much higher crude protein (CP) content of the leafy stage than the late heading herbage. High N fertilisation rate of grass forage (150 vs. 65 kg N/ha) was also in relation to reduced NUE owing to a 5 %-unit higher CP content of the silage with high N fertilisation level (Warner et al., 2016).

The positive DM intake and milk production responses of dairy cows to grass silage harvested at early stage of maturity are well established (e.g. Rinne, 2000; Harrison et al., 2003) as is also the concomitant high N content of silages leading to losses of N from animals (e.g. Rinne et al., 1997). Thus, extremely early harvest of grass forage such as that used by Warner et al. (2017) is not recommendable. However, using silages made from early cut primary growth grass improves FE, decreases CH_4 emission intensity and ensures good milk production level of high-producing dairy cows though with compromises in NUE. Decreases in CH_4 owing to harvest at early maturity stage seem not to be related to rumen fermentation pattern as effects of advancing maturity of ensiled grass on molar proportion of propionate have been small and inconsistent (Harrison et al., 2003; Warner et. al.,2016). High nitrate content in early maturity grass silage (Warner et al., 2016) or e.g. increased microbial growth (Knapp et al., 2014) due to high energy content in early maturity grass silage may possibly have served as an alternative H_2 sink to propionate thus explaining decreases in CH_4. High-sugar content ryegrass grass silage improved NUE in all-silage diet (Table 1; Staerfl et al., 2012) in agreement with beef cattle production studies (Merry et al., 2006), but reduced milk production parameters with minor effect on CH_4 emission intensity. According to Bertilsson et al. (2018) elevated WSC levels in high-sugar ryegrass silage were achieved at the expense of CP and fibre but their effects on dairy cow performance were minor. However, positive effects were attributed to a more favourable distribution of N in terms of more N to milk and faeces and less N to urine. Overall, the issue of improving low NUE of grass forage silages clearly warrants further research. Despite earlier positive production responses of silage inoculants to milk production (e.g. Muck et al., 2018), no such effect nor reduced CH_4 emission intensity owing to inoculated grass silage was seen in a study of Ellis et al. (2016) (Table 1).

2.3 Forage legume silages

Data on the effects of forage legume silages on CH_4 intensity in dairy production is scarce (Table 1). Replacing timothy with lucerne in dairy cow diets was not effective in reducing CH_4 emissions but led to increased DM intake, impaired FE and especially reduced NUE (Hassanat et al., 2014; Table 1). Inclusion of sainfoin (*Onobrychis viciifolia*) containing condensed tannins in grass-silage based silage increased DM intake and ECM yield but led only to a minor decrease in CH_4 emission intensity (Huyen et al., 2016; Table 1). Replacing mixture of timothy and red clover silage of 70:30 with a mixture of red clover and timothy silage of 70:30 in dairy cows did not affect DM intake, ECM production, FE or CH_4 emission intensity but led to a reduction in NUE (Gidlund et al., 2017; Table 1). This is in agreement with findings of Van Dorland et al. (2007) showing that replacing part of ryegrass silage with red or white clover silage did not affect CH_4 emissions but slightly enhanced N losses to the environment. Even so, according to a review of Phelan et al.

(2015) forage legumes have generally resulted in lower CH_4 emissions per kg of milk or meat produced when compared to grasses. However, they emphasise that this occurs only when the forage legume has had higher feed intake and ruminal passage rates than the grasses, or with legumes that contain condensed tannins.

Indeed, results from comparing forage legume and grass silage-based diets in general demonstrate the superior DM intake and milk production potential of forage legumes over grasses (Vanhatalo and Jaakkola, 2016). Moreover, forage legumes are often considered as an economically profitable alternative to grass and/or maize based forages owing to their ability to provide biologically fixed nitrogen, which serves as an effective means to reduce dependence on synthetic N fertilizers and thus fossil energy (Vanhatalo and Jaakkola, 2016). The higher intake characteristics of legume than grass silages despite lower digestibility have been attributed to their lower fibre content, more rapid fermentation and particle breakdown in the rumen, and higher passage from the rumen (Kuoppala et al., 2009; Kuoppala, 2010; Dewhurst, 2013). Contradictory results on CH_4 mitigation potential of forage legumes may be related to the varying silage fermentation quality and proportions of forage legume in the silages studied. It should be noted, that forage legumes are often grown in mixtures with grasses or other plants rather than as pure stands owing to higher annual herbage yield in mixtures (Phelan et al., 2015). Reduced NUE with forage legume-based diets rather than with grass silage diets stems from their inherently high CP concentrations, especially so with lucerne. However, there are also differences between the forage legume species such as red clover and lucerne in their N fractions, which may differently affect NUE as reviewed by Dewhurst (2013). Clearly, more research is needed on the potential of forage legumes to reduce CH_4 emission intensity and their effects on NUE.

2.4 Maize silages

According to Hatew et al. (2016) increasing maturity of whole-plant maize from very early (20% DM) to late stage (40% DM) at harvest effectively reduced CH_4 emission intensity on high-forage diet but did not affect DM intake, ECM yield, FE or NUE (Table 1). This was caused by the markedly increased starch content, decreased ruminal fractional rate of degradation of starch and decreased neutral detergent fibre (NDF) content with advancing maturity of maize crop. However, despite higher starch intake this was not attributable to decreased rumen pH and increased propionate as expected. Instead, the acetate to propionate ratio tended to increase with increasing maturity of maize. Their results suggest that harvesting whole-plant maize at a higher maturity instead of the currently recommended practise (30 to 35% DM; e.g. Khan et al., 2015) have potential to reduce enteric CH_4 emissions.

Maize cultivars developed for potentially higher cell wall digestibility and intake properties (Jung et al., 2011) relative to conventional maize cultivar have been compared recently (Hassanat et al., 2017; Table 1). It was shown that replacing conventional maize silage (DM 40%, starch 269 g/kg DM) with more digestible brown midrib maize silage (BMCS; DM 34%, starch 283 g/kg DM) on high-forage diet increased not only DM intake and ECM yield but improved NUE and reduced CH_4 emission intensity. Again, reduction in CH_4 was not attributed to rumen fermentation pattern, which was unchanged between the treatments. Moreover, it was demonstrated that by using BMCS N excretion in manure reduced and potential N volatilisation was avoided by shifting N excretion from urine to faeces. Nevertheless, increased volatile solid content (i.e. degradable organic matter excretion) in the manure was observed giving rise to CH_4 emissions from manure storage. However, maize silage type (Falkone vs. LG30224) was of little importance in terms of dairy cow performance and CH_4 emissions despite the lower rumen NDF digestibility and higher starch content of Falkone in a diet where proportion of maize in the forage was 65% (De Boever et al., 2016).

Replacing barley silage (DM 32%, starch 139 g/kg DM) completely with maize silage (DM 31%, starch 322 g/kg DM) increased considerably DM intake, milk yield and NUE, but did not affect FE or CH_4 emission intensity (Benchaar et al., 2014; Table 1). However, increasing proportion of maize silage at the expense of barley silage in the diet reduced CH_4 energy losses in association with lower ruminal acetate to propionate ratio. The improved NUE with increasing maize silage in the diet was due to decreased urinary losses suggesting low potential for N_2O and ammonia emissions from manure.

2.5 Maize silage vs. grass and forage legume silages

Because of the inherently high energy value and low CP concentration of the maize crop it has been of interest to study whether mixing or replacing grasses or legumes high in CP with maize silage in the diet leads to beneficial environmental effects in terms of increasing NUE and reducing CH_4 emission intensity. Replacing grass silage with maize silage (DM 32%, starch 322 g/kg DM) on restricted high-forage diet improved NUE and reduced CH_4 emission intensity but did not affect FE or milk production except for increased milk protein yield (Van Gastelen et al., 2015; Table 1). Reduction in CH_4 was not associated with acetate to propionate ratio, which was unchanged between the treatments. Replacing early cut or late cut grass silages in the high-forage diet with maize silage (DM 31%, starch

150 g/kg DM) had no major effect on DM intake or milk yield, but NUE was improved particularly in relation to early cut grass silage and CH_4 emission intensity reduced particularly in relation to late cut grass silage (Brask et al., 2013a; Table 1). Reductions in CH_4 were attributed to clearly decreased acetate to propionate ratio in the rumen and to lower ruminal fibre digestibility with maize silage diets.

Increasing proportion of maize in the mixture of grass and maize silage from 25% to 75% on 50:50 forage to concentrate ratio diet increased DM intake and milk yield and decreased CH_4 emission intensity, while effects on FE and NUE were variable and more inconsistent (Reynolds et al., 2010; Hammond et al., 2016; Table 1). However, using similar experimental setup Livingstone et al. (2015, Table 1) did not find differences between these silage treatments in any of the parameters mentioned above owing to the exceptionally low NDF content of the grass herbage. Using low-forage diet, Doreau et al. (2014) (Table 1) did neither find any differences in these parameters except for the lower CH_4 emission intensity with maize silage diets. Even so, ruminal acetate to propionate ratio was unchanged between the treatments.

Also red clover and lucerne silages have been replaced with maize silage on high-forage diets but with minor effects on DM intake, production parameters and CH_4 emission intensity while NUE was clearly improved with maize silages (Hassanat et al., 2013; Benchaar et al., 2015; Arndt et al., 2015; Table 1). In these studies, maize silage maturity varied in range of 36 to 38% DM and of 290 to 339 g/kg DM starch while starch contents of legumes were less than 18 g/kg DM (Hassanat et al., 2013; Benchaar et al., 2015). Despite unaffected CH_4 emission intensity, rumen pH and ruminal acetate to propionate ratios were clearly decreased when red clover or lucerne was replaced with maize silage in the diet (Hassanat et al., 2013; Benchaar et al., 2015; Arnd et al., 2015). The positive changes for lower urinary and faecal N with maize silage at the expense of forage legume silages in these studies would likely result in lower ammonia and N_2O emissions. Though, the reduced fibre digestion in the rumen with starch containing maize silage diets may lead to increased CH_4 emissions from manure storage (Hassanat et al., 2013).

It seems that replacing grass or legume forage silages with maize silage consistently leads to environmental benefits such as reducing CH_4 emission intensity on high-forage grass silage-based diets, and improved NUE especially on forage legume silages high in CP without compromises in milk production. However, despite increased amounts of starch with increasing proportion of maize silage in the high-forage grass diet, reductions in CH_4 emission intensity are not necessarily related to changes in rumen fermentation pattern with reduced pH and decreased acetate to propionate ratio as expected. For instance, replacing grass silage with maize silage maintained high rumen pH and increased rumen butyrate in high-forage diet (Van Gastelen et al. 2015). Thus, the reduction in rumen pH enhancing the production of propionate (Dijkstra et al. 2011) does not necessarily occur in high-forage diet. As maize starch is fairly resistant to rumen fermentation it is susceptible to enzymatic digestion in the small intestine (Owens et al. 1986). Consequently, a possible shift from acetate to butyrate in favour of less H_2 production in the rumen (e.g. Moss et al. 2000) and reduced H_2 production in the rumen owing to a partial shift of starch digestion to the lower tract may contribute to reduced CH_4 production with maize-containing diets. Nevertheless, a critical dietary concentration of starch is likely required to alter ruminal methanogenesis and decrease CH_4 production (Hassanat et al., 2013; Van Gastelen et al., 2015).

It is interesting that the clearly decreased acetate to propionate ratio with maize silage diets at the expense of forage legume diets (Hassanat et al., 2013; Benchaar et al., 2015; Arnd et al., 2015) did not lead to reduced CH_4 emission intensity. This may be related to the type of carbohydrate in forage NDF, which affects CH_4 emissions (Arnd et al., 2015). They found that fermentation of maize NDF yielded substantially more CH_4 than fermentation of lucerne NDF. Thus, the greater CH_4 emission expected from greater amount of NDF in lucerne was counterbalanced by a decreasing emission per gram of lucerne NDF fermented. Similar difference in NDF fermentation between maize and lucerne was found in the study of Hassanat et al. (2013). Moreover, in the study of Brask et al. (2013a) less NDF was digested in the rumen for maize silage than grass silage diets despite a comparable NDF concentration in the diets. Further research on the effects of forage carbohydrate type on CH_4 emissions and rumen digestion kinetics is needed. The studies should ideally simultaneously examine rumen fermentation, digestion kinetics and microbiota together with GHG and milk production to give in-depth view on mechanism affecting the rumen function and GHG formation. The positive changes on N losses owing to increasing proportion of maize in the diet suggest that the dietary shift in forage source from forage legumes to maize silage results in lower NH_3 and N_2O emissions from manure (Hassanat et al., 2013; Arndt et al., 2015).

Nevertheless, it should be emphasised that conclusions about the potential of a feeding strategy to reduce GHG emissions depend also on the level of analysis which may reveal if the strategy is feasible also on the farm and chain level and not only at animal level (Van Middelaar et al., 2013). They found that at animal level increasing maize silage at the expense of grass and grass silage in dairy cow diets is a promising strategy with an immediate effect on GHG emissions. However, application of this strategy to average intensive Dutch farms would lead to problems with EU regulations when reducing grassland area. On the other hand, applying this strategy to intensive farm that can reduce its area of grassland would lead to higher emissions owing to land use change i.e. ploughing grassland into maize land.

3 Role of concentrates

3.1 Lipids

Level of lipid supplementation. Lipid supplements are widely used to increase diet energy density to meet the energy requirements of high-producing dairy cows during early and mid-lactation and to improve energy utilization for milk production. However, dietary lipid content should not exceed 6-7% in DM (review by Beauchemin et al., 2008), otherwise a depression of DM intake, ruminal fibre digestibility and further milk production may occur thus hampering the advantages of increased diet energy density (Bayat et al., 2017; Halmemies-Beauchet-Filleau et al., 2017). Lipids are also one of the most effective and practicable means to improve milk or meat fatty acid composition and mitigate GHG emissions of ruminants in industrialized countries, but the effectiveness depends on multiple factors. These include level of lipid supplementation, fatty acid profile of lipid supplement (eg. chain length and level of unsaturation), form in which the lipid is given (eg. oil vs. full-fat seeds) and the type of basal diet (reviews by Eugène et al., 2008; Beauchemin et al., 2008; Shingfield et al., 2013; Table 2). To support long-term health of human consumers, the aim is to decrease the proportion of saturated fatty acids and to increase those of *cis*-monounsaturated fatty acids and omega-3 fatty acids, and improve the balance of omega-6 and omega-3 fatty acids in ruminant products (Shingfield et al., 2013).

Over a broad range of dietary conditions, Martin et al. (2010) reported a mean decrease in CH_4 emissions of 3.8% with each 1% addition of supplemental lipid in diet DM. One or more mechanisms may contribute to CH_4 mitigation potential of different lipids in the rumen. These include lower amount of organic matter (OM) fermented in the rumen (lipid decreases DM intake and/or replaces rumen fermentable ingredients in the ruminant diet), direct toxicity or inhibition of rumen cellulolytic bacteria, methanogens and/or attached protozoa, shift in ruminal fermentation from acetate to propionate that consume rather than produce H_2 and biohydrogenation of fatty acids in case of unsaturated lipid supplements (review by Martin et al., 2010).

Medium chain saturated fatty acids. Medium chain saturated fatty acid sources such as myristic acid (14:0) or coconut oil rich in lauric acid (12:0) and 14:0 have reduced ruminal CH_4 emission intensity up to 30% when fed at 3.3-5% in diet DM for lactating cows. The primary mechanism is probably through decrease in DM intake (Table 2) and fermentable OM content in the rumen (Bayat et al., 2018). Medium chain saturated fatty acids may also exhibit toxic effects on methanogens (Beauchemin et al., 2008) or protozoa (Hristov et al., 2011) and impair fibre digestion (Hollmann et al., 2012), but these effects are inconsistent between studies. Recently Bayat et al. (2018) reported only minor alterations in the diversity of specific microbial taxa and no effect on total quantities of bacteria, methanogenic archaea or ciliate protozoa or fibre digestion despite a significant decrease in ruminal CH_4 production. Inclusion of medium chain saturates in the diet in general improves FE and NUE in milk production, but concomitant sharp decrease in ECM yield

(Table 2) together with relatively high price of these lipid supplements make applications in commercial dairy farms unlikely. Furthermore, dietary 12:0 and 14:0 supplementation increase their incorporation in bovine milk fat (Odongo et al., 2007; Hollman et al., 2012; Bayat et al., 2018) that is a nutritionally undesirable change for human consumers.

Unsaturated fatty acids. Plant unsaturated fatty acids such as oleic acid (18:1n-9) and essential fatty acids linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are considered beneficial to human health. Thus, their use in dairy cow diets that typically results in increases of these omega-fatty acids in ruminant milk and meat together with a decrease in saturated fatty acids, could be a viable way for CH_4 mitigation.

Trials with lactating dairy cows clearly indicate that the effects of lipids on animal performance and rumen methanogenesis are proportional to the level of supply and unsaturation (Table 2). As for saturated fatty acids, the primary mechanism is probably through a decrease in DM intake (Table 2). A dose-response trial by Martin et al. (2016) with three levels of 18:3n-3 rich linseed supplementation up to 5.4% of lipid in the diet DM decreased ruminal CH_4 emission intensity up to 39% with inconsistent effects at lower levels inherent to differences in the composition of the basal diets. Besides lower level of DM intake, the decrease in enteric CH_4 production was attributed to a decreased ruminal acetate to propionate ratio and number of protozoa, whereas the number of ruminal methanogens and fibre digestibility remained unaltered. A modest lipid inclusion (1 to 2% in diet DM) do not suppress feed intake yet, but it already alters milk fatty acid composition (Halmemies-Beauchet-Filleau et al., 2011). At a 5% lipid inclusion level in diet DM, rapeseed rich in 18:1n-9 and 18:2n-6 sources safflowerseed, sunflowerseed and camelina seed have resulted in much less pronounced decreases in CH_4 emission intensity compared to linseed at best reaching a 23% decrease (Table 2). In most studies presented in Table 2 the experimental periods are of 4 to 6 weeks, but recently Alstrup et al. (2015) demonstrated that plant lipids suppress ruminal methanognesis throughout the entire lactation. However, more studies comprising entire lactation are needed to confirm the persistency of lipids to mitigate ruminal CH_4 emissions.

It is generally thought that intact oilseeds give a partial protection for oil against microbial metabolism or limits the effects of oil on ruminal microbes and nutrient digestibility or both. However, Martin et al. (2008) reported no difference in OM and fibre digestibility between whole intact linseed, extruded linseed and linseed oil diets. Though pure oil is often more effective to mitigate CH_4 production in the rumen, processed oilseeds (e.g. crushed by milling, extruded, pressed cakes) are preferred because of less adverse effects on DM intake and generally lower price (Beauchemin et al., 2008; Table 2). In addition, administering high levels of dietary unsaturated fatty acids as a part of total mixed ratio (TMR) results in a lower decrease in DM intake (Bayat et al., 2015) than incorporation into concentrates fed separately to forage (Halmemies-Beauchet-Filleau et al., 2017). This is probably due to a more sudden pulsitary release of unsaturated fatty acids in the rumen in separate feeding relative to TMR as high amounts of free unsaturated fatty acids are toxic to cellulolytic bacteria (Maia et al., 2007).

Basal diet greatly affects the production and enteric CH_4 emission response to lipids. On forage predominant diets (diets rich in fibre) based on 50% or more in diet DM of grass silage, red clover silage or grass hay, unsaturated lipid supplements up to 5% in diet DM in general maintain ECM yield and improve FE. Furthermore, there is a dose-dependent and consistent decrease in ruminal CH_4 emissions (Table 2). In contrast, on starchy diets (diets based on maize silage or rich in concentrate starch), the ECM yield and FE are often compromised already at low lipid inclusion levels (2-4% in diet DM) and the effects on ruminal CH_4 emissions are variable between studies (Table 2). At high lipid inclusion levels (4-5.5% in diet DM), ruminal CH_4 emission intensity is more consistently reduced, but the reduction is often accompanied by a decrease in ECM production (Table 2). Therefore, unsaturated lipid supplements to mitigate CH_4 emissions suit best for diets rich in fibre, but their use in starchy diets is of limited interest due to the negative effects on ECM yield. This is probably linked to a much more detrimental effect of unsaturated lipid on rumen fibre digestion on diets high in starch relative to high fibre diets leading to a decrease in acetate formation in the rumen and further milk fat content (Benchaar et al., 2015; Bayat et al., 2017). In addition, PUFA supplementation on starchy diet readily directs rumen biohydrogenation from *trans*-11 to *trans*-10 route, some *trans*-10 isomers being potential antilipogenic agents in the bovine mammary gland thus causing milk fat depression (MFD; review by Shingfield et al., 2010; Ventto et al., 2017).

Feed N use efficiency in milk protein synthesis is in general unaltered or slightly improved by unsaturated lipid supplements in the diet (Table 2). Though milk protein content or yield may in some cases decrease by lipid in the diet so do the feed and CP intakes (Benchaar et al., 2015; Halmemies-Beauchet-Filleau et al., 2017; Bayat et al., 2018), which explains the unaffected or improved conversion efficiencies. The possible decrease in milk protein synthesis due to lipid supplementation has been attributed to the negative effects on energy intake, limitation in glucose supply and microbial protein synthesis (review by Lock and Shingfield, 2004; Halmemies-Beauchet-Filleau et al., 2017).

Eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) present in fish oil or specific algae products have had a strong CH_4 supressing effect when tested *in vitro* (Martin et al., 2010). However, when fed at low levels (up to 1% in diet DM) to lactating dairy cows, ECM yield together with FE have already decreased without any improvement in ruminal CH_4 emissions. This suggest that lipid supplements rich in 20- and 22-carbon polyunsaturated fatty acids are not a useful tool to mitigate the GHG emissions of dairy cows in practice.

3.2 Carbohydrates

Level of concentrate supplementation. Decreasing forage to concentrate ratio (i.e. an increase in concentrate starch inclusion) in the dairy cow diet generally improves feed intake (Table 3) due to the reduced contribution of forage fibre with high bulk density (review of Allen, 2000). In addition, the greater proportion of concentrates in the diet may be associated with more extensive OM digestion in the rumen reflecting the greater inherent digestibility of NSC in concentrates (starch and sugars) relative to structural forage carbohydrates (hemicellulose and cellulose; Bayat et al., 2017). Consistent with this concentrate level in the diet is often an increase in the total tract digestibility of starch, but fibre digestibility is also often simultaneously impaired (Niu et al., 2016; Bayat et al., 2017) that may level out the overall impact of starchy concentrate on OM digestibility. The changes in feed intake are reflected in ECM production leading to unaffected FE except for lipid-rich diets (Table 3). There is a trend for an increase in milk protein and a decrease in milk fat in response to concentrate supplementation (Aguerre et al., 2011; Niu et al., 2016; Bayat et al., 2017). The increase in milk protein may be driven by greater inclusion of cereals with high metabolizable energy density in the diet. As the level of starchy concentrate in the diet increases, there is a concomitant decrease in forage intake and thus forage fibre. The ruminal fermentation of NDF results in lipogenic VFA in the rumen that may account for the decrease in milk fat on diets low in forage.

The low and high concentrate diets reported in Table 3 were all isonitrogenous (CP 15-18% in DM) except for Olijhoek et al. (2018), where high concentrate diet had a 20% higher CP content relative to a low forage diet leading to a reduced NUE. The general improvement in NUE on concentrate rich diets (Table 3) is probably a result of a better balance of degradable protein and energy available for microbes in the rumen as indicated by lower rumen ammonia concentrations in high concentrate diets (Bayat et al., 2017) and higher relative contribution of good quality protein feeds in dietary protein.

The reduction of ruminal CH_4 production at increasing levels of concentrate in the diet is well established (Table 3; Martin et al., 2010). Cattle CH_4 emissions are rather constant for diets containing up to 30-40% of concentrate in DM, then decreasing rapidly to low levels for diets containing 80-90% concentrate (Martin et al., 2010) common in some beef production systems. Replacing forage structural (fibrous) carbohydrates with NSC (starch and sugar) in concentrates drives changes in rumen physico-chemical environment and microbial populations favouring starch-fermenting microbes and propionate formation (Martin et al., 2010). However, even marked reductions in ruminal CH_4 formation are not always accompanied by a shift towards propionate in the rumen VFA (Aguerre et al., 2011). The forage in their study was a mixture of maize and grass silage and rumen fermentation pattern in cattle fed grass silage-based diets appears to be rather resistant to increased concentrate supplementation (Huhtanen et al., 2013). On the other hand, Bayat et al. (2017) reported a significant decrease in acetate to propionate ratio (-28%) and CH_4

emission intensity (-25%) in response to increased concentrate supplementation from 35 to 65% in the diet DM on grass silage-based diets. However, when the diet contained supplemental lipids there was no decrease in CH_4 despite a marked decrease in acetate to propionate ratio (-22%) that was similar to the decrease on unsupplemented diet. It should be noted that the VFA concentrations in the rumen fluid do not directly reflect the VFA production, but the ruminal balance of production and absorption of VFA. This may contribute to the apparent inconsistencies between the VFA profile in the rumen fluid and ruminal CH_4 production in some cases as speculated by Aguerre et al. (2011).

In addition to rumen fermentation pattern, the reduction of rumen pH in high concentrate diets may also contribute to decreased ruminal CH_4 production via the decrease of protozoal numbers (Martin et al., 2010) or direct inhibition of methanogenesis below pH 6 (Van Kessel and Russell, 1996) or both. Although inclusion of high levels of concentrate in the diet of dairy cows is an effective CH_4 mitigation strategy (Table 3), it has disadvantages associated with increased risk for sub-acute rumen acidosis (SARA, review by Krause and Oetzel, 2006), competition with human food sources, GHG emissions during grain production and high feed cost. In addition, fibrous forage rather than starchy concentrate is the predominant natural part of the cattle diet they are specialised to utilize.

Carbohydrate source. Fibrous, human inedible by-products of food and bioenergy industries to partly or totally replace starch-rich cereal grains in the diet of high-producing dairy cows could provide a cost-effective and ethically sound feeding strategy that promote also circular economy. In addition, fibre-rich diets promote rumen and animal health as starch and other readily fermentable carbohydrates are at high amounts known to greatly modify the rumen environment through a decrease in pH and, consequently, to predispose to SARA (Krause and Oetzel, 2006).

Feeding dairy cows a fibre-rich concentrate with pulps, soya bean hulls or to some extent cereal bran as main carbohydrate ingredient has resulted in similar DM intakes, ECM production, FE and NUE as concentrates rich in cereal starch under variable isonitrogenous dietary conditions in terms of forage to concentrate ratio and forage type (Table 4). It should, however, be noted that all these trials were made in mid- to late lactation and the ECM production was around 30 kg/d. Therefore, at higher milk production and nutrient demand levels in early and at peak lactation, the results on animal performance may be different. At early lactation (30 days in milk), Piccioli-Cappelli et al. (2014) reported no difference in DM intake or lactation performance between dairy cows fed diets low or high in readily fermentable carbohydrates (starch plus sugars 18 vs 25 % in diet DM, milk yield level 37 kg/d). However, the alterations in the concentrations of energy metabolites and hormones in blood together with body weight loss indicated mobilization of body reserves in low-starch diet relative to high-starch diet that in contrast resulted in positive energy balance and some body weight gain. It seems that a large proportion if not all cereal starch can be replaced by fibrous by-products of high digestibility such as sugar beet pulp and soya bean hulls up to milk production level of 30 kg/d without a significant decrease in dairy cow lactation performance. Furthermore, Cabezas-Garcia et al. (2017) demonstrated recently that it is possible to replace barley and late-cut grass silage with early-cut grass silage of high energy value without compromising ECM yield, NUE and CH_4 emission intensity. In their study, the proportion of concentrate in the diet DM incrementally decreased from 60 to 45% and the starch from 25 to 17%, whereas NDF gradually increased from 36 to 42%.

Though Benchaar et al. (2001) modelled that replacing fibrous concentrate with starchy one reduces CH_4 emissions, a critical dietary concentration of starch of 20 to 22 % in DM is required to mitigate ruminal methanogenesis (Tables 3 and 4; Hassanat et al., 2013). On lipid supplemented diets, however, even a dietary starch content of 28% in DM did not alter ruminal CH_4 formation (Pirondini et al., 2015 Table 4; Bayat et al., 2017 Table 3). The decrease in ruminal protozoa population and the shift of rumen fermentation towards propionate seem to be the main factors for reduced CH_4 emission intensity on starchy concentrate diets (Pirondini et al., 2015; Bougouin et al., 2018). Though high-starch diets are promising in mitigating CH_4 emission intensity of dairy cattle, the level of cereal starch needed to obtain significant reduction is very high. This contradicts with the uniqueness of ruminants to convert fibrous biomass inedible to monogastrics to high-quality human food.

3.3 Protein

Level of protein supplementation. Conventional good quality protein sources rapeseed and soya bean meals typically increase DM intake of dairy cows (meta-analysis of Huhtanen et al., 2011) though the effect has been negligible in some cases (Table 4). The increase in feed intake may at least in part be attributed to improved digestibility of dietary fibre and CP (Broderick, 2003; Jaakkola et al., 2009), but better amino acid balance of conventional protein feeds or increased nutrient demand due to higher milk production may also contribute (Gidlund et al., 2017). The improved ECM and milk protein yields in response to good quality CP supplementation probably results from higher supply of essentials amino acids to mammary gland (Gidlund et al., 2017). However, the increase in ECM production above dietary CP content of 14-15% is in general rather moderate and seem to level out or even decline at high dietary CP concentrations above 18-20% (Table 4; Broderick, 2003). Interestingly, the production responses of rapeseed meal have been similar irrespective of a wide range of forage CP concentration (Jaakkola et al., 2009; Gidlund et al., 2017 in Table 4). Replacing soya bean meal with dehydrated lucerne also resulted in lower (-7%) milk yield (Doreau et al., 2014). All this indicates the superiority of protein in rapeseed and soya bean to that in forage to enhance milk production.

Dietary CP concentration is the best predictor of NUE in dairy production (meta-analysis by Huhtanen and Hristov, 2009). Indeed, the linear decrease of NUE in response to incremental supply of dietary CP is consistently reported (Table 4) the efficiency typically ranging between 40 and 20% (Dijkstra et al., 2011). With low protein diets, faecal N excretion represents a larger proportion of N intake (up to 50%) than urine N (as low as 25%), but as dietary protein continue to increase the contribution of faecal N decreases and the environmentally labile urinary N exponentially increases up to 60% of N intake (Dijkstra et al., 2011). In part this is related to the failure of the rumen microbes to utilise the extra degradable protein since the primary route of removing excess ammonia from the rumen is conversion to urea in the liver, followed by excretion in urine (Castillo et al., 2000).

The most obvious reason why increased dietary level of CP could in theory reduce ruminal CH_4 emissions was that the fermentation of protein produces less CH_4 than that of carbohydrates (Bannink et al., 2006). The potential to decrease ruminal CH_4 emissions intensity by increased protein supplementation has been small in practise; the low to medium inclusion level of protein feed resulting in the smallest ruminal CH_4 emission intensity (at best around -15%) together with the biggest improvement in lactation performance (Table 4). The excess of dietary CP in dairy cow ratios is unnecessary and unwanted since the protein feeds are expensive and the improvement in milk and CH_4 production are diminishing or even inverse at the highest CP levels (Table 4) leading only to more significant N load to environment via manure and urine.

Protein source. Inclusion of rapeseed meal in dairy ratios generally increases DM intake, ECM yield and milk protein yield to a larger extent relative to soybean meal (review by Huhtanen et al., 2011; Table 4) and to other protein sources (review by Martineau et al., 2013). Huhtanen et al. (2011) suggested that the greater milk production responses with rapeseed meal is due to increased or more balanced amino acid supply (histidine in particular) or both, the greater energy demand for milk production pulling also DM intake. All this also results in slightly improved NUE in rapeseed supplemented diets relative to soya bean (Table 4). The reports comparing the effects of rapeseed meal to soya bean on enteric CH_4 production are scarce. Gidlund et al. (2015; Table 4) reported a marginal decrease in CH_4 emission intensity on rapeseed relative to soya bean meal across a wide range of dietary CP contents.

Grain legume faba bean (*Vicia faba*) and pea (*Pisum sativum*) seeds are relatively rich in protein (23-30% of DM) and starch (45-50 % of DM) making them an interesting home-grown protein and energy source for dairy cow feeding in the temperate areas. However, the protein of these alternative grain legumes is more rumen degradable and lower in methionine than that of rapeseed or soybean, which may limit the lactation performance of dairy cows (review by Halmemies-Beauchet-Filleu et al., 2018). Replacing protein in soya bean meal partially or completely with faba beans or peas has, however, resulted in rather similar bovine lactation performances (Halmemies-Beauchet-Filleau et al., 2018, Table 4).

In contrast, the milk production responses of alternative grain legumes are often inferior compared to the rapeseed meal (Halmemies-Beauchet-Filleau et al., 2018; Table 4). Puhakka et al. (2016) reported a decreased milk protein yield together with an increased milk urea concentration and the proportion of N excreted in urine suggesting less efficient use of protein in faba beans than in rapeseed leading to increased N emissions to environment. However, the NUE of alternative grain legumes seems rather similar to soya bean and rapeseed meal in most of the studies (Table 4). It can be speculated that the inclusion of faba beans or peas in the dairy cow diet could increase starch intake and shift rumen fermentation towards propionate thus mitigating ruminal CH_4 production, but in recent studies (Ramin et al., 2017; Cherif et al., 2018; Johnston et al., 2019) the effect on CH_4 emission intensity has been negligible (Table 4).

4 Case study: Effects of milled rapeseed on milk production, milk fat composition and ruminal CH_4 emissions of dairy cows in practical farm conditions

Introduction. Besides mitigating ruminal CH_4 production unsaturated lipids such as 18:1n-9 rich oil from rapeseeds have great potential to modify lipid composition of ruminant meat and milk by decreasing the proportion of saturated fatty acids and increasing that of unsaturated fatty acids inherent to lipid supplement. This is noteworthy as milk and dairy products contribute significantly to human 12:0, 14:0 and palmitic acid (16:0) consumption, excessive intake of these saturated fatty acids predisposing to cardiovascular disease as well as lowered insulin sensitivity (review by Shingfield et al., 2013). The form of lipid inclusion in the dairy cow diet affects lipid bioavailability and final product composition. Milling of rapeseeds was necessary to release lipids within seeds for efficient absorption (Kairenius et al., 2009). Furthermore, milled rapeseeds in the diet resulted in a similar fatty acid profile in bovine milk as free rapeseed oil with the exception of lower increase in *trans* fatty acids. In dairy farms, milling whole oilseeds according to consumption also minimises the risk for oxidative deterioration of unsaturated lipid during the feed component storage relative to pure oil. The aim of this study was therefore to examine the effects of milled rapeseed on milk fat composition and ruminal CH_4 emissions of dairy cows in practical farm conditions.

Materials and methods. The dairy cow study was conducted at the University of Helsinki, Viikki research farm in Finland (for details see Halmemies-Beauchet-Filleau et al., 2019). In brief, the whole Finnish Ayrshire herd in milk was fed a control diet for 3 weeks (Period 1) followed by rapeseed lipid-rich diet of 4 weeks (Period 2). After this, all cows were switched back to the control diet (3 weeks, period 3). Forage-rich dairy cow TMR based on high quality grass silage (digestible OM 696 g/kg DM, 60% in TMR DM) were fed *ad libitum*. The pre-wilted grass silage (predominantly timothy and meadow fescue) was of 1st cut and ensiled with formic acid-based additive in big bales. Concentrates in TMR (40% in TMR DM) comprised of home-grown cereals, rapeseed feeds as protein supplement, molassed sugar beet pulp and vitamins and minerals. Rapeseed protein was isonitrogenously supplied either as a lipid extracted meal (control diet) or full-fat seeds milled daily during TMR preparation using an ordinary hammer mill (sieve pore size 6 to 8 mm) (test diet). The amount of additional rapeseed lipids in the test diet was ca. 50 g/kg diet DM. Cereal in the control diet was barley and in the test diet oats. When visiting the milking-robot (Lely Astronaut A3, Lely, Maassluis, The Netherlands), cows producing less than 30, between 30 and 40 and over 40 kg of milk per day at the beginning of the trial received 3, 4 or 5 kg of standard concentrate per day throughout the study. The milking robot was equipped with GreenFeed system (C-Lock Inc., Rapid City, SD, USA) that measures ruminal CH_4, carbon dioxide and H_2 emissions.

Results and discussion. Cows had no health concerns when fed the test diet, but DM intake was decreased by on average 4% relative to control diet (for details see Halmemies-Beauchet-Filleau et al., 2019). This is not unexpected because lipid supplementation often suppresses DM intake at high inclusion rates (review of Huhtanen et al., 2008; Halmemies-Beauchet-Filleau et al., 2017). As ECM was unaffected by the test diet, the FE was marginally improved from 1.34 to 1.40 (ECM kg per DM intake kg) compared with control diet. Protein yield and milk urea content were also unaffected by the form of rapeseed protein in the diet. Though test diet had no effect on milk fat yield, it altered milk fat composition (Table 5). The total saturated fatty acid content of milk fat from the test diet was 17% lower than from the control diet (Table 5). Furthermore, the 10- to 16-carbon saturated fatty acids, regarded as the key blood cholesterol-increasing fatty acids in humans, were substantially lower in milk from the test than in the milk from the control diet. Indeed, increased supply of long-chain fatty acids is known to inhibit de novo synthesis of saturated fatty acids in the mammary gland (review of Shingfield et al., 2010). The total monounsaturated fatty acids were 58% higher in milk fat from the test diet than the control diet that principally originated from 18:1n-9. The effect of milled rapeseeds on polyunsaturated fatty acids in milk was marginal. Furthermore, milk fat and fat-rich dairy products with a high monounsaturated fatty acid content are less susceptible to oxidative deterioration (Lin et al., 1996) compared with milk fat enriched with polyunsaturated fatty acids (Havemose et al., 2006).

Ruminal CH_4, carbon dioxide and H_2 emissions were decreased by 18, 5 and 36%, respectively on the test diet relative to control. Thus, milled rapeseeds substantially decreased H_2 load and CH_4 formation in the rumen of dairy cows fed diets rich in highly digestible grass silage (Figure 1). The small decrease in DM intake cannot account for all the diminution in the ruminal H_2 and CH_4 emissions observed in the test diet. It is likely that the rumen fermentation pattern shifted towards propionate that increases H_2 utilisation. Rapeseed or other unsaturated lipids in the dairy cow diet have decreased ruminal acetate to propionate ratio in some (Hristov et al., 2011; Table 2), but not in all studies (Table 2). Though ruminal biohydrogenation of unsaturated fatty acids is an alternative H_2 sink as well, its significance to the overall ruminal methanogenesis is generally considered very low (Martin et al., 2010). The CH_4 emission intensity was 12.1 vs. 15.1 kg CH_4 per kg ECM for test and control diet, respectively. In an average dairy herd in Finland that produces 10 300 ECM per year per cow and has 45 dairy cows a change from control diet typical to the production system in the area to test diet would thus represent an annual decrease of 1 390 t in ruminal CH_4 emissions. This corresponds to the withdrawal of CH_4 production of the whole herd of about 2 months in a year.

Conclusions. Replacing rapeseed meal with milled rapeseeds (supplying 5% of lipid in diet DM) in a dairy cow diet based on highly digestible grass silage had no adverse effects on milk production, FE or animal health. Milled rapeseeds improved milk fat profile by decreasing the proportion of cholesterol-raising medium-chain saturated fatty acids and increasing that of 18:1n-9 in a whole herd level. In addition, milled rapeseeds fed at a commercially practical level substantially suppressed ruminal CH_4 production.

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5 Summary and future research perspectives

Nutritional strategies available for mitigating GHG emissions from dairy cow production include various rumen modifiers under development and forage and/or concentrate based dietary strategies currently more flexibly available and applicable in practice. Given the ambition for reducing human-edible feed ingredients such as grains in animal feeding forage-based dietary strategies should be stressed especially with ruminant animals specialized in fibre digestion. In temperate areas, major plant species available for silage making include grasses, forage legumes and maize crop but their availability in various areas depend a lot on local climatic conditions.

Grass silages. Altering forage maturity at harvest has the greatest potential to reduce environmental footprint of cool-season grass silages in dairy production. Harvesting grass herbage at early rather than late maturity stage has led to increased DM intake, ECM, FE and reduced CH_4 emission intensity with dairy cows, though at the expense of reduced NUE. The trade-offs between reduced CH_4 emissions and reduced NUE are complex and clearly warrant further research. Other management factors such as N fertilization rate, use of additives in ensiling or high-sugar grass cultivars were of minor importance.

Forage legume silages. Limited data on the effects of forage legume silages on CH_4 emission intensity in comparison to grasses together with reduced NUE suggest minor potential for forage legumes to reduce environmental footprint of dairy production. In contrast, literature suggests lower CH_4 emission intensity for forage legumes than grasses provided that higher DM intake potential and ruminal passage rates characteristic to forage legumes occur. Contrasting results may be attributable to practice of growing and feeding forage legumes in mixtures with grasses or other plants as well as large variation in silage nutritive and fermentation quality between years. Further research on potential of forage legumes to reduce environmental footprint of dairy production is needed to fully exploit their beneficial effects on forage production, feed DM intake and animal performance.

Maize silages. Starch containing maize silage can be characterized with high metabolizable energy and low CP contents, which makes it a highly valuable forage crop and compatible to be mixed with grasses and legumes higher in CP. The means available for reducing environmental footprint of maize silage include advancing maturity of maize crop at harvest to late stage (40% DM) and using maize cultivars developed for higher cell wall digestibility and intake properties such as brown midrib maize. These methods may have potential to reduce CH_4 emission intensity up to 10% on high-forage diets. Replacing grass or legume forage silages with maize silage consistently leads to environmental benefits in terms of reducing CH_4 emission intensity on high-forage grass silage based diets, and improving NUE especially on forage legume silages high in CP without compromises in milk production. Nevertheless, further research is needed to optimize the use of these forages in dairy production. Especially, more research on the effects of forage legume N fractions on NUE and forage carbohydrate type on CH_4

emissions are warranted. Moreover, chain level analyses comparing environmental effects of maize and perennial silage crops are needed.

Lipids in concentrate. Unsaturated plant lipids at inclusion levels up to 5% in diet DM have potential to mitigate ruminal CH_4 emissions in a dose dependent manner by 20-40% on diets based on conserved grass or forage legumes without negative effects on animal performance in terms of ECM yield and FE. The effect of lipids seems to persist throughout the entire lactation, but more long-term studies are needed to confirm the persistency. At high lipid inclusion rates, feeding lipid as a part of TMR is preferred to separate concentrate feeding. In contrast, on starchy diets (based on maize silage or rich in concentrate starch) lipid supplementation is of limited interest due to the negative effect on ECM yield. This is probably linked to more detrimental effect of unsaturated lipid on rumen fibre digestion when basal diet contains significant amounts of starch.

Carbohydrates in concentrate. Increasing the proportion of cereal starch in the dairy cow diet in general improves feed intake, ECM yield and NUE. A critical dietary concentration of starch of 20-22% in the diet DM is required to mitigate ruminal methanogenesis and decreases of 20-25% have been reported, when the starch content has reached 20-32% in the diet DM. However, high inclusion of readily fermentable carbohydrates from cereals predisposes to SARA and competes with human nutrition. Fibrous, human inedible by-products of food and bioenergy industries provide a cost-effective and ethically sound feeding strategy that promote also circular economy. Soya bean hulls, sugar beet pulp and cereal bran have partly or totally replaced starch-rich cereal grains in the diet of dairy cows without a decrease in animal performance or increase in ruminal CH_4. However, the production level of mid-lactation cows has not exceeded 30 kg/d in these studies, so more research is needed at higher levels of milk production and at early lactation to confirm these promising findings.

Protein in concentrate. Good quality protein sources rapeseed and soya bean meals typically increase DM intake of dairy cows though the effects have been negligible in some cases. The low to medium inclusion level of protein feed (dietary CP content of 15-18% depending on the CP of the basal forage) results in the smallest ruminal CH_4 emission intensity (at best around -15%) together with the biggest improvement in lactation performance. The excess of dietary CP in dairy cow ratios (CP above 18-20% in DM) is unnecessary and unwanted since the protein feeds are expensive and the improvement in milk and CH_4 production are diminishing or even inverse at the highest CP levels leading only to a more significant N load to environment via manure, urine in particular. Interestingly, the protein in conventional dairy cow protein feeds rapeseed and soya bean is superior to that in forage to enhance milk production. Furthermore, rapeseed protein is slightly superior to soya bean, and faba bean and pea in terms of lactation performance, but in ruminal CH_4 emissions the differences between these protein sources are negligible. Faba beans and peas are promising home-grown protein and energy sources for dairy cow feeding in the temperate areas due to their relatively high CP and starch content. More research is needed to find ways to improve the CP utilization of forage and alternative grain legumes to improve their NUE in milk production.

6 Where to look for further information

The following review articles or meta-analysis provide a good overview of the subject:

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Table 1. The effects of substituting basal forage with forage differing in plant maturity, ensiling method or plant species on dairy cow performance

				Change in % relative to control[1]					CH_4 emission intensity, g CH_4/kg ECM			
Basal forage[2]	Plant species / Variety	Substituting forage[2]	F:C[3]	DMI	ECM	FE	NUE	Rumen C2 / C3	Control	Test	Change in %	Reference
Grass silage												
Late cut	Ryegrass[4]	Early cut, Primary growth	65:35	9	11	2	-7	-2	16.9	14.7	-13	Brask et al. 2013a
Late cut	Timothy	Early cut, Primary growth	60:40	10	13	3	-17	NR	14.0	13.1	-7	Pang et al. 2018
Late cut, Low N	Ryegrass:Timothy	Early cut, Sec. growth	80:20	6	32	25	-13	18	15.9	12.5	-21	Warner et al. 2016
Late cut, High N		Early cut, Sec. growth		20	34	12	-8	-1	16.3	13.1	-20	
Low N		High N		-4	-2	2	-19	-4	15.2	14.9	-2	
Late cut[5]	Ryegrass:Timothy	Very early cut (leafy)	80:20	9	12	6	-35	NR	14.0	11.2	-20	Warner et al. 2017
Late cut[6]		Primary growth[7]		4	11	8	-35	NR	12.9	10.2	-21	
No additive	Ryegrass:Timothy	Inoculated grass	75:25	3	2	-1	4	NR	16.0	16.0	0	Ellis et al. 2016
Low sugar	Ryegrass	High sugar	100:0	-8	-15	-8	46	NR	16.5	17.2	4	Staerfl et al. 2012
Legume silage												
Grass	Timothy	Lucerne	60:40	9	-2	-10	-15	-1	12.8	13.4	5	Hassanat et al. 2014
Red clover:grass 30:70	NR:Timothy	Red clover:grass 70:30	60:40	-1	0	1	-10	NR	15.2	15.5	5	Gidlund et al. 2017
Grass:sainfoin:maize 86:0:14	NR	Grass:sainfoin:maize 42:42:16	70:30	5	7	2	-5	NR	15.0	13.9	-7	Huyen et al. 2016
Maize silage												
Early cut maize	LG30218	Late cut maize	80:20	0	0	0	-3	13	12.8	11.9	-7	Hatew et al. 2016
Conventional maize	NR	Brown midrib maize	65:35	6	8	1	5	-4	14.0	12.6	-10	Hassanat et al. 2017
Barley	Cut at soft dough	Maize, two-thirds at milkline	60:40	20	15	-4	6	-13	15.0	14.9	-1	Benchaar et al. 2014
Grass	NR	Maize	80:20	8	7	-1	18	6	16.6	15.0	-10	Van Gastelen et al. 2015
Early cut grass	Ryegrass[4]	Maize	65:35	-1	0	2	24	-22	14.7	13.8	-6	Brask et al. 2013a
Late cut grass				7	11	4	15	-23	16.9	13.8	-19	
Grass:maize 75:25	NR	Grass:maize 25:75	50:50	11	5	-5	-7	NR	15.0	14.3	-5	Reynolds et al. 2010
Grass:maize 75:25[8]	Third cut ryegrass	Grass:maize 25:75	50:50	28	9	-15	-10	NR	16.3	14.2	-13	Hammond et al. 2016
Same diets as above[9]				19	24	4	15	NR	16.9	16.2	-4	

Grass:maize 75:25	NR	Grass:maize 25:75	50:50	7	-2	-8	1	NR	12.9	12.0	7	Livingstone et al. 2015
Grass	Ryegrass: cocksfoot:fescue	Maize at vitreous stage	45:55	0	-2	-2	6	-5	14.9	13.4	-10	Doreau et al. 2014
Red clover	NR	Maize	60:40	0	2	2	7	-16	14.6	14.1	-4	Benchaar et al. 2015
Lucerne	NR	Maize	60:40	5	-1	-6	14	-26	13.9	14.4	3	Hassanat et al. 2013
Lucerne:maize 80:20	NR	Lucerne:maize 20:80	55:45	-1	0	1	15	-18	17.8	18.1	2	Arnd et al. 2015

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Table 2. The effects of supplemental lipid on dairy cow performance

					Change in % relative to unsupplemented control diet^{1}					CH_4 emission intensity, g CH_4 per kg ECM			
Lipid source	Form	Lipid dosage in diet DM	Basal forage component^{2}	F:C^{3}	DMI	ECM	FE	NUE	Rumen C2 / C3	Control diet	Lipid diet	Change in %	Reference
Medium chain saturated fatty acids													
Myristic acid	oil	5%	Maize silage:grass haylage:hay 55:35:10	60:40	-7	-10	-4	-1	NR	28.4	20.4	-28	Odongo et al. 2007
Myristic acid	methyl ester	5%	Grass silage	60:40	-31	-20	17	2	-6	22.7	18.8	-17	Bayat et al. 2018
Coconut	oil	1.3%	Maize:lucerne:grass silages 75:15:10	50:50	-7	4	12	9	NR	13.9	12.9	-7	Hollmann et al. 2012
		2.7%			-22	-18	4	11	NR	13.9	14.4	3	
		3.3%			-29	-24	8	22	NR	13.9	9.9	-29	
Monounsaturated fatty acids													
Rapeseed	cake	2-3%	Grass:maize silages 55:45	50:50	3	11	8	8	-1	14.6	13.6	-7	Brask et al. 2013b
	crushed				-2	-8	-6	4	-1	14.6	12.1	-17	
	oil				-14	4	21	6	7	14.6	12.0	-18	
Rapeseed	crushed	3%	Maize silage	65:35	-5	-10	-6	7	-2	13.7	14.0	3	Brask et al. 2013a
			Early grass silage^{4}		-2	-1	1	0	-1	15.6	13.9	-11	
			Late grass silage^{4}		1	3	3	3	1	17.8	16.1	-10	
Rapeseed	crushed	2%	Maize:grass silages 75:25	50:50	2	4	1	4	NR	14.7	14.2	-3	Kliem et al. 2019
Rapeseed	oil	5%	Grass silage	60:40	-12	3	17	11	3	22.7	17.5	-23	Bayat et al. 2018
Polyunsaturated fatty acids													
Safflower	oil	5%	Grass silage	60:40	-6	2	9	6	0	22.7	17.5	-23	Bayat et al. 2018
Soybean	oil	3.5%	Maize silage:lucerne haylage:hay 45:40:15	65:35	-3	0	4	NR	NR	18.3	18.2	0	Sauer et al. 1998
Sunflower	oil	5%	Grass silage	65:35	-2	-2	0	-6	-5	18.9	14.5	-23	Bayat et al. 2017
				35:65	-11	-16	-6	13	3	14.2	14.5	2	
Camelinaseed	oil	5%	Grass silage	50:50	-12	-16	-5	6	-5	15.4	13.0	-16	Bayat et al. 2015
Linseed	whole seed	5%	Maize silage:grass hay 90:10	65:35	-2	-1	0	-6	NR	17.7	15.9	-10	Martin et al. 2008
	extruded				-16	-16	-1	4	NR	17.7	13.1	-26	
	oil				-26	-26	0	12	NR	17.7	8.5	-52	

Linseed	extruded	1.8%	Maize silage:grass hay 90:10	60:40	-2	-15	-14	-2	-9	15.4	17.1	11	Ferlay et al. 2013
		3.6%			-5	-16	-12	-3	-14	15.4	15.9	4	Martin et al. 2016
		5.4%			-11	-5	7	12	-27	15.4	9.4	-39	
		1.8%	Grass hay	50:50	-9	-4	6	5	-6	19.8	17.4	-12	
		3.6%			-4	5	10	8	-6	19.8	15.3	-23	
		5.4%			-4	-3	1	12	-12	19.8	12.2	-39	
Linseed	extruded	2%	Maize:grass silages 75:25	50:50	0	2	2	4	NR	14.7	13.4	-9	Kliem et al. 2019
Linseed and palm mix	Ca-salts of oil mix	2%		50:50	-2	3	6	1	NR	14.7	12.8	-13	
Linseed	oil	4%	Maize silage	60:40	-9	-14	-6	1	-22	14.1	12.1	-14	Benchaar et al 2015
			Red clover silage	60:40	-2	2	4	5	-4	14.6	13.0	-11	
Linseed	oil	5%	Grass silage	60:40	-8	3	12	5	2	22.7	17.5	-23	Bayat et al. 2018
Fish	oil	0.8%	Maize silage:lucerne hay: grass hay 55:25:20	52:48[5]	4	8	4	-7	-1	13.5	12.6	-7	Pirondini et al. 2015
		0.8%		52:48[6]	-2	-3	-1	1	0	12.4	13.2	6	
Algae rich in 22:6n-3	meal	0.3%	Lucerne hay	74:26	0	-6	-6	3	2	21.8	23.9	10	Moate et al. 2015
		0.6%			-6	-14	-8	5	-1	21.8	25.7	18	
		1%			-11	-15	-5	0	4	21.8	24.1	11	
Algae rich in 22:6n-3	meal	0.3%[7]	Maize:grass silages 70:30	70:30	0	-12	-12	0	NR	9.5	11.1	16	Klop et al. 2016

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Table 3. The effects of concentrate level and diet starch content on dairy cow performance

						Change in % relative to control concentrate diet[1]					CH$_4$ emission intensity, g CH$_4$ per kg ECM			
Main ingredients of control concentrate (CC)	CC in diet DM, %	Main ingredient difference of substituting concentrate (SC)	SC in diet DM, %	Starch in SC vs CC diets DM, %	Basal forage component	DMI	ECM	FE	NUE	Rumen C2 / C3	CC diet	SC diet	Change in %	Reference
Maize grain, soya bean meal and hulls	32	More maize	39	23 vs 20	Maize-lucerne silage 50:50[2]	0	-1	0	3	-1	18.9	17.2	-9	Aguerre et al. 2011
			46	26 vs 20		3	3	0	3	-4	18.9	16.8	-11	
			53	29 vs 20		4	3	-1	7	4	18.9	15.2	-20	
Maize grain, soya bean meal, dry distillers grains	47	More maize and soya bean	63	32 vs 21	Lucerne hay	3	1	-2	10	NR	14.8	13.7	-8	Niu et al. 2016
Barley, wheat, rapeseed meal	35	More cereals	65	32 vs 14	Grass silage	23	15	-6	-4	-28	18.9	14.2	-25	Bayat et al. 2017
Same as above plus plant oil		Same as above plus plant oil		29 vs 11		11	-2	-12	15	-22	14.5	14.5	0	
Barley, rapeseed cake, soya bean meal	32	More barley	61	22 vs 11	Grass-clover silage	15	10	-4	-15	-31	15.3	12.7	-17	Olijhoek et al. 2018

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[2] Silage mixture containing 50% of maize and 50% of lucerne silage on a DM basis, Not reported and not calculable (NR)

Table 4. The effects of concentrate carbohydrate and protein source and protein level on dairy cow performance

					Change in % relative to control concentrate diet[1]					CH_4 emission intensity, g CH_4 per kg ECM			
Control concentrate (CC) main ingredients	Difference in substituting concentrate (SC)	In SC vs CC diet DM, %	Basal forage component[2]	F:C[3]	DMI	ECM	FE	NUE	Rumen C2 / C3	CC diet	SC diet	Change in %	Reference
Carbohydrate source		**Starch**											
Citrus and sugar beet pulps, soya bean hulls, palm kernel extract	Wheat, wheatfeed	15 vs 10	GS:MS 70:30	70:30	3	0	-3	-4	NR	13.0	13.3	2	Hart et al. 2015
		19 vs 14	GS:MS 30:70		2	-2	-4	3	NR	12.6	12.1	-4	
Maize meal, Soya bean hulls	Less maize meal, more soya bean hulls	28 vs 24[4]	MS:LH:GH 55:25:20	50:50	0	3	3	1	0	13.5	12.6	-8	Pirondini et al. 2015
		28 vs 24[5]	MS:LH:GH 55:25:20		-6	-8	-2	10	1	12.4	13.2	5	
Sugar beet pulp, wheat bran, palm kernel cake	Oats, barley, wheat	15 vs 3	Early GS	66:34	5	1	-4	-2	NR	13.1	13.0	1	Pang et al. 2018
		15 vs 3	Late GS		2	7	2	1	NR	14.0	13.9	1	
Beet pulp, soya bean hull, dried distillers maize grains, wheat bran	Wheat, maize grain, wheat middling, wheat starch	23 vs 6[6]	GS:GH 85:15	50:50	-3	-3	0	9	-13	14.9	13.3	-11	Bougouin et al. 2018
		23 vs 6[7]	GS:GH 85:15		-4	-1	3	1	-14	15.3	11.9	-22	
Protein level		**Protein**											
No protein feed	Soya bean meal	17 vs 15	GS	60:40	0	1	1	-9	NR	17.5	16.9	-3	Gidlund et al. 2015
		19 vs 15	GS		1	5	3	-18	NR	17.5	15.9	-9	
		21 vs 15	GS		0	3	3	-25	NR	17.5	17.8	2	
	Rapeseed meal	17 vs 15	GS		1	3	1	-8	NR	17.5	16.9	-3	
		18 vs 15	GS		4	7	2	-14	NR	17.5	16.1	-8	
		20 vs 15	GS		2	7	5	-19	NR	17.5	15.8	-10	
No protein feed	Rapeseed meal	16 vs 15	GS:RCS 70:30	60:40	5	8	3	-1	NR	16.7	15.6	-7	GIdlund et al. 2017
		17 vs 15	GS:RCS 70:30		10	10	0	-14	NR	16.7	15.2	-9	
		19 vs 15	GS:RCS 70:30		10	6	-4	-22	NR	16.7	14.9	-11	
		17 vs 16	GS:RCS 30:70		4	3	-1	-4	NR	17.8	15.0	-16	
		19 vs 16	GS:RCS 30:70		9	4	-5	-14	NR	17.8	16.4	-8	
		19 vs 16	GS:RCS 30:70		7	7	0	-20	NR	17.8	16.3	-8	
Less soya bean protein	More soya bean protein	16 vs 14	GS:MS 75:25	50:50	8	1	-6	-10	NR	16.7	15.6	-7	Reynolds et al. 2010[8]
		18 vs 14	GS:MS 75:25		0	6	6	-22	NR	16.7	15.2	-9	
Less soya bean protein, rapeseed meal	More soya bean protein, rapeseed meal	16 vs 14	GS:MS 25:75		2	0	-1	-13	NR	17.8	15.0	-16	
		18 vs 14	GS:MS 25:75		5	2	-3	-24	NR	17.8	16.4	-8	
Less soya bean meal	More soya bean meal	19 vs 15[9]	LH	45:55	0	3	3	-18	NR	14.4	14.0	-3	Niu et al. 2016
Protein source		**Protein**											
Soya bean meal	Rapeseed meal	19 vs 19	GS	60:40	2	2	0	5	NR	16.9	16.3	-4	Gidlund et al. 2015

Soya bean meal	Faba bean milled	16 vs 16	LS:MS 65:35	55:45	0	-2	-2	-3	1	15.0	15.2	1	Cherif et al. 2018
	Faba bean rolled	16 vs 16	LS:MS 65:35		1	-2	-3	-3	9	15.0	15.4	3	
Soya bean meal: rapeseed meal 65:35[10]	Faba bean 16%	16 vs 16	GS	60:40	0	5	5	5	NR	17.6	15.9	-9	Johnston et al. 2019
	33%	16 vs 16	GS		0	0	-1	2	NR	17.6	16.2	-8	
	47%	16 vs 16	GS		-1	1	2	-8	NR	17.6	16.9	-4	
Rapeseed meal	Faba bean	19 vs 19	GS	60:40	-2	-4	-3	4	NR	15.2	16.1	6	Ramin et al. 2017
	Pea	18 vs 19	GS		0	-6	-6	-6	NR	15.2	16.7	10	

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Table 5. Fatty acid composition of tank milk (adapted from Halmemies-Beauchet-Filleau et al., 2019)

Fatty acid, g/100 g total fatty acids	Control diet	Test diet	Change in %
10:0	3.9	2.0	-49
12:0	4.6	2.2	-52
14:0	13	8.5	-35
16:0	31	21	-31
18:0	9.7	18	+82
18:1n-9	16	28	+70
18:2n-6	1.3	1.1	
18:3n-3	0.4	0.4	
Total saturated fatty acids	74	61	-17
Total monounsaturated fatty acids	23	36	+58
Total polyunsaturated fatty acids	2.6	2.3	
Total *trans* fatty acids	3.6	5.0	

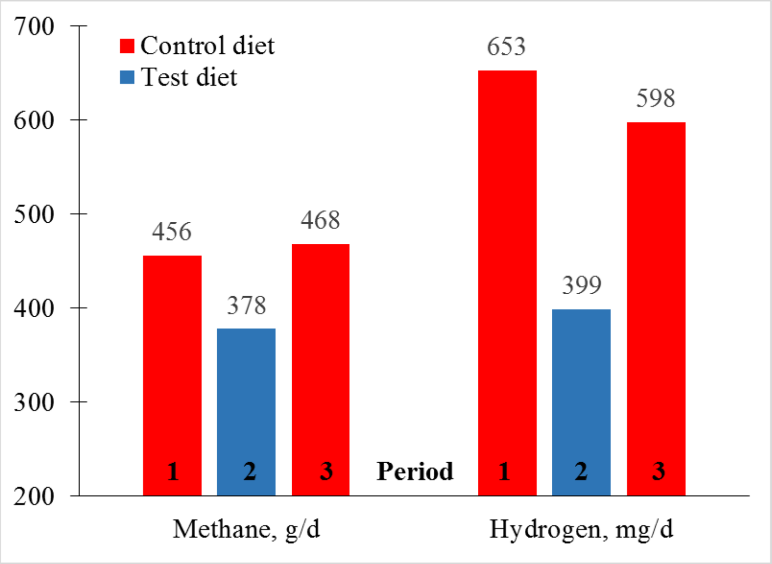

Figure 1. Ruminal gas emissions of dairy cows fed control diet in period 1, test diet in period 2 and again control diet in period 3 (adapted from Halmemies-Beauchet-Filleau et al., 2019)