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

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5

6 **Abstract**

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

19

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

21

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1 Introduction

Ruminant farm animals contribute significantly to global greenhouse gas (GHG) emissions including among others enteric methane (CH₄) and nitrous oxide (N₂O). 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 CH₄ 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 CH₄ emission intensity in terms of CH₄ 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

65 (VFA) fermentation pattern. Any change in dietary composition in favour of propionate
66 production reduces CH₄ owing to consuming reducing equivalents while diets in favour of
67 acetate and butyrate formation generate H₂ for methanogenesis and thus increase CH₄
68 production as reviewed by Knapp et al. (2014). Also other fermentation processes such as
69 rumen protein degradation and assimilation into microbial protein and biohydrogenation of
70 fatty acids taking place in the rumen contribute to the balance of H₂. The former results in
71 either a net consumption or net production of H₂, while the latter results in net consumption
72 of H₂.

73
74 As forages are the main component of dairy cow diets local production of high-quality
75 forage is of utmost importance to dairy farmers for maintaining and ensuring profitable milk
76 production. Therefore, the choice of adopting GHG mitigating nutritional strategy
77 appropriate at farm level depends a lot on geographical location of the farm, which largely
78 determines climatic conditions and the forage plant species available for silage making.
79 For instance, in hot regions silage crops are influenced by high temperatures negatively
80 affecting crop yield and nutritive value while in cold regions a short and cool growing
81 season may limit the use of crops such as maize sensitive to cool temperature as
82 reviewed by Bernardes et al. (2018). This is often the case in Northern areas such as in
83 Northern Europe, where grass species rather than use of maize predominate in silage
84 production. According to Bernardes et al. (2018) climatic conditions affect all stages of
85 silage production and utilization temperature being the most limiting factor. Silage
86 production is dependent both on controllable factors e.g. plant species and stage of
87 maturity, harvesting and ensiling methods and use of additives, and uncontrollable climate-
88 related factors being thus vulnerable to considerable annual variation in silage
89 fermentation quality and nutritive value.

90

91 Concentrate-based strategies include increasing proportion of concentrate in the diet
92 and/or altering the type of carbohydrate (e.g. fibre vs. starch) or type of lipid (e.g. fatty acid
93 profile) supplementation in the concentrate. Even though concentrate components such as
94 grains and oilseeds in the diet are not as sensitive to annual changes in nutritive value
95 relative to forages their contribution to dairy cow feed ration affects a lot on dry matter
96 (DM) intake of forages and digestibility of dietary components e.g. fibre in the diet.
97 According to Hristov et al. (2013) inclusion of concentrate feeds in the diet likely decreases
98 CH₄ emission intensity particularly when inclusion is above 40% of dietary DM and rumen
99 function is not impaired. On the hand, decreasing forage to concentrate ratio in dairy cow
100 diets contradicts with endeavours at reducing use of human-edible components such as
101 grains in the dairy cow feed rations. Sustainable dairy cow feeding strategy should make
102 the most of the unique ability of ruminant animals to convert human-inedible biomass to
103 high-quality animal-derived protein foods i.e. milk and meat.

104

105 In this chapter, we focus on reviewing recently accumulated literature from dairy cow
106 physiological or milk production studies conducted on silage and/or concentrate
107 supplemented diets including measurements on CH₄ production. We look at their dietary
108 effects on the production parameters, feed efficiency (FE), nitrogen use efficiency (NUE)
109 and CH₄ emission intensity in dairy cows in temperate zone conditions with emphasis in
110 Europe and North America. We deal among others with the potential of plant species and
111 stage of maturity of silage crops as well as dietary forage to concentrate ratio and
112 concentrate composition to reduce environmental footprint of dairy cow production without
113 compromising animal performance.

114

115 **2 Role of silages**

116

117 **2.1 Silage plant species**

118

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

122

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

140 biodegradable film in cultivation also enable earlier sowing and harvesting of maize in the
141 marginal areas of Northern Europe such as in Scandinavian countries.

142

143 Cool-season forage grasses are characterised with high digestibility attributed to low
144 temperature and long day length, which delay lignification of cell walls (Huhtanen et al.,
145 2013; Bernardes et al., 2018). As the decline in the rate of digestibility due to advancing
146 maturity is slower with legumes than grasses mixing of these plant species for silage
147 making is thus beneficial extending the optimal harvesting period of the herbages
148 (Kuoppala, 2010). However, concentration of non-structural carbohydrates (NSC) including
149 water soluble carbohydrates (WSC) and starch of these forages is often low and varies a
150 lot depending on the climatic conditions. It affects ensiling potential and feed value of
151 grass and legume forages and can be manipulated with harvesting time and ensiling
152 methods such as use of additives (Vanhatalo and Jaakkola, 2016). There are also so
153 called high-sugar ryegrass cultivars rich in WSC available for silage making (Moorby et al.,
154 2006).

155

156 **2.2 Grass silages**

157

158 Of the grass silage management factors altering forage maturity stage at harvest has the
159 greatest CH₄-mitigating potential while N fertilisation rate, use of additives or high-sugar
160 ryegrass cultivars have no effect (Table 1). Feeding ryegrass-timothy (Warner et al., 2016;
161 Warner et al., 2017), ryegrass (Brask et al., 2013a) or timothy (Pang et al., 2018) silage
162 harvested at early relative to late stage of maturity in high-forage dairy cow diets has
163 increased DM intake, energy-corrected milk yield (ECM) and FE considerably and
164 decreased CH₄ emission intensity in terms of g CH₄ per kg ECM up to 20% (Warner et al.,

2016; Warner et al., 2017). Moreover, reductions in CH₄ 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 CH₄ emission intensity and ensures good milk production level of high-producing dairy cows though with compromises in NUE. Decreases in CH₄ 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 H₂ sink to propionate thus explaining decreases in CH₄. High-sugar content ryegrass grass silage improved NUE

190 in all-silage diet (Table 1; Staerfl et al., 2012) in agreement with beef cattle production
191 studies (Merry et al., 2006), but reduced milk production parameters with minor effect on
192 CH₄ emission intensity. According to Bertilsson et al. (2018) elevated WSC levels in high-
193 sugar ryegrass silage were achieved at the expense of CP and fibre but their effects on
194 dairy cow performance were minor. However, positive effects were attributed to a more
195 favourable distribution of N in terms of more N to milk and faeces and less N to urine.
196 Overall, the issue of improving low NUE of grass forage silages clearly warrants further
197 research. Despite earlier positive production responses of silage inoculants to milk
198 production (e.g. Muck et al., 2018), no such effect nor reduced CH₄ emission intensity
199 owing to inoculated grass silage was seen in a study of Ellis et al. (2016) (Table 1).

200

201 **2.3 Forage legume silages**

202

203 Data on the effects of forage legume silages on CH₄ intensity in dairy production is scarce
204 (Table 1). Replacing timothy with lucerne in dairy cow diets was not effective in reducing
205 CH₄ emissions but led to increased DM intake, impaired FE and especially reduced NUE
206 (Hassanat et al., 2014; Table 1). Inclusion of sainfoin (*Onobrychis viciifolia*) containing
207 condensed tannins in grass-silage based silage increased DM intake and ECM yield but
208 led only to a minor decrease in CH₄ emission intensity (Huyen et al., 2016; Table 1).
209 Replacing mixture of timothy and red clover silage of 70:30 with a mixture of red clover
210 and timothy silage of 70:30 in dairy cows did not affect DM intake, ECM production, FE or
211 CH₄ emission intensity but led to a reduction in NUE (Gidlund et al., 2017; Table 1). This is
212 in agreement with findings of Van Dorland et al. (2007) showing that replacing part of
213 ryegrass silage with red or white clover silage did not affect CH₄ emissions but slightly
214 enhanced N losses to the environment. Even so, according to a review of Phelan et al.

215 (2015) forage legumes have generally resulted in lower CH₄ emissions per kg of milk or
216 meat produced when compared to grasses. However, they emphasise that this occurs only
217 when the forage legume has had higher feed intake and ruminal passage rates than the
218 grasses, or with legumes that contain condensed tannins.

219

220 Indeed, results from comparing forage legume and grass silage-based diets in general
221 demonstrate the superior DM intake and milk production potential of forage legumes over
222 grasses (Vanhatalo and Jaakkola, 2016). Moreover, forage legumes are often considered
223 as an economically profitable alternative to grass and/or maize based forages owing to
224 their ability to provide biologically fixed nitrogen, which serves as an effective means to
225 reduce dependence on synthetic N fertilizers and thus fossil energy (Vanhatalo and
226 Jaakkola, 2016). The higher intake characteristics of legume than grass silages despite
227 lower digestibility have been attributed to their lower fibre content, more rapid fermentation
228 and particle breakdown in the rumen, and higher passage from the rumen (Kuoppala et al.,
229 2009; Kuoppala, 2010; Dewhurst, 2013). Contradictory results on CH₄ mitigation potential
230 of forage legumes may be related to the varying silage fermentation quality and
231 proportions of forage legume in the silages studied. It should be noted, that forage
232 legumes are often grown in mixtures with grasses or other plants rather than as pure
233 stands owing to higher annual herbage yield in mixtures (Phelan et al., 2015). Reduced
234 NUE with forage legume-based diets rather than with grass silage diets stems from their
235 inherently high CP concentrations, especially so with lucerne. However, there are also
236 differences between the forage legume species such as red clover and lucerne in their N
237 fractions, which may differently affect NUE as reviewed by Dewhurst (2013). Clearly, more
238 research is needed on the potential of forage legumes to reduce CH₄ emission intensity
239 and their effects on NUE.

240

241 **2.4 Maize silages**

242

243 According to Hatew et al. (2016) increasing maturity of whole-plant maize from very early
244 (20% DM) to late stage (40% DM) at harvest effectively reduced CH₄ emission intensity on
245 high-forage diet but did not affect DM intake, ECM yield, FE or NUE (Table 1). This was
246 caused by the markedly increased starch content, decreased ruminal fractional rate of
247 degradation of starch and decreased neutral detergent fibre (NDF) content with advancing
248 maturity of maize crop. However, despite higher starch intake this was not attributable to
249 decreased rumen pH and increased propionate as expected. Instead, the acetate to
250 propionate ratio tended to increase with increasing maturity of maize. Their results suggest
251 that harvesting whole-plant maize at a higher maturity instead of the currently
252 recommended practise (30 to 35% DM; e.g. Khan et al., 2015) have potential to reduce
253 enteric CH₄ emissions.

254

255 Maize cultivars developed for potentially higher cell wall digestibility and intake properties
256 (Jung et al., 2011) relative to conventional maize cultivar have been compared recently
257 (Hassanat et al., 2017; Table 1). It was shown that replacing conventional maize silage
258 (DM 40%, starch 269 g/kg DM) with more digestible brown midrib maize silage (BMCS;
259 DM 34%, starch 283 g/kg DM) on high-forage diet increased not only DM intake and ECM
260 yield but improved NUE and reduced CH₄ emission intensity. Again, reduction in CH₄ was
261 not attributed to rumen fermentation pattern, which was unchanged between the
262 treatments. Moreover, it was demonstrated that by using BMCS N excretion in manure
263 reduced and potential N volatilisation was avoided by shifting N excretion from urine to
264 faeces. Nevertheless, increased volatile solid content (i.e. degradable organic matter

excretion) in the manure was observed giving rise to CH₄ emissions from manure storage. However, maize silage type (Falkone vs. LG30224) was of little importance in terms of dairy cow performance and CH₄ 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).

270

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 CH₄ emission intensity (Benchaar et al., 2014; Table 1). However, increasing proportion of maize silage at the expense of barley silage in the diet reduced CH₄ 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 N₂O and ammonia emissions from manure.

278

2.5 Maize silage vs. grass and forage legume silages

280

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 CH₄ emission intensity. Replacing grass silage with maize silage (DM 32%, starch 322 g/kg DM) on restricted high-forage diet improved NUE and reduced CH₄ 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 CH₄ 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

290 150 g/kg DM) had no major effect on DM intake or milk yield, but NUE was improved
 291 particularly in relation to early cut grass silage and CH₄ emission intensity reduced
 292 particularly in relation to late cut grass silage (Brask et al., 2013a; Table 1). Reductions in
 293 CH₄ were attributed to clearly decreased acetate to propionate ratio in the rumen and to
 294 lower ruminal fibre digestibility with maize silage diets.
 295
 296 Increasing proportion of maize in the mixture of grass and maize silage from 25% to 75%
 297 on 50:50 forage to concentrate ratio diet increased DM intake and milk yield and
 298 decreased CH₄ emission intensity, while effects on FE and NUE were variable and more
 299 inconsistent (Reynolds et al., 2010; Hammond et al., 2016; Table 1). However, using
 300 similar experimental setup Livingstone et al. (2015, Table 1) did not find differences
 301 between these silage treatments in any of the parameters mentioned above owing to the
 302 exceptionally low NDF content of the grass herbage. Using low-forage diet, Doreau et al.
 303 (2014) (Table 1) did neither find any differences in these parameters except for the lower
 304 CH₄ emission intensity with maize silage diets. Even so, ruminal acetate to propionate
 305 ratio was unchanged between the treatments.
 306
 307 Also red clover and lucerne silages have been replaced with maize silage on high-forage
 308 diets but with minor effects on DM intake, production parameters and CH₄ emission
 309 intensity while NUE was clearly improved with maize silages (Hassanat et al., 2013;
 310 Benchaar et al., 2015; Arndt et al., 2015; Table 1). In these studies, maize silage maturity
 311 varied in range of 36 to 38% DM and of 290 to 339 g/kg DM starch while starch contents of
 312 legumes were less than 18 g/kg DM (Hassanat et al., 2013; Benchaar et al., 2015).
 313 Despite unaffected CH₄ emission intensity, rumen pH and ruminal acetate to propionate
 314 ratios were clearly decreased when red clover or lucerne was replaced with maize silage

315 in the diet (Hassanat et al., 2013; Benchaar et al., 2015; Arnd et al., 2015). The positive
316 changes for lower urinary and faecal N with maize silage at the expense of forage legume
317 silages in these studies would likely result in lower ammonia and N₂O emissions. Though,
318 the reduced fibre digestion in the rumen with starch containing maize silage diets may lead
319 to increased CH₄ emissions from manure storage (Hassanat et al., 2013).

320 It seems that replacing grass or legume forage silages with maize silage consistently leads
321 to environmental benefits such as reducing CH₄ emission intensity on high-forage grass
322 silage-based diets, and improved NUE especially on forage legume silages high in CP
323 without compromises in milk production. However, despite increased amounts of starch
324 with increasing proportion of maize silage in the high-forage grass diet, reductions in CH₄
325 emission intensity are not necessarily related to changes in rumen fermentation pattern
326 with reduced pH and decreased acetate to propionate ratio as expected. For instance,
327 replacing grass silage with maize silage maintained high rumen pH and increased rumen
328 butyrate in high-forage diet (Van Gastelen et al. 2015). Thus, the reduction in rumen pH
329 enhancing the production of propionate (Dijkstra et al. 2011) does not necessarily occur in
330 high-forage diet. As maize starch is fairly resistant to rumen fermentation it is susceptible
331 to enzymatic digestion in the small intestine (Owens et al. 1986). Consequently, a possible
332 shift from acetate to butyrate in favour of less H₂ production in the rumen (e.g. Moss et al.
333 2000) and reduced H₂ production in the rumen owing to a partial shift of starch digestion to
334 the lower tract may contribute to reduced CH₄ production with maize-containing diets.

335 Nevertheless, a critical dietary concentration of starch is likely required to alter ruminal
336 methanogenesis and decrease CH₄ production (Hassanat et al., 2013; Van Gastelen et al.,
337 2015).

338

339 It is interesting that the clearly decreased acetate to propionate ratio with maize silage
340 diets at the expense of forage legume diets (Hassanat et al., 2013; Benchaar et al., 2015;
341 Arnd et al., 2015) did not lead to reduced CH₄ emission intensity. This may be related to
342 the type of carbohydrate in forage NDF, which affects CH₄ emissions (Arnd et al., 2015).
343 They found that fermentation of maize NDF yielded substantially more CH₄ than
344 fermentation of lucerne NDF. Thus, the greater CH₄ emission expected from greater
345 amount of NDF in lucerne was counterbalanced by a decreasing emission per gram of
346 lucerne NDF fermented. Similar difference in NDF fermentation between maize and
347 lucerne was found in the study of Hassanat et al. (2013). Moreover, in the study of Brask
348 et al. (2013a) less NDF was digested in the rumen for maize silage than grass silage diets
349 despite a comparable NDF concentration in the diets. Further research on the effects of
350 forage carbohydrate type on CH₄ emissions and rumen digestion kinetics is needed. The
351 studies should ideally simultaneously examine rumen fermentation, digestion kinetics and
352 microbiota together with GHG and milk production to give in-depth view on mechanism
353 affecting the rumen function and GHG formation. The positive changes on N losses owing
354 to increasing proportion of maize in the diet suggest that the dietary shift in forage source
355 from forage legumes to maize silage results in lower NH₃ and N₂O emissions from manure
356 (Hassanat et al., 2013; Arndt et al., 2015).

357
358 Nevertheless, it should be emphasised that conclusions about the potential of a feeding
359 strategy to reduce GHG emissions depend also on the level of analysis which may reveal
360 if the strategy is feasible also on the farm and chain level and not only at animal level (Van
361 Middelaar et al., 2013). They found that at animal level increasing maize silage at the
362 expense of grass and grass silage in dairy cow diets is a promising strategy with an
363 immediate effect on GHG emissions. However, application of this strategy to average

364 intensive Dutch farms would lead to problems with EU regulations when reducing
365 grassland area. On the other hand, applying this strategy to intensive farm that can reduce
366 its area of grassland would lead to higher emissions owing to land use change i.e.
367 ploughing grassland into maize land.

368

369 **3 Role of concentrates**

370

371 **3.1 Lipids**

372

373 ***Level of lipid supplementation.*** Lipid supplements are widely used to increase diet
374 energy density to meet the energy requirements of high-producing dairy cows during early
375 and mid-lactation and to improve energy utilization for milk production. However, dietary
376 lipid content should not exceed 6-7% in DM (review by Beauchemin et al., 2008),
377 otherwise a depression of DM intake, ruminal fibre digestibility and further milk production
378 may occur thus hampering the advantages of increased diet energy density (Bayat et al.,
379 2017; Halmemies-Beauchet-Filleau et al., 2017). Lipids are also one of the most effective
380 and practicable means to improve milk or meat fatty acid composition and mitigate GHG
381 emissions of ruminants in industrialized countries, but the effectiveness depends on
382 multiple factors. These include level of lipid supplementation, fatty acid profile of lipid
383 supplement (eg. chain length and level of unsaturation), form in which the lipid is given
384 (eg. oil vs. full-fat seeds) and the type of basal diet (reviews by Eugène et al., 2008;
385 Beauchemin et al., 2008; Shingfield et al., 2013; Table 2). To support long-term health of
386 human consumers, the aim is to decrease the proportion of saturated fatty acids and to
387 increase those of *cis*-monounsaturated fatty acids and omega-3 fatty acids, and improve

388 the balance of omega-6 and omega-3 fatty acids in ruminant products (Shingfield et al.,
389 2013).

390

391 Over a broad range of dietary conditions, Martin et al. (2010) reported a mean decrease in
392 CH₄ emissions of 3.8% with each 1% addition of supplemental lipid in diet DM. One or
393 more mechanisms may contribute to CH₄ mitigation potential of different lipids in the
394 rumen. These include lower amount of organic matter (OM) fermented in the rumen (lipid
395 decreases DM intake and/or replaces rumen fermentable ingredients in the ruminant diet),
396 direct toxicity or inhibition of rumen cellulolytic bacteria, methanogens and/or attached
397 protozoa, shift in ruminal fermentation from acetate to propionate that consume rather than
398 produce H₂ and biohydrogenation of fatty acids in case of unsaturated lipid supplements
399 (review by Martin et al., 2010).

400

401 **Medium chain saturated fatty acids.** Medium chain saturated fatty acid sources such as
402 myristic acid (14:0) or coconut oil rich in lauric acid (12:0) and 14:0 have reduced ruminal
403 CH₄ emission intensity up to 30% when fed at 3.3-5% in diet DM for lactating cows. The
404 primary mechanism is probably through decrease in DM intake (Table 2) and fermentable
405 OM content in the rumen (Bayat et al., 2018). Medium chain saturated fatty acids may also
406 exhibit toxic effects on methanogens (Beauchemin et al., 2008) or protozoa (Hristov et al.,
407 2011) and impair fibre digestion (Hollmann et al., 2012), but these effects are inconsistent
408 between studies. Recently Bayat et al. (2018) reported only minor alterations in the
409 diversity of specific microbial taxa and no effect on total quantities of bacteria,
410 methanogenic archaea or ciliate protozoa or fibre digestion despite a significant decrease
411 in ruminal CH₄ production. Inclusion of medium chain saturates in the diet in general
412 improves FE and NUE in milk production, but concomitant sharp decrease in ECM yield

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

417

418 ***Unsaturated fatty acids.*** Plant unsaturated fatty acids such as oleic acid (18:1n-9) and
419 essential fatty acids linoleic acid (18:2n-6) and α -linolenic acid (18:3n-3) are considered
420 beneficial to human health. Thus, their use in dairy cow diets that typically results in
421 increases of these omega-fatty acids in ruminant milk and meat together with a decrease
422 in saturated fatty acids, could be a viable way for CH₄ mitigation.

423

424 Trials with lactating dairy cows clearly indicate that the effects of lipids on animal
425 performance and rumen methanogenesis are proportional to the level of supply and
426 unsaturation (Table 2). As for saturated fatty acids, the primary mechanism is probably
427 through a decrease in DM intake (Table 2). A dose-response trial by Martin et al. (2016)
428 with three levels of 18:3n-3 rich linseed supplementation up to 5.4% of lipid in the diet DM
429 decreased ruminal CH₄ emission intensity up to 39% with inconsistent effects at lower
430 levels inherent to differences in the composition of the basal diets. Besides lower level of
431 DM intake, the decrease in enteric CH₄ production was attributed to a decreased ruminal
432 acetate to propionate ratio and number of protozoa, whereas the number of ruminal
433 methanogens and fibre digestibility remained unaltered. A modest lipid inclusion (1 to 2%
434 in diet DM) do not suppress feed intake yet, but it already alters milk fatty acid composition
435 (Halmemies-Beauchet-Filleau et al., 2011). At a 5% lipid inclusion level in diet DM,
436 rapeseed rich in 18:1n-9 and 18:2n-6 sources safflowerseed, sunflowerseed and camelina
437 seed have resulted in much less pronounced decreases in CH₄ emission intensity

438 compared to linseed at best reaching a 23% decrease (Table 2). In most studies
439 presented in Table 2 the experimental periods are of 4 to 6 weeks, but recently Alstrup et
440 al. (2015) demonstrated that plant lipids suppress ruminal methanogenesis throughout the
441 entire lactation. However, more studies comprising entire lactation are needed to confirm
442 the persistency of lipids to mitigate ruminal CH₄ emissions.

443

444 It is generally thought that intact oilseeds give a partial protection for oil against microbial
445 metabolism or limits the effects of oil on ruminal microbes and nutrient digestibility or both.
446 However, Martin et al. (2008) reported no difference in OM and fibre digestibility between
447 whole intact linseed, extruded linseed and linseed oil diets. Though pure oil is often more
448 effective to mitigate CH₄ production in the rumen, processed oilseeds (e.g. crushed by
449 milling, extruded, pressed cakes) are preferred because of less adverse effects on DM
450 intake and generally lower price (Beauchemin et al., 2008; Table 2). In addition,
451 administering high levels of dietary unsaturated fatty acids as a part of total mixed ratio
452 (TMR) results in a lower decrease in DM intake (Bayat et al., 2015) than incorporation into
453 concentrates fed separately to forage (Halmemies-Beauchet-Filleau et al., 2017). This is
454 probably due to a more sudden pulsitary release of unsaturated fatty acids in the rumen in
455 separate feeding relative to TMR as high amounts of free unsaturated fatty acids are toxic
456 to cellulolytic bacteria (Maia et al., 2007).

457

458 Basal diet greatly affects the production and enteric CH₄ emission response to lipids. On
459 forage predominant diets (diets rich in fibre) based on 50% or more in diet DM of grass
460 silage, red clover silage or grass hay, unsaturated lipid supplements up to 5% in diet DM in
461 general maintain ECM yield and improve FE. Furthermore, there is a dose-dependent and
462 consistent decrease in ruminal CH₄ emissions (Table 2). In contrast, on starchy diets (diets

463 based on maize silage or rich in concentrate starch), the ECM yield and FE are often
464 compromised already at low lipid inclusion levels (2-4% in diet DM) and the effects on
465 ruminal CH₄ emissions are variable between studies (Table 2). At high lipid inclusion levels
466 (4-5.5% in diet DM), ruminal CH₄ emission intensity is more consistently reduced, but the
467 reduction is often accompanied by a decrease in ECM production (Table 2). Therefore,
468 unsaturated lipid supplements to mitigate CH₄ emissions suit best for diets rich in fibre, but
469 their use in starchy diets is of limited interest due to the negative effects on ECM yield.
470 This is probably linked to a much more detrimental effect of unsaturated lipid on rumen
471 fibre digestion on diets high in starch relative to high fibre diets leading to a decrease in
472 acetate formation in the rumen and further milk fat content (Benchaar et al., 2015; Bayat et
473 al., 2017). In addition, PUFA supplementation on starchy diet readily directs rumen
474 biohydrogenation from *trans*-11 to *trans*-10 route, some *trans*-10 isomers being potential
475 antilipogenic agents in the bovine mammary gland thus causing milk fat depression (MFD;
476 review by Shingfield et al., 2010; Ventto et al., 2017).

477

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

486

487 Eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) present in fish oil or
488 specific algae products have had a strong CH₄ suppressing effect when tested *in vitro*
489 (Martin et al., 2010). However, when fed at low levels (up to 1% in diet DM) to lactating
490 dairy cows, ECM yield together with FE have already decreased without any improvement
491 in ruminal CH₄ emissions. This suggest that lipid supplements rich in 20- and 22-carbon
492 polyunsaturated fatty acids are not a useful tool to mitigate the GHG emissions of dairy
493 cows in practice.

494

495 **3.2 Carbohydrates**

496

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

512 concentrate in the diet increases, there is a concomitant decrease in forage intake and
513 thus forage fibre. The ruminal fermentation of NDF results in lipogenic VFA in the rumen
514 that may account for the decrease in milk fat on diets low in forage.

515

516 The low and high concentrate diets reported in Table 3 were all isonitrogenous (CP 15-
517 18% in DM) except for Olijhoek et al. (2018), where high concentrate diet had a 20%
518 higher CP content relative to a low forage diet leading to a reduced NUE. The general
519 improvement in NUE on concentrate rich diets (Table 3) is probably a result of a better
520 balance of degradable protein and energy available for microbes in the rumen as indicated
521 by lower rumen ammonia concentrations in high concentrate diets (Bayat et al., 2017) and
522 higher relative contribution of good quality protein feeds in dietary protein.

523

524 The reduction of ruminal CH₄ production at increasing levels of concentrate in the diet is
525 well established (Table 3; Martin et al., 2010). Cattle CH₄ emissions are rather constant for
526 diets containing up to 30-40% of concentrate in DM, then decreasing rapidly to low levels
527 for diets containing 80-90% concentrate (Martin et al., 2010) common in some beef
528 production systems. Replacing forage structural (fibrous) carbohydrates with NSC (starch
529 and sugar) in concentrates drives changes in rumen physico-chemical environment and
530 microbial populations favouring starch-fermenting microbes and propionate formation
531 (Martin et al., 2010). However, even marked reductions in ruminal CH₄ formation are not
532 always accompanied by a shift towards propionate in the rumen VFA (Aguerre et al.,
533 2011). The forage in their study was a mixture of maize and grass silage and rumen
534 fermentation pattern in cattle fed grass silage-based diets appears to be rather resistant to
535 increased concentrate supplementation (Huhtanen et al., 2013). On the other hand, Bayat
536 et al. (2017) reported a significant decrease in acetate to propionate ratio (-28%) and CH₄

537 emission intensity (-25%) in response to increased concentrate supplementation from 35
538 to 65% in the diet DM on grass silage-based diets. However, when the diet contained
539 supplemental lipids there was no decrease in CH₄ despite a marked decrease in acetate to
540 propionate ratio (-22%) that was similar to the decrease on unsupplemented diet. It should
541 be noted that the VFA concentrations in the rumen fluid do not directly reflect the VFA
542 production, but the ruminal balance of production and absorption of VFA. This may
543 contribute to the apparent inconsistencies between the VFA profile in the rumen fluid and
544 ruminal CH₄ production in some cases as speculated by Aguerre et al. (2011).

545

546 In addition to rumen fermentation pattern, the reduction of rumen pH in high concentrate
547 diets may also contribute to decreased ruminal CH₄ production via the decrease of
548 protozoal numbers (Martin et al., 2010) or direct inhibition of methanogenesis below pH 6
549 (Van Kessel and Russell, 1996) or both. Although inclusion of high levels of concentrate in
550 the diet of dairy cows is an effective CH₄ mitigation strategy (Table 3), it has
551 disadvantages associated with increased risk for sub-acute rumen acidosis (SARA, review
552 by Krause and Oetzel, 2006), competition with human food sources, GHG emissions
553 during grain production and high feed cost. In addition, fibrous forage rather than starchy
554 concentrate is the predominant natural part of the cattle diet they are specialised to utilize.

555

556 **Carbohydrate source.** Fibrous, human inedible by-products of food and bioenergy
557 industries to partly or totally replace starch-rich cereal grains in the diet of high-producing
558 dairy cows could provide a cost-effective and ethically sound feeding strategy that promote
559 also circular economy. In addition, fibre-rich diets promote rumen and animal health as
560 starch and other readily fermentable carbohydrates are at high amounts known to greatly

561 modify the rumen environment through a decrease in pH and, consequently, to predispose
562 to SARA (Krause and Oetzel, 2006).

563

564 Feeding dairy cows a fibre-rich concentrate with pulps, soya bean hulls or to some extent
565 cereal bran as main carbohydrate ingredient has resulted in similar DM intakes, ECM
566 production, FE and NUE as concentrates rich in cereal starch under variable
567 isonitrogenous dietary conditions in terms of forage to concentrate ratio and forage type
568 (Table 4). It should, however, be noted that all these trials were made in mid- to late
569 lactation and the ECM production was around 30 kg/d. Therefore, at higher milk production
570 and nutrient demand levels in early and at peak lactation, the results on animal
571 performance may be different. At early lactation (30 days in milk), Piccioli-Cappelli et al.
572 (2014) reported no difference in DM intake or lactation performance between dairy cows
573 fed diets low or high in readily fermentable carbohydrates (starch plus sugars 18 vs 25 %
574 in diet DM, milk yield level 37 kg/d). However, the alterations in the concentrations of
575 energy metabolites and hormones in blood together with body weight loss indicated
576 mobilization of body reserves in low-starch diet relative to high-starch diet that in contrast
577 resulted in positive energy balance and some body weight gain. It seems that a large
578 proportion if not all cereal starch can be replaced by fibrous by-products of high
579 digestibility such as sugar beet pulp and soya bean hulls up to milk production level of 30
580 kg/d without a significant decrease in dairy cow lactation performance. Furthermore,
581 Cabezas-Garcia et al. (2017) demonstrated recently that it is possible to replace barley
582 and late-cut grass silage with early-cut grass silage of high energy value without
583 compromising ECM yield, NUE and CH₄ emission intensity. In their study, the proportion of
584 concentrate in the diet DM incrementally decreased from 60 to 45% and the starch from 25
585 to 17%, whereas NDF gradually increased from 36 to 42%.

586

587 Though Benchaar et al. (2001) modelled that replacing fibrous concentrate with starchy
588 one reduces CH₄ emissions, a critical dietary concentration of starch of 20 to 22 % in DM
589 is required to mitigate ruminal methanogenesis (Tables 3 and 4; Hassanat et al., 2013).
590 On lipid supplemented diets, however, even a dietary starch content of 28% in DM did not
591 alter ruminal CH₄ formation (Pirondini et al., 2015 Table 4; Bayat et al., 2017 Table 3). The
592 decrease in ruminal protozoa population and the shift of rumen fermentation towards
593 propionate seem to be the main factors for reduced CH₄ emission intensity on starchy
594 concentrate diets (Pirondini et al., 2015; Bougouin et al., 2018). Though high-starch diets
595 are promising in mitigating CH₄ emission intensity of dairy cattle, the level of cereal starch
596 needed to obtain significant reduction is very high. This contradicts with the uniqueness of
597 ruminants to convert fibrous biomass inedible to monogastrics to high-quality human food.

598

599 **3.3 Protein**

600

601 ***Level of protein supplementation.*** Conventional good quality protein sources rapeseed
602 and soya bean meals typically increase DM intake of dairy cows (meta-analysis of
603 Huhtanen et al., 2011) though the effect has been negligible in some cases (Table 4). The
604 increase in feed intake may at least in part be attributed to improved digestibility of dietary
605 fibre and CP (Broderick, 2003; Jaakkola et al., 2009), but better amino acid balance of
606 conventional protein feeds or increased nutrient demand due to higher milk production
607 may also contribute (Gidlund et al., 2017). The improved ECM and milk protein yields in
608 response to good quality CP supplementation probably results from higher supply of
609 essentials amino acids to mammary gland (Gidlund et al., 2017). However, the increase in
610 ECM production above dietary CP content of 14-15% is in general rather moderate and

611 seem to level out or even decline at high dietary CP concentrations above 18-20% (Table
612 4; Broderick, 2003). Interestingly, the production responses of rapeseed meal have been
613 similar irrespective of a wide range of forage CP concentration (Jaakkola et al., 2009;
614 Gidlund et al., 2017 in Table 4). Replacing soya bean meal with dehydrated lucerne also
615 resulted in lower (-7%) milk yield (Doreau et al., 2014). All this indicates the superiority of
616 protein in rapeseed and soya bean to that in forage to enhance milk production.

617

618 Dietary CP concentration is the best predictor of NUE in dairy production (meta-analysis
619 by Huhtanen and Hristov, 2009). Indeed, the linear decrease of NUE in response to
620 incremental supply of dietary CP is consistently reported (Table 4) the efficiency typically
621 ranging between 40 and 20% (Dijkstra et al., 2011). With low protein diets, faecal N
622 excretion represents a larger proportion of N intake (up to 50%) than urine N (as low as
623 25%), but as dietary protein continue to increase the contribution of faecal N decreases
624 and the environmentally labile urinary N exponentially increases up to 60% of N intake
625 (Dijkstra et al., 2011). In part this is related to the failure of the rumen microbes to utilise
626 the extra degradable protein since the primary route of removing excess ammonia from
627 the rumen is conversion to urea in the liver, followed by excretion in urine (Castillo et al.,
628 2000).

629

630 The most obvious reason why increased dietary level of CP could in theory reduce ruminal
631 CH₄ emissions was that the fermentation of protein produces less CH₄ than that of
632 carbohydrates (Bannink et al., 2006). The potential to decrease ruminal CH₄ emissions
633 intensity by increased protein supplementation has been small in practise; the low to
634 medium inclusion level of protein feed resulting in the smallest ruminal CH₄ emission
635 intensity (at best around -15%) together with the biggest improvement in lactation

636 performance (Table 4). The excess of dietary CP in dairy cow ratios is unnecessary and
637 unwanted since the protein feeds are expensive and the improvement in milk and CH₄
638 production are diminishing or even inverse at the highest CP levels (Table 4) leading only
639 to more significant N load to environment via manure and urine.

640

641 **Protein source.** Inclusion of rapeseed meal in dairy ratios generally increases DM intake,
642 ECM yield and milk protein yield to a larger extent relative to soybean meal (review by
643 Huhtanen et al., 2011; Table 4) and to other protein sources (review by Martineau et al.,
644 2013). Huhtanen et al. (2011) suggested that the greater milk production responses with
645 rapeseed meal is due to increased or more balanced amino acid supply (histidine in
646 particular) or both, the greater energy demand for milk production pulling also DM intake.
647 All this also results in slightly improved NUE in rapeseed supplemented diets relative to
648 soya bean (Table 4). The reports comparing the effects of rapeseed meal to soya bean on
649 enteric CH₄ production are scarce. Gidlund et al. (2015; Table 4) reported a marginal
650 decrease in CH₄ emission intensity on rapeseed relative to soya bean meal across a wide
651 range of dietary CP contents.

652

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

661 In contrast, the milk production responses of alternative grain legumes are often inferior
662 compared to the rapeseed meal (Halmemies-Beauchet-Filleau et al., 2018; Table 4).
663 Puhakka et al. (2016) reported a decreased milk protein yield together with an increased
664 milk urea concentration and the proportion of N excreted in urine suggesting less efficient
665 use of protein in faba beans than in rapeseed leading to increased N emissions to
666 environment. However, the NUE of alternative grain legumes seems rather similar to soya
667 bean and rapeseed meal in most of the studies (Table 4). It can be speculated that the
668 inclusion of faba beans or peas in the dairy cow diet could increase starch intake and shift
669 rumen fermentation towards propionate thus mitigating ruminal CH₄ production, but in
670 recent studies (Ramin et al., 2017; Cherif et al., 2018; Johnston et al., 2019) the effect on
671 CH₄ emission intensity has been negligible (Table 4).

672

673 **4 Case study: Effects of milled rapeseed on milk production, milk fat composition** 674 **and ruminal CH₄ emissions of dairy cows in practical farm conditions**

675

676 **Introduction.** Besides mitigating ruminal CH₄ production unsaturated lipids such as 18:1n-
677 9 rich oil from rapeseeds have great potential to modify lipid composition of ruminant meat
678 and milk by decreasing the proportion of saturated fatty acids and increasing that of
679 unsaturated fatty acids inherent to lipid supplement. This is noteworthy as milk and dairy
680 products contribute significantly to human 12:0, 14:0 and palmitic acid (16:0) consumption,
681 excessive intake of these saturated fatty acids predisposing to cardiovascular disease as
682 well as lowered insulin sensitivity (review by Shingfield et al., 2013). The form of lipid
683 inclusion in the dairy cow diet affects lipid bioavailability and final product composition.
684 Milling of rapeseeds was necessary to release lipids within seeds for efficient absorption
685 (Kairenius et al., 2009). Furthermore, milled rapeseeds in the diet resulted in a similar fatty

686 acid profile in bovine milk as free rapeseed oil with the exception of lower increase in *trans*
687 fatty acids. In dairy farms, milling whole oilseeds according to consumption also minimises
688 the risk for oxidative deterioration of unsaturated lipid during the feed component storage
689 relative to pure oil. The aim of this study was therefore to examine the effects of milled
690 rapeseed on milk fat composition and ruminal CH₄ emissions of dairy cows in practical
691 farm conditions.

692

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

710 robot was equipped with GreenFeed system (C-Lock Inc., Rapid City, SD, USA) that
711 measures ruminal CH₄, carbon dioxide and H₂ emissions.

712

713 **Results and discussion.** Cows had no health concerns when fed the test diet, but DM
714 intake was decreased by on average 4% relative to control diet (for details see
715 Halmemies-Beauchet-Filleau et al., 2019). This is not unexpected because lipid
716 supplementation often suppresses DM intake at high inclusion rates (review of Huhtanen
717 et al., 2008; Halmemies-Beauchet-Filleau et al., 2017). As ECM was unaffected by the test
718 diet, the FE was marginally improved from 1.34 to 1.40 (ECM kg per DM intake kg)
719 compared with control diet. Protein yield and milk urea content were also unaffected by the
720 form of rapeseed protein in the diet. Though test diet had no effect on milk fat yield, it
721 altered milk fat composition (Table 5). The total saturated fatty acid content of milk fat from
722 the test diet was 17% lower than from the control diet (Table 5). Furthermore, the 10- to
723 16-carbon saturated fatty acids, regarded as the key blood cholesterol-increasing fatty
724 acids in humans, were substantially lower in milk from the test than in the milk from the
725 control diet. Indeed, increased supply of long-chain fatty acids is known to inhibit de novo
726 synthesis of saturated fatty acids in the mammary gland (review of Shingfield et al., 2010).
727 The total monounsaturated fatty acids were 58% higher in milk fat from the test diet than
728 the control diet that principally originated from 18:1n-9. The effect of milled rapeseeds on
729 polyunsaturated fatty acids in milk was marginal. Furthermore, milk fat and fat-rich dairy
730 products with a high monounsaturated fatty acid content are less susceptible to oxidative
731 deterioration (Lin et al., 1996) compared with milk fat enriched with polyunsaturated fatty
732 acids (Havemose et al., 2006).

733

734 Ruminant CH₄, carbon dioxide and H₂ emissions were decreased by 18, 5 and 36%,
735 respectively on the test diet relative to control. Thus, milled rapeseeds substantially
736 decreased H₂ load and CH₄ formation in the rumen of dairy cows fed diets rich in highly
737 digestible grass silage (Figure 1). The small decrease in DM intake cannot account for all
738 the diminution in the ruminal H₂ and CH₄ emissions observed in the test diet. It is likely that
739 the rumen fermentation pattern shifted towards propionate that increases H₂ utilisation.
740 Rapeseed or other unsaturated lipids in the dairy cow diet have decreased ruminal acetate
741 to propionate ratio in some (Hristov et al., 2011; Table 2), but not in all studies (Table 2).
742 Though ruminal biohydrogenation of unsaturated fatty acids is an alternative H₂ sink as
743 well, its significance to the overall ruminal methanogenesis is generally considered very
744 low (Martin et al., 2010). The CH₄ emission intensity was 12.1 vs. 15.1 kg CH₄ per kg ECM
745 for test and control diet, respectively. In an average dairy herd in Finland that produces 10
746 300 ECM per year per cow and has 45 dairy cows a change from control diet typical to the
747 production system in the area to test diet would thus represent an annual decrease of 1
748 390 t in ruminal CH₄ emissions. This corresponds to the withdrawal of CH₄ production of
749 the whole herd of about 2 months in a year.

750

751 **Conclusions.** Replacing rapeseed meal with milled rapeseeds (supplying 5% of lipid in
752 diet DM) in a dairy cow diet based on highly digestible grass silage had no adverse effects
753 on milk production, FE or animal health. Milled rapeseeds improved milk fat profile by
754 decreasing the proportion of cholesterol-raising medium-chain saturated fatty acids and
755 increasing that of 18:1n-9 in a whole herd level. In addition, milled rapeseeds fed at a
756 commercially practical level substantially suppressed ruminal CH₄ production.

757

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759 Innovations and Technology (EIT) (EIT Food Project 18095: Dairy products with reduced
760 saturated fatty acids) and made in co-operation with Valio Ltd and the University of
761 Reading.

762

763 **5 Summary and future research perspectives**

764

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

773

774 **Grass silages.** Altering forage maturity at harvest has the greatest potential to reduce
775 environmental footprint of cool-season grass silages in dairy production. Harvesting grass
776 herbage at early rather than late maturity stage has led to increased DM intake, ECM, FE
777 and reduced CH₄ emission intensity with dairy cows, though at the expense of reduced
778 NUE. The trade-offs between reduced CH₄ emissions and reduced NUE are complex and
779 clearly warrant further research. Other management factors such as N fertilization rate,
780 use of additives in ensiling or high-sugar grass cultivars were of minor importance.

781

782 **Forage legume silages.** Limited data on the effects of forage legume silages on CH₄
783 emission intensity in comparison to grasses together with reduced NUE suggest minor
784 potential for forage legumes to reduce environmental footprint of dairy production. In
785 contrast, literature suggests lower CH₄ emission intensity for forage legumes than grasses
786 provided that higher DM intake potential and ruminal passage rates characteristic to forage
787 legumes occur. Contrasting results may be attributable to practice of growing and feeding
788 forage legumes in mixtures with grasses or other plants as well as large variation in silage
789 nutritive and fermentation quality between years. Further research on potential of forage
790 legumes to reduce environmental footprint of dairy production is needed to fully exploit
791 their beneficial effects on forage production, feed DM intake and animal performance.

792

793 **Maize silages.** Starch containing maize silage can be characterized with high
794 metabolizable energy and low CP contents, which makes it a highly valuable forage crop
795 and compatible to be mixed with grasses and legumes higher in CP. The means available
796 for reducing environmental footprint of maize silage include advancing maturity of maize
797 crop at harvest to late stage (40% DM) and using maize cultivars developed for higher cell
798 wall digestibility and intake properties such as brown midrib maize. These methods may
799 have potential to reduce CH₄ emission intensity up to 10% on high-forage diets.
800 Replacing grass or legume forage silages with maize silage consistently leads to
801 environmental benefits in terms of reducing CH₄ emission intensity on high-forage grass
802 silage based diets, and improving NUE especially on forage legume silages high in CP
803 without compromises in milk production. Nevertheless, further research is needed to
804 optimize the use of these forages in dairy production. Especially, more research on the
805 effects of forage legume N fractions on NUE and forage carbohydrate type on CH₄

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

808

809 ***Lipids in concentrate.*** Unsaturated plant lipids at inclusion levels up to 5% in diet DM
810 have potential to mitigate ruminal CH₄ emissions in a dose dependent manner by 20-40%
811 on diets based on conserved grass or forage legumes without negative effects on animal
812 performance in terms of ECM yield and FE. The effect of lipids seems to persist
813 throughout the entire lactation, but more long-term studies are needed to confirm the
814 persistency. At high lipid inclusion rates, feeding lipid as a part of TMR is preferred to
815 separate concentrate feeding. In contrast, on starchy diets (based on maize silage or rich
816 in concentrate starch) lipid supplementation is of limited interest due to the negative effect
817 on ECM yield. This is probably linked to more detrimental effect of unsaturated lipid on
818 rumen fibre digestion when basal diet contains significant amounts of starch.

819

820 ***Carbohydrates in concentrate.*** Increasing the proportion of cereal starch in the dairy cow
821 diet in general improves feed intake, ECM yield and NUE. A critical dietary concentration
822 of starch of 20-22% in the diet DM is required to mitigate ruminal methanogenesis and
823 decreases of 20-25% have been reported, when the starch content has reached 20-32% in
824 the diet DM. However, high inclusion of readily fermentable carbohydrates from cereals
825 predisposes to SARA and competes with human nutrition. Fibrous, human inedible by-
826 products of food and bioenergy industries provide a cost-effective and ethically sound
827 feeding strategy that promote also circular economy. Soya bean hulls, sugar beet pulp and
828 cereal bran have partly or totally replaced starch-rich cereal grains in the diet of dairy cows
829 without a decrease in animal performance or increase in ruminal CH₄. However, the
830 production level of mid-lactation cows has not exceeded 30 kg/d in these studies, so more

831 research is needed at higher levels of milk production and at early lactation to confirm
832 these promising findings.

833

834 ***Protein in concentrate.*** Good quality protein sources rapeseed and soya bean meals
835 typically increase DM intake of dairy cows though the effects have been negligible in some
836 cases. The low to medium inclusion level of protein feed (dietary CP content of 15-18%
837 depending on the CP of the basal forage) results in the smallest ruminal CH₄ emission
838 intensity (at best around -15%) together with the biggest improvement in lactation
839 performance. The excess of dietary CP in dairy cow rations (CP above 18-20% in DM) is
840 unnecessary and unwanted since the protein feeds are expensive and the improvement in
841 milk and CH₄ production are diminishing or even inverse at the highest CP levels leading
842 only to a more significant N load to environment via manure, urine in particular.
843 Interestingly, the protein in conventional dairy cow protein feeds rapeseed and soya bean
844 is superior to that in forage to enhance milk production. Furthermore, rapeseed protein is
845 slightly superior to soya bean, and faba bean and pea in terms of lactation performance,
846 but in ruminal CH₄ emissions the differences between these protein sources are negligible.
847 Faba beans and peas are promising home-grown protein and energy sources for dairy
848 cow feeding in the temperate areas due to their relatively high CP and starch content.
849 More research is needed to find ways to improve the CP utilization of forage and
850 alternative grain legumes to improve their NUE in milk production.

851

852 **6 Where to look for further information**

853

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

855

- 856 Dewhurst, R. J. (2013), Milk production from silage: comparison of grass, legume and
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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

Basal forage ²	Plant species / Variety	Substituting forage ²	F:C ³	Change in % relative to control ¹					CH ₄ emission intensity, g CH ₄ /kg ECM			Reference
				DMI	ECM	FE	NUE	Rumen C2 / C3	Control	Test	Change in %	
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

¹Dry matter intake (DMI), Energy corrected milk (ECM) calculated according to Sjaunja et al. (1991), Feed efficiency (FE) calculated as ECM / DM intake, Nitrogen use efficiency (NUE) calculated as N output in milk/N intake, Ruminal acetate to propionate ratio (rumen C2 / C3) calculated from their molar proportions in the rumen fluid, Not reported and not calculable (NR), ² For forage mixtures the proportions of components (%) on a DM basis are given, ³Forage to concentrate ratio on a DM basis (F:C), ⁴Contained clover <10%, ⁵Cows 96 days in milk; ⁶Cows 218 days in milk, ⁷Treatment consisting leafy stage grass contained 5% of chopped wheat straw, ⁸Methane measured with GreenFeed, ⁹Methane measured with respiratory chamber

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

Lipid source	Form	Lipid dosage in diet DM	Basal forage component ²	F:C ³	Change in % relative to unsupplemented control diet ¹					CH ₄ emission intensity, g CH ₄ per kg ECM			Reference
					DMI	ECM	FE	NUE	Rumen C2 / C3	Control diet	Lipid diet	Change in %	
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 crushed oil	2-3%	Grass:maize silages 55:45	50:50	3	11	8	8	-1	14.6	13.6	-7	Brask et al. 2013b
					-2	-8	-6	4	-1	14.6	12.1	-17	
					-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 extruded oil	5%	Maize silage:grass hay 90:10	65:35	-2	-1	0	-6	NR	17.7	15.9	-10	Martin et al. 2008
					-16	-16	-1	4	NR	17.7	13.1	-26	
					-26	-26	0	12	NR	17.7	8.5	-52	

Linseed	extruded	1.8%	Maize silage:grass hay	60:40	-2	-15	-14	-2	-9	15.4	17.1	11	Ferlay et al. 2013 Martin et al. 2016
		3.6%			-5	-16	-12	-3	-14	15.4	15.9	4	
		5.4%	Grass hay	50:50	-11	-5	7	12	-27	15.4	9.4	-39	
		1.8%			-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 Linseed and palm mix	extruded Ca-salts of oil mix	2%	Maize:grass silages	50:50	0	2	2	4	NR	14.7	13.4	-9	Kliem et al. 2019
		2%	75:25	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:	52:48 ⁵	4	8	4	-7	-1	13.5	12.6	-7	Pirondini et al. 2015
		0.8%	grass hay 55:25:20	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	0	-12	-12	0	NR	9.5	11.1	16	Klop et al. 2016

¹Dry matter intake (DMI), Energy corrected milk (ECM) calculated according to Sjaunja et al. (1991), Feed efficiency (FE) calculated as ECM / DM intake, Nitrogen use efficiency (NUE) calculated as N output in milk/N intake, Ruminal acetate to propionate ratio (rumen C2 / C3) calculated from their molar proportions in the rumen fluid, Not reported and not calculable (NR), ²For forage mixtures the proportions of components (%) on a DM basis are given, ³Forage to concentrate ratio on a DM basis (F:C), ⁴Contains <10% in DM clover, ⁵Concentrate low in starch, ⁶Concentrate high in starch, ⁷Supplemental DHA % in diet DM

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

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	Change in % relative to control concentrate diet ¹					CH ₄ emission intensity, g CH ₄ per kg ECM			Reference
						DMI	ECM	FE	NUE	Rumen C2 / C3	CC diet	SC diet	Change in %	
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 Same as above plus plant oil	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		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

¹Dry matter intake (DMI), Energy corrected milk (ECM) calculated according to Sjaunja et al. (1991), Feed efficiency (FE) calculated as ECM / DM intake, Nitrogen use efficiency (NUE) calculated as N output in milk/N intake, Rumenal acetate to propionate ratio (rumen C2 / C3) calculated from their molar proportions in the rumen fluid,

²Silage 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

Control concentrate (CC) main ingredients	Difference in substituting concentrate (SC)	In SC vs CC diet DM, %	Basal forage component ²	F:C ³	Change in % relative to control concentrate diet ¹					CH ₄ emission intensity, g CH ₄ per kg ECM			Reference
					DMI	ECM	FE	NUE	Rumen C2 / C3	CC diet	SC diet	Change in %	
Carbohydrate source													
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													
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													
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	

¹Dry matter intake (DMI), Energy corrected milk (ECM) calculated according to Sjaunja et al. (1991), Feed efficiency (FE) calculated as ECM / DM intake, Nitrogen use efficiency (NUE) calculated as N output in milk/N intake, Ruminal acetate to propionate ratio (rumen C2 / C3) calculated from their molar proportions in the rumen fluid, Not reported and not calculable (NR), ²Grass (G), Hay (H), Lucerne (L), Maize (M), Red clover (RC), Silage (S), for forage mixtures the proportions of components (%) on a DM basis are presented in the appearing order, ³Forage to concentrate ratio in DM basis (F:C), ⁴Without fish oil in the diet, ⁵With fish oil in the diet, ⁶Without bicarbonate in the diet, ⁷With bicarbonate in the diet, ⁸Personal communication of C.K. Reynolds for diet formulation and milk composition, ⁹Mean of two F:C ratios 53:47 and 38:62, ¹⁰Protein feed mixture containing 65% of soya bean meal and 35% of rapeseed meal on a DM basis

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 <i>trans</i> 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)