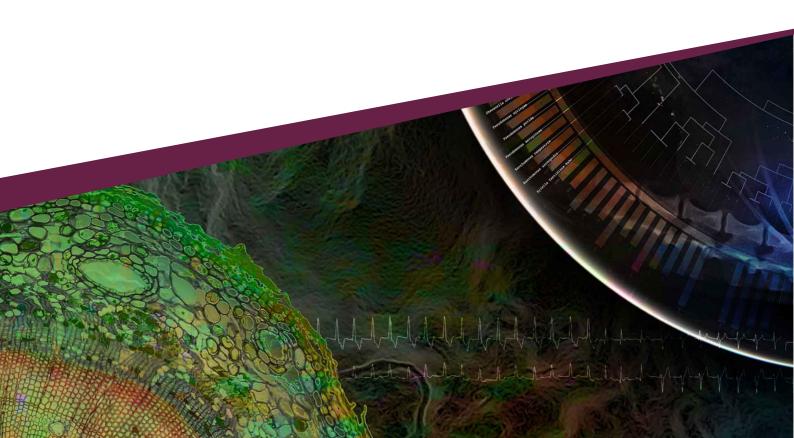


Leys from a Nordic perspective

- A knowledge compilation

David Parsons, Elisabet Nadeau, Dannylo Sousa, Magdalena Åkerfeldt, Gun Bernes, Per Hansson, Birgitta Åhman, Cecilia Palmborg, Valentin Picasso, Brooke Micke, Martin Knicky, Katarina Arvidsson Segerkvist, Sanna Bergqvist, Bengt-Ove Rustas, Mårten Lidfeldt, Sebastian Remvig, Johanna Wallsten



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Summary

The project involved creating a popular scientific review of the cultivation and use of leys for livestock. Target groups are the agricultural sector and industry. The project had four work packages (WP): crop production, harvest and conservation, feed value for different animals, and economy. Within each work package, search strings were developed and inclusion criteria defined, and a database for each WP developed through searching in online databases. Each selected article was assessed first on the title (include or exclude), second based on reading the abstract, and third based on reading the article. The review provides an extensive list of literature related to ley production and use, a description of the most important findings, and suggestions for future research.

Sammanfattning

Projektet gick ut på att göra en populärvetenskaplig kunskapssammanställning om odling av vall och användning till djur. Målgruppen är lantbruksnäringen i Sverige. Projektet hade fyra delar (WP); växtodling, skörd och konservering, fodervärde till olika djurslag och ekonomi. Inom varje WP utvecklades söksträngar för att finna relevanta artiklar, kriterier för inkludering definierades och en databas för varje WP skapades genom sökning i onlinedatabaser. Varje utvald artikel bedömdes först utifrån titeln (inkludera eller exkludera), för det andra efter läsning av sammanfattningen och för det tredje utifrån att läsa artikeln. Sammanställningen har därmed resulterat i en omfattande lista med litteratur relaterad till produktion och användning av vall, en beskrivning av de viktigaste fynden samt förslag för framtida forskning.

Populärvetenskaplig rapport

Gräsmarker spelar en viktig roll för att säkra en hållbar framtid för jordbruket i hela världen. De behövs både som foder för idisslare och för att tillhandahålla ekosystemtjänster, såsom att öka jordens mullhalt, förbättra markens bördighet, fixera atmosfäriskt kväve, minska användningen av bekämpningsmedel och öka den biologiska mångfalden. En utmaning för vallproduktionen i framtiden är att behålla den ekologiska hållbarheten och samtidigt förbättra avkastning och kvalitet i ett förändrat klimat.

Ett projekt har genomförts med syfte att skapa en översikt av relevant nationell och internationell forskning om vall som foder till idisslare (nötkreatur, får och ren) och grisar i Sverige, med lantbrukare, rådgivare och andra intressenter inom näringen i åtanke. Ett annat syfte var att identifiera kunskapsluckor inom de olika ämnesområdena. Projektet har utmynnat i en rapport och arbetet finansierades av Stiftelsen Lantbruksforskning.

Sammanställningen omfattar endast skördad vall, inte bete, inom forskningsområdena växtodling, skörd och konservering, fodervärde för djur samt ekonomi. En systematisk kartläggningsmetod användes för att hitta relevanta vetenskapliga artiklar och rapporter. Inom vart och ett av de utvalda ämnesområdena utvecklades sökord och kriterier definierades för om en artikel skulle tas med eller inte. Utvärderingen omfattar fält- och stallförsök av hög kvalitet som genomförts i Norden och Baltikum från år 2000 fram till dess att projektet inleddes i slutet av 2021. Några viktiga slutsatser nämns nedan.

Odling av grovfoder börjar med artval, som utvecklas med växtförädling. Att vara alltför beroende av ett litet antal arter är i sig riskabelt. Med ett förändrat klimat ökar risken för sjukdomar, skadeinsekter och ogräs, tillsammans med abiotiska faktorer såsom ökad förekomst av isbränna eller minskad vattentillgång. I rapporten utpekas vikten av mångfald, och ytterligare forskning behövs för att utveckla och utvärdera alternativa arter som kan klara dessa utmaningar, med fokus på foderkvalitet, näringseffektivitet och motståndskraft. Gödsling är nödvändig för att ersätta näringsämnen som förs bort med sålda produkter från gården. Nivåerna för mineralgödsel bör baseras på markkartering av makronäringsämnen, stallgödseltillgång och kvävefixering av baljväxter. Att undvika markpackning är viktigt. Kalkning och bevattning är ibland nödvändigt men rätt artsammansättning för gårdens förhållanden är allra viktigast.

Många faktorer påverkar kvaliteten på det lagrade ensilaget, bl.a. artsammansättning, mognadsstadium vid skörd, flytgödselspridning, förtorkning, skörde- och lagringsmetoder samt ensileringsmedel. Det är viktigt att alla steg är väl planerade och genomförda. Bioraffinaderi identifieras som ett område för framtida forskning, med potential att använda den raffinerade fraktionen för enkelmagade djur och biprodukten för idisslare.

Mjölkkor har dominerat den vallinriktade husdjursforskningen, med särskilt fokus på att formulera foderstater med olika grovfoderandel eller näringsinnehåll. För köttdjur och får är frågor om val av arter, antal skördar och skördetidpunkt, samt effekterna på smältbarhet och fiberhalt särskilt relevanta. För renar finns det många okända faktorer när det gäller hur man bedömer ett grovfoder som lämpligt och använder det i foderstaten. Framförallt har vallensilage potential att vara en del av

dräktiga suggors foder, om proteinhalten är hög och fiberhalten låg. Vallensilage kan även ingå i foderstaten till växande grisar, men då i en mindre mängd.

Begränsad forskning har bedrivits om ekonomiska faktorer när det gäller vall. Det finns behov av mångsidiga system och modeller som kan hantera beräkningar, mätningar och analyser i olika produktionssystem.

Vallproduktionen kommer även fortsättningsvis att vara viktig i animalieproduktionen och för att bidra till att upprätthålla jordbruksekosystemens funktion i Sverige. Författarna till denna sammanställning hoppas att den kommer att vara ett användbart dokument för att ge en översikt över tidigare forskning, lyfta fram viktiga resultat och fungera som ett verktyg för att vägleda framtida forskning.

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mix

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List of plant species

Common name

Scientific name

meadow foxtail Alopecurus pratensis cicer milkvetch Astragalus cicer liquorice milkvetch Astragalus glycyphyllos smooth brome Bromus inermis Alaska brome Bromus sitchensis caraway Carum carvi chicory Cichorium intybus cocksfoot Dactylis glomerata couch grass Elymus repens fodder galega Galega orientalis tall fescue Festuca arundinacea (syn. Lolium arundinaceum) meadow fescue Festuca pratensis (syn. Lolium pratense) festulolium × Festulolium annual ryegrass Lolium multiflorum Italian ryegrass Lolium multiflorum perennial ryegrass Lolium perenne hybrid ryegrass Lolium x boucheanum birdsfoot trefoil Lotus corniculatus greater lotus Lotus pedunculatus russel lupin Lupinus polyphyllus yellow lucerne Medicago falcata black medic Medicago lupulina lucerne Medicago sativa Medicago sativa x varia variegated lucerne white sweetclover Melilotus albus yellow sweetclover Melilotus officinalis sainfoin Onobrychis viciifolia reed canary grass Phalaris arundinacea boehmer's cat's-tail Phleum phleoides timothy Phleum pratense ribwort plantain Plantago lanceolata Kentucky bluegrass Poa pratensis salad burnet Sanguisorba minor kura clover Trifolium ambiguum alsike clover Trifolium hybridum zig-zag clover Trifolium medium red clover Trifolium pratense white clover Trifolium repens talish clover Trifolium tumens

1. Materials and Methods

For the analysis, a systematic mapping approach was applied. Systematic mapping is a transparent, robust, and repeatable method used to identify, collect, and assort relevant literature concerning a particular research topic. It provides a structured overview of the research area, but without aiming to synthesize or further process the study results. Instead, the method serves to identify and gather the published knowledge within a given research area and to indicate knowledge gaps where future research is needed. An outline of the methodology is presented in Figure 1.

Through an iterative process involving project members and relevant stakeholders the search strings for each WP to be used in the respective research area were developed and refined. The final search strings are contained in Appendix 1. To find relevant papers, the online databases Scopus, Web of Science Core Collection, CABI Cab Abstracts, Google Scholar and SLUpub were used. The searches were restricted to literature published from January 1, 2000 until the start of the project in late 2021. Further, to be included in the systematic mapping, the papers should originate from the Nordic and Baltic countries. Selected studies were those of high quality, with sufficient description of the methods. If the study was designed in a way where the results could be questioned or if they had not confirmed the results using statistics, then the article was discarded. The focus was on field or barn studies - if the study was performed in a green house, phytotron, climate chamber or other kind of controlled environment the article was discarded. Studies involving genetically modified organisms were not included. Regarding the crop production section, articles involving green manure crops, cover crops, bioenergy, seed crop production, turf grasses, or grazing were not included. If the full article was not accessible, or insufficient information was available in English or Scandinavian, then the study was discarded.

In some cases, grey literature, e.g. popular scientific papers and information material, was obtained from SLUpub, websites of other Nordic universities and websites of organisations such as Government offices of Sweden, Jordbruksverket, Växa Sverige, Hushållningssällskapen and Lantmännen. Method development

- Search strings
- Inclusion criteria

Searching

• Saving results

Screening of titles and abstracts

- Removal of duplicates
- Screening for relevance to topic

Screening of titles

- Removal of duplicates
- Screening for relevance to topic

Screening of abstracts

• Screening for relevance to topic

Retrieval and screening of full text

• Screening for relevance to topic

Development of searchable database

• Development of categories and coding keywords for database

Writing report

Dissemination

Figure 1 Stages in a systematic mapping (adapted from Clapton, Rutter and Sharif, 2009¹).

¹ Clapton, J., Rutter, D., & Sharif, N. (2009). SCIE Systematic mapping guidance. London: SCIE.

2. Crop Production

2.1 Ley species and mixes

David Parsons, Department of Crop Production Ecology, SLU

2.1.1 Introduction

In northern Europe the environmental conditions are challenging, and the number of forage species that are suitable for cultivation and actively used in agriculture is small. Species for ley production need to be able to survive the winter, persist under harvesting, persist under adverse conditions such as drought or waterlogging, be compatible with companion species, be resistant to pests and diseases, and produce forage of both high yield and quality. An additional characteristic that is beneficial is the ability of legume species to supply nitrogen.

A single forage species does not have all of these characteristics, so selecting species to use is a compromise. These limitations can be minimised by using mixtures, thus spreading risk, and potentially achieving results that are more positive than what is possible by using any single species. For this reason, single species are rarely sown, and ley seed mixtures commonly contain multiple species. Risks can also be minimized by sowing different species or mixtures in different fields on the same farm.

This section addresses the very important topic of forage species – how they perform in comparison with each other, how they perform in multi-species mixtures, and attempts to implement new species. Even within species, cultivars can differ in their characteristics and their suitability for different situations.

Much work has been done assessing forage species, and a lot of the literature pre-dates the timeframe of this review. Work is also ongoing, and the variety trials research program tests new cultivars in comparison with established cultivars at various sites across Sweden. This review does not attempt to include this ongoing work. For the latest variety trial information, people should consult the official results. For detailed specific information about individual forage species, there are various sources online, including from seed companies, Swedish Board of Agriculture, and the book "Vallväxter till slåtter och bete samt grönfoderväxter"².

2.1.2 Cultivar trials

Development of new cultivars is an ongoing exercise. Plant breeding is time consuming and many years are needed to bring a new perennial forage cultivar to the market. It is important that cultivars are thoroughly assessed across various locations and years, to draw conclusions from large and diverse datasets. New cultivars are tested in Sweden through official variety trials, and results are published annually by The Rural Economy and Agricultural Society in the "Sverigeförsöken" book and also in "Vallväxter till slåtter och bete samt grönfoderväxter". For information about the performance of cultivars in Swedish conditions, the reader should primarily refer to these sources.

Because of the official variety trials process, limited Nordic and Baltic research is published in peer-reviewed literature. However, the official variety trials mostly focus on yield, persistence, and basic forage quality. Thus, additional research comparing cultivars has often been conducted in order to focus on additional characteristics (e.g. water-soluble carbohydrates), compare across species, or provide more detail on specific agronomic or physiological aspects (e.g. light interception).

A research project that included one location in Uppsala compared two cultivars of perennial ryegrass (*Lolium perenne*), one of which had been bred for high levels of water-soluble carbohydrates (WSC). Although there were differences between cultivars, the results were not consistent between sites and years [1, 2], and strong conclusions could not be drawn. A related project with a research site in Norway [3] found that the cultivar differences in WSC were consistent across different nitrogen application rates. Another project involving perennial ryegrass cultivars focused on winter survival across Nordic countries, and included Lännäs as a Swedish node [4]. The project found that there were large interactions between genotype and environment, emphasising the need to develop cultivars with specific adaptations to regions, highlighting the needs for localised variety trials.

A range of timothy (*Phleum pratense*) and perennial ryegrass cultivars were compared for differences in light interception, which correspondingly affects yield [5, 6]. There were some differences between cultivars, and it was hypothesised that cultivars with more heterogeneous leaf orientation had greater interception than cultivars with erect leaves. This has implications for plant selection and breeding.

Eight lucerne (*Medicago sativa*) cultivars were compared with red clover (*Trifolium pratense*) in terms of yield and forage quality [7]. Trends in yield were not consistent between first and second year leys, and because the experiment had

² https://publications.slu.se/?file=publ/show&id=112152

one site and two years, it is difficult to draw conclusions about differences between cultivars. In general, red clover had lower crude protein (CP) and neutral detergent fibre (NDF) concentrations, and greater in vitro organic matter digestibility, than the lucerne cultivars.

A study conducted in Norway [8] assessed the effects of red clover with different rates of phenological development. They found that red clover cultivars with earlier development had greater re-growth yields, but lower CP and higher NDF. The study aimed to see whether it was possible to select cultivars with higher spring growth rate, but they were not successful. In Norway, most red clover cultivars are diploids. An experiment comparing red clover cultivars in grass mixtures found that some tetraploids had higher yield than diploids, and that the ranking of cultivars in mixtures was similar to monoculture variety trials [9]. This has interesting implications for Swedish red clover variety trials, which are also typically conducted as monocultures. In Lithuania, a study with a wide range of cultivars [10] found no significant difference in yields between diploids and tetraploids, however the average seed yield was 16.3% higher for diploids.

Research in Lithuania [11] compared 46 lucerne and hybrid lucerne (*Medicago sativa* x *varia*) cultivars of different geographic origin, sown with grass in both fertile and acidic infertile soils. As expected, lucerne did not persist well when cultivated in an acidic soil. Cultivars from Baltic countries were least affected by downy mildew (there were no Nordic cultivars).

An experiment in Norway [12] compared the effect of different timothy cultivars in mixed swards with meadow fescue (*Festuca pratensis*). Compared with cultivars 'Noreng' and 'Vega', 'Grindstad' had more generative tillers (shoots), i.e. that lead to flower formation, and consequently higher yields, lower quality, and lower proportion of the companion species meadow fescue. Experiments such as this can help to understand the mechanisms for differences between the behaviour of cultivars. Country of origin can effect performance – research in Estonia in 2020 [13] found that four recent Finnish cultivars of timothy were higher yielding and of better quality than two Estonian cultivars.

Tetraploid Italian ryegrass (*Lolium multiflorum*) can produce more biomass than their diploid progenitors, especially in drought periods [14], suggesting that increasing ploidy level is a tool for conferring drought resistance in this species. Leafier perennial ryegrass cultivars can contain less fibre [15]. In Estonia, a comparison of a diploid and a tetraploid perennial ryegrass [16] found a similar response to fertiliser application and cutting frequency, with slightly greater yield potential for the tetraploid averaged over different management. An experiment in Lithuania [17] found that 8 annual ryegrass (*Lolium multiflorum*) cultivars differed in their yield and stability across environmental conditions, and that two ('Rapid' and 'Elunaria') were higher yielding than the standard cultivar.

2.1.3 Species comparisons

Festuloliums

The possibilities for improving yield, persistence and quality of forages are motivations for comparing different grass and legume species. Of particular note has been comparison of festulolium hybrids (× *Festulolium*), through crossing *Lolium* species, which have high productivity and quality, with *Festuca* species, which have greater tolerance to stresses such as drought and cold.

Festuloliums can vary a lot, depending on the parent material. In general, festuloliums have better persistence than perennial ryegrass [18]. In a series of experiments across southern and central Sweden, the yield stability of festulolium hybrids was compared to that of perennial ryegrass, by examining the change in yield of the first harvest. The decline in first harvest yield of festulolium hybrids was less than for perennial ryegrass [19]. With increasing latitude, the festuloliums suffered more winter damage.

In Sweden, cultivar 'Hykor', which has tall fescue as a parent, had different forage quality characteristics to 'Paulita' and 'Perun', which have meadow fescue as a parent [20]. The energy content of the crop was always poorer for 'Hykor', and thus it should be harvested earlier to maintain the same level of digestibility. These results are supported by research in Norway [21].

A number of studies focused on comparing festulolium hybrid and hybrid ryegrass (*Lolium x boucheanum*) cultivars in Latvia. Both species yielded greater but were of lower digestibility than perennial ryegrass [22, 23]. A related study found no significant difference in crude protein between the different festulolium species [24]. Later heading hybrid ryegrass cultivars are more appropriate for Baltic conditions [25].

A Swedish study including multiple sites and years found that festulolium (cultivar 'Hykor') and tall fescue (*Festuca arundinacea*, cultivar 'Swaj') had lower fibre and greater fibre digestibility than timothy ('Switch') [26]. A similar multi-year and site study in Finland [27] compared multiple cultivars of festulolium, tall fescue, and meadow fescue. There were many differences between species and cultivars that were not fully explored in the study. In general, meadow fescue had the highest first cut yields, but tall fescue had the highest overall yields. Meadow fescue had the highest NDF and lowest digestibility. The authors concluded that it is important to know the genetic constitution of the cultivar to schedule the harvest time in order to obtain high forage quality.

Grass yield and quality comparisons

A range of research projects have compared different grass species across environments, including Latvia [28], Lithuania [29], Iceland [30], Norway [31] and in one study across multiple countries [32]. It is extremely hard to draw conclusions that are relevant to Sweden from such studies.

Two studies from Finland are of particular interest, because of the consistent results across multiple sites and years. The first [33] compared tall fescue cultivar 'Retu' with multiple cultivars of meadow fescue. Tall fescue was slower to establish, but over a 3-year rotation produced on average 12% higher yield than meadow fescue. It also had good persistence in Finnish growing conditions. The second study [34] compared the tiller characteristics of tall fescue and timothy. They differ in their flowering response. Timothy flowers when the critical daylength is exceeded, even in re-growth, whereas tall fescue requires vernalization (a period of cold weather) followed by long days, before it flowers. These differences have implications for the type of tillers produced by the different species, with timothy tending to produce more generative tillers. In contrast, tall fescue has more vegetative tillers, which leads to it being leafier, especially during re-growth. If everything else was equal, this should result in tall fescue having higher forage quality than timothy; however, in practice this is not necessarily the case. It is important to compare forage quality at similar phenological stages, and similar levels of biomass, which is not easy to do practically. Tall fescue is notably faster to re-grow after harvest, due to the larger number of vegetative tillers present at harvest, which can continue their growth after defoliation. In addition, senesced plant material in regrowth can affect the nutritive value, and this increases with time since the previous harvest. These concepts make it difficult to compare nutritive value of characteristics of different species, even if compared at similar phenological stages. [29]. In addition, forage quality comparisons at the first harvest should not be extrapolated to subsequent harvests.

Grass persistence

Timothy can be grown in virtually all agricultural areas of the Nordic countries, due to its high persistence [31]. Compared with perennial ryegrass, timothy is more resistant to both frost and ice encasement [35]. Timothy cultivars differ in their tolerances, and cultivars that are more frost resistant also tend to be more tolerant of ice encasement. Festuloliums are more cold resistant than perennial ryegrass, however there is a wide range of responses, depending on the genetics [31]. In a set of experiments across Nordic countries, the order of winter tolerance was tall fescue > meadow fescue > festulolium > hybrid and perennial ryegrass [31]. Timothy was not part of the experiments. In a similar set of experiments [30], the order of winter tolerance was the most winter tolerant, and cocksfoot (*Dactylis gomerata*) was comparable to meadow fescue. In Latvia, a study showed that timothy, meadow fescue, and cocksfoot were more persistent than perennial ryegrass and festulolium [28].

Research in Latvia showed that festulolium swards with tall fescue as a parent had better tolerance of snow mould [36].

Legume yield and persistence

Legumes are an important component of leys, and should not be ignored, despite usually constituting a lower proportion of the sward. An experiment in Denmark showed that the choice of companion grass had less effect on yield and nitrogen use efficiency than the choice of companion legume [37]. Red clover or lucerne in mixes gave overall higher yield and N yield.

A study focusing on yield stability in a 3-cut silage system [38] included 3 sites in Finland and 3 sites in Sweden (Rådde, Uppsala, and Lilla Böslid). Compared with red clover, lucerne had a greater sensitivity to the site (i.e. the environment) and white clover (*Trifolium repens*) had a lower sensitivity. This means that site selection is more critical for lucerne than red or white clover. However, lucerne tended to maintain yield in older leys, and for locations that it was suited to it had the greatest yields.

There is substantial evidence that the content of red clover in mixed leys declines across harvesting years, often beginning in the third year [39], and this is particularly evident from the 4th production year [40]. In comparison, white clover tends to constitute a lower proportion of swards, but remains fairly constant over years.

2.1.4 Alternative species

Yellow lucerne (*Medicago falcata*) and hybrid lucerne are lesser-known species that have the potential to grow in conditions which are colder or less fertile than lucerne (commonly referred to as blue lucerne in Sweden). Because they often have rhizomes, they have the potential to recover from stresses that damage or kill some of the plants in a population. Yellow and hybrid lucerne tend to have greater autumn dormancy than blue lucerne cultivars [41] and are subsequently more winter tolerant [42]. A non-specified cultivar from Estonia was cultivated in Finland, and yielded well [43]. In Sweden, yellow and hybrid lucerne were identified as potential species for northern Sweden, or areas where blue lucerne is not well-adapted [44]. Research to identify the most suited cultivars in northern Sweden is ongoing.

Fodder galega (*Galega orientalis*) has been well studied in the Nordic and Baltic countries, yet remains a less important species. It is high yielding [45-48] and persistent [45] in many different environments, reportedly surviving for 25 years [45]. Mixtures with grass are more productive than monocultures [49] and galega can grow well in a sward mixture with timothy and perennial ryegrass [50]. Another experiment found festulolium to be the most productive companion species [51]. In an experiment in Estonia, grass mixes with galega yielded more than those with

white clover or lucerne [52]. In Sweden and Finland, galega had good persistence, especially in third year sites [38], but had a lower percentage of legume in mixed stands with grass, in comparison with mixtures of grass and white clover, red clover or lucerne. Some research claims that galega can be of high forage quality [45] but this is not a consistent finding. It depends not only on the stage of growth, but also on the companion species and level of nitrogen fertilization. At later growth stages, a high protein concentration is maintained [45], but NDF concentration can be high [53, 54] and digestibility is low (e.g. 58.2% at full bloom) [45]. Averaged across sites in Finland and Sweden, galega had similar forage quality to red clover [38].

Previous work has examined the potential of birdsfoot trefoil (*Lotus corniculatus*) as an alternative legumes species, although not as much work has been done in recent years. A study across multiple sites in Finland and Sweden showed that it has low yield and persistence at all sites, and had a low content in grass mixtures [38]. Its forage quality was similar to red clover. A study in Denmark found that grass-legume mixtures with birdsfoot trefoil were lower yielding than those with red clover, lucerne, or white clover [37]. It remains an interesting species due to the presence of condensed tannins, which can have an anti-parasitic effect, reduce bloat, and improve protein uptake.

There are a range of other perennial legumes that have been studied, but there is insufficient information for this review to discuss them in detail – these include white sweetclover (*Melilotus albus*), yellow sweetclover (*Melilotus officinalis*), russel lupin (*Lupinus polyphyllus*), alsike clover (*Trifolium hybridum*), greater lotus (*Lotus pedunculatus*), kura clover (*Trifolium ambiguum*), zig-zag clover (*Trifolium medium*), sainfoin (*Onobrychis viciifolia*), liquorice milkvetch (*Astragalus glycyphyllos*), cicer milkvetch (*Astragalus cicer*), black medic (*Medicago lupulina*) and talish clover (*Trifolium tumens*) [44, 55-57]. Forb species of interest include salad burnet (*Sanguisorba minor*), caraway (*Carum carvi*), ribwort plantain (*Plantago lanceolata*), and chicory (*Cichorium intybus*) [57]. To some extent, experiences with these species are detailed in earlier literature that is not covered within the time range of this review.

There are various grass species that have potential for cultivation. Brome species are known for their drought tolerance, but are rarely used in Sweden. Smooth brome (*Bromus inermis*) has been tested in the past (for example the cultivar Leif), whereas Alaska brome (*Bromus sitchensis*) is a more recently introduced species. Preliminary research in Estonia [58] has compared the two species and found that Alaska brome can be higher yielding than smooth brome [58] and tall fescue [59], and is a good companion species to lucerne. Research comparing Timothy to the related and lesser known boehmer's cat's-tail (*Phleum phleoides*) [60]. Boehmer's cat's-tail had low yield, but good quality and resistance to drought stress, which makes it interesting for development through plant breeding. Additional grass species which are not commonly used or for which there is limited information in

leys include meadow foxtail (*Alopecurus pratensis*), Kentucky bluegrass (*Poa pratensis*), and reed canary grass (*Phalaris arundinacea*) [61, 62].

2.1.5 The benefits of increasing species diversity

The simplest and most common forage species mixture is a grass and a legume. There is abundant evidence that even simple mixtures can yield greater than monocultures [41, 63-71], although the suitability of particular combinations can vary greatly between sites [72]. In addition to being more productive, and having greater yield stability [73, 74], mixtures can have higher forage quality, and less weeds than monocultures [75-80].

Mixtures more biodiverse than a binary grass-legume sward can be advantageous, and can include additional grass, legume, or non-leguminous forbaceous species (referred to here as forbs for simplicity). A biodiversity experiment in northern Sweden [81] with plots including from 1 to 12 species showed that biomass increased with increasing species richness. Results in Lithuania also showed a positive relationship between species richness and yield [82].

Even increasing the number of species, from 2 to 3, can result in improvements. For example, adding red clover to perennial ryegrass and white clover mixes in Denmark resulted in higher yields, particularly in the first two years [83]. In addition to the complementarity across years, there was also complementarity within years, where the red clover dominated the legume proportion in the first and third cuts, and white clover dominated in the second and fourth cuts. An experiment in Latvia on three soil types found that mixtures containing two species of legumes had higher yields [84, 85]. Legume-containing mixtures also had higher levels of crude protein [86]. More evidence is needed as to whether combining multiple cultivars of the same species can be beneficial [87].

Increased species diversity can promote ecosystem function [88]. As species richness increases, particularly when there are multiple functional groups (e.g. grass, legume, forb), there are numerous benefits. The sward becomes more resistant to invasion from unwanted species [89-91], due to increased resource use [92]. In Sweden, inclusion of cocksfoot and white clover can reduce invasion, whereas leys with red clover are more commonly invaded [91]. Including a deeprooted forb such as lucerne or chicory also reduces weed invasion [93].

Increased biodiversity often results in yield increases, potentially due to increased light capture by complex mixes, compared with pure or simple swards [94]. Yield stability can also be increased by adding an additional legume to a sward [95, 96], potentially offering increased resilience to changing environmental conditions [97]. When a deep-rooted legume such as lucerne is added to a sward, it can improve yield and consequent nitrogen fixation [98-100]. Legumes differ in the amount of atmospheric N fixed and their ability to transfer N to neighbouring plants

[101]. For example, compared with red and white clover, lucerne typically derives a higher proportion of its nitrogen from fixation. However, because its root architecture is quite different to companion species, it typically does not transfer as much nitrogen to its neighbours.

Adding deep rooting grasses like tall fescue to swards can also potentially improve both yield and quality of leys [102]. Adding rhizomatous grasses to mixes can improve winter hardiness (e.g. in an experiment in Latvia [103]). An alternative approach is to add a productive but less winter hardy grass such as perennial ryegrass to mixtures, which can increase yield in the establishment year, and reduce weed invasion [104]. It is important to consider the compositions of mixes to enable stable swards, because some species are sensitive to the companion species. For example, experiments with white clover showed large differences in white clover yield and development, depending on the companion grass [105, 106].

Non-leguminous forbs are seldom used as companion species in sown leys in Sweden. Adding forbs species such as chicory and ribwort plantain to clover-grass mixes increased yield by 10-14% in experiments in Denmark [89]. Ribwort plantain, chicory, and caraway can be included in biodiverse mixes to increase yield [107] without reducing red clover performance [108]. They can also improve the mineral composition of swards [109]. However, because forbs can reduce the amount of red clover in the total biomass, and thus affect the amount of N-fixation, mixtures should not include a high seeding proportion of chicory [110]. Forbs vary in their competitiveness, and their proportion in a ley depends strongly on management [111].

An additional way to increase biodiversity is to include more diversity of species on field margins, either by allowing naturally occurring species to persist, or by sowing them. An experiment in Lithuania [112] sowed a wide range of native species on field margins. The species formed dense swards with a long flowering period, and the authors concluded that many are suitable for establishment of field margins on land used intensively for agriculture.

2.1.6 Conclusions and knowledge gaps

The selection of ley species, how they are combined in seed mixtures, and how they are managed, is clearly important. Commonly used species such as timothy and red clover provide a solid foundation for forage production. However, being overly reliant on a small number of species is inherently risky. With a changing climate comes the potential for different effects of diseases, insect pests, and weeds, along with abiotic factors such as increased ice encasement or reduced water availability. During the year of writing this review (2023) the early growing season was extremely dry over much of Sweden, and there was much discussion of whether the most commonly grown species are well suited to drier conditions. Indeed, species such as lucerne and tall fescue have deep roots and well-known resilience to drought, and including them in ley mixes could provide greater resilience and yield stability. To do this, expert knowledge is required. Lucerne could potentially be cultivated over a larger area than it is currently; however site selection (pH, drainage, soil type) can be critical. Tall fescue has a reputation for being difficult to manage, and if treated the same way as timothy it will likely result in forage of poorer quality.

Forbs are another group of plants that could potentially be used more extensively, and may contribute to resilience in dry conditions. However, although the species are useful, the available cultivars have not been developed for Nordic countries, and likely perform below their potential. The review identified ley biodiversity as an area of development, to increase resilience and improve yield stability. This requires more testing of diverse seed mixes, and development of methods to appropriately manage them. Breeding programs for minor species, particularly perennial legumes, would also be beneficial in the long-term. An interesting research question is whether the breeding and variety trial processes should include assessment in polycultures, rather than just monocultures.

2.1.7 References

- Halling, M.A., et al. Changes in water soluble carbohydrates for perennial ryegrass in the first growth in Norway and Sweden. in EGF. 2008. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- Halling, M.A., et al. Accumulation of water soluble carbohydrates in two perennial ryegrass cultivars at nine European sites. in EGF. 2004. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich.
- Conaghan, P., et al., Yield and Quality Response of Perennial Ryegrass Selected for High Concentration of Water-Soluble Carbohydrate to Nitrogen Application Rate. Crop Science, 2012. 52(6): p. 2839-2851.
- 4. Helgadottir, A., et al. New cultivars needed to ensure survival of perennial ryegrass across the northern region. in EGF. 2016. Ås, Norway: Nibio.
- Jonsson, H.A. Light interception and green matter yield in a grass trial. in Proceedings of the 23rd Meeting of the Fodder Crops and Amenity Grasses Section of EUCARPIA. 2001. Azores, Portugal: Department of Agricultural Science, University of Azores.
- Jonsson, H.A. Light interception and dry matter yield in grass/legume mixtures.
 2001. Sao Pedro, Brazil: Fundacao Estudos Agrarios Luiz Queiroz (Fealq).
- Sousa, D.O., et al., A two-year comparison on nutritive value and yield of eight lucerne cultivars and one red clover cultivar. Grass and Forage Science, 2019.
 75(1): p. 76-85.
- Nadeem, S., et al., Variation in rate of phenological development and morphology between red clover varieties: Implications for clover proportion and feed quality in mixed swards. Grass and Forage Science, 2019. 74(3): p. 403-414.

- 9. Nesheim, L. and A. Langerud, Improving sown grasslands through breeding and management. Proceedings of the Joint 20th Symposium of the European Grassland Federation and the 33rd Meeting of the EUCARPIA Section 'Fodder Crops and Amenity Grasses', Zurich, Switzerland, 24-27 June 2019, 2019: p. 506-508.
- Liatukas, Ž. and J. Bukauskaitė, Differences in Yield of Diploid and Tetraploid Red Clover in Lithuania. Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences, 2012. 66(4-5): p. 163-167.
- Liatukiene, A., et al., Evaluation of agro-biological traits of *Medicago sativa* and *M. varia* in a Cambisol and Retisol. Zemdirbyste-Agriculture, 2020. 107(1): p. 41-48.
- 12. Nesheim, L. and A.K. Bakken. The importance of timothy variety for feed quality development in regrowths of swards mixed with meadow fescue. in EGF. 2008. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- 13. Aavola, R. and P. Pechter. Modern cultivars of timothy produce more herbage with enhanced feeding value. in EGF. 2020. Helsinki, Finland: Luke.
- Akinroluyo, O.K., et al., Drought stress response of Westerwolths ryegrass (*Lolium multiflorum* ssp. *multiflorum*) cultivars differing in their ploidy level. Zemdirbyste-Agriculture, 2020. 107(2): p. 161-170.
- Skuodiene, R. and R. Repsiene. Productivity and quality of perennial ryegrass (*Lolium perenne* L.) in western Lithuania. in EGF. 2008. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- Aavola, R. The yield potential of Estonian perennial ryegrass (*Lolium perenne* L.) cultivars at different mineral fertilisation levels and cutting frequencies. in EGF. 2005. Tartu, ESTONIA: Estonian Grassland Soc-Egs.
- 17. Kemesyte, V. and P. Tarakanovas. Genotype * environment influence on first-cut dry matter yield stability of annual ryegrass cultivars. in EGF. 2008. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- Berzins, P., et al. Performance of *Lolium* spp., *Festuca* spp. and their mutual hybrids in Latvian conditions. in EGF. 2018. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre.
- Halling, M.A. Yield stability of Festulolium and perennial ryegrass in southern and central Sweden. in EGF. 2012. Lublin, POLAND: Polish Grassland Soc-Polskie Towarzystwo Lakarskie.
- Frankow-Lindberg, B.E. and K.F. Olsson. Digestibility and fibre content of leaves and straw of three Festulolium hybrids during spring regrowth. in EGF. 2008. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- Østrem, L., et al. Winter survival, yield performance and forage quality of Festulolium cvs. for Norwegian farming. in EGF. 2008. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- Gutmane, I. and A. Adamovich. Analysis of Festulolium and hybrid ryegrass (*Lolium * boucheanum*) dry matter yield stability. in EGF. 2008. Uppsala, Sweden: Swedish University of Agricultural Sciences.

- 23. Gutmane, I. and A. Adamovich. Productivity and yield quality of Festulolium and *Lolium * boucheanum* varieties. in EGF. 2004. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich.
- Gutmane, I. and A. Adamovics. Crude protein yield and quality of festulolium (* Festulolium Asch. & Graebn.) and hybrid ryegrass (*Lolium * boucheanum* Kunth.). in EGF. 2011. Irdning, Austria: Agricultural Research and Education Center (AREC) Raumberg-Gumpenstein.
- 25. Gutmane, I. and A. Adamovics. Productivity aspects of Festulolium and *Lolium* * *boucheanum* cultivars. in EGF. 2006. Madrid, Spain: Sociedad Espanola para el Estudio de los Pastos (SEEP).
- 26. Halling, M.A. Fibre quality in timothy, Festulolium and tall fescue around first cut. in International Grassland Congress. 2013. Orange, Australia: New South Wales Department of Primary Industry.
- 27. Niemelainen, O., M. Niskanen, and L. Jauhiainen. Productivity and quality of meadow fescue, tall fescue and festulolium in silage cutting regime in Finland. in International Silage Conference. 2012. Helsinki, Finland: MTT Agrifood Research Finland.
- 28. Berzins, P., et al. The evaluation of perennial grass cultivars in Latvia condition. in NJF. 2015. Riga, LATVIA: Nordic Assoc Agricultural Scientists-Njf.
- 29. Butkute, B. and V. Paplauskiene. Comparison of the variability in quality of the LIA-bred perennial grasses, relationships between quality parameters. in EGF.
 2006. Madrid, Spain: Sociedad Espanola para el Estudio de los Pastos (SEEP).
- 30. Thorvaldsson, G., et al. Climatic adaptation of species and varieties of grass and clover in the West Nordic countries. in EGF. 2016. Ås, Norway: Nibio.
- Østrem, L., et al. Environmental impact on winter survival and production in non-native grasses in the Nordic countries. 2015. Riga, Latvia: Nordic Assoc Agricultural Scientists-Njf.
- Aavola, R., et al. Yield performance of forage grasses on both shores of the Baltic Sea. in EGF. 2013. Borgarnes, Iceland: Agricultural University of Iceland.
- 33. Niemelainen, O., L. Jauhiainen, and E. Miettinen, Yield profile of tall fescue (*Festuca arundinacea*) in comparison with meadow fescue (*F. pratensis*) in Finland. Grass and Forage Science, 2001. 56(3): p. 249-258.
- Virkajarvi, P., et al., Tiller Characteristics of Timothy and Tall Fescue in Relation to Herbage Mass Accumulation. Crop Science, 2012. 52(2): p. 970-980.
- 35. Jørgensen, M., et al. Effects of ice encasement during winter on different cultivars of *Phleum pratense* and *Lolium perenne* in Norway. in International Grassland Congress 2008. Guangzhou, China: Guangdong People's Publishing House.
- Gutmane, I. and A. Adamovich. Productivity and persistency of Festulolium and *Lolium * boucheanum* swards. in EGF. 2007. Merlbeke, Belgium: Belgian Society for Grassland and Forage Crops.
- Elgersma, A. and K. Soegaard. Productivity and herbage quality in two-species grass-legume mixtures under cutting. in EGF. 2015. Wageningen, Netherlands: Wageningen Academic Publishers.

- Halling, M.A., et al. Forage legumes productivity and composition. in Legume silages for animal production : LEGSIL. 2001. Braunschweig, Germany: Bundesforschungsanstalt Landwirtschaft (Fal).
- Mela, T., Red clover grown in a mixture with grasses: yield, persistence and dynamics of quality characteristics. Agricultural and Food Science in Finland, 2003. 12(3-4): p. 195-212.
- 40. Halling, M.A., Changes in sward botanical composition in a ley cutting system over four years. Improving sown grasslands through breeding and management. Proceedings of the Joint 20th Symposium of the European Grassland Federation and the 33rd Meeting of the EUCARPIA Section 'Fodder Crops and Amenity Grasses', Zurich, Switzerland, 24-27 June 2019, 2019: p. 57-59.
- 41. Tamm, U. and S. Tamm. Effect of timothy sowing ratio on yield and nutritive value of alfalfa/timothy bi-crops. in EGF. 2005. Tartu, Estonia: Estonian Grassland Soc-Egs.
- 42. Linke, A. and R. Lillak. Formation of yielding ability and cold resistance of three red clover and alfalfa cultivars. in EGF. 2005. Tartu, Estonia: Estonian Grassland Society.
- Mela, T., H. Ihamaki, and A. Matilainen. Yellow-flowered lucerne (*Medicago falcata* L.) A potential forage legume for the north. in International Grassland Congress 2001. Sao Pedro, Brazil: Fundacao Estudos Agrarios Luiz Queiroz (Fealq).
- 44. Parsons, D. Potential new forage legumes for Northern Sweden. in EGF. 2020. Helsinki, Finland: Luke.
- 45. Adamovich, A. and O. Adamovicha. Forage quality dynamics and productivity of fodder galega-grass swards. in EGF. 2007. Merlbeke, Belgium: Belgian Society for Grassland and Forage Crops.
- 46. Balezentiene, L., Bio-morphological peculiarities of new cultivars of fodder galega (*Galega orientalis* Lam.). Agronomijas Vestis, 2008(10): p. 82-87.
- Meripold, H., et al. The effect of different fodder galega-grass mixtures and nitrogen fertilization on forage yield and chemical composition. in EGF. 2014.
 Aberystwyth, UK: IBERS, Aberystwyth University.
- 48. Balezentiene, L., Comparison of the ecological applicability and yield capacity of long-term legumes and their mixtures. Vagos, 2003(57): p. 12-15.
- 49. Balezentiene, L. and E. Klimas. Variation in soil microbiological activity in relation to the yield of fodder galega (*Galega orientalis* Lam.) swards. in EGF. 2003. Pleven, Bulgaria: Bulgarian Association for Grassland and Forage Production (BAGFP).
- 50. Balezentiene, L. and E. Klimas. Ability of grasses to compete in galega swards. in EGF. 2008. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- 51. Meripõld, H., et al. Yields and feed value of different fodder galega-grass mixtures. in EGF. 2016. Ås, Norway: Nibio.
- 52. Laidna, T. Adaptation of long-term forage legumes for growing on light acid soil of south Estonia. in EGF. 2003. Pleven, Bulgaria: Bulgarian Association for Grassland and Forage Production (BAGFP).

- 53. Meripõld, H., et al., Fodder galega (*Galega orientalis* Lam) grass potential as a forage and bioenergy crop. Agronomy Research, 2017. **15**(4): p. 1693-1699.
- 54. Meripõld, H., et al. The effect of different grass species and fertilization level in fodder galega mixtures. in EGF. 2015. Wageningen, Netherlands: Wageningen Academic Publishers.
- 55. Bender, A., S. Tamm, and R. Aavola. Biomass allocation to shoots and roots, and nutrient content in herbage legumes. in EGF. 2017. Sassari, Italy: Cnr-Ispaam.
- 56. Butkute, B., et al., Phytochemical composition of temperate perennial legumes. Crop & Pasture Science, 2018. **69**(10): p. 1020-1030.
- 57. Elgersma, A., K. Søegaard, and S.K. Jensen, Herbage dry-matter production and forage quality of three legumes and four non-leguminous forbs grown in single-species stands. Grass and Forage Science, 2014. **69**(4): p. 705-716.
- 58. Tamm, S., et al. Productivity of Alaska brome and smooth brome in pure stand and in mixture with lucerne. in EGF. 2020. Helsinki, Finland: Luke.
- 59. Tamm, U., et al. The nutritive value of Alaska brome and tall fescue forage using different growing technologies. in EGF. 2018. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre.
- Jonaviciene, K., V. Paplauskiene, and G. Brazauskas. Comparison of *Phleum phleoides* versus *Phleum pratense* species for forage potential. in EGF. 2013. Borgarnes, Iceland: Agricultural University of Iceland.
- 61. Adamovics, A., O. Adamovica, and M. Beca. Rational use of legume/grass swards for high-quality forage production. in EGF. 2006. Madrid, Spain: Sociedad Espanola para el Estudio de los Pastos (SEEP).
- 62. Adamovics, A. and R. Platace. Influence of nitrogen fertilizers on yield and digestibility of grass. in EGF. 2014. Aberystwyth, UK: IBERS, Aberystwyth University.
- 63. Bender, A., S. Tamm, and R. Aavola. Tetraploid red clover in a pure stand or mixed with timothy? in EGF. 2009. Zubri, Czech Republic: Organising Committee of the 15th European Grassland Federation Symposium.
- 64. Ergon, A., et al. Species mixtures dry matter yield and herbage quality as affected by harvesting frequency under low N supply. in EGF. 2013. Borgarnes, Iceland: Agricultural University of Iceland.
- 65. Kadziuliene, Z., L. Sarunaite, and L. Kadziulis. Investigation of some factors accelerating formation of protein-rich yield of legume/grass swards from the sowing year. in EGF. 2005. Tartu, Estonia: Estonian Grassland Soc-Egs.
- 66. Li, H.H., et al., Advantages of grass-legume mixture for improvement of crop growth and reducing potential nitrogen loss in a boreal climate. Agricultural and Food Science, 2019. **28**(4): p. 176-189.
- 67. Slepetys, J., The productivity and persistency of pure and mixed forage legume swards. Agronomijas Vestis, 2008(11): p. 276-282.
- Slepetys, J. and A. Slepetiene. Perennial legume swards for organic farming system in Lithuania. in International Grassland Congress 2013. Orange, Australia: New South Wales Department of Primary Industry.

- 69. Slepetys, J. and A. Slepetiene. The benefits of long-term legume swards in an organic farming system. in EGF. 2010. Duderstadt, Germany: Mecke Druck und Verlag.
- 70. Soegaard, K. and M.R. Weisbjerg. Herbage quality and competitiveness of grassland legumes in mixed swards. in EGF. 2007. Merlbeke, Belgium: Belgian Society for Grassland and Forage Crops.
- 71. Zarinna, L., L. Dzedule, and A. Vaivode. Potential of aboveground biomass of perennial grasses depending on the proportion of Leguminosae in the sward under organic farming conditions. in Zinātniski praktiskās konferences. 2021. Jelgava, Latvia: Latvijas Lauksaimniecibas universitate (LLU).
- 72. Gierus, M., et al. Influence of contrasting environments on forage quality of ryegrass and four legumes growing in binary swards. in EGF. 2007. Merlbeke, Belgium: Belgian Society for Grassland and Forage Crops.
- 73. Helgadottir, A., T.O. Gylfadottir, and T.A. Kristjansdottir. Changes in species proportion over time in grass-legume mixtures under northern conditions. in EGF. 2013. Borgarnes, Iceland: Agricultural University of Iceland.
- 74. Helgadottir, A., et al. Grass-legume mixtures sustain yield advantage under cool maritime growing conditions over a period of five years. in EGF. 2017. Sassari, Italy: Cnr-Ispaam.
- Helgadottir, A., T.A. Kristjansdottir, and T.O. Gylfadottir. Plant diversity enhances productivity in grasslands under marginal growing conditions. in EGF.
 2012. Lublin, POLAND: Polish Grassland Soc-Polskie Towarzystwo Lakarskie.
- 76. Helgadottir, A., et al., Grass-legume mixtures sustain strong yield advantage over monocultures under cool maritime growing conditions over a period of 5 years. Ann Bot, 2018. **122**(2): p. 337-348.
- 77. Elgersma, A. and K. Søegaard, Effects of species diversity on seasonal variation in herbage yield and nutritive value of seven binary grass-legume mixtures and pure grass under cutting. European Journal of Agronomy, 2016. **78**: p. 73-83.
- 78. Sturludottir, E., et al., Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. Grass and Forage Science, 2014. 69(2): p. 229-240.
- 79. Finn, J.A., et al. Four-species grass-clover mixtures demonstrate transgressive overyielding and weed suppression in a 3-year continental-scale experiment. in EGF. 2012. Lublin, Poland: Polish Grassland Soc-Polskie Towarzystwo Lakarskie.
- Sebastia, M.T., et al. Higher yield and fewer weeds in grass/legume mixtures than in monocultures - 12 sites of COST action 852. in EGF. 2004. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich.
- 81. Palmborg, C., et al., Inorganic soil nitrogen under grassland plant communities of different species composition and diversity. Oikos, 2005. **110**(2): p. 271-282.
- 82. Sidlauskaite, G. and Z. Kadziuliene, The influence of different grass-legume mixtures on the productivity of first-year spring cutting. 2020, Helsinki, Finland: Organising Committee of the 28th General Meeting of the European Grassland Federation & Natural Resources Institute Finland (Luke). 135-137.

- 83. Eriksen, J., M. Askegaard, and K. Soegaard, Complementary effects of red clover inclusion in ryegrass-white clover swards for grazing and cutting. Grass and Forage Science, 2014. **69**(2): p. 241-250.
- 84. Adamovics, A. and I. Gutmane. Productivity and quality of multicomponent grass swards on three soil types. in EGF. 2016. Ås, Norway: Nibio.
- 85. Adamovics, A., I. Gutmane, and M. Katamadze, The influence of nitrogen fertilisation on the productivity of multi-species swards in three production years. Improving sown grasslands through breeding and management. Proceedings of the Joint 20th Symposium of the European Grassland Federation and the 33rd Meeting of the EUCARPIA Section 'Fodder Crops and Amenity Grasses', Zurich, Switzerland, 24-27 June 2019, 2019: p. 36-38.
- 86. Adamovics, A. and I. Gutmane. The influence of nitrogen fertiliser and legume content on the quality of multi-species swards. in EGF. 2018. Fermoy, Irish Republic: Teagase, Animal & Grassland Research and Innovation Centre.
- Sidlauskaite, G., M. Toleikiene, and Z. Kadziuliene, Productivity potential of three tetraploid ryegrass cultivars and their mixture in new swards with clovers. Zemdirbyste-Agriculture, 2021. 108(3): p. 241-246.
- 88. Brophy, C., et al., Major shifts in species' relative abundance in grassland mixtures alongside positive effects of species diversity in yield: a continental-scale experiment. Journal of Ecology, 2017. **105**(5): p. 1210-1222.
- 89. Cong, W.F., et al., Species interactions between forbs and grass-clover contribute to yield gains and weed suppression in forage grassland mixtures. Agriculture Ecosystems & Environment, 2018. **268**: p. 154-161.
- Cong, W.E., K. Soegaard, and J. Eriksen. Diversity promotes production of ryegrass-clover leys through inclusion of competitive forb species. in EGF. 2016. Ås, Norway: Nibio.
- 91. Frankow-Lindberg, B.E., et al., Biodiversity effects on yield and unsown species invasion in a temperate forage ecosystem. Ann Bot, 2009. **103**(6): p. 913-21.
- 92. Frankow-Lindberg, B.E., Grassland plant species diversity decreases invasion by increasing resource use. Oecologia, 2012. **169**(3): p. 793-802.
- 93. Frankow-Lindberg, B.E. Richness of unsown plant species in a sown ley crop. in EGF. 2010. Duderstadt, Germany: Mecke Druck und Verlag.
- 94. Frankow-Lindberg, B.E. and N. Wrage-Monnig, Light availability is improved for legume species grown in moderately N-fertilized mixtures with non-legume species. Basic and Applied Ecology, 2015. **16**(5): p. 403-412.
- 95. Frankow-Lindberg, B.E., et al., Yield and stability of yield of single- and multiclover grass-clover swards in two contrasting temperate environments. Grass and Forage Science, 2009. **64**(3): p. 236-245.
- 96. Jing, J., et al., Species Diversity Effects on Productivity, Persistence and Quality of Multispecies Swards in a Four-Year Experiment. PLoS One, 2017. **12**(1): p. e0169208.
- 97. Kykkanen, S., et al. Effects of increasing plant diversity on yield of grass and grass-legume leys in Finland. in EGF. 2020. Helsinki, Finland: Luke.

- 98. Frankow-Lindberg, B.E. and A.S. Dahlin, N-2 fixation, N transfer, and yield in grassland communities including a deep-rooted legume or non-legume species. Plant and Soil, 2013. 370(1-2): p. 567-581.
- Kadziuliene, Z. and L. Kadziulis, Nitrogen accumulation and efficiency in herbage depending on legume species in grassland sward. Biologija, 2007. 53(1): p. 54-59.
- 100. Kadziulis, L. and Z. Kadziuliene. Lucerne in leys and pastures: economic and ecological aspects. in EGF. 2003. Pleven, Bulgaria: Bulgarian Association for Grassland and Forage Production (BAGFP).
- 101. Pirhofer-Walzl, K., et al., Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. Plant and Soil, 2012. 350(1-2): p. 71-84.
- 102. Niemelainen, O., et al. Timothy and tall fescue mixtures increase forage yield and improve yield quality in comparison with pure timothy in Finland. in EGF.
 2004. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich.
- Jansone, B., et al., Evaluation of perennial grass mixtures of different species and varieties in the central part of Latvia introduced from other European countries. Agronomy Research, 2010. 8: p. 563-572.
- 104. Jørgensen, M., et al., Effects of including perennial ryegrass (*Lolium perenne* L.) in different species mixtures on yield, feed quality and botanical composition in first year of ley. Improving sown grasslands through breeding and management. Proceedings of the Joint 20th Symposium of the European Grassland Federation and the 33rd Meeting of the EUCARPIA Section 'Fodder Crops and Amenity Grasses', Zurich, Switzerland, 24-27 June 2019, 2019: p. 152-154.
- 105. Sprainaitis, A. and V. Paplauskiene, Development of white clover cv. 'Suduviai' in communities of various grass species. Biologija, 2001(2): p. 108-110.
- 106. Helgadottir, A., T.O. Gylfadottir, and T.A. Kristjansdottir, The effects of grass species and nitrogen fertilizer on white clover growth and mixture yield in a northern maritime environment. Icelandic Agricultural Sciences, 2005(18): p. 75-84.
- 107. Dhamala, N.R., K. Soegaard, and J. Eriksen. Competitive forbs in highproducing temporary grasslands with perennial ryegrass and red clover can increase plant diversity and herbage yield. in EGF. 2015. Wageningen, Netherlands: Wageningen Academic Publishers.
- 108. Dhamala, N.R., et al. Nitrogen fixation in red clover grown in multi-species mixtures with ryegrass, chicory, plantain and caraway. in EGF. 2016. Ås, Norway: Nibio.
- 109. Pirhofer-Walzl, K., et al., Forage herbs improve mineral composition of grassland herbage. Grass and Forage Science, 2011. **66**(3): p. 415-423.
- 110. Dhamala, N.R., et al., Effects of including forbs on N-2-fixation and N yield in red clover-ryegrass mixtures. Plant and Soil, 2018. **424**(1-2): p. 525-537.
- Mortensen, T., K. Soegaard, and J. Eriksen. Effect of seed mixture composition and management on competitiveness of herbs in temporary grasslands. in EGF. 2012. Lublin, Poland: Polish Grassland Soc-Polskie Towarzystwo Lakarskie.

112. Arlauskiene, A., et al. Wild species flowering margins for improved biodiversity in intensive agricultural areas. in EGF. 2017. Sassari, Italy: Cnr-Ispaam.

2.2 Forage breeding and genetics

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2.2.1 Species and environments

Breeding forage species for high forage productivity and adaptation to the environmental conditions of the Nordic and Baltic countries is of great importance for livestock production, resilience to climate change and provision of ecosystem services. The most researched grass species were perennial ryegrass (*Lolium perenne*), timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*), and festulolium (x *Festulolium*), while legume species were red clover (*Trifolium pratense*) and white clover (*Trifolium repens*). The full list of forage species included in field studies in the region is summarized in Table 1.

Table 1 Summary of forage species evaluated in field studies in the Nordic and Baltic countries, common and scientific names, number of publications in this literature review including the species (#), countries where the field studies were established, and references.

Common name	Species name	#	Countries and references
Grasses			
Perennial ryegrass	Lolium perenne	23	Denmark [1-3], Lithuania [4-10], Latvia [11, 12], Finland [13], Sweden [14-19]
Timothy	Phleum pratense	16	Finland [13, 20-23], Norway [24], Latvia [25], Lithuania [26], Sweden [14-19]
Meadow fescue	Festuca pratensis	16	Lithuania [27-30], Latvia [25, 31], Finland [13], Norway [32], Sweden [14-19]
Festulolium	x Festulolium	16	Norway [33, 34], Lithuania [35, 36], Latvia [31, 37], Sweden [14-19]
Cocksfoot	Dactylis glomerata	10	Latvia [38], Lithuania [39-42], Sweden [14-18]
Other grasses	Festuca arundinacea, Lolium multiflorum, Dactylis polygama, Poa pratensis	11	Lithuania [30, 43], Finland [13], Sweden [14- 18]
Legumes	-		
Red clover	Trifolium pratense	18	Lithuania [44-47], Latvia [48, 49], Estonia [50, 51], Norway [52], Sweden [14-18, 52], Iceland [53], Denmark [54]
White clover	Trifolium repens	16	Lithuania [55-60], Iceland [61, 62], Latvia [63], Norway [64-66], Sweden [14-18, 67]
Lucerne	Medicago sativa	9	Lithuania [68-72], Estonia [73], Sweden [14-18]
Alsike clover	Trifolium hybridum	5	Lithuania [59, 60, 68], Sweden [14-18]
Birdsfoot trefoil	Lotus corniculatus	4	Sweden [14-18]
Other legumes	T. medium, T. montanum, T. alpestre, T. pannonicum, T. fragiferum, T. ambiguum, T. ochroleucum, M. varia	3	Lithuania [59, 60, 68]

Most studies found in this literature review consisted of a phenotypic characterization and evaluation of cultivars, breeding lines, hybrids, ecotypes, or wild accessions of forage species. Many studies report high variability in traits that can be used for breeding of different forage species from wild populations, ecotypes, and cultivars (e.g., [12, 32, 37, 52]). These studies provide relevant information about germplasm availability for the future breeding of forage species. It is noteworthy that results from forage variety trials in Sweden are seldom published in peer-reviewed literature. Therefore, non-peer reviewed Swedish publications that include statistical analysis of results were included in this review. Multi-environment field evaluations are critical to determine the performance of species and genotypes across environments and to assess genotype by environment (GxE) interactions, which are common (e.g., [1-3], [54]). However, only 18 publications (25% of the total) conducted evaluations in more than one location, and only 6 in more than one country. This is a critical limitation, which is discussed later. Swedish breeding trials were conducted at 11 sites across Sweden [14]. Comparing specific phenotypic performance within or across species is beyond the scope of this review. Nevertheless, the full list of publications per species is provided in Table 1 for reference.

2.2.2 Traits of interest

Breeding traits of interest include forage productivity, resistance to diseases, winter survival, regrowth ability, and forage nutritive value (quality). Traits evaluated in pre-breeding or breeding field studies are summarized in Table 2. As expected, for all species forage dry matter yield is the most frequently evaluated trait. Surprisingly, forage quality is reported in much less frequency, although is a highly important trait, and ranking of cultivars based on digestible forage yield does not always correspond with total forage yield (e.g., [34]). About half of the grass species studies report some indicator of forage quality (e.g., protein, fibre, digestibility), while two thirds of the legume species studies report on forage quality (Table 2).

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Species	Forage Yield	Forage Quality	Winter survival	Disease	Seed yield	Plant height	Re- growth	Develop.	Other	Total
Per. ryegrass	17	12	17	7	3	5	7	10	6	23
Timothy	12	8	9	2	0	2	2	10	7	16
M. fescue	11	8	8	3	0	1	1	9	3	16
Festulolium	14	11	8	2	0	1	1	7	3	16
Cocksfoot	6	6	5	1	0	1	0	5	1	10
Other grasses	6	6	5	0	2	2	1	5	0	11
Red clover	13	11	10	3	6	3	0	8	5	18
White clover	10	9	10	1	3	4	1	8	10	16
Lucerne	9	6	6	2	2	2	1	5	2	9
Other legumes	8	7	6	2	2	2	0	6	0	8

Table 2 Summary of number of publications reporting different traits evaluated in field studies by forage species, and total number of publications per species. Traits are forage yield (kg DM/ha), forage quality (one or more chemical parameters, such as CP, NDF).

Persistence, defined as stand longevity (e.g., number of years), is a trait of great importance, but no study reported persistence explicitly. Phenotypic evaluation for persistence is complex, as many factors affect persistence. In the Nordic countries, the main limiting factors are the stresses that affect the plants during the winter, hence winter survival is an important trait evaluated in most studies [52]. Winter survival is also a complex trait, which depends on the resistance mechanisms to the various stresses that plants face during winter, such as freezing, ice encasement, soil movements, waterlogging, drought, fungal pathogens, and lack of light [52, 64]. High GxE interaction for winter survival was found in a study evaluating 48 accessions of red clover across Nordic countries, and freezing tolerance was correlated with winter survival only in one environment, suggesting that other winter stresses are also important [52]. Freezing tolerance is positively associated with total non-structural carbohydrates in timothy, while resistance to the fungal pathogen, snow mould (Typhula), is positively associated with fructan content, and some cultivars are resistant to both stressors [22]. In perennial forages, the physiological preparation for overwintering that occurs in autumn (i.e., acclimation) is driven by temperature and daylength and is critical for winter survival. When white and red clover plants cannot acclimate due to fast temperature drop in autumn, population genetic shift is observed [67]. Several studies on white clover provide evidence that it is possible to simultaneously select for winter survival/winter hardiness and higher forage yield under northern conditions [62, 66]. A study of 166 white clover crosses in Iceland and Norway [62] found large variation in leaf size, winter survival, and fatty acid profiles between families. Another study of eleven populations of white clover in two locations in Norway [66] revealed through path coefficient analysis that foliage height had positive direct and indirect effects on forage dry matter yield, winter survival, and internode length.

Several diseases have been evaluated in these breeding or pre-breeding field studies. For red clover, frost and snow mould fungi [53], and anthracnose, rust, and mildew [44] were reported. In Lucerne (*Medicago sativa*), sclerotinia, crown and stem rust, downy mildew, black stem leaf spot, and *Phoma* were evaluated [69, 71]. In grass studies, crown rust, stem rust, and leaf spot were the main diseases evaluated, e.g. [1-3, 26]. High heritability was found for rust resistance in perennial ryegrass across seven locations throughout Europe (from Denmark to France) [2]. High performing ryegrass varieties showed high resistance to crown and stem rust, and overall, tetraploid varieties performed better than diploid ones [3].

Other traits evaluated included plant morphological traits (length and weight of leaves, stems, stolons, rhizomes, leaf/stem ratio, roots, etc.) and biochemical traits (e.g., HCN cyanogenic glycosides in white clover [56, 57], fatty acid profile in stolons of white clover [74], fructans in perennial ryegrass [1], non-structural

carbohydrates in timothy [22], and vernalization effects in timothy [13, 20, 21]). Additionally, tolerance to abiotic stresses, such as drought or flooding, has not been reported.

2.2.3 Breeding approaches

Few studies focused on evaluation of breeding approaches [33, 34, 54], genetic variance characterization [2, 65], association mapping [4], or genomic selection [1, 3]. The relative success of different breeding approaches was evaluated for some forage species. For instance, red clover cultivars developed by polycross selections showed 19% higher yields than those obtained from recurrent phenotypic selection across three locations (Denmark, Czech Republic, and France), even though GxE was significant [54]. Festulolium (hybrids of fescue and ryegrass) developed by an amphitetraploid breeding approach of perennial ryegrass and meadow fescue achieved higher yields and winter hardiness than the hybrid diploids and the parents across two locations in Norway [34]. A breeding approach combining freezing tests with measurements of high photosynthetic activity during cold acclimation (by chlorophyll-a fluorescence) was the best option for selection of winter hardy and well performing populations of festulolium in Nordic environments [33]. Finally, implementation of genomic selection in ryegrass breeding programs was feasible [2] given the high heritabilities observed, and high accuracies for genomic prediction were further observed [1]. Using GxE interaction analysis and the identification of mega-environments where varieties perform in a similar ranking, it was possible to predict performance of ryegrass varieties with high accuracy for disease resistance [3].

2.2.4 Conclusions and knowledge gaps

Several strong areas, as well as research gaps were identified in this literature review. First, there is capacity and experience in all countries for phenotypic characterization of forage species, cultivars and populations. However, coordinated international multi-environment trials to better characterize GxE in complex quantitative traits are generally lacking for most species. Second, forage breeding research in the region has focused on major species such as perennial ryegrass, red clover, white clover, timothy, meadow fescue, and festulolium. Research on evaluating adaptation and productivity of new species, especially legumes, to diversify forage production is another gap. Third, forage yield, winter survival, and disease resistance have been the main focus of the previous studies. Integrating forage quality into forage breeding studies is another relevant missing aspect. Finally, understanding winter survival has rightfully been a major focus of research, but drought and flood resistance are also important issues to breed for persistence in a changing climate.

2.2.5 References

- 1. Fe, D., et al., Accuracy of Genomic Prediction in a Commercial Perennial Ryegrass Breeding Program. Plant Genome, 2016. **9**(3): p. 12.
- 2. Fe, D., et al., Genetic and Environmental Variation in a Commercial Breeding Program of Perennial Ryegrass. Crop Science, 2015. **55**(2): p. 631-640.
- Fois, M., et al., Genomic Prediction and Genotype-by-Environment Interaction Analysis of Crown and Stem Rust in Ryegrasses in European Multi-Site Trials. Agronomy-Basel, 2021. 11(6): p. 19.
- Aleliunas, A., et al., Association of single nucleotide polymorphisms in LpIRI1 gene with freezing tolerance traits in perennial ryegrass. Euphytica, 2015. 204(3): p. 523-534.
- 5. Kemesyte, V., et al., Evaluation of *Lolium perenne* L. wild ecotypes of Ukrainian origin in Lithuania. Agronomy Research, 2010. **8**: p. 615-624.
- 6. Kemešytė, V., et al., Morphological variation between perennial ryegrass (*Lolium perenne* L.) natural ecotypes. 2014. 81-84.
- 7. Nekrosas, S., Comparison of early and late diploid breeding lines and varieties of perennial ryegrass. Zemes ukio Mokslai, 2003(3): p. 53-60.
- 8. Nekrosas, S., Perennial ryegrass herbage, dry matter yield and other parameters in relation to plant disease incidence. Biologija, 2003(2): p. 62-64.
- 9. Nekrosas, S., Use of self-pollination in perennial ryegrass breeding. Zemes ukio Mokslai, 2005(4): p. 44-51.
- 10. Dabkeviciene, G., et al., Autopolyploids in fodder grass breeding: induction and field performance. Spanish Journal of Agricultural Research, 2017. **15**(4): p. 7.
- 11. Rancane, S., et al., Assessment of *Lolium perenne* tetraploid clones produced from a diverse diploid breeding population. Zemdirbyste-Agriculture, 2021.
 108(1): p. 35-42.
- Rancane, S., et al., Assessment of perennial ryegrass (*Lolium perenne* L.) genotypes under Latvia agro-ecological conditions. Annual 27th International Scientific Conference: "Research for Rural Development 2021", Jelgava, Latvia, 12-14 May 2021, 2021. 36: p. 7-14.
- Seppanen, M.M., et al., The role of vernalization in freezing tolerance and tiller composition of forage grasses. 2013, Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland. Borgarnes, Iceland: Agricultural University of Iceland. 297-299.
- 14. Halling, M., et al., Vallväxter till slåtter och bete samt grönfoderväxter : sortval för södra, mellersta och norra Sverige 2020/2021. 2021, SLU.
- Halling, M. and S. Larsson, Vallväxter till slåtter och bete samt grönfoderväxter.
 2017, SLU.
- 16. Halling, M., Vallväxter till slåtter och bete samt grönfoderväxter. Sortval för södra och mellersta Sverige 2012/2013. 2012, SLU.
- Halling, M., Sortval i ekologisk vallodling 2004-2009. Sortförsök i timotej, ängssvingel, rörsvingel, rörsvingelhybrid, engelskt rajgräs och rajsvingel. 2010, SLU.

- 18. Halling, M., Vallväxter till slåtter och bete samt grönfoderväxter. Sortval för södra och mellersta Sverige 2008/2009. 2008, SLU.
- 19. Halling, M. and L. Wigh, Sortval i ekologisk valloding. Sortförsök 2003-2007 i timotej, ängsvingel, rajsvingel och engelskt rajgräs 2008, SLU.
- Seppanen, M.M., et al., Genotypic variation in vernalisation response and autumn growth in forage grass species. 2015, Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland. Wageningen, Netherlands: Wageningen Academic Publishers. 316-318.
- Seppanen, M.M., et al., Vernalization response of Phleum pratense and its relationships to stem lignification and floral transition. Ann Bot, 2010. 106(5): p. 697-707.
- 22. Isolahti, M. and O. Nissinen, Role of storage carbohydrates in hardening and resistance of timothy genotypes to frost and Typhula spp. 2004, Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich. 434-436.
- 23. Virkajarvi, P., et al., Does tiller type distribution explain the differences in yield and nutritive value of timothy genotypes? 2010, MTT Agrifood Research Finland, FIN-31600 Jokioinen, Finland, Finland. Duderstadt, Germany: Mecke Druck und Verlag. 572-574.
- Larsen, A., et al., Evaluation of local populations for timothy breeding. 2001, The Norwegian Crop Research Institute, Norway. Azores, Portugal: Department of Agricultural Science, University of Azores. 46-50.
- Bumane, S. and P. Berzins, Development of new forage grass varieties at the Skriveri Research Institute of Agriculture. Agronomijas Vestis, 2008(11): p. 43-46.
- 26. Lemeziene, N. and E. Lemezis, Evaluation of morphological characters and valuable agronomic traits of common timothy (*Phleum pratense* L.) R0 and R1 regenerants. Biologija, 2003(1): p. 66-68.
- Kanapeckas, J., Resistance of meadow fescue wild ecotypes to foliar diseases. Biologija, 2001(3): p. 24-26.
- 28. Kanapeckas, J., P. Tarakanovas, and N. Lemeziene, Variability, heritability and correlations of genetic resources in meadow fescue. Biologija, 2005(3): p. 10-14.
- Kanapeckas, J., Development of meadow fescue late breeding material based on local populations. Latvijas Lauksaimniecibas Universitate - Raksti, 2006(16): p. 25-29.
- Butkute, B. and V. Paplauskiene, Forage quality potential of perennial grasses Daugiameciu varpiniu zoliu pasarines vertes potencialas. Zemdirbyste, Mokslo Darbai, 2006. 93(3): p. 172-187.
- Berzins, P., et al., Current events in perennial grass breeding. 2014, Latvijas Lauksaimniecibas Universitates Agentura "Zemkopibas Zinatniskais Instituts", Jelgava, Latvia. Jelgava, Latvia: Latvijas Lauksaimniecibas Universitate (LLU). 87-92.
- Fjellheim, S., et al., Phenotypic variation in local populations and cultivars of meadow fescue potential for improving cultivars by utilizing wild germplasm.
 Plant Breeding, 2007. 126(3): p. 279-286.

- Østrem, L., et al., Chlorophyll a fluorescence and freezing tests as selection methods for growth cessation and increased winter survival in * Festulolium. Frontiers in Plant Science, 2018. 9: p. 1200.
- 34. Østrem, L., B. Volden, and A. Larsen, Morphology, dry matter yield and phenological characters at different maturity stages of X Festulolium compared with other grass species. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2013. 63(6): p. 531-542.
- 35. Nekrosas, S. and A. Sliesaravicius, Investigation of intergeneric ryegrass-fescue hybrids produced by various crossing combinations. Zemes ukio Mokslai, 2004(3): p. 20-27.
- 36. Nekrosas, S., P. Tarakanovas, and A. Sliesaravicius, The new Festulolium varieties. Zemdirbyste (Agriculture), 2007. **94**(1): p. 150-159.
- 37. Berziņš, P., et al., Yield and genetic composition of Latvian × Festulolium cultivars and breeding material. 2018. 62-66.
- Rancane, S., et al., Breeding of cocksfoot (*Dactylis glomerata* L.) with improved forage quality. 2016, LLU Institute of Agriculture, Zemkopibas inst., Latvia. Ås, Norway: Nibio. 314-316.
- Tarakanovas, P., Dry matter yield stability in cocksfoot varieties. Zemes ukio Mokslai, 2004(3): p. 8-14.
- 40. Tarakanovas, P. and M. Chomiak, Evaluation of dry matter yield and its stability for cocksfoot varieties (Agriculture), 2008. **95**(1): p. 153-161.
- Tarakanovas, P., J. Kanapeckas, and N. Lemeziene, Studies of intertrait correlations in cocksfoot (*Dactylis glomerata* L.). Zemdirbyste, Mokslo Darbai, 2006. 93(4): p. 306-313.
- 42. Tarakanovas, P., S. Nekrosas, and V. Kemesyte, Assessment of cocksfoot (*Dactylis glomerata* L.) varieties differing in the rhythm of development from the viewpoint of breeding. Zemdirbyste, Mokslo Darbai, 2006. **93**(3): p. 119-130.
- 43. Dabkeviciene, G., et al., Production of slender cocksfoot (*Dactylis polygama* H.) tetraploid populations and their assessment for agromorphological characteristics. Zemdirbyste-Agriculture, 2013. **100**(3): p. 303-310.
- 44. Bukauskaite, J., et al., Resistance of diploid and tetraploid red clover (*Trifolium pratense* L.) to mildew (*Erysiphe trifolii*), anthracnose (*Kabatiella caulivora*) and rust (*Uromyces trifolii*). Zemes ukio Mokslai, 2014. **21**(3): p. 109-119.
- 45. Petrauskas, G., E. Norkeviciene, and K. Jaskune, Breeding of red clover for ingredient in functional food. AASW'19, 18th Alps-Adria Scientific Workshop, Cattolica, Italy, 1-6 April 2019. Abstract book, 2019: p. 128-129.
- 46. Petrauskas, G., V. Stukonis, and E. Norkeviciene, Defining a phenotypic variability and productivity in wild type red clover germplasm. Journal of Agricultural Science (Toronto), 2020. **12**(9): p. 52-61.
- Vilcinskas, E., et al., The productivity of early type red clover (*Trifolium pratense*) varieties and breeding lines. 2008, Lithuanian Institute of Agriculture, Plant Breeding, Akademija, Lithuania. Uppsala, Sweden: Swedish University of Agricultural Sciences. 350-352.

- Jansone, B., et al., Wild populations of red clover and their agro-morphological characteristics in Latvia climate condition. 2008, Research Institute of Agriculture, Skriveri, Zemkopibas Inst. 7, LV-5125, Latvia. Valencia, Spain: Editorial Universidad Politecnica de Valencia. 143-146.
- Rebane, A., S. Rancane, and A. Jansons, The evaluation of red clover plants obtained in field conditions by using biotehnological methods. 2019, Jelgava, Latvia: Latvijas Lauksaimniecibas universitate, Lauksaimniecibas fakultate. 62-66.
- 50. Bender, A., et al., Characteristics of red clover (*Trifolium pratense* L.) populations collected in West- and North-West Estonia. 2012, Jogeva Plant Breeding Institute, Jogeva 48 309, Estonia. Poznan, Poland: Polskie Towarzystwo Lakarskie (Polish Grassland Society). 338-340.
- Tamm, S. and A. Bender, Assessment of a tetraploid red clover collection in Estonia. 2007, Wageningen, Netherlands: European Association for Research on Plant Breeding (EUCARPIA). 226-229.
- 52. Zanotto, S., et al., Trait characterization of genetic resources reveals useful variation for the improvement of cultivated Nordic red clover. Journal of Agronomy and Crop Science, 2021. **207**(3): p. 492-503.
- Helgadóttir, Á., et al., Prebreeding of Red Clover (*Trifolium pratense* L.) for Northern Areas. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 2000. 50(3): p. 187-190.
- 54. Jaluvka, L., et al., Comparison of breeding methods for forage yield in red clover. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2009. 57(2): p. 45-52.
- Butkute, B., et al., Forage yield and quality potential of different white clover forms. 2007, Lithuanian Institute of Agriculture, LT-58344 Akademija, Kedainiai Distr., Lithuania. Merlbeke, Belgium: Belgian Society for Grassland and Forage Crops. 158-161.
- 56. Paplauskiene, V. and B. Butkute, The contents of feeding value components and cyanogenic glycosides in white clover forms and genotypes. Zemdirbyste, Mokslo Darbai, 2006. 93(3): p. 158-171.
- 57. Paplauskiene, V. and A. Sprainaitis, Variability of cyanogenic glucoside content in white clover plants. Biologija, 2003(1): p. 85-88.
- 58. Tarakanovas, P. and A. Sprainaitis, Yield stability of white clover (*Trifolium repens* L.) cultivars and breeding populations. Vagos, 2006(72): p. 61-65.
- 59. Vilcinskas, E. and G. Dabkeviciene, Qualitative and quantitative characteristics of clover (*Trifolium* spp.) species in the first year of growing. Zemdirbyste-Agriculture, 2009. **96**(4): p. 170-180.
- 60. Vilcinskas, E. and G. Dabkeviciene, Yield structure and dry matter qualitative characteristics of clover (*Trifolium* spp.) species. Zemes ukio Mokslai, 2010. 17(1): p. 18-24.
- 61. Helgadóttir, A. and S. Dalmannsdóttir, Adaptation of white clover (*Trifolium repens* L.) to a cool and short growing season. 2004, Agricultural Research

Institute (RALA), Keldnaholt, IS-112 Reykjavik, Iceland. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich. 395-397.

- 62. Helgadóttir, A., et al., Combining winter hardiness and forage yield in white clover (*Trifolium repens*) cultivated in northern environments. Ann Bot, 2008. 102(5): p. 825-34.
- Dzene, I., et al., The study of variability of agro-morphological characteristics of white clover accessions from other countries in Latvian climate conditions. Agronomy Research, 2010. 8: p. 537-544.
- 64. Finne, M.A., O.A. Rognli, and I. Schjelderup, Genetic variation in a Norwegian germplasm collection of white clover (*Trifolium repens* L.) 1. Population differences in agronomic characteristics. Euphytica, 2000. **112**(1): p. 33-44.
- 65. Finne, M.A., O.A. Rognli, and I. Schjelderup, Genetic variation in a Norwegian germplasm collection of white clover (*Trifolium repens* L.) 2. Genotypic variation, heritability and phenotypic stability. Euphytica, 2000. **112**(1): p. 45-56.
- 66. Finne, M.A., O.A. Rognli, and I. Schjelderup, Genetic variation in a Norwegian germplasm collection of white clover (*Trifolium repens* L.) 3. Correlation and path coefficient analyses of agronomic characters. Euphytica, 2000. **112**(1): p. 57-68.
- 67. Collins, R.P., et al., Temporal changes in population genetic diversity and structure in red and white clover grown in three contrasting environments in northern Europe. Ann Bot, 2012. **110**(6): p. 1341-50.
- 68. Liatukiene, A., Genetic variability of the most important traits in alfalfa cultivars under Lithuanian conditions Breeding Grasses and Protein Crops in the Era of Genomics. 2018. 67-72.
- Liatukiene, A. and Z. Liatukas, Downy Mildew Reaction of Alfalfa Accessions of Different Geographical Origin under Lithuanian Conditions. International Journal of Agriculture and Biology, 2014. 16(5): p. 905-910.
- Liatukiene, A., Z. Liatukas, and V. Ruzgas, Winterhardiness as the key factor for selecting accessions of *Medicago sativa* L. high-yielding germplasm. Biologija, 2008. 54(2): p. 129-133.
- Liatukiene, A., Z. Liatukas, and V. Ruzgas, Reaction of the Lithuanian Alfalfa Breeding Populations to Phoma Medicaginis under Cool Temperate Climate Conditions. Pakistan Journal of Botany, 2015. 47(3): p. 919-925.
- 72. Svirskis, A., The performance of lucerne varieties and accessions of Estonian origin in Lithuanian agroclimatical conditions. Zemdirbyste (Agriculture), 2007.
 94(4): p. 20-28.
- 73. Bender, A. and S. Tamm, Natural lucerne populations of Estonia: yielding ability, herbage quality and prospective ways of use. 2010, Jogeva Plant Breeding Institute, 48 309, Jogeva, Estonia. Duderstadt, Germany: Mecke Druck und Verlag. 199-201.
- 74. Collins, R.P., et al., Variation amongst survivor populations of white clover collected from sites across Europe: growth attributes and physiological responses to low temperature. Ann Bot, 2002. **89**(3): p. 283-92.

2.3 Climate related growth and stresses

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2.3.1 Introduction

Climate change leads to longer growing seasons at Nordic latitudes. This gives the potential for increasing number of ley harvests, cultivation of species and cultivars from more southern areas, and higher total harvested biomass yields per year. However, extreme weather is predicted to increase with longer droughts and heat spells as well as larger risks of heavy rains. Also, large scale changes in the earth system could lead to tipping points such as a possible collapse of the Gulf stream with subsequent colder climate in the Nordic countries. The winter survival of perennial crops could both increase and decrease since it depends on several factors such as light and temperature conditions during winter hardening, snow cover, winter temperatures, and sometimes ice. One factor to keep in mind is that lately climate scientists have noticed that the warming of the globe has been faster than their models. This makes agriculture adaptation to climate change an even more important issue and we have to prepare for many different scenarios.

2.3.2 Contemporary consequences of climate variation and change

One way to assess climate change consequences is to look at the consequences of historical weather variations and recent climate changes. In Tromsø, northern Norway, temperature in May had increased significantly from 1989 to 2014, but this did not affect grassland yields significantly [1]. In the same area, hay yields were strongly negatively affected by ground ice thickness, but not by soil freezing depth [2]. Icy, low snow conditions are predicted to become more frequent in the area. In contrast, climate changes occurring 1980-2017 caused a dramatic increase, more than double, in timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) yields in Rovaniemi, Northern Finland. This was caused by both better winter survival and warmer growing seasons with more growing degree days [3].

For all agricultural areas in Finland the Finnish variety testing data 1979-2012 was used to get an overview of the yield consequences of different climatic conditions [4] [5]. For red clover (*Trifolium pratense*), and some southern timothy cultivars, a high growing season temperature was positive, but it was negative for meadow fescue and variable for tall fescue (*Festuca arundinacea*) and festulolium hybrids (× *Festulolium*). A rainy frost hardening period was positive for festulolium and tall fescue but negative for timothy, while red clover responded positively to a rainy regrowth period and a cold frost hardening period. More than six days with temperatures over 25°C after the first cut were negative to most species but not to

red clover, and for the other species the response was dependent on soil type with the most negative responses on clay soils that are more common in southern Finland [6].

In a database of legume variety trials in Scandinavia, Baltic countries, Germany and Great Britain, growing degree days during the regrowth period explained much of the total yield of white clover, with higher total yields at warmer sites and seasons. The total yield of red clover was explained both by accumulated growing degree days which was positive and ley age, which was negative. Red clover was the least persistent and lucerne (*Medicago sativa*) the most persistent legume in the study [7].

2.3.3 Predicted future climate consequences

Climate simulations until 2099 predict that timothy yields will increase at sites all over Norway. This was caused by increasing temperature sums, precipitation, and number of cuts during the growing season. However, the biomass could be more difficult to harvest at some sites due to shorter dry periods and high precipitation especially in the later cuts [8]. In another study, timothy yields were predicted to increase to 2070-2099 for three sites spread over Norway both because a third cut will become profitable and, at some sites, because of decreased drought stress in the first cut [9].

The winter hardening period at six Norwegian sites was predicted to shorten in three different climate change scenarios but the risk of frost injuries would still be less than now because of less severe frost periods. The risk of ice encasement increased in one location only. The models predicted that perennial ryegrass (*Lolium perenne*) will become possible to grow in a larger area than now [10].

Climate change scenarios from 14 sites in the Nordic and Baltic countries 2040-2065 showed increasing growing season temperature and potential evapotranspiration sums for all sites and increasing precipitation sum for all sites but one. The optimal time for the first harvest was 8-20 days earlier than the reference period and one more harvest could be taken at all sites. The start of the winter hardening was 5-20 days later and the duration of >10 cm snow cover shorter at all sites with the largest differences in the north. The risk of winter damage in timothy increased in some eastern sites because of less snow, while the results for perennial ryegrass were very variable with both increased and decreased risks [11].

2.3.4 Drought tolerance

Grass species differ in their abilities to tolerate drought. Forage grasses evaluated in a Danish experiment with rain-protected plots and irrigated controls showed that the most high-yielding cultivars under control conditions, cockfoot (*Dactylis* glomerata cv. 'Sevenop') and tall fescue cvs. 'Jordane' and 'Kora', also were most high yielding under drought. Festulolium cv. 'Hykor' and cocksfoot cv. 'Amba' were less productive in the irrigated control but did not lose much growth under drought. All grasses showed compensatory growth after the drought, giving in total increased water use efficiency in the drought treated plots [12]. Not all festuloliums are drought tolerant. Two Festulolium cultivars originating from crosses between meadow fescue and perennial ryegrass were sensitive to drought and had poor winter survival during the second winter. However, long autumns allowed for a fourth cut, giving high yields in the first harvest season [13].

Tetraploid cultivars of both Italian ryegrass (*Lolium multiflorum*) [14] and perennial ryegrass [15] have been shown to be more high yielding under drought stress than diploid cultivars.

2.3.5 Winter damages/tolerance

Comparisons between species

Mechanisms of frost tolerance are important when climate change models are evaluated. Timothy had a higher tolerance of ice encasement and frost than perennial ryegrass and, in both species, cultivars with a more northern origin were also more winter tolerant. In timothy, this was linked to a slower depletion of water soluble carbohydrates during ice encasement [16]. The most frost tolerant cultivar, 'Engmo', had higher frost tolerance also after de-hardening [17]. Perennial ryegrass and timothy sown in July had a better frost tolerance in the following spring than when sown in May in an experiment in western Norway. This seemed to be due to more sucrose in the younger, plants especially during early spring [18]. Frost treatments triggered compensatory regrowth in roots and herbage [19].

Mechanisms for winter adaptation were studied in ten cultivars of meadow fescue, perennial ryegrass and Festulolium. Winter survival was negatively correlated to leaf elongation rate in autumn at a more southern site in Norway, but not further north. At both sites, winter survival the first year was positively correlated to photosynthetic activity before winter [20]. Likewise, at more northern sites in Scandinavia, meadow fescue and tall fescue survived winter better than most ryegrass hybrids because the latter did not stop growing early enough in the autumn. However, the Festulolium cultivar 'Hykor' that is closest to tall fescue showed good winter survival at all sites but Iceland. Ryegrass hybrids with meadow and tall fescue showed a higher production potential in the more favourable sites (Sweden, Denmark and to some extent Norway). [21].

Several grasses and clovers were tested across sites from Greenland to mid Sweden. The tested timothy and cocksfoot cultivars were winter hardy at all sites, while perennial ryegrass, festulolium and tall fescue survived only in the more favourable sites [22]. Peat soils can offer particular challenges for winter survival. Most grasses, especially fescues, festulolium and reed canary grass (*Phalaris arundinacea*) cultivars, grown on peat soil in Estonia did not survive a very cold winter. Only smooth brome grass (*Bromus inermis*) survived very well and in mixture it can take over when the timothy decreases after 3 years [23].

Perennial ryegrass

Perennial ryegrass is very productive and adds good quality to the forage where it can be grown. However, in many parts of the Nordic and Baltic countries it is not considered winter hardy. At a peat site in Estonia perennial ryegrass showed poor winter survival [23]. Twenty-two cultivars of ryegrass were tested on five sites at the margin area of its cultivation in five Nordic countries. Regionally developed cultivars and diploid cultivars had better winter survival and higher yield than more southern cultivars. Yield was positively correlated with growing season temperatures, while winter survival was negatively correlated to warm periods and rain during the winter [24]. A gene producing a protein that protects cell membranes from freezing damage was studied in ryegrass in Lithuania. Variation in this gene can be used in plant breeding to increase winter tolerance [25].

In a simulated climate change experiment with open top chambers the perennial ryegrass cultivar 'Riikka' was more frost tolerant than 'Gunne' and 2°C higher temperature during autumn reduced frost tolerance in December (by 1°C); however, increased CO2 concentration had no effect on frost tolerance [26].

Festulolium

Festulolium cultivars 'Felina' and 'Hykor' had the highest yield and good winter survival in a three year experiment in Latvia. Yields were higher than for the ryegrass hybrids (*Lolium* x *boucheanum*). The first harvest year gave higher yields for all cultivars in a three cut system [27, 28].

Timothy

The variation in yield on a timothy field in Finland was largely caused by winter damage caused by ice from a snowmelt in December. Plots with a 68% winter damage gave about half yield in the first cut compared to plots without winter damage, but the difference was less in the second cut [29]. In timothy, vernalization, cold stimulating flowering shoots, occurred simultaneously with cold acclimation. The northernmost timothy cultivar required vernalization to form flowering tillers, in contrast to other timothy cultivars. [30]. Deep soil frost and low temperatures could affect timothy growth and protein content negatively even if the cultivar is winter tolerant [31].

Legumes

Six white clover (*Trifolium repens*) populations were subjected to field conditions in northern Norway and lab winter conditions. The survival rates in the lab were a good estimation of the survival rates in the field [32]. Mechanisms for frost tolerance at Iceland for a Norwegian white clover population compared to the cultivar 'Aberherald' was connected to higher content of the fatty acid 18:2 and higher content of sucrose in stolons in January [33]. White clover could be adapted to the Icelandic climate by cross breeding between Norwegian cultivars and more high yielding cultivars of more southerly origin [34].

One method for winter tolerance acquisition in populations could be survival of the fittest individuals during field experiments. Survival populations of Norwegian cultivars of white clover grown in Iceland had better long-term performance than the original populations [35]. In Iceland and southern Sweden, populations of white and red clovers changed genetically during three years, but did not lose genetic diversity. The largest genetic change was recorded in Sweden, where the autumn temperatures were lower [36].

Competition with other species also affects winter survival. Experiments with two cultivars of white clover grown together with perennial ryegrass at three Nordic sites showed that the clover leaf area in autumn is essential for spring growth and that too much competition from the grass was negative for clover cover in the spring [37, 38].

Four lucerne cultivars were subjected to both field (Northern Finland) and lab winter conditions. All four had a good winter survival in the field, but the cultivar that had the highest frost tolerance in the lab produced better in the second harvest years. Frost tolerance was linked to allocation of biomass to the roots and a higher concentration of total non-structural carbohydrates in the winter [39]. A standard test to classify winter hardiness in lucerne is used in Latvia [40]. In this test, cultivars from the Baltic countries were more winter hardy than cultivars from the USA.

Lucerne had better persistence and yield the following year if the last cut was not made between August 28 and September 10 in Estonia. This period was critical under a three cut system and a later third harvest gave better growth the following year [41].

2.3.6 Conclusions and knowledge gaps

The plant materials used now in the Nordic and Baltic countries have varied responses to drought stress and winter conditions. This is promising for the possibilities of future adaptation to changing climate conditions. However, most studies have focused on winter stresses and there were few studies on drought tolerance and none on flooding from the Nordic and Baltic countries that were identified through this review process. The growing season in 2023, when large areas of Sweden first had a long drought period in spring and early summer, followed by heavy rains and flooding of important agricultural areas later in summer, shows that drought and flooding tolerance in perennial forage species is an important future research area. Reed canary grass is a species that has good tolerance for both drought and flooding, but unfortunately, the forage quality is low.

2.3.7 References

- Golinski, P., et al., Relationship Between Climate Trends and Grassland Yield Across Contrasting European Locations. Open Life Sci, 2018. 13(1): p. 589-598.
- Bjerke, J.W., et al., Impacts of snow season on ground-ice accumulation, soil frost and primary productivity in a grassland of sub-Arctic Norway. Environmental Research Letters, 2015. 10(9): p. 14.
- 3. Niemelainen, O., et al., Increase in perennial forage yields driven by climate change, at Apukka Research Station, Rovaniemi, 1980-2017. Agricultural and Food Science, 2020. **29**(2): p. 139-153.
- 4. Mäkinen, H., et al., Managing resilience of forage crops to climate change through response diversity. Field Crops Research, 2015. **183**: p. 23-30.
- Mäkinen, H., et al., Gaps in the capacity of modern forage crops to adapt to the changing climate in northern Europe. Mitig Adapt Strateg Glob Chang, 2018.
 23(1): p. 81-100.
- 6. Mäkinen, H., et al., Shifts in soil-climate combination deserve attention. Agricultural and Forest Meteorology, 2017. **234**: p. 236-246.
- Halling, M.A., C.F.E. Topp, and C.J. Doyle, Aspects of the productivity of forage legumes in Northern Europe. Grass and Forage Science, 2004. 59(4): p. 331-344.
- Persson, T. and M. Höglind, Impact of climate change on harvest security and biomass yield of two timothy ley harvesting systems in Norway. Journal of Agricultural Science, 2014. 152(2): p. 205-216.
- Baadshaug, O.H., A.O. Skjelvag, and M. Trnka. Ley production potential as affected by climate change at high latitudes. in EGF. 2018. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre.
- Thorsen, S.M. and M. Höglind, Assessing winter survival of forage grasses in Norway under future climate scenarios by simulating potential frost tolerance in combination with simple agroclimatic indices. Agricultural and Forest Meteorology, 2010. 150(9): p. 1272-1282.
- Höglind, M., S.M. Thorsen, and M.A. Semenov, Assessing uncertainties in impact of climate change on grass production in Northern Europe using ensembles of global climate models. Agricultural and Forest Meteorology, 2013. 170: p. 103-113.

- Korup, K., et al., Biomass production and water use efficiency in perennial grasses during and after drought stress. Global Change Biology Bioenergy, 2018.
 10(1): p. 12-27.
- Kemesyte, V., K. Jaskune, and G. Statkeviciute, Festulolium field performance under fluctuating growing conditions in Lithuania. Biologia Plantarum, 2020. 64: p. 821-827.
- Akinroluyo, O.K., et al., Drought stress response of Westerwolths ryegrass (*Lolium multiflorum* ssp. *multiflorum*) cultivars differing in their ploidy level. Zemdirbyste-Agriculture, 2020. 107(2): p. 161-170.
- 15. Kemesyte, V., G. Statkeviciute, and G. Brazauskas, Perennial Ryegrass Yield Performance under Abiotic Stress. Crop Science, 2017. **57**(4): p. 1935-1940.
- Höglind, M., et al., Tolerance to frost and ice encasement in cultivars of timothy and perennial ryegrass during winter. Grass and Forage Science, 2010. 65(4): p. 431-445.
- Jørgensen, M., L. Østrem, and M. Höglind, De-hardening in contrasting cultivars of timothy and perennial ryegrass during winter and spring. Grass and Forage Science, 2010. 65(1): p. 38-48.
- Østrem, L., et al., Effect of developmental stage on carbohydrate accumulation patterns during winter of timothy and perennial ryegrass. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2011. 61(2): p. 153-163.
- 19. Østrem, L., et al., Impact of frost and plant age on compensatory growth in timothy and perennial ryegrass during winter. Grass and Forage Science, 2010. 65(1): p. 15-22.
- Østrem, L., et al., Influences of growth cessation and photoacclimation on winter survival of non-native *Lolium-Festuca* grasses in high-latitude regions. Environmental and Experimental Botany, 2015. 111: p. 21-31.
- 21. Østrem, L., et al. Environmental impact on winter survival and production in non-native grasses in the Nordic countries. in NJF. 2015. Riga, LATVIA: Nordic Assoc Agricultural Scientists-Njf.
- 22. Thorvaldsson, G., et al. Climatic adaptation of species and varieties of grass and clover in the West Nordic countries. in EGF. 2016. Ås, Norway: Nibio.
- 23. Aavola, R. and J. Karelson, The dynamics of botanical composition of pure and mixed grass swards on peaty soil. Agronomy Research, 2010. **8**: p. 513-522.
- 24. Helgadottir, A., et al., Adaptability and phenotypic stability of *Lolium perenne* L. cultivars of diverse origin grown at the margin of the species distribution. Journal of Agronomy and Crop Science, 2018. **204**(5): p. 493-504.
- Aleliunas, A., et al., Association of single nucleotide polymorphisms in LpIRI1 gene with freezing tolerance traits in perennial ryegrass. Euphytica, 2015. 204(3): p. 523-534.
- Höglind, M., et al. Effect of increased autumn temperature and CO2 concentration on frost hardening and winter survival in *Lolium perenne*. in International Grassland Congress 2008. Guangzhou, China: Guangdong People's Publishing House.

- Gutmane, I. and A. Adamovich. Analysis of Festulolium and hybrid ryegrass (*Lolium * boucheanum*) dry matter yield stability. in EGF. 2008. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- Gutmane, I. and A. Adamovich. Productivity and persistency of Festulolium and Lolium * boucheanum swards. in EGF. 2007. Merlbeke, Belgium: Belgian Society for Grassland and Forage Crops.
- 29. Niemelainen, O., et al. Variability in herbage mass and chemical composition within a timothy sward. in International Grassland Congress. 2001. Sao Pedro, Brazil: Fundacao Estudos Agrarios Luiz Queiroz (Fealq).
- Seppanen, M.M., et al. The role of vernalization in freezing tolerance and tiller composition of forage grasses. in EGF. 2013. Borgarnes, Iceland: Agricultural University of Iceland.
- Gustavsson, A.M. and K. Martinsson, Analysis of growth and nutrition value in timothy using a dynamic model. Agricultural and Forest Meteorology, 2001.
 107(2): p. 83-101.
- Rapp, K. and O. Junttila, Heritability estimates of winter hardiness in white clover based on field and laboratory experiments. Acta Agricultura Scandinavica. Section B, Soil and Plant Science, 2000. 50(3): p. 143-148.
- Dalmannsdottir, S., A. Helgadottir, and B.E. Gudleifsson, Fatty acid and sugar content in white clover in relation to frost tolerance and ice-encasement tolerance. Annals of Botany, 2001. 88: p. 753-759.
- 34. Helgadottir, A. and S. Dalmannsdottir. Adaptation of white clover (*Trifolium repens* L.) to a cool and short growing season. in EGF. 2004. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich.
- Helgadottir, A., S. Dalmannsdottir, and R.P. Collins, Adaptational changes in white clover populations selected under marginal conditions. Annals of Botany, 2001. 88: p. 771-780.
- 36. Collins, R.P., et al., Temporal changes in population genetic diversity and structure in red and white clover grown in three contrasting environments in northern Europe. Ann Bot, 2012. **110**(6): p. 1341-50.
- Wachendorf, M., et al., Overwintering and growing season dynamics of *Trifolium repens* L. in mixture with *Lolium perenne* L.: A model approach to plant-environment interactions. Annals of Botany, 2001. 88: p. 683-702.
- 38. Wachendorf, M., et al., Overwintering of *Trifolium repens* L. and succeeding growth: Results from a common protocol carried out at twelve European sites. Annals of Botany, 2001. **88**: p. 669-682.
- Seppanen, M.M., et al., Growth, freezing tolerance, and yield performance of alfalfa (*Medicago sativa* L.) cultivars grown under controlled and field conditions in northern latitudes. Canadian Journal of Plant Science, 2018. 98(5): p. 1109-1118.
- 40. Gaile, Z., et al. Evaluation of winterhardiness in lucerne in Latvian conditions using an accelerated test. in EGF. 2003. Pleven, Bulgaria: Bulgarian Association for Grassland and Forage Production (BAGFP).

41. Lillak, R. and A. Linke. The influence of cutting system and soil conditions influence on persistence and productivity of lucerne. in EGF. 2003. Pleven, Bulgaria: Bulgarian Association for Grassland and Forage Production (BAGFP).

2.4 Diseases and weeds

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2.4.1 Root rot

Root rot is a significant disease of forages in the Nordic countries, and is caused by infection from a range of soil-borne pathogens; commonly *Fusarium avenaceum*, *Phoma medicaginis* and *Cylindrocarpon destructans* [1]. The root rot complex is widespread, and is a significant cause of poor red clover persistence, with yield and nitrogen fixation declining over time. It is possible to reduce root rot effects through fungicides or fumigants [2] however this is not a typical practice with leys.

The effect of root rot on white clover (*Trifolium repens*), red clover (*Trifolium pratense*) birdsfoot trefoil (*Lotus corniculatus*) and lucerne (*Medicago sativa*) was compared in mixed grass-legume swards in a field experiment [3]. Root rot was already present in the autumn of the establishment year, and after two years, infection of red clover plants was widespread. Although the severity of the disease was high, there was still a strong presence of red clover in the plots; however by the third production year the red clover significantly decreased. The disease incidence of the other legume species was less than for red clover.

A later field experiment examined the effect of root rot on persistence of the same legumes [4, 5]. The pathogens were present in all species, but disease severity was greatest in red clover. Birdsfoot trefoil and lucerne were more tolerant of the pathogens, whereas white clover maintained persistence through continuous growth of new stolons. The third harvest year (2018) was extremely dry, and lucerne was much higher yielding than other species. The combination of drought tolerance and root rot resistance make lucerne an interesting species that can potentially be grown in a wider area than it is currently in Sweden.

In a long term experiment in Northern Sweden [6], red clover was included in a grass-clover mixture in different cropping systems across three sites. Barley was followed by two, three, or five years of leys. For the three- and five-year ley treatments, there was a strong decrease in clover content in the third ley production year, with no significant difference between the systems when comparing within harvest years. If this third-year decline was due to root rot, as was hypothesised, then having a longer ley-free period in the rotation is not sufficient to reduce the effect on red clover persistence in leys.

2.4.2 Clover rot

Clover rot is a common cause of winter damage, caused by the fungus *Sclerotinia trifoliorum*. Although the infections form in late summer and autumn,

it is during the winter that the fungus grows systemically through the plants and into the root system. It commonly spreads to nearby plants, creating patches of dead plants in the field. It is possible to control the disease with fungicides, but no compound is currently allowed for this purpose in Sweden [7]. Clover rot weakens red clover plants, decreasing yield, and also kills plants, reducing the density of plants [7]. A study at multiple sites in Sweden [7] compared 10 red clover cultivars, and clover rot was recorded at all sites. Resistance was associated with tetraploidy, late flowering, and previous selection for resistance. Red clover yields were affected by gaps in crop stands where plants had died. At the location near Östersund, there was very serious clover rot damage, at a site that had not had red clover for more than 8 years. It was hypothesised that the infestation may have been maintained by unsown white clover.

Clover rot can potentially be minimised through a crop rotation that doesn't include sown or unsown species that maintain the infestation; however the previously mentioned study suggests that this management practice is not always effective. Plant breeding will continue to remain an extremely important mechanism for reducing the impact of infection. Biological control of clover rot has also been explored [8], using the fungus *Coniothyrium minitans*, traded as Contans WG. The research found that application of the biocontrol agent in the early summer doubled the number of surviving red clover plants in the following year. Contans WG is currently registered in Sweden for "Behandling av jord mot bomullsmögel" before, during, or after sowing (Odla 2023, Lantmännen).

Clover rot is also the main disease affecting red clover in Lithuania. Field research [9] found that development of clover rot was highly dependent on the weather conditions – favourable conditions for disease development were a humid autumn and a warm humid winter. It is unclear how future weather conditions in Sweden will change the importance of clover rot. As with the studies in Sweden, late or medium-late flowering types were more resistant. The researchers found that tetraploid cultivars were more resistant in the first production year, but not in the second year. It is not clear whether or not there is a consistent difference between tetraploids and diploids.

2.4.3 Other diseases

Spring black stem and leaf sport is a fungal disease in lucerne caused by *Phoma medicaginis*. A range of genotypes of lucerne, yellow lucerne (*Medicago falcata*) and hybrid lucerne (*Medicago x varia*) were compared in a field experiment in Lithuania [10]. The genotypes showed a range of partial resistance, which could be useful in resistance breeding.

A similar experiment in Lithuania assessed 100 lucerne genotypes for downy mildew (*Peronospora trifoliorum*) resistance [11]. Downy mildew causes leaf blotches, and is usually identified by the downy grey growth on the underside of

leaves. The genotypes ranged from 10% to 80% susceptible, and suggest potential for resistance breeding. The most resistance genotypes came from countries near Lithuania, including Sweden.

Crown and stem rust, caused by *Puccinia* species, are diseases that primarily affect ryegrass species. They are most predominantly diseases of seed crops, but can also affect plants in the vegetative phase. In an experiment with Italian ryegrass (Lolium multiflorum), hybrid ryegrass (Lolium x boucheanum) and perennial ryegrass (Lolium perenne), stem rust occurred almost exclusively on perennial ryegrass [12]. Crown rust was more commonly observed, and for all three plant species. A study by the Norwegian seed company Graminor [13] examined plant material of perennial ryegrass, hybrid ryegrass, meadow fescue (Festuca pratensis), and festulolium (× Festulolium) at three locations, including in Denmark, with the greatest incidence of crown rust at a site in France. In general, all plant materials were susceptible to rust. Across sites, festulolium was least affected by rust, and the least affected genotype was a cross with tall fescue (*Festuca arundinacea*). The two meadow fescue genotypes were from Nordic material and did not have tolerance to rust. Experiments that test plant material outside of their range of origin are useful, as they can help to separate differences in tolerances of breeding material, particularly when it comes from locations where the disease is not common. It is expected that with climate change, the conditions for rust may become more common, and thus rust should not be ignored in breeding programs.

Snow mould refers to a group of fungi that can cause diseases in plants during winter. The most relevant type in the Nordic countries is pink snow mould (Microdochium nivale). Pink snow mould infects different parts of the plant, both above and below the soil surface, and the infection process is influenced by microclimatic conditions, especially ambient air temperature and humidity. However, unlike other snow moulds, pink snow mould does not require snow cover to cause damage. Testing resistance to snow mould is complicated, due to the difficulty in separating it from other winter-related stresses (biotic and abiotic), and there is no direct correlation between resistance to snow mould and these other stressors. In mild winters, resistance to snow mould can be the most important factor affecting winter survival, and it is unclear how the disease will be affected by climate change. In a study in Northern Norway [14] a range of festulolium, meadow fescue, and perennial ryegrass were assessed for resistance. In the field trial, entries of both perennial ryegrass and festulolium that had been exposed to natural selection in northern Norway (above 65°N) showed good levels of winter survival. Resistance to snow mould in non-hardened plants tested under controlled conditions was not correlated with winter survival in the field. This may indicate that cold acclimation exerts a major influence on the expression of snow mould resistance. Plant breeding will likely remain the most important defence against pink snow mould.

2.4.4 Weeds

There were few scientific papers that focused on weeds in ley crops, and they tended to focus on perennial weeds. Perennial weeds can be particularly important in ley crops because tillage is not used during the ley phase. They are more of a problem in parts of the country where leys dominate the crop rotation. A guide to non-chemical management of perennial weeds can be found in the publication 'Rotogräsens När Var Hur'³.

One approach to address the perennial weed couch grass (Elymus repens) was to fragment rhizomes using a machine with vertical coulter discs [15]. The machine was used before and/or after sowing. Fragmentation was combined with repeated mowing. The sown species were combinations of white clover and Italian ryegrass, and the fragmentation process had a positive effect on the crop. Both early and late rhizome fragmentation reduced couch grass biomass, by 38%, and the effect was somewhat additive, with two treatments reducing couch grass biomass by 63%. Repeated mowing reduced couch grass biomass (by 75%) and there was no benefit of combining mowing with rhizome fragmentation [15]. A similar process, but with manual rhizome fragmentation (using a spade) was tested in a white clover crop, again combined with mowing [16]. When done in autumn, rhizome fragmentation and mowing reduced couch grass shoot biomass, but not rhizome biomass or number of shoots. However, when done in early summer, rhizome fragmentation reduced the couch grass rhizome biomass by up to 60%, and repeated mowing reduced it by up to 95%. Rhizome fragmentation is potentially more effective in the early summer because the couch grass has less stored energy resources.

An experiment in Finland [17] examined the best methods for terminating long term leys in a way which suppresses couch grass. The treatments included: i) three passes of stubble cultivation after one forage harvest; ii) stubble cultivation after two forage harvests; iii) ploughing in September after two forages harvests; iv) ploughing in October, just before winter; v) ploughing in spring. Treatments i and ii reduced the number of couch grass shoots, with three stubble cultivations (treatment i) having the greatest effect.

A field experiment in Southern Sweden [18] examined the effects of increasingly more complex ley mixtures on the observation of unsown species. The sown species included timothy (*Phleum pratense*), perennial ryegrass, red clover, chicory (*Cichorium intybus*), and lucerne. Fewer unsown species were recorded in mixtures compared to monocultures. Among the monoculture plots, perennial ryegrass had the least unsown species. These results highlight the importance of ley species diversity to help suppress weeds.

³ https://www.slu.se/globalassets/ew/org/centrb/epok/dokument/ograsskrift_web.pdf

2.4.5 Conclusions and knowledge gaps

The most significant plant diseases are a problem for red clover in particular. It is important that we don't overly rely on a single species, as it makes ley production vulnerable to such effects as new diseases or changes in climate. There is a need for diversity – diversity in crop rotations, crops, ley mixtures, species, and cultivars. As described in the chapter on ley species, there are many advantages of having more diverse ley mixtures.

Crop rotation cannot be relied on to control the two most important legume diseases, root rot and clover rot; however it is a part of the solution. The other parts involve including diversity as much as possible, and continued investment in plant breeding to develop more resistant cultivars. As emphasised in other sections, it is essential that new plant cultivars for the Nordic countries are developed and field tested in the Nordic countries.

Crop rotation is also an important part of weed management, particularly for perennial weeds that are problematic in leys. As much as possible, weeds need to be controlled in the annual cropping phase. Rhizome fragmentation and mowing in the ley phase can also reduce perennial weeds such as couch grass.

The great unknown for both diseases and weeds is the future effect of climate, which may change the prevalence of existing diseases and weeds, and potentially introduce new threats.

2.4.6 References

- 1. Almquist, C., E. Stoltz, and A.C. Wallenhammar. Incidence of root pathogens associated to clover root rot in Sweden. in EGF. 2016. Ås, Norway: Nibio.
- Lager, J. and A.C. Wallenhammar, Crop loss from soil-borne pathogens in white clover stands assessed by chemical treatments. Journal of Plant Diseases and Protection, 2003. 110(2): p. 120-128.
- 3. Wallenhammar, A.C., et al. Influence of root rot on the sustainability of grass/legume leys in Sweden. in EGF. 2008. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- 4. Wallenhammar, A.C., et al. Challenges in ley farming systems based on legumes in Sweden. in EGF. 2020. Helsinki, Finland: Luke.
- Wallenhammar, A.C., et al., Production capacity of forage legumes and persistence to root rot in organic mixed swards, in EGF and EUCARPIA. 2019, Organising Committee of the Joint 20th Symposium of the European Grassland Federation and the 33rd Meeting of the EUCARPIA: Zurich, Switzerland. p. 99-99.
- 6. Bergqvist, S., et al. Crop rotation effect on red clover persistence in mixed grassclover leys. in EGF. 2022. Caen, France: Wageningen Academic Publishers.
- Öhberg, H., P. Ruth, and U. Bang, Differential responses of red clover cultivars to *Sclerotinia trifoliorum* under diverse natural climatic conditions. Plant Pathology, 2008. 57(3): p. 459-466.

- Öhberg, H. and U. Bang, Biological control of clover rot on red clover by *Coniothyrium minitans* under natural and controlled climatic conditions. Biocontrol Science and Technology, 2010. 20(1): p. 25-36.
- Mikaliuniene, J., et al., Evaluation of red clover (*Trifolium pratense* L.) resistance to Sclerotinia crown and root rot (*Sclerotinia trifoliorum*) in the laboratory and field conditions. Zemdirbyste-Agriculture, 2015. 102(2): p. 167-176.
- Liatukiene, A., Z. Liatukas, and V. Ruzgas, Reaction of lucerne (*Medicago* spp.) to fungal diseases caused by *Phoma medicaginis*. Zemes ukio Mokslai, 2010. 17(1): p. 25-31.
- 11. Liatukiene, A., Z. Liatukas, and V. Ruzgas, Investigation of downy mildew in lucerne crop Zemes ukio Mokslai, 2010. 17(3): p. 87-92.
- 12. Schubiger, F.X., et al., Susceptibility of European cultivars of Italian and perennial ryegrass to crown and stem rust. Euphytica, 2010. 176(2): p. 167-181.
- Østrem, L., et al., Low crown rust resistance in Norwegian material of *Lolium* perenne and × Festulolium, in Breeding Grasses and Protein Crops in the Era of Genomics, G.B.e. al., Editor. 2018, Springer International Publishing AG. p. 145-149.
- 14. Abdelhalim, M., et al., Snow mould resistance under controlled conditions and winter survival in the field in populations of perennial ryegrass, meadow fescue, and Festulolium are partly dependent on ploidy level and degree of northern adaptation. Canadian Journal of Plant Science, 2016. 96(4): p. 579-589.
- 15. Ringselle, B., et al., Rhizome Fragmentation by Vertical Disks Reduces *Elymus repens* Growth and Benefits Italian Ryegrass-White Clover Crops. Front Plant Sci, 2017. 8: p. 2243.
- Bergkvist, G., et al., Control of *Elymus repens* by rhizome fragmentation and repeated mowing in a newly established white clover sward. Weed Research, 2017. 57(3): p. 172-181.
- 17. Kakriainen-Rouhiainen, S., et al. Terminating ley with mid-summer bare fallow controls *Elymus repens*. in EWRS Workshop on Physical and Cultural Weed Control. 2004. Lillehammer, Norway: European Weed Research Society.
- Frankow-Lindberg, B.E. Richness of unsown plant species in a sown ley crop. in EGF. 2010. Duderstadt, Germany: Mecke Druck und Verlag.

2.5 Plant-microbe and plant-fungal symbioses in leys

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2.5.1 Introduction

Many plant species form mutually beneficial symbiotic relationships with bacteria and fungi. Through these relationships, the plant can gain fitness advantages, such as increased stress tolerance or improved plant development.

Systemic endophytic fungi within the *Epichloë* and *Neotyphodium* complex often colonize grasses within the subfamily *Pooideae*. *Pooideae* grasses, such as meadow fescue (*Festuca pratensis*) and tall fescue (*Festuca arundinacea*), are agriculturally important crops in northern Europe, often cultivated as forage species in multi-year leys. These species represent key components of mixed leys in the Nordic and Baltic countries due to their high nutritive value and productivity. Endophytic fungal symbionts have been shown to have variable impacts on the performance of their grass hosts, depending on environmental conditions.

The mutual symbiosis between rhizobia, a group of soil bacteria, and forage legume species allows legume plants to convert atmospheric nitrogen into a form usable by plants through biological nitrogen fixation. Rhizobia interact with legume roots to form nodules containing nitrogen-fixing bacteriods, which provide nitrogen to the plant and the surrounding system. This production of usable nitrogen greatly benefits the agricultural system, as it reduces the need for the application of nitrogen fertilizer.

2.5.2 Endophytic fungal mutualism

The endophytic fungus *Neotyphodium* commonly forms a symbiosis with meadow fescue cultivars used in the Nordic countries. The natural infection rate of *N. uncinatum* on meadow fescue ranges significantly, with infection rate increasing throughout the growing season [1]. Studies on the performance of meadow fescue with and without *N. uncinatum* colonization have demonstrated a variable impact of endophyte presence on grass productivity. In northern Sweden, plant productivity did not differ between endophyte-infected and non-infected plants [2]. However, in northern Finland endophyte-infected fescue had improved productivity in high-nutrient soils and decreased productivity in low-nutrient soils, indicating environmental influence on the impact of endophyte presence on plant growth [3]. The productivity of endophyte-infected meadow fescue can also have implications for its competitiveness and subsequently its ability to supress weeds. Stands of meadow fescue with and without *N. uncinatum* infection were evaluated in southern Finland to determine if endophyte symbiosis can assist in biological weed suppression [4]. Results from the study demonstrated that colonized fescue

stands had denser cover and increased survival, leading to improved weed suppression.

N. uncinatum may also impact the winter survival of their host grass through altered resistance to snow mould pathogens. Endophyte-infected and endophyte-free meadow fescue cultivars grown in northern Finland were evaluated for infection with speckled snow mould (*Typhula ishikariensis*) and pink snow mould (*Microdochium nivale*) and their subsequent winter survival [5]. Endophyte infection resulted in greater susceptibility to speckled snow mould. However, endophyte-infected plants had improved growth in spring despite higher snow mould infection rates, indicating that endophyte infection may support snow mould tolerance in their host grass and improve the grass' winter survival rate despite increased snow mould infection rates.

Tall fescue forms a symbiosis with another species of endophytic fungus, *Epichloë coenophiala*. A common American cultivar of tall fescue (Kentucky-31) and three wild accessions from Sweden and Finland were evaluated in a field experiment in southern Finland to determine the impact of *E. coenophiala* colonization on tall fescue productivity and winter survival [6]. Results demonstrated a positive influence of endophyte infection on biomass accumulation and reproductive performance for all accessions, though endophyte symbiosis had no impact on the grasses' winter survival. A similar experiment was established in southern Finland using the same American cultivar and wild accessions of tall fescue, as well as a common Finnish cultivar (Retu), to evaluate the influence of *E. coenophiala* on the occurrence of leaf blotch (*Rhynchosporium* sp.) [7]. Endophyte colonization had a variable impact on leaf blotch infection rate depending on the origin of the host grass, indicating that endophytic plant genotype drives pathogen resistance.

A third fescue grass-endophyte symbiosis (*Festuca rubra* and *Epichloë festucae*), was studied to examine the interaction between red fescue, the endophytic fungus, a grass pathogen (*Claviceps purpurea*), and aphids (*Sitobion* sp.) [8]. Endophyte colonization increased pathogen infection rate, however had no direct influence on aphid infestation. However, grasses infected with the pathogen had significantly lower rates of aphid herbivory, demonstrating the complexity of interactions between plants, fungi, and herbivores.

2.5.3 Nitrogen fixation

Nitrogen fixation rates can vary greatly between fields of the same species due to variables such as legume percentage, genotype of the legume and/or rhizobia, management, and environmental conditions. The nitrogen fixation rate of red clover grown in mixed leys was found to be highest in July and August, demonstrating the clover's higher need for nitrogen during periods of regrowth after the first harvest [9, 10]. Additionally, high N concentrations were present in the stubble, thus

providing considerable nitrogen fertilization effects for regrowth or for the subsequent crop in the rotation. The age of a ley can also have implications for nitrogen fixation rates, due mainly to the low persistence of red clover (Trifolium pratense). Nitrogen fixation rates from a Finnish field trial were found to decrease with increasing ley age, owing heavily to a decreasing clover content [11]. This study also reported high variability in nitrogen fixation dependent on soil fertility parameters, demonstrating the importance of soil characteristics on the nitrogen fixation potential of red clover. A legume's nitrogen fixation activity can also be impacted by the fertilization rate and the type of fertilizer applied. The results of two Danish studies demonstrated no effect of fertilizer type (mineral fertilizer or slurry) on white clover (Trifolium repens) nitrogen fixation rates [12, 13]. However, the nitrogen fixation rates of mixed leys containing both red and white clover were negatively impacted by the addition of mineral N fertilizer, indicating variable impacts of fertilizer type on nitrogen fixation depending on the legume species [12]. Similarly, fertilizer rate had a variable impact on the nitrogen fixation activity of different legume species [14]. The addition of cattle slurry fertilizer had no impact on the nitrogen fixation rates of birdsfoot trefoil (Lotus corniculatus) and lucerne (Medicago sativa), but lowered the nitrogen fixation rates of red and white clover. The variability in nitrogen fixation activity between legume species can have implications for optimum fertilizer application rates of a mixed ley. Species capable of high nitrogen fixation rates can maintain high productivity with minimal inputs. A Lithuanian study compared nitrogen fixation rates of white clover, red clover, and lucerne when grown in mixed stands with grass and reported the highest rates of nitrogen fixation and N transfer to the companion grass for lucerne [15]. Understanding how the species composition of a ley influences the nitrogen fixation rate of the legume component can provide useful insight in determining optimal species mixtures. Two studies evaluating the nitrogen fixation rate of legumes included in species-rich mixtures containing grasses and forbs reported no negative impact of increased species diversity on the nitrogen fixation of red and white clover [16, 17]. The nutrient availability in mixed stands of legumes and grasses may also impact the nitrogen fixation efficiency of the legume component. Competition for phosphorus and potassium was shown to decrease the nitrogen fixation rate of white clover in leys containing ryegrass, demonstrating the importance of sufficient nutrient application to maximize nitrogen fixation efficiency [18].

The method used to quantify nitrogen fixation rates and the plant organs used in the analysis will influence the reported nitrogen fixation rate for forage legumes in the field, making it essential to employ highly accurate methods. ¹⁵N isotopic dilution and ¹⁵N natural abundance methods for nitrogen fixation analysis were compared in northern Sweden on mixed leys containing red clover on above- and below-ground plant organs [19]. Results demonstrated similar nitrogen fixation rate

estimates for both methods throughout the season, though the ¹⁵N isotopic dilution method gave higher estimates when nitrogen fixation rates were at their highest. Additionally, aboveground plant organs provided accurate rate estimates, indicating that the time-consuming root analysis is unnecessary. The spatial distribution of sampling must also be optimized to ensure accurate estimation of nitrogen fixation rates within a field. A study in Finland concluded that a maximum sampling distance of 60 m is sufficient for nitrogen fixation rate estimation [20].

The nitrogen fixation activity of legumes is directly correlated to the strain of the legume's rhizobial symbiont and its subsequent symbiotic efficiency. Rhizobia can naturally be found in the soil, though inoculation using a specific strain may be necessary to achieve the most efficient symbiosis depending on the cultivated legume species. Acidic soils from Lithuania were evaluated for the presence of rhizobia, with the clover specific biovar, Rhizobium leguminosarum bv. trifolii, having the largest distribution [21]. The nitrogen fixation rate of inoculated red clover was further improved through soil liming, demonstrating the impact of soil pH on symbiotic efficiency [21, 22]. In addition to the impact of soil pH, rhizobia efficiency has also been shown to be positively impacted by the addition of the phytohormone heteroauxin [23]. The competitiveness of rhizobia strains of a single biovar can vary greatly, as can the symbiotic efficiency. Inoculation with highly competitive, yet effective strains is essential to achieve high rates of nitrogen fixation. A study on the interaction of white clover and various strains of Rhizobium leguminosarum bv. trifolii in Iceland identified a dominant rhizobia strain (20-15) which accounted for 90% of the tested nodules [24]. Additionally, a positive effect of inoculation with multiple rhizobia strains on plant yield was found, demonstrating the complex interaction between rhizobia genotype and plant performance.

A major benefit of including legumes in leys is their ability to provide nitrogen to the system via biological nitrogen fixation. The transfer of nitrogen from legumes into the soil and subsequently to the companion ley species or the next crop in the rotation improves the productivity of the system and decreases its dependence on the input of nitrogen fertilizer. A Danish study explored the nitrogen transfer of mixed leys containing white or red clover [25]. Results demonstrated that the transfer of biologically fixed nitrogen from white clover to the grass component was higher than that from red clover. The accumulation of biologically fixed nitrogen into the soil from stubble and post-harvest residues was evaluated in mixed leys containing red clover and lucerne [26]. The most efficient nitrogen accumulation occurred in three cut systems for both species mixtures, though lucerne accumulated higher amounts of nitrogen than red clover. Biologically fixed nitrogen can also be accumulated in the soil through rhizodeposits (material lost from plant roots). Results from a Danish study on mixed leys containing red or white clover demonstrated that nitrogen rhizodeposition can account for even higher amounts of nitrogen accumulation in the soil than that accumulated in the stubble, making it an important aspect of nitrogen transfer in leys [27].

2.5.4 Conclusions and knowledge gaps

The influence of endophyte-plant symbiosis on the plant's response to biotic stresses, such as pathogens and herbivores, can have important implications for the persistence and productivity of fescue grasses in leys. The impact of endophyte colonization on the fitness of fescue grasses is not concrete, with both positive and negative implications observed in variable environmental conditions. The interaction between these symbiotic partners is clearly complex and additional work is necessary to determine how environment influences endophyte-fescue mutualism.

The nitrogen fixation efficiency of legumes varies throughout a single season and over time, with the highest rates occurring post-harvest and in the earlier years of production. As the addition of nitrogen fertilizer can have negative impacts on nitrogen fixation efficiency, management decisions regarding fertilizer application should take into account periods of high nitrogen fixation activity. Fertilization rates should be decreased accordingly during periods of high fixation efficiency to maximize biological nitrogen fixation and its nitrogen contribution to the system. Additionally, fertilization decision making should take in account the cultivated legume species, as some species (e.g. red clover) are more susceptible to the negative impact of nitrogen fertilizer addition on nitrogen fixation.

Assuring inoculation with competitive and effective strains of the correct rhizobia is essential to secure the high nitrogen fixation rates necessary to improve productivity of the system. These rhizobia can be found naturally in some soils, but when absent, the inoculation of seed becomes an important aspect of ley management that should be prioritized by seed companies and farmers. The importance of biological nitrogen fixation is apparent not only due to nitrogen transfer from legumes to their companion grasses, but also due to the accumulation of nitrogen in the soil and its subsequent impact on the next crop in rotation. Ensuring high nitrogen fixation rates is thus essential to improve the productivity of the ley system, as well as the productivity of crops sown after the ley portion of the rotation. Accurate estimation of the accumulation of biologically fixed nitrogen following ley cultivation in Nordic and Baltic regions can provide insight into how fertilization rates can be adjusted for the subsequent crops to avoid over or under application of nitrogen.

2.5.5 References

- Puentes, A., D.R. Bazely, and K. Huss-Danell, Endophytic fungi in *Festuca* pratensis grown in Swedish agricultural grasslands with different managements. Symbiosis, 2007. 44(1-3): p. 121-126.
- 2. Bylin, A.G., et al., Influence of nitrogen fertilization on growth and loline alkaloid production of meadow fescue (*Festuca pratensis*) associated with the fungal symbiont *Neotyphodium uncinatum*. Botany, 2014. **92**(5): p. 370-376.
- 3. Wäli, P.R., et al., Endophyte infection, nutrient status of the soil and duration of snow cover influence the performance of meadow fescue in sub-arctic conditions. Grass and Forage Science, 2008. **63**(3): p. 324-330.
- 4. Saikkonen, K., et al., Fungal endophytes help prevent weed invasions. Agriculture Ecosystems & Environment, 2013. **165**: p. 1-5.
- Wali, P.R., et al., Susceptibility of endophyte-infected grasses to winter pathogens (snow molds). Canadian Journal of Botany-Revue Canadienne De Botanique, 2006. 84(7): p. 1043-1051.
- 6. Gundel, P.E., et al., Neotyphodium fungal endophyte in tall fescue (*Schedonorus phoenix*): a comparison of three Northern European wild populations and the cultivar Kentuky-31. Fungal Diversity, 2013. **60**(1): p. 15-24.
- Kauppinen, M., et al., Epichloe endophyte effects on leaf blotch pathogen (*Rhynchosporium* sp.) of tall fescue (*Schedonorus phoenix*) vary among grass origin and environmental conditions. Plant Ecology & Diversity, 2018. 11(5-6): p. 625-635.
- Laihonen, M., et al., Epichloe Endophyte-Promoted Seed Pathogen Increases Host Grass Resistance Against Insect Herbivory. Front Microbiol, 2021. 12: p. 786619.
- 9. Huss-Danell, K., et al., Nitrogen fixation and nitrogen allocation in red clovergrasslands. 2008, Dept of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umea, Sweden. Uppsala, Sweden: Swedish University of Agricultural Sciences. 562-564.
- Huss-Danell, K., E. Chaia, and G. Carlsson, N2 fixation and nitrogen allocation to above and below ground plant parts in red clover-grasslands. Plant and Soil, 2007. 299(1): p. 215-226.
- Riesinger, P. and I. Herzon, Symbiotic nitrogen fixation in organically managed red clover-grass leys under farming conditions. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2010. 60(6): p. 517-528.
- Kristensen, R.K., et al., Contrasting effects of slurry and mineral fertilizer on N2fixation in grass-clover mixtures. European Journal of Agronomy, 2022. 133: p. 10.
- Kristensen, R.K., J. Rasmussen, and J. Eriksen, Biological N-2 -fixation in grassclover ley in response to N application in cattle slurry vs. mineral fertilizer. Plant and Soil, 2022. 471(1-2): p. 629-641.
- Rasmussen, J., et al., N2-fixation and residual N effect of four legume species and four companion grass species. European Journal of Agronomy, 2012. 36(1): p. 66-74.

- Kadziuliene, Z., et al., Lucerne, white clover and red clover in leys for efficient N use. 2004, Lithuanian Institute of Agriculture, Dotnuva-Akademija, Kedainiai, LT-5051, Lithuania. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich. 492-494.
- 16. Dhamala, N.R., et al., Effects of including forbs on N-2-fixation and N yield in red clover-ryegrass mixtures. Plant and Soil, 2018. **424**(1-2): p. 525-537.
- Carlsson, G., et al., N2 fixation in three perennial *Trifolium* species in experimental grasslands of varied plant species richness and composition. Plant Ecology, 2009. 205(1): p. 87-104.
- 18. Hogh-Jensen, H. and J.K. Schjoerring, Interactions between nitrogen, phosphorus and potassium determine growth and N-2-fixation in white clover and ryegrass leys. Nutrient Cycling in Agroecosystems, 2010. **87**(3): p. 327-338.
- Huss-Danell, K. and E. Chaia, Use of different plant parts to study N(2) fixation with (15)N techniques in field-grown red clover (*Trifolium pratense*). Physiologia Plantarum, 2005. 125(1): p. 21-30.
- Nykanen, A., et al., Field-scale spatial variation in yields and nitrogen fixation of clover-grass leys and in soil nutrients. Agricultural and Food Science, 2008.
 17(4): p. 376-393.
- 21. Lapinskas, E., Biological nitrogen fixation in acid soils of Lithuania. Zemes ukio Mokslai, 2008. **15**(3): p. 67-72.
- Lapinskas, E. and L. Piaulokaite-Motuziene, The influence of soil acidity on symbiotic and non-symbiotic nitrogen fixation. Zemdirbyste, Mokslo Darbai, 2006. 93(4): p. 210-220.
- Lapinskas, E., Efficiency of combination of phytohormones and R. leguminosarum bv. trifolii and S. meliloti strains for clover and lucerne. Biologija, 2001(2): p. 28-30.
- 24. Svenning, M.M., et al., Competition for nodule occupancy between introduced strains of *Rhizobium leguminosarum* biovar Trifolii and its influence on plant production. Annals of Botany, 2001. **88**: p. 781-787.
- 25. Hogh-Jensen, H. and J.K. Schjoerring, Below-ground nitrogen transfer between different grassland species: Direct quantification by N-15 leaf feeding compared with indirect dilution of soil N-15. Plant and Soil, 2000. **227**(1-2): p. 171-183.
- 26. Kadziulis, L., et al., Some factors affecting efficient nitrogen accumulation in herbage yield and soil in Lithuania. 2004, Lithuanian Institute of Agriculture, Dotnuva-Akademija, Kedainiai, LT-5051, Lithuania. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich. 466-468.
- Hogh-Jensen, H. and J.K. Schjoerring, Rhizodeposition of nitrogen by red clover, white clover and ryegrass leys. Soil Biology & Biochemistry, 2001. 33(4-5): p. 439-448.

2.6 Modelling and remote sensing

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2.6.1 Introduction

Broadly defined, a model is a representation or simplification of reality. Models of agricultural systems attempt to represent the various components and how they interact. The main reasons for doing this are firstly to understand the implications of changes to the system, and secondly, to forecast the long term development of the system, for example in relation to climate change. There are different types of models, which relate to the way they are structured and the way calculations are done. A specific type of model is called a simulation models, because they simulate reality. They can also be referred to as mechanistic models, because the model structure (the different elements of the model and how they interact) represents our understanding of the mechanisms that occur in reality – for example, photosynthesis, or leaching of nutrients.

Remote sensing can be defined in different ways, but can broadly mean to acquire information about an object without making physical contact with the object. In this review, we include satellites, and drone-mounted and handheld devices. Unlike simulation modelling, remote sensing is not able to forecast what will happen in the future. The strength of remote sensing lies in its ability to monitor what happened in the past (when data is available) and what is happening in the present moment.

2.6.2 Development and testing of crop simulation models

A range of crop models have been developed to simulate Nordic and Baltic forage systems. The variables of focus include biomass, growth rate, forage quality, N concentration, and water balance. The most common type of models are agroecosystem models that include a high number of variables, and simulate the cropsoil environment in response to inputs such as weather and management decisions. These models include APSIM [1], BASGRA [2-5], CATIMO [2, 3, 6-9], LINGRA [10], and STICS [2, 3, 9],

Only one of the studies [1] focused on red clover (*Trifolium pratense*). The remaining studies focused on timothy (*Phleum pratense*). No other grass or legume species were included.

One study compared the BASGRA, CATIMO and STICS models in relation to yield [2] and forage quality [3]. All models tended to underestimate the yield of the first harvest. The models were similar in performance for simulation of yield and response to N, however overall the STICS model was slightly better. Forage quality

simulation included crude protein (CP), neutral detergent fibre (NDF), and neutral detergent fibre digestibility (NDFD), and models differed in their ability to simulate the different parameters. In general, the prediction accuracy was similar to that for biomass.

Less complex than the full agro-ecosystem models, a number of studies used mechanistic models similar to the agro-ecosystem models, but with more limited scope and a sharper focus. The QUAL-model [11] was developed to model growth and changes in CP concentration and digestibility of timothy, and responds to temperature, solar radiation, nitrogen, and soil water limitations. A different model was used to simulate grass swards [12] and dynamics of mixed species swards, based on radiation use efficiency [13, 14].

Simpler modelling methods have also been used to model forages, including using "expolinear" growth equations [15] for timothy and meadow fescue (*Festuca pratensis*). Other models have defined the relationship between temperature and changes in forage quality, specifically digestibility [16, 17].

2.6.3 Applications of crop simulation modelling

The main application of forage simulation models has been in assessing future climate scenarios. The Lingra model was used to assess the impact of climate change on timothy yield and harvest security in Norway [18]. The results suggest that in general, yield will be greater under future climates, due to increased temperature. The average number of harvests also increased, and in general there was no evidence of potential negative effects of climate change on grass production. The same authors used the Lingra model to assess the effect of future climates on timothy yields with different soil types [19]. The main aim of the study was to determine whether detailed soil characterizations are necessary for assessing future climate scenarios. The results suggested that a few representative soils are sufficient if the aim is to simulate the average regional yield. A geographically wider study of the effects of climate change on northern Europe (14 sites in Nordic and Baltic countries) also used the Lingra model of timothy, with projections from 15 different global climate models [20]. The simulation results suggests that grass yield will increase, due to increasing temperature, with greater potential for increase when coupled with irrigation. The risk of frost damage in timothy would remain similar for most locations, including for the Swedish locations (Skara, Umeå, and Uppsala). A similar study using the BASGRA model in 4 Norwegian locations [21] also projected an increase in timothy yield under future climate scenarios. A related modelling study [22] suggests that breeding ryegrass for increased tolerance to frost will be an important climate change adaptation.

It should be noted that conclusions about the effect of future scenarios should be made hesitantly, as there are a range of possible future global CO₂ and warming

scenarios, and how this is translated into regional climate effects will continue to be an area of scientific development.

2.6.4 Remote sensing

The studies vary in the type of sensor use, including: i. RGB, i.e. red green blue – an image defined as a mixture of red, green and blue light, as would typically be used in an ordinary digital camera. ii. MS, multispectral – a collection of a few image layers collected at different wavelengths of the electromagnetic spectrum (spectral wavebands). iii. HS, hyperspectral – a collection of many image layers collected at different wavelengths.

The studies also vary in the type of analysis methods used. Spectral wavebands are often used to calculate vegetation indices (VI), such as NDVI (Normalized Difference Vegetation Index). The relationship between spectral data and physical data is established using various types of regression and classification models, including machine learning (ML) methods.

An early (2000) paper used SPOT satellite images to develop yield maps [23], and confirmed that multispectral satellites have potential to be useful in agricultural management. Despite this, no other published manuscripts were identified by the literature search, although there are ongoing research projects in this area.

The application of drones for assessing forages is comparatively more common; however, again there are few published articles. A study in Estonia [24] used a MS camera and various regression methods to estimate biomass of mixed red clover and meadow fescue leys. The study found strong relationships between spectral indices and forage biomass, using ML methods; however, the dataset was small, and only based on one site and two sampling dates. A study in Norway [25] used a drone with an RGB camera to estimate the biomass of timothy-dominated and perennial ryegrass (*Lolium perenne*)-dominated leys. An additional analysis method included construction of three-dimensional (3D) point clouds. Again, the results were promising but the number and diversity of samples was limited.

In Finland, a study [26] showed that improved biomass models could be built by integrating 3D models, RGB, and VI features. A following study [27] compared a range of different sensors, including RGB, MS, HS, and 3D point clouds processed using the RGB data. The experimental plots of timothy and meadow fescue mixtures at a single location were sampled 4 times during primary growth, and 3 times during re-growth, during the one year of the experiment. In general, HS models were better than MS models, and results were improved when combined with 3D point clouds. Accurate results were obtained for both yield and quality, and confirm the potential for use of drones to also assess Swedish ley fields.

There are various handheld instruments that have been used to assess forages, the most common of which is a field spectrometer, which measures light reflectance in multiple wavebands. The earliest work in Sweden [28] used a Hydro handheld N-sensor, which includes 8 spectral bands, to assess yield, CP, metabolizable energy (ME), digestibility, and NDF of forages, and showed that with a large enough dataset, good predictive models could be developed for yield and CP, but not for other forage quality parameters. Further work in Sweden [29] used a handheld Yara N-sensor, which includes 60 wavebands. This study assessed yield, CP, and nitrogen uptake, and the models worked well across two different locations, and for a range of species mixtures. These researchers continued with the Yara N-sensor [30] to develop models for CP, digestibility, NDF, and NDFD, based on a wide range of field samples. In contrast with previous studies, the models did not work well for CP, and worked the best for NDF. A more complex and expensive spectrometer, the Fieldpec 4, was also used to scan the same fields [31], with similar results to those of the Yara N-sensor. The Yara N-sensor has also been used to assess the pre-harvest botanical composition, with reasonable accuracy [32].

An application of remote sensing that is quite unrelated to other studies, involved using a handheld NDVI device to detect differences between plant types in their ability to acclimate before winter in Iceland [33].

Image analysis has been applied to assess botanical composition of leys, using RGB images, e.g. from a typical digital camera. Methods were developed in Denmark [34, 35] to classify images, and then estimate botanical composition on a dry matter basis, with promising accuracy. A further development involved mounting a camera and flash on an ATV to create maps of the botanical composition of large fields [36, 37]. The maps showed high variability in clover content between and within fields, enabling site-specific N fertilization strategies. A grass-clover image dataset has been published to facilitate further research in this area [38].

2.6.5 Conclusions and knowledge gaps

The use of agro-ecosystem models to study forages in Northern Europe has been limited. The important species in Sweden (such as timothy and red clover) are less important in warmer regions of Europe and the world, and thus they have had less development. Most of the work reported in this review focused on timothy, with very little work on other species, and even less on mixed species leys. Understanding how companion species interact and respond to events such as harvesting or fertilization, is important if the models are to be realistic and useful. The models have included the change in quality; however, this needs further development. It could be extremely useful if models were able to simulate changes in digestibility before harvesting. Models also need further development to better represent winter survival, and the decline in sown species over time.

An important application of simulation models will continue to be the impact of climate change, and the effects of various adaptation strategies. For such results to

be useful, the models need to be able to accurately simulate current systems, and the future climate data need to be realistic. The results of climate change simulations will offer the most value to policy makers, and to a lesser extent farmers, due to the long time horizons of such studies. It is also possible to develop decision support systems for farmers based on simulation models, however the practical value of such systems has been limited in other parts of the world – they tend to be either too generic to be useful, or require too much data to set up the models.

In the area of remote sensing, there is much potential for these technologies, but limited application in Northern Europe. Satellite applications in particular are under-represented. An advantage of satellites is that the end-user does not have to be involved in the data collection – everything can be available in a web-based application. However, there are downsides, including lack of spatial resolution, and the influence of clouds. Based on other satellite studies, it is likely that useful algorithms can be developed for biomass and nitrogen content; however, it can be difficult to accurately assess complex traits like digestibility. Further studies should also include the use of available radar satellite data, and hyperspectral satellite data, which is currently not widely accessible but holds future promise.

Drones have been shown to be useful for estimating biomass and quality, with some success. However, there are obstacles to making them practically useful. The cheapest drones have RGB cameras, which are not capable of estimating quality, and more capable of estimating biomass through complex computation involving development of 3D models. Multispectral and hyperspectral cameras have more wavebands with which to build models for quality, however they are more expensive (particularly hyperspectral cameras) and the data is more complex to work with. Drones also require suitable weather conditions, a pilot license, and technical competence.

Handheld spectrometers can capture detailed spectral information. A limitation is their cost, and the lack of spatial information, unless mounted on a tractor, such as with the Yara N-sensor. The most current version of the Yara N-sensor uses an active light source, so that it can be used at any time of day; however, the equipment captures much less spectral information than the spectrometers used in the studies described above, and there are no published studies using an active light source sensor to assess forages. Despite their cost, handheld spectrometers have potential to assess the digestibility of forages, and aid in harvest time decision making.

A limitation of many of the remote sensing studies is that they have been developed using narrow datasets. To be useful, remote sensing models, whether based on satellites, drones or spectrometers, need to work across a range of species, locations, harvests, and other conditions. Models need to be based on large datasets and cover a wide range of situations, to be robust. The strength of remote sensing is to get a real-time assessment of the status of a field, in contrast to agro-ecosystem models, which can simulate future conditions. The strength of both approaches could be harnessed by using remote sensing to assess the current or recent situation, and simple crop models to simulate from the recent past to the present, and from the present to the near future.

2.6.6 References

- Ahmed, M., D. Parsons, and A. Gustavsson. Modelling radiation use efficiency of red clover (*Trifolium pratense* L.) under the Nordic climate. in EGF. 2020. Helsinki, Finland: Luke.
- 2. Korhonen, P., et al., Modelling grass yields in northern climates a comparison of three growth models for timothy. Field Crops Research, 2018. 224: p. 37-47.
- 3. Persson, T., et al., Simulation of timothy nutritive value: A comparison of three process-based models. Field Crops Research, 2019. 231: p. 81-92.
- 4. Höglind, M., et al., BASGRA_N: A model for grassland productivity, quality and greenhouse gas balance. Ecological Modelling, 2020. 417: p. 13.
- 5. Oijen, M., et al., Process-Based Modeling of Timothy Regrowth. Agronomy Journal, 2005. 97(5): p. 1295-1303.
- 6. Jing, Q., et al., Simulating the Nutritive Value of Timothy Summer Regrowth. Agronomy Journal, 2013. 105(3): p. 563-572.
- 7. Jing, Q., et al., Regrowth simulation of the perennial grass timothy. Ecological Modelling, 2012. 232: p. 64-77.
- 8. Jing, Q., et al. New modules for the simulation of timothy yield and nutritive value: regrowth and harvest index. in EGF. 2012. Lublin, POLAND: Polish Grassland Soc-Polskie Towarzystwo Lakarskie.
- Korhonen, P., et al., Intercomparison of models for simulating timothy yield in Northern countries. 2016, Natural Resources Institute Finland (Luke), Viikinkaari 4, 00790 Helsinki, Finland. Ås, Norway: Nibio. 762-764.
- 10. Persson, T., et al., Evaluation of the LINGRA timothy model under Nordic conditions. Field Crops Research, 2014. 161: p. 87-97.
- Gustavsson, A.M. and K. Martinsson, Analysis of growth and nutrition value in timothy using a dynamic model. Agricultural and Forest Meteorology, 2001. 107(2): p. 83-101.
- Eckersten, H., et al., Modelling biomass, water and nitrogen in grass ley:
 Estimation of N uptake parameters. European Journal of Agronomy, 2007. 27(1):
 p. 89-101.
- 13. Torssell, B., et al., Modelling carbon dynamics in mixed grass-red clover swards. Agricultural Systems, 2007. 94(2): p. 273-280.
- Riesinger, P., et al., Radiation-use efficiency in leys: influences of growth period and clover proportion. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2009. 59(5): p. 465-474.

- Bonesmo, H., Modelling Spring Growth of Timothy and Meadow Fescue by an Expolinear Growth Equation. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 1999. 49(4): p. 216-224.
- Tamm, U., S. Tamm, and J. Kadaja. The influence of effective temperatures on the nutritive value of fodder. 2007. Sacu, Estonia: Estonian Research Institute of Agriculture.
- Hyrkas, M., et al. Grass growth models for estimating digestibility and dry matter yield of forage grasses in Finland. in EGF. 2018. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre.
- Persson, T. and M. Höglind, Impact of climate change on harvest security and biomass yield of two timothy ley harvesting systems in Norway. Journal of Agricultural Science, 2014. 152(2): p. 205-216.
- Persson, T., S. Kvaerno, and M. Höglind, Impact of soil type extrapolation on timothy grass yield under baseline and future climate conditions in southeastern Norway. Climate Research, 2015. 65: p. 71-86.
- Höglind, M., S.M. Thorsen, and M.A. Semenov, Assessing uncertainties in impact of climate change on grass production in Northern Europe using ensembles of global climate models. Agricultural and Forest Meteorology, 2013. 170: p. 103-113.
- 21. Gulzari, S.O., et al., Combining models to estimate the impacts of future climate scenarios on feed supply, greenhouse gas emissions and economic performance on dairy farms in Norway. Agricultural Systems, 2017. 157: p. 157-169.
- 22. Höglind, M., T. Persson, and M.v. Oijen. Identifying target traits for forage grass breeding under a changing climate in Norway using the BASGRA model. in International Grassland Congress. 2013. Orange, Australia: New South Wales Department of Primary Industry.
- Rydberg, A. and M. Söderstrom. Potential crop growth assessment from remotely sensed images compared to ordinary yield maps. in International conference on precision agriculture. 2000. Madison, USA: American Society of Agronomy.
- 24. Li, K.Y., et al., The Application of an Unmanned Aerial System and Machine Learning Techniques for Red Clover-Grass Mixture Yield Estimation under Variety Performance Trials. Remote Sensing, 2021. 13(10): p. 22.
- Rueda-Ayala, V.P., et al., Comparing UAV-Based Technologies and RGB-D Reconstruction Methods for Plant Height and Biomass Monitoring on Grass Ley. Sensors (Basel), 2019. 19(3): p. 17.
- 26. Viljanen, N., et al., A Novel Machine Learning Method for Estimating Biomass of Grass Swards Using a Photogrammetric Canopy Height Model, Images and Vegetation Indices Captured by a Drone. Agriculture, 2018. 8(5): p. 28.
- 27. Oliveira, R.A., et al., Machine learning estimators for the quantity and quality of grass swards used for silage production using drone-based imaging spectrometry and photogrammetry. Remote Sensing of Environment, 2020. 246: p. 20.
- 28. Börjesson, T., et al., Remote sensing to predict dry matter yield and nutritional quality in forage. DIAS Report, Plant Production, 2003(100): p. 153-155.

- Zhou, Z.J., et al., Estimation of yield and quality of legume and grass mixtures using partial least squares and support vector machine analysis of spectral data. Computers and Electronics in Agriculture, 2019. 162: p. 246-253.
- 30. Morel, J., et al., Estimation of the nutritive value of grasslands with the Yara N-sensor field spectrometer. The Plant Phenome Journal, 2022. 5(1).
- Sun, S., et al., Estimation of biomass and nutritive value of grass and clover mixtures by analyzing spectral and crop height data using chemometric methods.
 Computers and Electronics in Agriculture, 2022. 192: p. 10.
- 32. Morel, J., et al. Estimation of the botanical composition of leys using field spectroscopy and chemometric models. in EGF. 2020. Helsinki, Finland: Luke.
- 33. Helgadottir, A. and T.A. Kristjansdottir. Leaf spectroscopy: a surrogate measurement of autumn growth cessation of non-adapted grasses at high latitudes. in EGF. 2013. Borgarnes, Iceland: Agricultural University of Iceland.
- Mortensen, A.K., et al., Preliminary Results of Clover and Grass Coverage and Total Dry Matter Estimation in Clover-Grass Crops Using Image Analysis. Journal of Imaging, 2017. 3(4): p. 16.
- 35. Skovsen, S., et al., Estimation of the Botanical Composition of Clover-Grass Leys from RGB Images Using Data Simulation and Fully Convolutional Neural Networks. Sensors 2017. 17(12): p. 18.
- Skovsen, S., et al., Species distribution mapping of grass clover leys using images for targeted nitrogen fertilization, in Precision agriculture '19, J.V. Stafford, Editor. 2019, Wageningen Academic Publishers
- Skovsen, S.K., et al., Robust Species Distribution Mapping of Crop Mixtures Using Color Images and Convolutional Neural Networks. Sensors, 2020. 21(1): p. 28.
- Skovsen, S., et al. The GrassClover Image Dataset for Semantic and Hierarchical Species Understanding in Agriculture. in CVPR workshop. 2019. Long Beach, CA.

2.7 Management of soil properties

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2.7.1 Introduction

Successful ley cropping requires that the species in the ley fit the soil conditions. Different species have different demands regarding pH, availability of oxygen, water and nutrients in the soil. Crop management affects these properties and how they interact with each other. Fertilization effects on soil nutrients are covered in the Fertilization chapter. Manure, however can affect soil in other ways than as a fertilizer, and thus some manure studies also fit into this section. However, long-term effects of including leys in the crop sequence and short-term green manure effects of leys are not included in this review.

2.7.2 Manure application

Much of the nitrogen in manure is organic and can contribute to soil organic matter. In a 42-year experiment with application of 20-120 tonnes of manure to potatoes every seven years, soil organic matter increased by almost 0.1 % per 10 tonnes of manure/rotation both on acid and limed soil. The higher manure application, >80 tonnes manure per rotation, combined with liming gave more stable soil aggregates, higher soil moisture and lower soil bulk density [1]. This resulted in significant yield increases in the grass/clover leys, even though the manure was applied two years before the ley in the crop rotation [2].

Cattle slurry has less organic nitrogen than solid manure, especially if digested in a biogas plant before spreading. In organic farming there have been concerns that digestate could be less beneficial for soil fertility and biology than undigested manure because of lower organic matter concentrations. A comparison between three years of high (40+20 tonnes/ha per year) and low (20+10 tonnes/ha per year) applications of digested or non-digested cattle slurries to a phosphorous-poor loamy sand showed a higher soil organic matter content in the upper 20 cm (12.3%) with the high slurry application than with the low application (10.3-10.5%) and the unfertilized control (10.2%), both for digested and undigested slurry. The low slurry application rates enhanced yields compared to the control in all years but the high slurry application only gave a significantly higher yield than the low in one year out of three. The digested slurry gave higher yields than the undigested in the second year. The ley yield increased more compared to the unfertilized control each year with slurry application, with 22-28% first year and 97-128% the third year [3]. The high application rates negatively affected earthworms and surface dwelling springtail in the days after application, but not in the long run [4].

Manure also affects soil pH in a positive way. On a clay loam in Finland, organic fertilization with cattle manure and urine did not increase soil carbon content compared to fertilization with the same amount of soluble N as CaNO₃ fertilizer. However, soil pH was significantly higher after the manure/urine fertilization than after CaNO₃ even though the latter is the least acidifying mineral fertilizer [5]. Likewise, pH was enhanced by manure application both in acid and limed soil in a long term experiment [1].

2.7.3 Liming

Liming is a well-established measure to increase the pH and create good conditions for crops sensitive to acidity such as red clover and lucerne. Liming with two forms of Dolomite and limestone meal on established leys (mainly timothy and meadow fescue) gave immediate changes in soil pH of the upper 25 mm of soil. Plant growth increased all years on soil where the initial pH_(H2O) was below 5.3, and years 2-4 for all soils. Dolomite lime increased forage yields of macronutrients including magnesium (Mg), decreasing the risk of grass tetany, especially on low pH-soils. However, the effect on micronutrients was variable, with significant concentration decreases in manganese (Mn) and zinc (Zn), and increase of molybdenum (Mo). Small applications of dolomite each year instead of the quadruple amount every four years was beneficial, since it avoided large pH-increases in the root zone that can be negative for plant uptake of some micronutrients [6].

Liming a moraine-loam retisol with 7000 kg of CaCO₃ per ha increased the $pH_{(KCI)}$ from 3.9 to 5.0. The ley was undersown with barley, which had a denser biomass on the limed soil. Mixed leys with timothy and smooth meadow grass and either lucerne, red, clover, alsike clover or white clover grown on the limed soils produced 2.4 times less root biomass and 2.2 times less root length than in the acid soils during the establishment year [7]. This was explained by increased belowground root growth to alleviate nutrient stress in the acid soil. However, aboveground biomass also was smaller after liming because of competition with denser barley [8]. In the second year, aboveground biomass and number of nitrogen fixing nodules were larger in the limed plots but belowground biomass was similar in acid and limed soil. Liming increased earthworm biomass. [8, 9].

Liming once per rotation increased timothy/red clover yields by 1800 kg DM/ha when there was no manure application. Manure applications gave more yield increases on acid soil than on limed soil and the manure applications also contributed to a rise in pH [2].

Since crop growth is promoted by liming, weed invasions can be reduced. A long-term experiment with increasing amounts of CaCO3 given at the start showed

that with soil pH above 5.7, clover was dominating, while below pH 5.7, unsown forbs were dominating in the two-year leys. Yields were greatly enhanced in soils with higher pH, especially where PK-fertilization was low [10].

2.7.4 Irrigation

When fertilized with 200 kg N (first year) and 320 kg N (second year) as $Ca(NO_3)_2$ split between fertilization in spring and before each regrowth, daily irrigation increased the aboveground biomass yield of a four-species grass ley by 30% over the two years. Distributing the fertilizer daily in the irrigation water gave insignificant aboveground biomass increase, but a decrease in live root biomass (0-100 cm). Live root biomass was highest under the periodically fertilized treatments, regardless of irrigation [11].

Irrigation with 25 mm whenever needed (five times during year 1) of a white clover/perennial ryegrass sward on sandy soil increased the white clover percentage in the summer but decreased it in the autumn. Total yield increased from 7.9 t/ha to 11.9 t/ha the first year but there were no significant differences between the irrigation treatments in the second year. This could partly be due to N-leaching since an irrigation event right after N-fertilization was followed by a heavy rain [12].

Irrigation experiments at one site with higher than normal and one with normal precipitation in normally dry areas showed larger differences between irrigated and non-irrigated leys when drought sensitive species (timothy, meadow fescue and red clover) were used than with the drought tolerant species (cocksfoot and lucerne). Irrigation gave higher second and third cut yields at both sites and for both species compositions, but higher first cut yield only at the drier site [13].

2.7.5 Wheel compaction

Tractor traffic from fertilization and harvesting causes direct damages on the vegetation and compacts the soil. Tractors, slurry tanks, and harvest equipment also have increased in size over time, giving higher wheel loads to consider. The effects of traffic are more severe if the soil is wet, which is often the case in spring. Increasing the wheel load from 2865 to 4745 kg in March in Denmark decreased the yield from 88% to 81% of the yield in the control without wheel tracks in a perennial ryegrass/red clover/white clover ley, while increasing tire pressure only had a small effect [14]. Ley with brome grass on sandy loam compressed with 118 kPa after the third harvest in September in Estonia showed higher bulk density in the top soil (0-10 cm) and lower root length by 48-37% (0-30 cm), with the largest root density decrease in the upper soil [15]. Likewise, wheel tracks from harvest machinery with full load 12000 kg and axle load of 3500 kg on a lucerne ley made

in the end of September had higher soil bulk density down to 10 cm depth, causing changes in soil structure and 10 % decreased air conductivity [16].

Soil texture and structure also determine risk of traffic damages on leys. Timothy-dominated leys on peat soils were more vulnerable to tractor traffic conducted both in spring and by the harvests than on sandy soils. On peat soils, light tractor traffic (medium axle load 2800 kg) caused only 7-10 % yield reduction, compared to 14-16 % reduction using medium tractor traffic (medium axle load 4300 kg) [17]. On the other hand, another study showed no significant decreases of yield or clover contents due to light or heavy tractor traffic after the cuts at three sites in Norway [18]. This shows that in summer, leys are sometimes not as sensitive to tractor traffic.

2.7.6 Conclusions and knowledge gaps

Manure and slurry application, liming, and irrigation can be beneficial to leys, but the success depends on the real need for the specific management. Manure application is more beneficial to yields when there is no other fertilization or liming, liming is more successful in more acidic soils, and irrigation is more successful under very dry conditions. In addition, different ley species tolerate low pH and drought differently and selecting the right species composition for the soil and climate is essential. Like fertilization, liming should be planned after careful soil mapping and using calculation tools [19].

Heavy tractor traffic should, if possible be avoided early in spring, when the soil is wet. Damage is caused both by direct damage to the plants and by soil compaction in the root zone that hinders root growth and soil aeration.

Climate change will likely cause more extreme events, both droughts and floods. More research is needed to further develop recommendations of measures, particularly for irrigation.

2.7.7 References

- 1. Repsiene, R. and D. Ozeraitiene, Manuring effect on the soil properties and crop rotation yield. Zemdirbyste, Mokslo Darbai, 2006. 93(4): p. 199-209.
- Repsiene, R., D. Ozeraitiene, and R. Skuodiene. The effects of long-term manure application on DM yield of perennial grasses and soil chemical properties. in EGF. 2005. Tartu, Estonia: Estonian Grassland Soc-Egs.
- Løes, A.K., Animal manure reduced quality by anaerobic digestion?, in 'Building Organic Bridges', at the Organic World Congress 2014. 2014, Organic World Congress: Istanbul. p. 891-894.

- 4. Johansen, A., et al. Anaerobic digestion of animal manure implications for crop yields and soil biota in organic farming. in NJF. 2015. Uppsala: Nordic Assoc Agricultural Scientists-Njf.
- 5. Li, H.H., et al., Advantages of grass-legume mixture for improvement of crop growth and reducing potential nitrogen loss in a boreal climate. Agricultural and Food Science, 2019. 28(4): p. 176-189.
- 6. Fystro, G. and A.K. Bakken, Soil reaction, yields and herbage element content as affected by lime applied on established leys in a multi-site field trial. Journal of Agricultural Science, 2005. 143: p. 407-420.
- 7. Tomchuk, D., R. Skuodiene, and J. Aleinikoviene. Soil acidity effect on agrocenoses with perennial grasses. in EGF. 2016. Ås, Norway: Nibio.
- 8. Tomchuk, D., R. Skuodiene, and J. Slepetys, The influence of soil acidity on development of forage legumes in the sowing year Dirvozemio rugstingumo itaka pupiniu daugiameciu zoliu augimui ir vystymuisi sejos metais. Zemes ukio Mokslai, 2015. 22(2): p. 81-92.
- Skuodiene, R., D. Tomchuk, and J. Aleinikoviene, Plant root morphology and soil biological indicators under primary development of various swards. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2017. 67(5): p. 435-443.
- 10. Nekrosiene, R., G. Tulabiene, and R. Repsiene. Changes of perennial grasses agrocenosis depending on the soil reaction and nutrients. in EGF. 2006. Madrid, Spain: Sociedad Espanola para el Estudio de los Pastos (SEEP).
- 11. Meurer, K.H.E., et al., Shoot and root production in mixed grass ley under daily fertilization and irrigation: validating the N productivity concept under field conditions. Nutrient Cycling in Agroecosystems, 2019. 115(1): p. 85-99.
- 12. Søegaard, K., M. Andersen, and F. Plauborg. Irrigation of grass/clover under grazing and cutting. in EGF. 2006. Madrid, Spain: Sociedad Espanola para el Estudio de los Pastos (SEEP).
- Wesström, I., N. Nilsdotter-Linde, and A. Joel, Supplemental irrigation a measure to sustain yield in mixed grass-legume ley systems during drought periods in EGF, L. Delaby, et al., Editors. 2022, Wageningen Academic Publishers: Caen, France. p. 500-502.
- Jørgensen, R.N., et al., Estimating impact on clover-grass yield caused by traffic intensities, in Precision Farming 09, C. Lokhorst and P.W.G. Groot Koerkamp, Editors. 2009, Wageningen Academic Publishers: Wageningen, the Netherlands. p. 143-147.
- 15. Krebstein, K., et al., The effect of tractor wheeling on the soil properties root growth of smooth brome. Plant Soil and Environment, 2014. 60(2): p. 74-79.
- Krebstein, K., et al., Soil compaction in a Cambisol under grassland in Estonia. Zemdirbyste-Agriculture, 2013. 100(1): p. 33-38.
- Volden, B., et al., Effects of traffic and fertilization levels on grass yields in northern Norway. Agricultural and Food Science in Finland, 2002. 11(3): p. 219-231.

- Rivedal, S., et al. The impact of contrasting management conditions on clover performance. in EGF. 2013. Borgarnes, Iceland: Agricultural University of Iceland.
- Jordbruksverket, Rekommendationer för gödsling och kalkning. 2023, Jordbruksverket: Jönköping.

2.8 Establishment of ley crops

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2.8.1 Introduction

Leys are by definition established from seeds more or less regularly. A successful and even establishment of ley crops with a small amount of weeds is essential for the ley production. The success is dependent on sowing time, seeding rate and establishment technique.

2.8.2 Sowing time

Establishment in the spring has been recommended for grass/legume leys, however more frequent spring droughts have motivated experiments with autumn seeding. Red clover (*Trifolium pratense*) cultivars 'Vicky', 'Rozeta', 'Taifun' and 'Ares'; white clover (*Trifolium repens*) 'Hebe'; and blue lucerne (*Medicago sativa*) 'Nexus' were successfully established three years in a row in Skåne and Västergötland in August and in Skåne in mid-September [1]. In Örebro, however, the plant winter survival was only satisfactory in one year out of three after sowing in mid-August. Sowing in October gave poor winter survival at all three sites.

Sowing time affected the biomass of Festulolium (\times *Festulolium*) varieties 'Punia' and 'Vetra' differently, when established in two consecutive years [2]. 'Punia' had higher shoot biomass production in the first production year after sowing late in June or early in July than with earlier sowing. 'Vetra', on the other hand, had higher shoot biomass in one of the years, when sown in the beginning of May compared to later.

2.8.3 Seeding rate

Seed amount, when varied between 25 and 125 % of the standard for monoculture, did not affect the yield in the first harvest year of red clover cultivar 'Ilte' [3]. The less dense swards had more tillers per plant. When red clover cultivars 'Ilte' and 'Varte' were undersown in early spring wheat, the optimum seeding rate of red clover in monoculture was 4-6 kg per ha [4].

Different seeding strategies for red clover/timothy (*Phleum pratense*)/meadow fescue (*Lolium pratense*) ley undersown in barley were tested on a fine sand moraine [5]. A seeding rate of 100 clover seeds per m² (approx. 3 kg per ha) was enough to get a red clover content of 60 % in the second harvest year, which also maximized dry matter yield. In the first harvest year, however, there were no significant yield differences even with the lowest seeding rate, 50 seed per m². Neither seed coating with lime/Rhizobium nor sowing with clover seeds in early spring in the harvest years affected the clover content of the ley.

2.8.4 Establishment technique

Using an annual protection crop is a common technique used to get a harvest in the establishment year and avoid excessive weed growth when forage plants are small. Seeding red clover/meadow fescue with spring barley can be done in one operation [6]. When broadcasting ley seeds after the barley sowing tines and using press-wheels or long tined harrow to cover the seed- on clay loam soils, establishment was successful and there was no excess weed growth. This method could replace the previously recommended sowing of the barley and the ley seeds in separate operations. Separate rolling after the seeding operation was beneficial only in one year out of four.

Red clover/perennial ryegrass (*Lolium perenne*) leys were equally well established under barley or pea, as well as without a protection crop. However, the total yield in establishment year plus first ley year was highest when undersown with barley both years; whereas when undersown with pea, the system was more productive than without protection crop in a dry establishment year, but not a wetter year. For lucerne/perennial ryegrass, undersowing in pea or barley only gave higher total yield than without protection crop in the dry establishment year [7].

2.8.5 Resowing/renovation of leys

It is difficult to determine when resowing of winter damaged leys could be successful. Resowing of red clover to increase yield and protein content in the ley is most common, since red clover is vulnerable to winter damage, but the success depends on the sward density [8]. A series of damaged leys with 0-100 % plant cover of timothy or perennial ryegrass on sandy loam soil were created by glyphosate treatment. The red clover seeds were sown with a direct drill after cutting of the swards either in summer or in spring. In a timothy sward, red clover establishment was successful when the initial grass coverage was 30% or less in late summer and 50 % or less in early spring. The ryegrass had a better capacity than timothy to fill bare spots of soil and thus an initial grass cover of 15 % or less was necessary for successful clover establishment in the perennial ryegrass swards. Another study showed that flowering aboveground grass biomass, especially from timothy and perennial ryegrass, can inhibit the germination of red clover seeds. Thus, sowing of red clover seeds was more successful in early May or August, than in late May [9]. In northern Norway, red clover establishment in grass swards was most successful when spring seeded at 10 kg per ha [10].

Anaerobic conditions under ice can lead to butyrate accumulation in the soil, causing inhibition of seed germination. When resowing after ice encasement has killed the previous crop, this may justify a 1-2 week delay of sowing, even though this delay would also reduce available soil water for germination [11].

2.8.6 Conclusions and knowledge gaps

The establishment of a ley is an investment for several years. It should be done thoroughly with adaptations for the local soils and climate. There are too few and scattered papers on ley establishment in this material to draw general conclusions. However, seed companies do a lot of development regarding seeding rates and good technologies for establishment are available. The biggest risk is that farmers take shortcuts and apply methods that only work for crops with larger seeds, such as direct sowing without ploughing, or too deep sowing, thus getting a poor establishment of the much smaller ley seeds. Because of this, it is important to follow recommendations regarding seeding rates and choose appropriate technologies for seedbed preparation and seeding. More research on alternative sowing times of ley species is needed for adaptation to the spring and summer droughts that have become frequent, especially in south eastern Sweden. Resowing on soils that have been flooded and maybe also covered with sediments is another climate challenge that might demand new solutions and ley species with higher flooding tolerance.

2.8.7 References

- Hallin, O., Tidpunkt och art för insådd av vallbaljväxter på hösten, in Sverigeförsöken. 2022, Hushållningssällskapen. p. 98-102.
- Leliuniene, J., et al., Impact of fertilization and sowing time on formation of productivity seedling elements of Festulolium. Journal of Food, Agriculture & Environment, 2014. 12(2): p. 515-519.
- 3. Geherman, V., R. Viiralt, and A. Parol, The yield ability of red clover depending on year of productivity and seed rate. Transactions of the Estonian Agricultural University, Agronomy, 2005(220): p. 141-143.
- 4. Bender, A., Impact of the seeding and nitrogen fertilizer rates of spring wheat that is used as a cover crop on the yielding ability of tetraploid red clover stand established at different seeding rates. Agronomy Research, 2017. 15(1): p. 33-43.
- Nykanen-Kurki, P., et al. Effect of sowing strategy on clover performance in an organic red clover-grass mixture. in EGF. 2004. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich.
- 6. Känkänen, H.J., H.J. Mikkola, and C.I. Eriksson, Effect of sowing technique on growth of undersown crop and yield of spring barley. Journal of Agronomy and Crop Science, 2001. 187(2): p. 127-136.
- Sarunaite, L., Z. Kadziuliene, and L. Kadziulis. Nutritive value and early yield formation of legume-grass swards in a crop rotation. in EGF. 2012. Lublin, Poland: Polish Grassland Soc.
- 8. Rueda-Ayala, V.P. and M. Höglind, Determining Thresholds for Grassland Renovation by Sod-Seeding. Agronomy-Basel, 2019. 9(12): p. 15.

- Kryzeviciene, A., et al. Allelopathic effect of forage grasses on red clover (*Trifolium pratense* L) seed germination. in EGF. 2003. Pleven, Bulgaria: Bulgarian Association for Grassland and Forage Production (BAGFP).
- Sturite, I. and T. Lunnan. Persistence and establishment of red clover under extensive forage production systems in Norway. in NJF. 2015. Riga, Latvia: Nordic Assoc Agricultural Scientists-NJF.
- Brandsæter, L.O., et al., Identification of phytotoxic substances in soils following winter injury of grasses as estimated by a bioassay. Canadian Journal of Plant Science, 2005. 85(1): p. 115-123.

2.9 Fertilization

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2.9.1 Introduction

Fertilization recommendations are based on soil analysis of available nutrients for all nutrients except nitrogen. The standard methods for soil nutrient analysis and classification differ between countries. Because of this, it is difficult to compare studies from different countries. Thus, this review will not give fertilization recommendations for phosphorous, P, potassium, K and other nutrients but instead summarize effects on yield, nutrient uptake, botanical composition, forage quality and diseases in perennial ley crops. When available, we refer to soil levels of nutrients analyzed with the Swedish standard extraction methods; ammonium lactate extraction for plant available P (P_{AL}) or K (K_{AL}) and hydrochloric acid extraction for long term available soil pools of P (P_{HCl}) and K (K_{HCl}). Swedish national fertilization guidelines are found in "Rekommendationer för gödsling och kalkning" (Jordbruksverket 2023) [1]. All fertilization levels are nutrient per ha and year, unless specified otherwise.

Nutrient demands also differ between different ley species. The most fundamental difference is between legumes, which fix their own nitrogen in symbiosis with *Rhizobium* bacteria, and other plants. Because of this, studies of grass leys and leys that include legumes are reviewed separately.

2.9.2 Grass leys

Nitrogen

Forage grasses are selected for being fast growing and with high nutrient content. The nutrient that most often limits growth rate is nitrogen (N), since the photosynthesis is conducted by nitrogen-rich proteins. Most fertilization studies in grass leys involve giving increasing amounts of N either in mineral form, combined with other nutrients, or as an organic fertilizer. N-fertilization generally increases the growth rate of forage grasses. A Swedish experiment with four grass species showed up to a five-fold increase in biomass with 200 kg N [2]. Potential yield, however, is mainly determined by the grasses' access to water [2], temperature, light, and their genetic potential for growth [3]. Damage caused by tractor traffic can also limit the yield response from increasing N-fertilization [4]. Sites with more optimal environmental conditions and grasses with potential to grow more biomass need higher N-fertilization to realize the yield potential. The fertilization response, most often, is a curve with decreasing marginal responses (yield increase per additional kg N) above 100-200 kg N [3, 5, 6]. An analysis of marginal fertilization responses in six experiments with combinations of timothy (*Phleum pratense*) and

meadow fescue (*Festuca pratensis*) or tall fescue (*Festuca arundinacea*) hybrids found decreasing marginal response from 23-48 kg DM/kg added N for 90 kg N to 7-28 kg DM/kg added N for 270 kg N. The marginal responses were generally higher in mixtures with tall fescue hybrids (x *Festulolium*) [7]. In the same experiments crude protein concentration in the grass increased with increasing Nfertilization, but only in the first two harvests. Often, low N-fertilization only increases the yield, not the crude protein concentration [8-10]. For timothy and meadow fescue in Finland, crude protein concentrations increased only with fertilization higher than 150 kg N at one site and 200 kg N at the other site [3]. Crude protein can also be linked to other forage parameters, for example fatty acid concentrations correlated positively with crude protein content even though they were not directly affected by N-fertilization [11]. On the other hand, at high Nfertilization, non-protein nitrogen such as nitrate, that can be harmful at high concentrations, can accumulate [6, 12]. Because of this, excess N-fertilization is not recommended.

The effects of N-fertilization on fibre quality are not consistent. Forage digestibility decreased with increasing N-fertilization in three-cut timothy [12] and perennial ryegrass (*Lolium perenne*) [13] but digestibility was not significantly affected by N-fertilisation in perennial ryegrass and ryegrass hybrids (*Lolium x boucheanum*) in Latvia [14]. Both crude protein and crude fibre content in perennial ryegrass were positively correlated to N-fertilization [15]. A plausible reason for higher NDF or lower digestibility could be increasing stem proportion, found in the second and third cut of timothy at high N-fertilization in Finland [12]. Digestible energy was lower in the first harvests with higher N-fertilisation, but higher in the second and third harvest of timothy and meadow fescue or tall fescue hybrid [16]. Similarly, increasing nitrogen fertilization decreased NDF-concentration in second cut [11] and increased NDF-degradation with two-cut timothy [8].

NPK

When the basic N-demand of the grass is covered, P or K fertilization is needed on soils low in plant available P or K. Studies of K or P demand of the grasses often give a basic fertilization of mineral nitrogen or, in studies of organic leys as organic fertilizers. Studies of P and K fertilization are often conducted on soils that have low P or K status.

On sandy soils in Denmark, reed canary grass (*Phalaris arundinacea*) and tall fescue in a three-cut system could not utilize the N-fertilization fully in the second and third harvest year without P and K-fertilization [17]. In perennial ryegrass, correlation between yield and N fertilization was twice as large as the correlation to P and K fertilization. The K effect was usually larger than the P effect [15]. On soil that had received no K or P fertilization during the last 30 years, perennial ryegrass fertilized with 120 kg N increased the yield with K fertilization the first

year and with P fertilization the second year [18]. Perennial ryegrass in Estonia with 4-6 cuts, with as much as 400-500 kg N in balanced mineral fertilizer (N 18, P 4, K 7.5 plus Ca, Mg, S, B and Se) gave a good N-recovery in harvested grass (59-70 % of applied N)[20].

Lack of nutrients can also effect the botanical composition. Balanced fertilization with NPK favoured both sown grasses and couch grass in a long term ley (36 years) [19].

Phosphorous

Phosphorous (P) in soil is not very soluble. It is bound to calcium, iron, aluminium and organic particles. The solubility is pH-dependent, with maximum solubility between pH 6 and 7. A Finnish study showed increased timothy yield after P fertilization only in early June in the third ley year, even though the P-fertilized grass had increased uptake of P at four sampling occasions in June [21]. Only one out of three Norwegian fields with timothy/meadow fescue, chosen to be low in soil P, showed a significant yield effect of P fertilization, even at a high N-fertilization level [22]. A Finnish study of two soils with low plant available P showed that the grass could extract enough P for growth from a loam soil but not from a peat soil [23]. Similarly, long term fertilization with 45 kg P gave increased ley yields in an acidic loam rich in organic matter and poor in P, but not in a clay and P rich soil in Finland [24]. In two studies of grass roots in Finland, long-term P fertilization was positive for root DW and root area [25, 26].

Potassium

The potassium (K) demand of leys is high. Potassium, is easily soluble in fertilizers and soils and K bound in primary minerals is released through weathering (clay soils 35-65 kg K and sandy soils 8 kg K). Plant uptake of potassium also competes with uptake of other positively charged ions and excess K fertilization can lead to decreased uptake of, for example, magnesium, Mg. The quotient K_{AL}/Mg_{AL} should not exceed 3 in the soil. The plant access to K and Mg is dependent on the mineralogy of the soil and ultimately the K and Mg fertilization needs evaluation by forage analyses (Jordbruksverket 2023) [1]. The desired K concentration in forage is >20g*kg dry weight (DW)⁻¹ and the K/Mg ratio in forage should be approximately 4-5 for pregnant ruminants and 5-6 for other ruminants to minimize the risk of grass tetany and milk fever [27].

Potassium is most often not yield limiting in grass, but if the grass is only N-fertilized, the K pools in soil can become depleted since the K offtake with harvests is large and K is not returned naturally with urine as in grazing systems. This is most apparent in sandy soils low in K, where yield reductions were recorded when K concentration in herbage was lower than 18 g/kg DM [28]. On clay and silt soils, K is more seldom growth limiting. A Finnish study showed that the present K

fertilization recommendations, 130-170 kg K, for three sites with high-medium or low K_{HCl} in soil were too high and 50 kg K would be enough for the grass growth [29]. In a study, where Mg and Ca were also reported, fertilization with more than 120 kg K increased the ratio of K/Mg+Ca in first cut grass above the critical 2.2 where there is increased risk of grass tetany on most of the clay soils and high P sandy soils [28].

Rock- and ash-based fertilizers

Alternative P and K sources have been investigated for organic farming. Crushed minerals but not mine tailings released K over the long term, but were not able to supply as much K to the crop as KCl. The grass removed 30 % of the K in the best crushed rock, carbonatite [30]. Wood ash could replace mineral PK-fertilization to reed canary grass or festulolium at a sandy loam in Latvia without lowering the yield [31]. In reed canary grass, wood ash fertilization also increased the N-uptake in a two-cut system.

Organic fertilizers

Organic fertilizers also give several nutrients, often with a good balance. However, in cattle manure a large part of the nitrogen is organic and not directly plant available. Often solid manure is applied before ley establishment. Manure application in spring directly before ley establishment was more beneficial for the perennial ryegrass ley than autumn or winter application [32]. Dried pig manure, that was either untreated, digested, or amended with wood biochar given before establishment, increased yields and soil P and K in the second harvest year in timothy or tall fescue fertilised with NPK on a sandy soil in Norway [33]. Composted household waste/sewage sludge at very high application rate (820 kg N/ha) before ley establishment + NK fertilization in the harvest years could replace mineral P fertilizer to grass ley and even enhanced the yields compared to NK fertilization the second year [34].

In harvest years, the inorganic nitrogen is easily lost as ammonia emission to the air if the manure is surface spread. Cattle slurry N utilization can be improved by anaerobic digestion followed by separation of the liquid fraction in two steps. In three years and at two sites in Finland, the liquid fraction of digestate achieved almost the same grass yield in cuts 2+3 as mineral N fertilizers [35]. Digestate from pig manure and perennial grasses increased the yield of cocksfoot (*Dactylis glomerata*) when applied repeatedly for five years in a row. In the last year, the biogas residue gave higher yield than mineral N at the same application rate of total N [36]. This is not always the case since, in another study, mineral NPK gave 2 tonnes higher DM yield of reed canary grass or festulolium than the digestate. [31]. When slurry was spread on a rather unfertile soil, digested and undigested cattle slurry gave higher yields than mineral N, even though the ammonium in the

digestate probably was lost as ammonia emission to a high extent because of high pH and spreading by broadcasting [37]. Cattle slurry with added seeds has been tested in Norway for resowing of grass but was, most often, not more successful than conventional resowing [38].

Other nutrients

Fertilization of grass leys with other nutrients than NPK is only reported in a few studies. Chloride fertilization could decrease the dietary cation and anion difference (DCAD) and thus decrease the risk for hypocalcaemia after calving and the risk of milk fever. The lowest DCAD were found in reed canary grass and perennial ryegrass [39]. Low rates of K supply combined with Cl-fertilization lower the DCAD [40]. Mg and S supplementation to NPK fertilization affected leaf spot and crown rust disease in perennial ryegrass, but the effect was not always negative on the disease [41].

2.9.3 Legume and grass/legume leys

Nitrogen

Having one or more legume species in the ley greatly decreases the need for Nfertilization. Yield increases from N-fertilization of grass/legume are generally lower than of pure grass [42]. Unfertilized, PK-fertilized, or only organic fertilized grass/legume or pure legume swards often give as much or more yield as Nfertilized grass swards [6, 42-47]. The reason for this is both decreased competition for nitrogen since the legume largely relies on symbiotically fixed nitrogen and transfer of N from legume to non-legumes. In grass/clover stands the Nrhizodeposition can be in the same order of magnitude as the N-offtake in harvest [48]. However, too high legume contents can lead to larger harvest losses in hay making, need for chemical additives in ensiling due to high buffering capacity and a need to complement the high protein content of the ley with carbohydrate rich feed, such as whole crop cereal or maize silage (see other sections of this report). Nitrogen fertilization can give a better balance between grass and clover. Without N-fertilization the legumes often tend to dominate, at least during the first years. Also, the yield in unfertilized grass/clover swards can decrease in the third or fourth harvest year [45, 47] most likely because of clover diseases, especially in red clover (Trifolium pratense). Including white clover (Trifolium repens) in the mixture can give a more long-lasting clover content. Over five years moderately (20-70 kg) Nfertilized four-species swards with white clover gave higher yields than monoculture grass or clover stands [49]. Lucerne (Medicago sativa) and/or fodder galega (Galega orientalis), together with grasses also give a more stable yield in long lasting leys [44, 50].

The effect of N-fertilization is very dependent on the legume content and on the legume species. Determination of clover content via image analysis can be a useful tool to optimize N-fertilization in mixed swards [51]. N-fertilization affects the competition between species. Increased grass biomass shades the legumes giving less energy to the nitrogen fixation. Fertilization with increasing N amounts from 100-400 kg N increased the yields, but decreased clover and crude protein contents in grass clover swards with both white and red clover the first harvest year [51]. In the second harvest year, yield increase with increasing N-fertilization was minor since clover contents were high (53-62 %) in unfertilized plots. The same pattern was also observed in a 5-species grass/clover ley: N-fertilization was profitable the first year, but due to a lower yield response and lower clover content in N-fertilized plots, not in the second year [52]. Semi-natural meadows and grass/clover leys at 700 m above sea level in Norway gave increased yields in a two-cut system with N-fertilization, especially up to 120 kg N/ha. However, clover occurrence decreased with increasing N-fertilization and white clover almost disappeared at 180 kg N/ha [53]. Thus, consideration of the effect of N-fertilization strategy over the entire duration of the ley is needed. In a compilation of 15 experiments with Nfertilization, the marginal N-responses in grass/clover leys were only large enough to be profitable when clover contents were $\leq 20\%$. Then 210-250 kg N, given mainly to the first harvest, was necessary to achieve high enough crude protein for high producing cattle. With clover contents above 30% in the spring, there was generally no need for N-fertilization [7]. However, then crude protein levels were too high for horses. The same study suggested that N-fertilization to the first harvest only could be a good strategy to be able to use the grasses' strong growth potential in spring and the clovers' N-fixing ability and growth in the summer. In an experiment with both grass leys and grass/clover leys, the grass/clover leys were always more productive and had higher crude protein content at the same N-fertilization level [54]. Similarly, some studies show that swards with galega and grass often do not benefit at all from N-fertilization [55-57]. In Estonia, galega swards with timothy or smooth brome (Bromus inermis) gave increased yields with up to 100 kg N but not galega/meadow fescue [57]. However, in both pure galega and grass/ galega mixes, yields increased with 50 kg N at the start of the growing season in Estonia [50]. Several different lucerne varieties in Latvia benefitted from fertilization with P and K but did not need N-fertilization[58].

Forage quality can be improved by N-fertilization of grass/legume leys, but it is not always the case. Crude protein content of the yield is strongly dependent on the legume content, since legumes most often have higher crude protein content than grasses. Crude protein content of grass/legume leys can increase with Nfertilization [59], but sometimes decrease [60, 61] because of decreased legume content in the yield. Neutral detergent fibre (NDF) content increased after Nfertilization in some galega/grass mixes but decreased in other [50]. Other studies show no effect of N-fertilization on NDF [8, 50]. The digestibility was not affected by the N-fertilization in multispecies swards [59]. In another study, 250 kg N increased the digestibility of a five species ley at only one harvest occasion out of six [52]. Inclusion of clovers decreased the digestible energy content in mixtures with timothy and meadow fescue or tall fescue hybrid [16].

NPK

Regular and high NPK fertilization enhanced ley yield in unfavourable sites even to the level of moderate NPK fertilized leys in favourable sites in long term experiments in southern Sweden [62]. A good PK status due to long term PK fertilization in Lithuania increased the yields of red clover dominated leys, but also increased some fungal diseases due to denser swards [63]. Similarly, in Estonia long term balanced NPK fertilization increased timothy/red clover yields 1.3- 2.6 times, where the higher number is from the second harvest year when the leys were fertilized [64].

Phosphorous

Legumes often have a higher need for P than grasses. White clover growth was P limited both with and without N fertilization, while perennial ryegrass was mainly N limited but more P limited when grown in mixture with clover [18]. The P demand, however can be covered by application of P to other, more P demanding crops in the rotation [64].

Potassium

In studies with a basic N or NP fertilization, K can become limiting for plant growth on sandy soils. In Norwegian sandy soils with K soluble in HNO3 < 249 mg/kg, the K release from soil decreased from the first to the third harvest year. In the second and third harvest year, zero K fertilization had lower yield than fertilization with 60 kg K, but to reach >2 g K/kg DW in forage 120 kg K was needed in the second and 180 kg K in the third harvest year. No yield response was found in years when K_{AL} was > 80 mg K/kg soil. However, K_{AL} –data, was not enough to predict plant uptake of K since approximately half of the K in plants was released from K reserves [65]. In studies without N-fertilizers, K is most often not limiting for plant growth. However, K concentrations in the forage can be too low [66]. In a comparison between conventional and organic systems at the same sites, both yields and K concentrations in forage were most often lower in organic systems that had lower inputs of K. However, both systems had a much higher offtake than input of K and negative trends over 16 years in K concentrations. This shows that with mineral N fertilizer inputs, increasing K fertilization is needed to compensate for the higher K offtake [67]. In another Norwegian study on organic fields, both grass and red clover growth were K-limited on a peaty soil, but ley yield

was N-limited on sandy soils when 60 kg K as manure was given each year [68]. A long-term study, where a crop rotation with leys and manure application were compared to a crop rotation without leys showed a larger offtake of K with the harvests with ley. Soils with more clay could maintain K concentrations > 2 g/ kg DW in grass/clover without K fertilization while the sandy loam and loamy sand needed mineral K fertilization for this. However, only the loamy sand site needed K fertilization at a level equal to the K in the harvested biomass to optimize the grass/clover yields in the long run [69]. Swedish National recommendations for K fertilization to leys is to distribute the fertilization to each harvest and crop. However, an Estonian study including red clover in a crop sequence showed that it was more profitable to give extra K to the more K demanding crops (potatoes and red clover) and not to the grain crops, instead of distributing the K to all crops [70]. Cattle slurry was not enough to replace P and K removed by harvests during the conversion to organic farming in two experiments simulating a dairy farm and a beef suckler cow farm in Norway [71].

Organic fertilizers

Regular manure application enhances ley yields compared to unfertilized [72, 73]. However, in the shorter perspective, manure application does not always enhance yields. Compost of biowaste or cattle manure decreased clover yield more than they increased grass yield in the first harvest year, with small differences in total yield the second year [34]. All composts had lower nitrogen use efficiency at the higher application rate. With good clover establishment, application of manure before ley establishment gave almost as high grass/clover yield the first harvest year as NPK fertilization but in the second and third harvest years the yields decreased without fertilization [74]. Cow manure application at establishment followed by cow urine application in the harvest years gave lower grass/clover harvests but similar or higher N yields than mineral NPK-fertilizer on a clay loam in Finland [75]. Organic farming in Denmark on a loamy sand increased the grass/clover yield (5-18 %) by application of 200 kg N in cattle manure in spring and after first cut [72]. A similar experiment with 2 or 3-species grass-clover leys gave increased production of both perennial ryegrass and white and red clover by injection of 200 kg N in cattle slurry twice a year. The inclusion of red clover increased the yields in the first three harvest years, but gave decreased yield in the fourth year [76]. Manure and dried lake sediments gave a long term improvement of ley production especially when combined with moderate NPK fertilization [77]. Balanced NPK fertilisation increased the ley yield in northern Estonia regardless of if the PK came from only mineral fertilizer or both mineral fertilizer and manure [64].

Cattle slurry has a higher ammonium concentration than solid or composted manure. Ammonia losses can be high, so techniques to enhance nitrogen use efficiency are often used. Application seasons were compared in Norway: Four experiments with cattle slurry to multispecies leys showed that slurry application in spring during the harvest years increased the relative growth rate of the sward more than cattle slurry application after the first cut. In the second cut, clover proportions of the swards were negatively affected by slurry application after the first cut. Ammonium concentrations of the slurries were positively correlated to growth rate [78]. Acidification of slurry with sulphuric acid is a technique to avoid ammonia evaporation that also enhances yields. Acidified cattle slurry spread with a trailing hose can often give similar or higher grass/clover yields than mineral N fertilization on sandy soils, sometimes with less negative effect on clover content [79, 80]. Incorporation of cattle slurry decreases ammonia emissions, but also damages the sward. Cattle slurry injection gave lower yields compared to mineral N-fertilization [81]. Different slurry incorporation equipment was tested without slurry. The least damage was caused by double disc tine open slot and the most by combined vertical and horizontal knives [82]. A Norwegian comparison found similar yield with digested or undigested cattle slurry when the same amount of N in the slurry was applied. Clover content in the sward was negatively affected by digested slurry, especially when the application rate was 220 kg N in slurry [83].

Organic farms without animals can still benefit from ley production by selling the forage. In these cases, fertilization of leys with commercial organic fertilizers can be beneficial: A Finnish study showed increased growth and mineral content of the grass component of the ley using organic animal-waste fertilisers [84]. In most cases, however, organic farms rely on local manure fertilization.

Non-legume herbs

There are few fertilization studies including non-legume herbs. The total yield increased after application of 200 kg N applied as cattle slurry due to increased grass growth in a multispecies sward, but due to lower abundance of legumes, the mineral concentrations in the yield decreased [85]. Caraway (*Carum carvi*) and chicory (*Cichorium intybus*), however, increased in abundance after slurry application but ribwort plantain (*Plantago lanceolata*) decreased in the same experiment [86]. In a similar experiment with different amounts of herbs in the ley, 200 kg N with cattle slurry increased yields and decreased perennial legume abundance. A mixture of deep rooted herbs and legumes outperformed perennial ryegrass and white clover both with and without slurry [87].

Other nutrients

There are few studies of other nutrients than NPK in legume-containing leys. Low sulphur, S, concentrations in organic leys in Norway have been found, but experimental S fertilisation only increased yields at one out of three sites even though 60 kg S increased S concentrations in the crop on all three sites [88]. Zn fertilization with 3 kg Zn increased yield of red clover but decreased timothy in a mixed ley. Total yield and root rot in clover was not affected. Mn applied in the same experiment had no significant effects [89]. Boron fertilization in B deficient soil increased the proportion of white clover in organic grass/clover leys, but did not affect other clover or grass species or the total yield [90].

2.9.4 Conclusions and knowledge gaps

Multispecies leys including several species of grasses and legumes and other herbs need less nitrogen fertilization than leys with fewer species. The seed mixture and the fertilization has to be adapted to the forage quality suitable for the animal that is going to eat the forage, the climate, and the soil. Climate change will also lead to more extreme weather situations, both drought and floods, and including more species can be an insurance against weather variability. Also, with fluctuating prices of fertilizers and farm products, it is difficult to calculate the economically optimal fertilization rates. Thus, the farmer is facing a very complex reality, but always needs strategies to reduce costs without decreasing production. Fertilization is necessary to replace nutrients removed with sold products from the farm. However, in animal production, the manure is the most abundant source of nutrients and it should be used in an optimal way. Most studies of fertilization responses in plants are, for convenience, made with mineral fertilizers. To achieve more sustainable farming, research needs to focus on how to use the manure resource combined with the nitrogen fixing ability of the legumes in the best way. The different pieces in the puzzle are often ready, but we have to make them fit together in more holistic and interdisciplinary research, adapted to local conditions using both modern technology and knowledge from the past. Variable quality of forage from multispecies leys due to temporal variation in botanical composition is another challenge that needs local solutions, also considering the competitiveness of species and varieties. Researchers cannot do all this alone, but need help from innovative farmers. I hope that some farmers will become inspired to experiment with leys with more species, especially legumes, and decrease N-fertilization to promote the N-fixation in the legumes. If the seed mixture is suitable for the location and the intended forage quality, this would benefit both economy and sustainability of ley farming.

2.9.5 References

- Jordbruksverket, Rekommendationer för gödsling och kalkning. 2023, Jordbruksverket: Jönköping.
- Meurer, K.H.E., et al., Shoot and root production in mixed grass ley under daily fertilization and irrigation: validating the N productivity concept under field conditions. Nutrient Cycling in Agroecosystems, 2019. 115(1): p. 85-99.

- 3. Kykkanen, S., et al. New nitrogen fertilisation experiments challenge the old yield responses of forage grasses in Finland. in *EGF*. 2018. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre.
- Volden, B., et al., Effects of traffic and fertilization levels on grass yields in northern Norway. Agricultural and Food Science in Finland, 2002. 11(3): p. 219-231.
- 5. Adamovics, A., et al., The efficiency of nitrogen fertilizer on the dry matter yield of tall fescue and festulolium grown as feedstock for combustion. Agronomy Research, 2019. **17**(6): p. 2146-2157.
- 6. Thers, H. and J. Eriksen, Annual protein yield and extractable protein potentials in three legumes and two grasses. J Sci Food Agric, 2022. **102**(9): p. 3742-3751.
- 7. Frankow-Lindberg, B., *Uppdatering av kvävegödslingsrekommendationer för vall, in Rapport* 2017, Institutionen för växtproduktionsekologi. p. 25.
- 8. Nordheim-Viken, H. and H. Volden, Effect of maturity stage, nitrogen fertilization and seasonal variation on ruminal degradation characteristics of neutral detergent fibre in timothy *(Phleum pratense L.)*. Animal Feed Science and Technology, 2009. **149**(1-2): p. 30-59.
- 9. Gutmane, I. and A. Adamovich. Productivity and yield quality of *Festulolium* and *Lolium * boucheanum* varieties. in *EGF*. 2004. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich.
- Gutmane, I. and A. Adamovics. Crude protein yield and quality of *festulolium (* Festulolium* Asch. & Graebn.) and hybrid ryegrass (*Lolium * boucheanum* Kunth.). in *EGF*. 2011. Irdning, Austria: Agricultural Research and Education Center (AREC) Raumberg-Gumpenstein.
- Arvidsson, K., K. Martinsson, and A.M. Gustavsson, Fatty acid concentrations in timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.): Effects of seasonal variations at different nitrogen fertilization levels. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2013. 63(4): p. 314-321.
- Termonen, M., et al., Effects of nitrogen application rate on productivity, nutritive value and winter tolerance of timothy and meadow fescue cultivars. Grass and Forage Science, 2020. 75(1): p. 111-126.
- Conaghan, P., et al., Yield and Quality Response of Perennial Ryegrass Selected for High Concentration of Water-Soluble Carbohydrate to Nitrogen Application Rate. Crop Science, 2012. 52(6): p. 2839-2851.
- Gutmane, I. and A. Adamovics, Influence of nitrogen fertilization rates on *Festulolium* and *Lolium * boucheanum* forage and seed yield and quality. Agraarteadus, 2011. 22(2): p. 13-20.
- 15. Bumane, S., The influence of NPK fertilization on *Lolium perenne* L. forage quality. Agronomy Research, 2010. **8**: p. 531-536.
- Frankow-Lindberg, B., Kväveintensitet i långliggande vall med rörsvingelhybrid.
 2010. p. 88-100.
- 17. Larsen, S.U., U. Jørgensen, and P.E. Lærke, Biomass yield and N uptake in tall fescue and reed canary grass depending on N and PK fertilization on two

marginal sites in Denmark, in Perennial Biomass Crops for a Resource-Constrained World, S. Barth, Editor. 2016, Springer International Publishing Switzerland. p. 233-242.

- Høgh-Jensen, H. and J.K. Schjorring. Shoot nutrient indices as indicators of nitrogen, phosphorus and potassium deficiencies in grassland. in Regional IPI Workshop. 2000. Akademija, Lithuania: Lithuanian Institute of Agriculture.
- Berzins, P., S. Raneane, and A. Svarta. The productive longevity of perennial grasses swards depending on the npk fertilizer rates. in Proceedings of the 8th International Scientific and Practical Conference. 2011. Rezekne, Latvia: Rezekne Higher Educ Inst-Rezeknes Augstskola.
- 20. Aavola, R. and M. Kärner, Nitrogen uptake at various fertilization levels and cutting frequencies of *Lolium* species. Agronomy Research, 2008. **6**(1): p. 5-14.
- Belanger, G., et al., Shoot growth and phosphorus-nitrogen relationship of grassland swards in response to mineral phosphorus fertilization. Field Crops Research, 2017. 204: p. 31-41.
- 22. Nesheim, L. and G. Fystro. Effects of fertiliser phosphorus on soils initially low in phosphorus. in *EGF*. 2006. Madrid, Spain: Sociedad Espanola para el Estudio de los Pastos (SEEP).
- 23. Kykkanen, S., et al. Forage grasses effectively use soil P-pools in low-PAAC soils in Finland. in *EGF*. 2018. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre.
- 24. Saarela, I., Y. Salo, and M. Vuorinen, Effects of repeated phosphorus fertilisation on field crops in Finland I. Yield responses on clay and loam soils in relation to soil test P values. Agricultural and Food Science, 2006. **15**(2): p. 106-123.
- Knuutila, K., et al. Differences in root morphological features and shoot P accumulation at different soil P levels. in *EGF*. 2020. Helsinki, Finland:
 Organising Committee of the 28th General Meeting of the European Grassland Federation & Natural Resources Institute Finland (Luke).
- 26. Knuutila, K., et al. The root growth of forage grasses at different soil P levels. in EGF. 2019. Zürich, Switzerland: University of Helsinki.
- 27. Västra, H. Så här tolkar du din grovfoderanalys. 2023 2023-10-17]; Available from: <u>https://laboratorium.hushallningssallskapet.se/sa-har-tolkar-du-din-grovfoderanalys/</u>.
- Lunnan, T., A.F. Øgaard, and T. Krogstad, Potassium fertilization of timothybased cut grassland-Effects on herbage yield, mineral composition and critical K concentration on soils with different K status. Grass and Forage Science, 2018.
 73(2): p. 500-509.
- 29. Kykkanen, S., et al. The effect of potassium on dry matter production and nutritive value of grass on three different soil types. in *EGF*. 2015. Wageningen, Netherlands: Wageningen Academic Publishers.
- 30. Bakken, A.K., et al., Crushed rocks and mine tailings applied as K fertilizers on grassland. Nutrient Cycling in Agroecosystems, 2000. **56**(1): p. 53-57.
- 31. Rancane, S., et al. The effect of fermentation residue and wood ash fertilisers on the productivity of grasses. in *EGF*. 2017. Sassari, Italy: Cnr-Ispaam.

- 32. Hansen, E.M., I.K. Thomsen, and M.N. Hansen, Optimizing farmyard manure utilization by varying the application time and tillage strategy. Soil Use and Management, 2004. **20**(2): p. 173-177.
- 33. Persson, T., et al., Impacts of organic soil amendments on forage grass production under different soil conditions. Agricultural and Food Science, 2020.
 29(5): p. 482-493.
- 34. Tontti, T., A. Nykanen, and M. Kuisma, Waste composts as nitrogen fertilizers for forage leys. Agricultural and Food Science, 2009. **18**(1): p. 57-75.
- 35. Jarvenranta, K., et al. Nitrogen concentrate from slurry digestate reaches mineral nitrogen efficiency as fertiliser for grass. in *EGF*. 2020. Helsinki, Finland: Natural Resources Institute Finland (Luke).
- 36. Tilvikiene, V., A. Slepetiene, and Z. Kadziuliene, Effects of 5years of digestate application on biomass production and quality of cocksfoot (*Dactylis glomerata* L.). Grass and Forage Science, 2018. **73**(1): p. 206-217.
- 37. Tampere, M. and R. Viiralt, The Efficiency of Biogas Digestate on Grassland Compared to Mineral Fertilizer and Cattle Slurry. Research for Rural Development 2014. 1: p. 89-94.
- 38. Nesheim, L. Slurry seeding in grassland in Norway. in *EGF*. 2010. Duderstadt, Germany: Mecke Druck und Verlag.
- Nesheim, L., et al., Effect of chloride fertilisation on dietary cation-anion difference forage species. 2015, Norwegian Institute for Agricultural and Environmental Research, Kvithamar, 7012 Stjordal, Norway. Wageningen, Netherlands: Wageningen Academic Publishers. 274-276.
- 40. Nesheim, L., et al., Controlling dietary cation-anion differences in forages by chloride fertilisation. Agricultural and Food Science, 2020. **29**(1): p. 18-28.
- Aavola, R., et al. Magnesium and sulfur fertilisation effects on foliar disease infection in perennial ryegrass. in *EGF*. 2018. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre.
- 42. Adamovics, A., I. Gutmane, and M. Katamadze. The influence of nitrogen fertilisation on the productivity of multi-species swards in three production years. in *EGF*. 2019. Zurich, Switzerland: European Grassland Federation
- 43. Adamovics, A. and I. Gutmane. Productivity and quality of multicomponent grass swards on three soil types. in *EGF*. 2016. Ås, Norway: Nibio.
- 44. Gutmane, I., A. Adamovics, and I. Gulbe. The productivity of multi-species swards using three nitrogen fertilisation rates. in *EGF*. 2018. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre.
- 45. Adamovics, A. and I. Gutmane. Productivity and persistency of multicomponent swards with different legume contents, using two nitrogen fertilisation rates. in *EGF*. 2020. Helsinki, Finland: Natural Resources Institute Finland (Luke).
- 46. Rivedal, S., et al. The impact of contrasting management conditions on clover performance. 2013. Borgarnes, Iceland: Agricultural University of Iceland.
- 47. Kadziuliene, Z. and L. Kadziulis, Nitrogen accumulation and efficiency in herbage depending on legume species in grassland sward. Biologija, 2007. 53(1): p. 54-59.

- Høgh-Jensen, H. and J.K. Schjoerring, Rhizodeposition of nitrogen by red clover, white clover and ryegrass leys. Soil Biology & Biochemistry, 2001. 33(4-5): p. 439-448.
- Helgadottir, A., et al., Grass-legume mixtures sustain strong yield advantage over monocultures under cool maritime growing conditions over a period of 5 years. Ann Bot, 2018. 122(2): p. 337-348.
- 50. Meripõld, H., et al., Fodder galega (*Galega orientalis* Lam) grass potential as a forage and bioenergy crop. Agronomy Research, 2017. **15**(4): p. 1693-1699.
- 51. Eriksen, J., et al. Nitrogen fertilization of grass-clover leys. in *EGF*. 2019. Zurich, Switzerland: European Grassland Federation.
- 52. Frankow-Lindberg, B.E. and L. af Geijersstam. Nitrogen application strategies to mixed grass-legume leys. in *EGF*. 2014. Aberystwyth, UK: IBERS, Aberystwyth University.
- 53. Lunnan, T. How does resowing of natural meadows affect yield, forage quality and botanical composition? in *EGF*. 2004. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich.
- Gustavsson, A.M., Kväve och klöver påverkar vallens avkastning och råproteinhalt. Svenska Vallföreningen. *1, 1-3*. Svenska Vallbrev, 2023(1): p. 1-3.
- 55. Adamovich, A., et al. Forage quality dynamics and productivity of fodder galegagrass swards. in *EGF*. 2007. Merlbeke, Belgium: Belgian Society for Grassland and Forage Crops.
- 56. Adamovich, A. and O. Adamovicha. Productivity and forage quality of Festulolium/legume mixed swards in response to cutting frequency. in *EGF*.
 2003. Pleven, Bulgaria: Bulgarian Association for Grassland and Forage Production (BAGFP).
- 57. Meripold, H., et al. The effect of different fodder galega-grass mixtures and nitrogen fertilization on forage yield and chemical composition. in *EGF*. 2014. Aberystwyth, UK: IBERS, Aberystwyth University.
- 58. Agafonova, L., Impact of fertilizer rates on the yield and quality of alfalfa on a sod podzolic loami sand. Agronomijas Vestis, 2008(11): p. 191-195.
- 59. Adamovics, A. and I. Gutmane. The influence of nitrogen fertiliser and legume content on the quality of multi-species swards. in *EGF*. 2018. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre.
- 60. Søegaard, K. Effect of N-fertilization on growth and quality of intensively managed, cut and grazed grass/clover. in *EGF*. 2003. Pleven, Bulgaria: Bulgarian Association for Grassland and Forage Production (BAGFP).
- Søegaard, K., Nitrogen fertilization of grass/clover swards under cutting or grazing by dairy cows. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2009. 59(2): p. 139-150.
- 62. Carlgren, K., Is it possible by use of heavy fertilization to boost the small yields normally obtained from arable land with unfavourable natural qualities to the yield levels of favourable land? Archives of Agronomy and Soil Science, 2001.
 46(3-4): p. 289-295.

- 63. Nekrosiene, R., G. Tulabiene, and R. Repsiene. Changes of perennial grasses agrocenosis depending on the soil reaction and nutrients. in *EGF*. 2006. Madrid, Spain: Sociedad Espanola para el Estudio de los Pastos (SEEP).
- 64. Loide, V., The results of an NPK-fertilisation trial of long-term crop rotation on carbonate-rich soil in Estonia. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2019. **69**(7): p. 596-605.
- 65. Øgaard, A.F., T. Krogstad, and T. Lunnan, Ability of some Norwegian soils to supply grass with potassium (K) soil analyses as predictors of K supply from soil. Soil Use and Management, 2002. **18**(4): p. 412-420.
- 66. Øgaard, A.F. and S. Hansen, Potassium uptake and requirement in organic grassland farming. Nutrient Cycling in Agroecosystems, 2010. **87**(1): p. 137-149.
- Andrist-Rangel, Y., et al., Long-term K dynamics in organic and conventional mixed cropping systems as related to management and soil properties.
 Agriculture Ecosystems & Environment, 2007. 122(4): p. 413-426.
- 68. Arstein, A., et al. Requirement of potassium in organic meadow cultivation. in EGF. 2004. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich.
- 69. Andersson, S., et al., Response of soil exchangeable and crop potassium concentrations to variable fertilizer and cropping regimes in long-term field experiments on different soil types. Soil Use and Management, 2007. 23(1): p. 10-19.
- 70. Masauskas, V. and A. Masauskiene, The impact of every four years PK fertilizer application on the crop rotation productivity and economy. Zemdirbyste, Mokslo Darbai, 2006. **93**(4): p. 190-198.
- Haraldsen, T.K., et al., Nutrient balances and yields during conversion from conventional to organic cropping systems on silt loam and clay soils in Norway. Biological Agriculture & Horticulture, 2000. 17(3): p. 229-246.
- Eriksen, J., M. Askegaard, and K. Søegaard. Productivity and N-leaching in organic dairy grass-arable crop rotations. in *EGF*. 2008. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- 73. Repsiene, R., D. Ozeraitiene, and R. Skuodiene. The effects of long-term manure application on DM yield of perennial grasses and soil chemical properties. in EGF. 2005. Tartu, ESTONIA: Estonian Grassland Soc-Egs.
- 74. Hakala, K., et al., *Y*ields and greenhouse gas emissions of cultivation of red clover-grass leys as assessed by LCA when fertilised with organic or mineral fertilisers. Biomass & Bioenergy, 2012. **46**: p. 111-124.
- 75. Li, H.H., et al., Advantages of grass-legume mixture for improvement of crop growth and reducing potential nitrogen loss in a boreal climate. Agricultural and Food Science, 2019. **28**(4): p. 176-189.
- 76. Eriksen, J., M. Askegaard, and K. Søegaard, Complementary effects of red clover inclusion in ryegrass-white clover swards for grazing and cutting. Grass and Forage Science, 2012. 69(2): p. 241-250.
- 77. Baksiene, E. and R. Asakaviciute, The Effects of Organic Lake Sediments on the Crop Rotation Yield and Soil Characteristics in Southeast Lithuania. Applied Ecology and Environmental Research, 2013. 11(4): p. 557-567.

- 78. Steinshamn, H., Effects of cattle slurry on the growth potential and clover proportion of organically managed grass-clover leys. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2001. **51**(3): p. 113-124.
- Kristensen, R.K., et al., Contrasting effects of slurry and mineral fertilizer on N2-fixation in grass-clover mixtures. European Journal of Agronomy, 2022. 133: p. 10.
- 80. Kristensen, R.K., J. Rasmussen, and J. Eriksen, Biological N-2 -fixation in grassclover ley in response to N application in cattle slurry vs. mineral fertilizer. Plant and Soil, 2022. **471**(1-2): p. 629-641.
- 81. Tampere, M., et al. Influence of undigested and digested cattle slurry on grassland yield compared to mineral fertilizer. in EGF. 2015. Wageningen, Netherlands: Wageningen Academic Publishers.
- Rodhe, L. and M.A. Halling, Grassland yield response to knife/tine slurry injection equipment benefit or crop damage? Grass and Forage Science, 2015. 70(2): p. 255-267.
- 83. Johansen, A., et al. Anaerobic digestion of animal manure implications for crop yields and soil biota in organic farming. in NJF. 2015. UPPSALA: Nordic Assoc Agricultural Scientists-Njf.
- Nurmi, E., P. Kurki, and J. Kivela. Fertilisation effect of recycled nutrients on organic feed barley and grass-clover mixture. in EGF. 2020. Helsinki, Finland: Natural Resources Institute Finland (Luke).
- 85. Pirhofer-Walzl, K., et al., Forage herbs improve mineral composition of grassland herbage. Grass and Forage Science, 2011. **66**(3): p. 415-423.
- 86. Søegaard, K., J. Eriksen, and M. Askegaard. Herbs in high producing organic grasslands - effect of management. in Proceedings of the Third Scientific Conference of the International Society of Organic Agriculture Research (ISOFAR). 2011. Namyangju, Korea Republic.
- 87. Eriksen, J., T. Mortensen, and K. Søegaard. Root biomass and carbon storage in differently managed multispecies temporary grasslands. 2012. Lublin, Poland: Polish Grassland Soc-Polskie Towarzystwo Lakarskie.
- 88. Bakken, A.K. and S. Hansen. Red clover yields and nitrogen content as related to sulphur supply. in *EGF*. 2004. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich.
- Stoltz, E. and A.C. Wallenhammar. Effects of Mn and Zn on root rot and biomass production in red clover (*Trifolium pratense L.*). in *EGF*. 2018. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre.
- Linse, L., et al., Boron fertilisation of organically managed grass-clover swards on coarse-textured soils: effects on botanical and element composition.
 Agricultural and Food Science, 2015. 24(4): p. 261-272.

2.10 Harvest strategy

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2.10.1 Introduction

A good harvest strategy is one of the corner stones to getting the quality and quantity that one wants from a ley. An early harvest will often yield a higher quality with higher concentrations of crude protein, lower fibre content, and higher digestibility. Over the years it has become increasingly common to take three or more harvests in a season even in northern parts of Sweden. Several studies have been performed on the subject to try to estimate the optimal number of cuts depending on species and the desired nutritional composition. The maturity stage of plants at harvest and the timing of the harvests taken also have important implications for the quality and productivity of the harvested forage.

2.10.2 Harvest frequency

The optimal number of harvests varies depending on the species grown and the region of cultivation. Harvest frequency selection has major implications for the yield and nutritive value of the harvested forage. In Estonia, frequent harvesting (4 -6 harvests per season) was effective at obtaining high crude protein yields from perennial ryegrass (Lolium perenne) swards under high fertilization rates (500 kg N ha⁻¹) [1]. Additionally, perennial ryegrass and Italian ryegrass (Lolium *multiflorum*) under high harvest frequencies in Estonia should be harvested at early heading to achieve a high nutritive value [2, 3]. In Denmark, swards of tall fescue (Festuca arundinacea) and festulolium (× Festulolium) under a two-cut harvest strategy with a delayed first harvest demonstrated superior biomass yields compared to both early and traditional two-cut systems and required lower inputs than a three-cut system [4]. The influence of harvest frequency on the nutrient use efficiency of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) swards was evaluated in field trials in Finland [5]. Results indicate that harvest frequency has only a minor impact on nutrient use efficiency, as the majority of nutrient uptake occurs well before typical harvest windows.

Selecting the correct harvest frequency for pure legume stands can help ensure stable yields and high nutritional value. Pure stands of lucerne (*Medicago sativa*) cultivated in Latvia produced the highest biomass yields when harvested using a three-cut system, with harvest timing selected based on phenology (1st cut at bud stage and 2nd/3rd cut at early to full bloom stage) [6]. However, higher nutritive value was achieved using two- or three-cut systems with a fixed cut schedule, where harvest date was predetermined irrespective of phenology. Harvest frequency was also shown to have implications for weed management in pure lucerne stands grown

in Estonia [7]. Decreasing the harvest frequency to two-three times per season and delaying the final cut to late September to early October achieved improved weed suppression.

The optimal harvest frequency for forage grass and legume mixtures can vary greatly between cultivation regions. For mixtures of festulolium and a single forage legume (red clover (Trifolium pratense), hybrid lucerne (Medicago × varia), fodder galega (Galega orientalis), or white clover (Trifolium repens)) harvested in Latvia two or four times per season, biomass yields were highest in two-cut systems for all species mixtures [8]. Nutritive value (crude protein, neutral detergent fibre, and digestibility), however, was superior in mixtures under a four-cut system. Grass leys and mixed leys containing grass and white clover subjected to two- or four-cut systems in Latvia had superior yields under two-cuts and higher nutritive value under four-cuts [9]. Similar results were observed in a meta-analysis of Norwegian mixed grass-legume leys subjected to two- or three-cut systems, with higher yields achieved in two-cut systems and higher nutritive value in three-cut systems [10]. Frequent harvest also had a negative impact on the productivity of mixed stands of grass and fodder galega. Latvian field trials spanning 20 years demonstrated higher biomass and CP yields under two- or three-cut systems, compared to an intensive four-cut system [11-13]. The trend of higher yields with fewer harvests was also demonstrated under more intensive harvest frequencies in Denmark, in which mixed grass-clover leys had higher biomass yields under four-cut systems, but higher nutritive value under five-cut systems [14].

Pure and mixed stands of perennial ryegrass, tall fescue, red clover, and white clover were harvested three or five times in Norway to determine the effect of species interaction on yield and nutritive value under varying harvest frequencies [15, 16]. Though pure grass stands produced higher yields in a three-cut system, less yield discrepancy between harvest frequencies was observed in the pure legume stands and grass-legume mixtures, indicating a positive influence of species diversity on yield stability across different harvest frequencies. Species diversity also had a positive impact on nutritive value, decreasing the intra-annual variability under both harvesting frequencies. Mixed leys can also include a third-functional group, forbs, to increase species diversity. Various forbs species were included in mixed leys alongside white clover and perennial ryegrass in Denmark to evaluate the impact of species diversity on biomass yields harvested using four- and six-cut systems [17]. Biomass yields increased with increasing species diversity under a four-cut system, while no impact of species diversity was observed under a six-cut system. Harvest frequency can also have implications for the environmental sustainability of leys. A Danish study on the carbon input of three forage grasses (tall fescue, festulolium, and reed canary grass (Phalaris arundinacea)) concluded that two-cut systems provide the highest contribution towards soil organic carbon stocks [18].

2.10.3 Harvest maturity

The nutritive value and productivity of forage grasses are strongly related to the maturity of the grass when harvested. The regrowth rate of timothy and meadow fescue was studied across Norway and the results demonstrated that the highest regrowth rates were obtained when the grasses were in an early vegetative stage [19]. Regrowth rates decreased by 50% when the plants matured to the jointing stage. A Lithuanian study on the influence of maturity stage on the nutritive value and digestible protein yield of timothy, reed canary grass, and smooth brome (Bromus inermis) reported the highest crude protein and lowest crude fibre values for plants harvested at a vegetative stage [20]. Additionally, the highest digestible protein yields were obtained at varying maturity stages for each species, occurring at the vegetative stage for timothy, at the beginning of heading for smooth brome, and at the end of heading for reed canary grass. Different varieties of timothy were evaluated for the influence of harvest maturity on various nutritive value parameters in different plant organs in Lithuania [21]. Variation between varieties mainly occurred in concentrations of water soluble carbohydrates, while the variation between plant organs was highest for neutral detergent fibre concentrations and dry matter digestibility in the stems, indicating that breeding efforts should focus on obtaining leafy varieties with high stem quality. Harvesting timothy at an early vegetative stage in northern Swedish leys was found to produce forage with an optimal nutritive value (high CP, low NDF, and high degradability of organic matter and NDF) for growing lambs [22, 23]. The high nutritive value achieved in early stage timothy promoted high voluntary intake and liveweight gains. The amino acid content of forage grasses is of great importance in the formulation of ruminant diets. A Swedish field trial determined that plant maturity influences the amino acid profile of timothy and meadow fescue, with the highest amino concentrations achieved when plants were in the leaf sheath elongation stage [24].

Similar to grasses, the harvest maturity of forage legumes impacts their nutritive value and productivity. A Swedish study on the phytoestrogen concentration of red clover at varying maturity stages demonstrated that phytoestrogen content changes as red clover matures, with the highest concentrations achieved at early stages of development [25]. As forage legumes are generally cultivated in mixed leys with grasses in the Nordic region, it is important to understand how the maturity of both functional groups impacts the nutritive value and productivity of the sward. In Finland, the nutritive value of timothy and red clover mixed leys declined as the plants matured [26]. This was particularly apparent for timothy, demonstrating the importance of balancing the harvest date according to the maturity stage of the dominant species in the sward. A Danish study explored the influence of plant maturity on complex species mixtures of various grass and legume species [27]. The study reported decreasing digestibility and increasing fibre concentration with increasing plant maturity, with fibre increasing more quickly in legumes. The

maturity stage at first harvest for mixed stands of meadow fescue, red clover, and timothy was evaluated at sites across Norway [28]. The nutritive value of red clover and timothy decreased more drastically with increasing plant age in comparison to meadow fescue. A Norwegian study compared the phenological development of early- and late-flowering red clover varieties and the subsequent impact on harvest forage quality [29]. No significant difference in phenological development was observed between varieties, though the early-flowering cultivar had inferior nutritive value across all harvests.

2.10.4 Harvest timing

Selecting the optimal harvest timing for forage grasses is essential to obtain forage within the targeted yield and nutritive value parameters. Changes in the harvest window for timothy were evaluated in Estonia and revealed that a three day delay in the first cut increased biomass yields, while a six day delay in the second harvest decreased yields (likely influenced by a period of drought between the normal and delayed second harvests) [30]. Additionally, harvest delays decreased the digestibility of timothy, though even forage from delayed cuts was within the targeted digestibility values. A Norwegian study evaluated the impact of early and delayed harvest on the regrowth and tiller survival of timothy and found that early cutting had a negative impact on tiller survival, while late cutting improved regrowth rates [31]. Due to the longer growing seasons occurring in northern Europe as a result of rising temperatures, the inclusion of an additional final cut of timothy swards was evaluated throughout Norway [32]. Results demonstrated that this additional late cut was low yielding and negatively impacted the productivity of the sward in the subsequent year. The optimal timing of first harvest was evaluated for timothy stands in Finland, with results suggesting that timothy should be harvested roughly five days after heading begins to ensure appropriate nutritive value [33].

When multiple grass species are included, harvest timing should take into account the optimal timing of both species. Mixed stands of timothy and meadow fescue were subjected to a delayed second harvest in Finland to determine the influence of harvest timing on nutritive value [34]. Delaying the second harvest had a negative influence on the nutritive value of timothy, while meadow fescue remained relatively unaffected. Two Finnish studies explored the influence of delaying the second and/or third cut of a three-cut system on the yield and nutritive value of mixtures of timothy and meadow fescue and found that delaying the second cut increased yields and decreased digestibility, while a delayed third cut had little influence on stand characteristics [35, 36].

The impact of early or delayed harvest on perennial ryegrass was also evaluated in two studies. A delayed first harvest increased the biomass in northern and southern Sweden, while an early first harvest improved yields in the subsequent year in northern Sweden and decreased it in southern Sweden [37]. In Denmark, delayed harvest of perennial ryegrass resulted in higher yields, but a lower nutritive value [38].

Dead plant material in harvested forage can become an issue in later harvests if it accounts for a substantial proportion of the biomass, as it can decrease the nutritive value of the sward. Two studies in Finland evaluated the amount of dead material in mixed stands of timothy, meadow fescue, and tall fescue [39, 40]. In both studies, the proportion of dead material was as high as 25% of the dry matter yield, with the highest proportions occurring with a prolonged interval between harvests.

The nutritive value and productivity of pure legume stands are also sensitive to harvest timing. Pure lucerne stands were evaluated in Finland to determine optimal harvest timing for regrowth [41]. Results indicate that cutting should take place 5-6 weeks after the previous harvest, though timing can be variable depending on the cultivar. Harvest timing can also impact the persistence of forage legume stands. In Finnish field trials, the persistence of pure lucerne stands and their yield in the subsequent year were best maintained when the last cut was delayed to the end of September [42].

Like with the other harvest strategies covered in this report, harvest timing of grass-legume mixes should take into account both functional groups. Mixtures of various grasses and legumes were subjected to variable timings of the spring cut of a five-cut system in Denmark [43]. Delaying the spring cut negatively impacted the forage's nutritive value, particularly in mixtures containing red clover. A Danish study evaluated the influence of timing of the spring cut on mixed leys containing various grass, legume, and forb species [44]. Little influence of harvest date on biomass yield was observed, however delayed harvest negatively impacted forage nutritive value. A study comparing the effect of early harvest, optimal harvest, or delayed harvest (early and delayed harvests were 1 week from optimal harvest) on the nutritive value of grass and legume mixtures in Denmark demonstrated that digestibility decreased throughout the two weeks, while fibre concentrations increased [45]. Additionally, fibre concentrations of the legume species increased more quickly across the harvest period than in grasses. The change in degradability of red clover and timothy across varying harvest dates was studied in northern Sweden, with harvests occurring in two week intervals from the first harvest date [46]. The results demonstrated a higher decline in degradability in timothy in the spring growth, while degradation rates were similar between red clover and timothy in the summer growth. A Swedish studied explored the possibility of widening the harvest of window of grass-clover leys by comparing the nutritive value and yield of late species and variety mixes with a later harvest date with early species and variety mixes with an early harvest date [47]. A wider harvest window was possible

depending on the mixtures used, as results showed no difference in nutritive value or yield between the two strategies.

Antioxidants play an important role in ruminant nutrition, making it important to understand how their concentration in forages changes under different harvest timings. A Swedish study evaluated the influence of harvest date on antioxidant concentrations in mixed stands of red clover, birdsfoot trefoil (*Lotus corniculatus*), timothy, and meadow fescue [48]. In some cases a later harvest date was correlated to lower concentrations of antioxidants, though the interaction between harvest date and other factors such as, maturity and leaf to stem ratio, likely influence forage antioxidants.

Harvest timing can also influence the concentration of extractable protein in forages, making it an important consideration for forage biorefinery. A study of timing of first harvest of red clover, white clover, perennial ryegrass, and tall fescue in Denmark concluded that optimum first harvest timing was late May for white clover, early June for red clover, and mid-June for both grasses [49]. A study by the same authors looked at protein extractability of the four species in the previous paper, as well as lucerne [50]. The results indicated that early first harvests produced legumes with the highest extractable protein, while grass extractable protein increased at later harvest dates. The selection of harvest timing becomes more complex with increasing species diversity.

2.10.5 Cutting height

Cutting height can also influence the productivity of a ley over the season and in subsequent years. Results from a Finnish study demonstrated that timothy and meadow fescue were most productive when the cutting height was increased to 9 cm compared to treatments using 3 and 6 cm cutting heights [51].

2.10.6 Conclusions and knowledge gaps

Harvest strategies are complex, requiring farmers to consider many aspects of how management impacts the yield and quality of their leys. The studies included in this report demonstrate how the region of cultivation and the species sown have major implications for the selection of optimal harvest strategies. It is thus essential for researchers and advisors to collaborate with farmers to establish harvest strategies suited to regional ley production. Harvest frequency and timing, as well as plant maturity at harvest drive the yield and nutritive value potential of leys, making it important for farmers to understand how they can utilize management to achieve their forage targets. Considerable work on understanding the implications of harvest strategy has been done in the Nordic and Baltic regions, however, the changing climate will require researchers and farmers to evaluate how to adapt harvest strategies to their region's future climatic conditions.

2.10.7 References

- Aavola, R., et al., The yield potential of Estonian perennial ryegrass (*Lolium perenne* L.) cultivars at different mineral fertilisation levels and cutting frequencies. Vol. 10. 2005, Aavola, R (corresponding author), Jogeva Plant Breeding Inst, EE-48309 Jogeva, Estonia. Tartu, ESTONIA: Estonian Grassland Soc-Egs. 449-453.
- 2. Aavola, R. and M. Karner, Nitrogen uptake at various fertilization levels and cutting frequencies of *Lolium* species. Agronomy Research, 2008. **6**(1): p. 5-14.
- Aavola, R., D. Rosellini, and F. Veronesi, Forage quality improvement of Italian and perennial ryegrass through enhanced mineral fertilization and cutting frequencies. 2007, Jogeva Plant Breeding Institute, 48309, Jogeva, Estonia. Wageningen, Netherlands: European Association for Research on Plant Breeding (EUCARPIA). 185-189.
- 4. Kandel, T.P., L. Elsgaard, and P.E. Lærke, Influence of harvest managements of festulolium and tall fescue on biomass nutrient concentrations and export from a nutrient-rich peatland. Ecological Engineering, 2017. **109**: p. 1-9.
- Pakarinen, K., et al., Nutrient use efficiency in different harvesting strategies of silage swards based on timothy and two fescue species. 2012, MTT Agrifood Research Finland, Halolantie 31 A, 71750 Maaninka, Finland. Helsinki, Finland: MTT Agrifood Research Finland. 130-131.
- 6. Gaile, Z., et al., Harvest management effects on productivity and forage quality of ten lucerne varieties. 2004, Research and Study farm 'Vecauce' of Latvia University of Agriculture, Auce, LV-3708, Latvia. Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich. 924-926.
- Lillak, R., et al., Invasion of broadleaved weeds into alfalfa stand during time of utilization of alfalfa stands in low-input farming system. Agronomy Research, 2005. 3(1): p. 65-72.
- Adamovich, A., et al., Productivity and forage quality of Festulolium/legume mixed swards in response to cutting frequency. 2003, Latvia University of Agriculture, 2 Liela iela, LV 3001, Jelgava, Latvia. Pleven, Bulgaria: Bulgarian Association for Grassland and Forage Production (BAGFP). 453-456.
- Adamovics, A., et al., Productivity and yield quality of white clover grass mixed swards depending on cutting frequency. 2014, Latvia Univ Agr, Liela Iela 2, LV-3001 Jelgava, Latvia. Albena, BULGARIA: Stef92 Technology Ltd. 417-424.
- Steinshamn, H., et al., Yield and herbage quality from organic grass clover leys—a meta-analysis of Norwegian field trials. Organic Agriculture, 2015. 6(4): p. 307-322.
- Adamovich, A., Influence of cutting regime and nitrogen fertilization on productivity of fodder galega-grass swards. DIAS Report, Plant Production, 2002(84): p. 157-161.
- 12. Adamovich, A., et al., Forage quality dynamics and productivity of fodder galega-grass swards. 2007, Latvia University of Agriculture, 2 Liela iela,

Jelgava, LV-3001, Latvia. Merlbeke, Belgium: Belgian Society for Grassland and Forage Crops. 181-184.

- Adamovics, A., et al., Galega for fodder and biogas production. 2008, Latvia University of Agriculture, 5, Cakstes blvd., LV-3001 Jelgava, Latvia. Slovakia: Slovak Agricultural Research Centre, Research Institute of Animal Production, Nitra. 170-171.
- Søegaard, K., A. Helgadottir, and A. Hopkins, Cutting strategy as a tool for manipulating the seasonal nutritive value and production in grass-clover. 2013, Department of Agroecology, Aarhus University, Tjele, Denmark. Borgarnes, Iceland: Agricultural University of Iceland. 216-218.
- 15. Ergon, A., et al., Species interactions in a grassland mixture under low nitrogen fertilization and two cutting frequencies: 1. dry-matter yield and dynamics of species composition. Grass and Forage Science, 2016. **71**(4): p. 667-682.
- Ergon, A., et al., Species interactions in a grassland mixture under low nitrogen fertilization and two cutting frequencies. II. Nutritional quality. Grass and Forage Science, 2017. 72(2): p. 333-342.
- Eriksen, J., et al., Root biomass and carbon storage in differently managed multispecies temporary grasslands. Vol. 17. 2012, Aarhus Univ, Dept Agroecol, POB 50, DK-8830 Tjele, Denmark. Lublin, Poland: Polish Grassland Soc-Polskie Towarzystwo Lakarskie. 610-612.
- Nielsen, C.K., U. Jørgensen, and P.E. Laerke, Root-To-Shoot Ratios of Flood-Tolerant Perennial Grasses Depend on Harvest and Fertilization Management: Implications for Quantification of Soil Carbon Input. Frontiers in Environmental Science, 2021. 9: p. 14.
- Bonesmo, H. and A.O. Skjelvåg, Regrowth Rates of Timothy and Meadow Fescue Cut at Five Phenological Stages. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 1999. 49(4): p. 209-215.
- Jatkauskas, J., et al., Changes in the yield, chemical composition, and digestibility of different perennial grasses at maturation. 2003, Lithuanian Institute of Animal Science, Baisogala, Radviliskis distr., Lithuania. Pleven, Bulgaria: Bulgarian Association for Grassland and Forage Production (BAGFP). 204-206.
- Jonaviciene, K., et al., Quality of timothy (*Phleum pratense* L.) and causality of its variation. 2008, Lithuanian Institute of Agriculture, Akademija, Kedainiai distr., Lithuania. Uppsala, Sweden: Swedish University of Agricultural Sciences. 471-473.
- 22. Bernes, G., M. Hetta, and K. Martinsson, Effect of maturity in timothy on silage quality and lamb performance. Options Mediterraneennes. Serie A, Seminaires Mediterraneens, 2007(74): p. 73-76.
- Bernes, G., M. Hetta, and K. Martinsson, Effects of harvest date of timothy (*Phleum pratense*) on its nutritive value, and on the voluntary silage intake and liveweight gain of lambs. Grass and Forage Science, 2008. 63(2): p. 212-220.
- 24. Arvidsson, K., K. Martinsson, and A.M. Gustavsson, Fatty acid concentrations in timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.):

Effects of seasonal variations at different nitrogen fertilization levels. Acta Agriculturae Scandinavica Section B-Soil and Plant Science, 2013. **63**(4): p. 314-321.

- Bernes, G., et al., Phytoestrogen concentration in red clover (*Trifolium pratense* L.) varieties. 2016, Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umea, Sweden. As, Norway: Nibio. 254-256.
- 26. Rinne, M. and A. Nykanen, Timing of primary growth harvest affects the yield and nutritive value of timothy-red clover mixtures. Agricultural and Food Science in Finland, 2000. **9**(2): p. 121-134.
- 27. Elgersma, A., et al., Effects of harvest delay on nutritive value in four grass and four legume species in binary mixtures. 2016, Wageningen, Netherlands. As, Norway: Nibio. 225-228.
- Bakken, A.K., et al., Quality development in regrowths of timothy, meadow fescue and red clover. 2008, Norwegian Institute for Agricultural and Environmental Research, Grassland and Landscape Division, Stjordal, Norway. Uppsala, Sweden: Swedish University of Agricultural Sciences. 444-449.
- 29. Nadeem, S., et al., The relationship between phenological development of red clover and its feed quality in mixed swards. 2018, Norwegian Institute of Bioeconomy Research (NIBIO), 1431 As, Norway. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre. 69-71.
- 30. Aavola, R. and P. Pechter, Modern cultivars of timothy produce more herbage with enhanced feeding value. 2020, Helsinki, Finland: Organising Committee of the 28th General Meeting of the European Grassland Federation & Natural Resources Institute Finland (Luke). 209-211.
- Hoglind, M., H.M. Hanslin, and M. Van Oijen, Timothy regrowth, tillering and leaf area dynamics following spring harvest at two growth stages. Field Crops Research, 2005. 93(1): p. 51-63.
- 32. Jorgensen, M., et al., Longer growing season with warmer autumn at northern latitudes - can farmers take an extra cut? 2018, Norwegian Institute of Bioeconomy Research, NIBIO, Ås, Norway. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre. 255-257.
- 33. Nissinen, O., et al., Effective growing degree days as a critical factor for yield and qualitative values of timothy in subartic growing conditions. 2001, Agr Res Ctr, Lapland Res Stn, Tutkijantie 28, FIN-96900 Saarenkyla, Finland. Sao Pedro, Brazil: Fundacao Estudos Agrarios Luiz Queiroz (Fealq). 396-397.
- 34. Termonen, M., P. Korhonen, and P. Virkajarvi, Nutritional values of leaf and stem fractions in the second growth of timothy and meadow fescue. 2020, Helsinki, Finland: Organising Committee of the 28th General Meeting of the European Grassland Federation & Natural Resources Institute Finland (Luke). 147-149.
- 35. Hyrkas, M., et al., Different harvesting strategies and cultivar mixtures for grass silage production in Finland. 2015, Natural Resources Institute Finland (Luke),

Green technology, Halolantie 31 A, 71750 Maaninka, Finland. Wageningen, Netherlands: Wageningen Academic Publishers. 239-241.

- 36. Hyrkas, M., et al., The development of yield and digestibility of the third cut of grass silage in Finland. 2016, Natural Resources Institute Finland (Luke), Green technology, Halolantie 31 A, 71750 Maaninka, Finland. As, Norway: Nibio. 498-500.
- 37. Halling, M.A., A. Helgadottir, and A. Hopkins, Persistence and yield of Lolium perenne in different harvest systems. 2013, Department of Crop Production Ecology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. Borgarnes, Iceland: Agricultural University of Iceland. 198-200.
- 38. Hansen, N.P., et al., Effect of regrowth period for perennial ryegrass on yield and nutritive value of grass. 2020, Helsinki, Finland: Organising Committee of the 28th General Meeting of the European Grassland Federation & Natural Resources Institute Finland (Luke). 267-269.
- 39. Kykkanen, S., et al., The role of fungal diseases in the accumulation of dead tissue in timothy and meadow fescue swards. 2016, Natural Resources Institute Finland (Luke), Green technology, Halolantie 31 A, 71750 Maaninka, Finland. Ås, Norway: Nibio. 492-494.
- Virkajarvi, P., et al., Importance of senescence and dead material on nutritive value of grass silage. 2012, MTT Agrifood Research Finland, Halolantie 31 A, 71750 Maaninka, Finland. Helsinki, Finland: MTT Agrifood Research Finland. 188-189.
- Makiniemi, K., et al., Optimization of the harvesting time of pure lucerne (Medicago sativa L.) swards in Finland. 2016, Natural Resources Institute Finland (Luke), Alapaantie 104, 61400 Ylistaro, Finland. Ås, Norway: Nibio. 290-292.
- Lillak, R., et al., The influence of cutting system and soil conditions influence on persistence and productivity of lucerne. 2003, Department of Grassland Science and Botany, Estonian Agricultural University, Tartu, Estonia. Pleven, Bulgaria: Bulgarian Association for Grassland and Forage Production (BAGFP). 357-360.
- 43. Søegaard, K., Cutting strategy of a five-cut system in different grassland mixtures. 2014, Aberystwyth, UK: IBERS, Aberystwyth University. 576-579.
- Søegaard, K., et al., The effect of cutting strategy on production and quality of high-yielding multispecies grasslands. 2015, Department of Agroecology, Aarhus University, Fjele, Denmark. Wageningen, Netherlands: Wageningen Academic Publishers. 319-321.
- 45. Elgersma, A. and K. Soegaard, Changes in nutritive value and herbage yield during extended growth intervals in grass-legume mixtures: effects of species, maturity at harvest, and relationships between productivity and components of feed quality. Grass and Forage Science, 2018. **73**(1): p. 78-93.
- 46. Hetta, M., et al., In vitro degradation characteristics of timothy and red clover at different harvest times. Acta Agriculturae Scandinavica Section a-Animal Science, 2004. 54(1): p. 20-29.

- 47. Nilsdotter-Linde, N., et al., Widening the harvest window with contrasting grassclover mixtures. 2016, Swedish University of Agricultural Sciences, Dept. of Crop Production Ecology, Sweden. Ås, Norway: Nibio. 191-193.
- 48. Nadeau, E., et al., Variations in alpha-tocopherol and beta-carotene concentrations in forage legumes and grasses harvested at different sites and maturity stages. 2013, Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, Sweden. Orange, Australia: New South Wales Department of Primary Industry. 643-646.
- Solati, Z., U. Jorgensen, and K. Søegaard, Protein productivity and extractability of legume and grass species during spring growth. 2016, Ås, Norway: Nibio. 116-118.
- 50. Solati, Z., et al., Dry matter yield, chemical composition and estimated extractable protein of legume and grass species during the spring growth. J Sci Food Agric, 2017. **97**(12): p. 3958-3966.
- 51. Virkajarvi, P., Effects of defoliation height on regrowth of timothy and meadow fescue in the generative and vegetative phases of growth. Agricultural and Food Science in Finland, 2003. **12**(3-4): p. 177-193.

3. Harvest and conservation

3.1 Harvest and Conservation

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3.1.1 Introduction

Forage species differ in ensiling characteristics depending on variations in concentrations of water-soluble carbohydrates (WSC) and crude protein (CP) and in variations in buffering capacity (BC). Legumes, such as clovers and lucerne, are more difficult to ensile because of less WSC, more CP and a higher buffering capacity than grasses. Good harvesting techniques with quick and uniform wilting of the forage in the field to an appropriate dry matter (DM) content for the silo or bale are important to maintain the WSC content and to minimize break-down of the protein by plant enzymes in the field, thereby decreasing DM losses. The BC of forages can be defined as the degree to which forage material resists changes in pH. Fresh forage with a high buffering capacity will require more acid to reduce its pH from 6 to 4 than forage with a low buffering capacity. The anions of organic acids accounts for most of the BC of the plant, whereas the plant proteins account for a smaller proportion. Furthermore, fresh forage can have higher contents of organic acids and, therefore, have higher BC than wilted forage.

3.1.2 Forage species, maturity, management and conservation

Forages are good sources of protein, fatty acids and vitamins but contains also phytoestrogens to various degrees that need further attention. All these plant constituents are affected by plant species, plant maturity and ensiling. Arvidsson et al. [1] studied the effect of ensiling on the fatty acid composition in timothy and found that the ensiling process did affect the fatty acid proportions. After ensiling there were lower proportions of i.e. α -linolenic acid (18:3n-3) and higher proportions of linoleic acid (18:2n-6) in ensiled material compared to fresh herbage. Further, Arvidsson et al. [2] found declining concentrations of total fatty acids, α - linolenic and linoleic acids with increasing maturity of timothy and meadow fescue. In addition, Koivunen et al. [3] compared the lipid content and fatty acid composition in fresh and ensiled red clover and of a mixture of timothy/meadow fescue. Concentrations of α -linolenic acid (10.6 vs. 9.7 g/kg DM) and total fatty acids (15.5 vs. 13.3 g/kg DM) were higher for red clover than for grass and the concentrations decreased with advancing maturity stages, with a peak at the vegetative stage, which could be related to the highest leaf:stem ratio. The majority of fatty acids in fresh herbage was esterified, whereas non-esterified fatty acids predominated in silage. This study confirmed previous studies that red clover has higher polyphenol oxidase (PPO) activity than grasses that seemed to decrease the proteolysis during ensiling of red clover. Polyphenol oxidase is an enzyme that oxidizes o-diphenol to o-quinone. The o-quinone can react with functional groups of proteins, forming protein-bound phenolics. There is only scarce information on vitamins and phytoestrogens in forages. Regarding vitamins, Lindqvist et al. [4] evaluated the fat-soluble vitamins of legume-grass mixtures. They found greater concentrations of α -tocopherol in a mixture of birdsfoot trefoil and timothy compared to a mixture of red clover and timothy and greater β -carotene concentration than in red clover mixed with timothy or meadow fescue. Phytoestrogens are present in legumes, where they have biological effects similar to those of oestrogens in animals. Sarelli et al. [5] compared the phytoestrogens in birdsfoot trefoil and red clover. They found nearly no phytoestrogens in birdsfoot trefoil, whereas fresh and ensiled red clover contained 0.7 to 1.2% of DM with the lower concentrations when harvested at flowering compared to budding stage [5].

Also, the microbial counts in the fresh and ensiled forage could be influenced by management system of the sward harvested at early and late maturity stages during the summer. One intensively managed and one extensively managed grass sward, both harvested as first cut in May, June and August (45, 51 and 55% DM, respectively) were investigated by Müller [6]. The extensively managed grass sward was approximately 20 years old, consisted mostly of timothy and meadow fescue but also of couch grass and dandelions and had been harvested twice a year for silage and hay. The intensively managed grass sward was two years old and consisted mostly of timothy and meadow fescue but also of perennial ryegrass and red clover. Ensiling occurred in 25-liter stainless steel silos. Sward type had less effect than harvest date on microbial counts, both in fresh and ensiled forage. Later harvest date resulted in increased counts of yeast and enterobacteria, while clostridia spore counts remained unchanged in the herbage. Lactic acid bacteria (LAB) and mould counts were highest in the herbage in August, when there was more dead material. Most LAB occurred in the stem base, where there was dead material and no sunlight but moisture. Later harvest dates resulted in increased yeast and LAB counts, while counts of enterobacteria decreased in the haylage. Haylage harvested in May had the highest ethanol content, the lowest lactic acid content and the highest DM losses. Haylage harvested in August had the lowest pH and ethanol concentration but the highest LAB count and the longest aerobic stability. Schenk and Müller [7] did a similar study in an intensively managed sward harvested for haylage (55% DM) in primary growth in June, July and August. Counts of yeast, enterobacteria and LAB in fresh and ensiled herbage increased with delayed harvest time. Number of mould species in herbage decreased during ensiling. Storage DM losses were greatest in August and smallest in July but aerobic stability of the haylage was not affected by harvest time.

An important factor affecting the fermentation process is naturally occurring microbiological contamination of forage. Using a variety of organic fertilizers in ley production is common. However, the application of organic fertilizers is associated with the contamination of ley crops with undesirable microorganisms when the application is not done directly after the previous harvest. Several studies investigated the application of organic fertilizers on silage quality and factors eliminating a negative effect of organic fertilization on silage quality. Gismervik et al. [8] evaluated LAB inoculant, acid-based additive, and nitrite with organic acid salt on silage quality of ley contaminated with slug populations of Arion vulgaris shortly before ensiling. Results showed that increasing slug contamination reduced the microbial quality of silages by increasing C. tyrobutyricum levels. Only silages treated with the nitrite-containing additive had non-detectable mean levels of C. tyrobutyricum and increasing slug contamination increased LAB enumerations in silages. Similar results were obtained by Randby et al. [9] where acid-based additive and an additive containing nitrite with organic acid salts reduced freshly applied slug populations of A. vulgaris in silages wilted to 37% DM. Such an effect was not obtained by bacterial inoculation. Johansson et al. [10] presented that wilting of grass to above 39% DM contaminated with a variety of bacteria and viruses negatively affected the preserving abilities of the crop. Bacterial and fungal spores and the viruses studied were not inactivated significantly by ensiling at these high DM contents. However, Müller et al. [11] concluded that the use of anaerobic digestion residue as fertilizer on grassland applied 7 weeks before harvest had no effect on microbial composition of silages. Furthermore, Pauly and Tham [12] investigated the survival of Listeria monocytogenes in crop inoculated shortly prior to ensiling. Increasing the DM content from 200 to 540 g/kg did not reduce listeria counts. Both bacterial inoculant and formic acid effectively reduced L. monocytogenes in wet silages (200 g DM/kg), but only the bacterial additive reduced listeria counts in the wilted silages (430 g DM/kg).

3.1.3 Wilting

During wilting of forages before ensiling, plant enzymes start to break down protein to ammonia-nitrogen and other simple nitrogen compounds. A longer wilting time in rainy weather, especially when wilting occurs in big swaths, increases this proteolysis. Therefore, rapid and uniform wilting, by use of wide spreading or narrow single swaths, is important to limit this breakdown of protein during wilting [13, 14]. Wide-spreading increased wilting rate, gave higher DM content, improved silage fermentation and decreased DM losses [13, 14, 15]. Wilting up to 24 hours in sunny and warm weather even increases the protein quality of grass and legume swards. There are very limited published results available on the effect of wilting on forage protein quality and other nutrients. Sousa et al. [16] evaluated the effect of wilting on the protein quality of a lucerne/white clover (85:15) mixture during the first regrowth. Rapid wilting for six hours decreased the soluble true protein, while increasing the digestible, slowly degradable protein and rumen undegradable protein, which shows that wilting did not only decrease the proteolysis but also improved the protein quality by converting soluble true protein into slowly degradable true protein, of which a major part is rumen undegradable protein (RUP). Part of this soluble protein was bounded to the NDF. These results are in agreement with results by Nadeau et al. [17] when wilting grass/legume forage for 23 hours by wide-spreading in the field to 35% DM. This improved protein quality might be caused by synthesis of proteinbound phenolics that could be catalyzed during wilting by the PPO enzyme. Ensiling of the wilted forage decreased the true protein by increasing the nonprotein nitrogen and decreasing rumen undegradable protein. This proteolysis during anaerobic conditions is caused by bacteria, which deaminate amino acids to ammonia. However, Bakken et al. [18] found that the effect of wilting rate on the content of soluble crude protein was affected by the harvest number and had only minor influence on the protein value calculated as amino acids absorbed in the small intestine and estimated as effective utilizable protein. In contrast, Steinshamn et al. [19] found greater AAT20-value of wilted compared to unwilted perennial ryegrass. Forages in both experiments were wilted indoors.

Wilting outside can also affect other nutrients, such as the vitamin content in forages. Lindqvist et al. [4] reported a 30% decrease in the α -tocopherol concentration of a birdsfoot trefoil/timothy mixture, whereas the α -tocopherol concentration of a red clover/timothy mixture and a red clover/meadow fescue mixture were not affected by wilting from 13% DM to 27% DM. There was no effect of wilting and ensiling on the β -carotene concentrations of the forage mixtures. Ensiling increased the α -tocopherol concentration in the birdsfoot trefoil/timothy mixture by 38% but did not affect the α -tocopherol in the red-clover mixtures [4]. Arvidsson et al. [1] studied the effect of wilting on the fatty acid concentrations in timothy but did not see any differences in wilted grass (35% DM) compared to fresh herbage.

Wilting of forage in the field can affect the rumen fermentation in cows. Kokkonen et al. [20] observed an increased passage rate of small particles from the rumen, thus, shorter rumen retention time of wilted compared to direct cut grass silage. Regarding rumen organic acids, the proportion of propionate tended to be higher whereas the proportion of butyrate was lower in wilted than in direct-cut silage. Rumen NH₃-N concentration was higher with direct-cut silage, which probably was a consequence of higher NH₃-N concentration in the silage.

Haymaking requires wilting in the field to a DM content of at least 60% followed by barn drying to 85% DM. Over the years, there has been a switch from hay to silage for dairy cows and growing cattle as the DM intake usually is greater for silage. This difference in DM intake could be related to a greater fibre concentration of hay than of silage when the forage is harvested later as hay than for silage [21, 22]. The different storage methods could also affect the contents of fatty acids and vitamins in the milk. Shingfield et al. [21] reported more poly-unsaturated fatty acids but lower riboflavin, α -tocopherol and β -carotene concentrations in milk from cows fed hay compared to silage of a primary growth of a timothy/meadow fescue ley. However, Halmemies-Beauchet-Filleau et al. [22] found decreased fatty acid content, primarily due to losses of linoleic acid and a-linolenic acid in hay compared to silage of a primary growth timothy/meadow fescue sward. Furthermore, hay decreased lipolysis and biohydrogenation of unsaturated fatty acids, rumen volatile fatty acids (VFA) and molar proportion of butyrate but increased molar proportion of acetate in the rumen [22]. Furthermore, in a study with ewes fed silage or hay, the intake of β -carotene was significantly lower in the hay group, which was also reflected in blood vitamin A levels, which were higher in the silage group from mid pregnancy [23]. The silage-fed ewes had higher levels of blood alpha-tocopherol in mid pregnancy, compared to hay-fed ewes.

3.1.4 Harvest method

There are different mowing techniques of leys that affect the wilting rate of the grass. Nadeau et al. [24, 25] found a higher DM concentration (42%) of grass/clover forage mowed with a rotary conditioner compared to grass/clover forage mowed with a rotary mower (32%) after 21 hours of wilting, when forage was broadcasted and swathed in the field. Mowing with a rotary mower decreased the wilting rate in the swath and, therefore, evened out the DM content during chopping/cutting of the forage. The forage was chopped with a precision chopper wagon (Taarup 480) to a mean length of 27 mm or cut with a rotor cutter wagon (Pöttinger Jumbo 6010) to a mean length of 85 mm. When the forage was mowed by a rotary mower, chopping improved silage characteristics by decreasing concentrations of butyric acid and ethanol, thereby decreasing DM losses (3.2 vs. 3.7%) compared to cut forage. When the forage was mowed by a rotary mower does had similar qualities, except for more acetic acid in the cut than in the chopped silage (10.0 vs. 7.3 g/kg DM). Similarly, Lingvall and Knicky [26] found no differences in silage quality between a precision chopper wagon and a rotor cutter wagon, when a grass

ley had been mowed with a rotary conditioner. Consequently, choice of harvesting technique can to some extent affect the quality of the silage but Nadeau et al. [24, 25] concluded that the use of different types of silage additives (salt, acid and bacterial inoculant) that were tested in the study, had greater effect than the harvest method on silage quality. Furthermore, results from studies where fermentation characteristics of long vs. chopped grass silages have been compared, the acidification rate, measured as a pH decrease after 3 days of fermentation, has been improved in chopped forage. However, differences have been relatively small in comparison to the positive effects of silage additives on fermentation characteristics and DM losses after a storage time of at least 90 days [27, 28, 29]. Likewise, Müller [30] found no differences in pH, fermentation characteristics, DM loss and aerobic stability between cut and uncut haylage (55% DM) in bales. However, Feng et al. [31] observed that chopping tall fescue from 130 mm to 24 mm improved silage fermentation in concrete clamps in terms of clostridial fermentation elimination. This was probably a result of more intensive fermentation in chopped silages in silos than in longer cut silage in bales, ensiled at the same DM, as demonstrated by Slottner and Bertilsson [32].

Shredding is a type of processing where the particles of forage plants are crushed and broken between rollers rotating with different speed. Compared to precision chopping, shredding attempts to disrupt the forage particles to increase the surface area for microbial adhesion and thereby facilitate silage fermentation and rate of rumen digestion [33]. Lucerne, red clover, perennial ryegrass and grass-clover mixtures at DM contents of 19-25% were ensiled in vacuum bags as-is or shredded 1x or 4x before ensiling [34]. Shredding occurred in a laboratory shredder and 4x meant four passages through the shredder. Shredding improved initial density and fermentation quality of perennial ryegrass and red clover silages while reducing fermentation weight losses. The lucerne silage was not affected by shredding but had clostridial fermentation, causing high pH [34]. In another study, Hansen et al. [33] evaluated the effect of shredding on fermentation quality of a grass-clover sward before baling and wrapping. The sward was harvested early (May 14) or late (May 29) in the first cut and wilted to 40-50% DM before shredding in the field with a tractor-driven machine. Similar to the results on perennial ryegrass and red clover by Samarasinghe et al. [34], shredding improved silage fermentation by increasing lactate and acetate while decreasing propionate and butyrate, resulting in decreased silage pH. Also, shredding decreased soluble N [33].

3.1.5 Storage method

There are two main storage technologies: hay making and ensiling. Hay is commonly stored in bales without plastic wrapping or loosely with final drying indoors, whereas silages are stored in a variety of silos that can be classified into several main categories; stack or clamp silos without retaining walls, bunker silos, tower silos and tube silos. Another common storage system for silage is plastic covered silage bales. Studies comparing storage technologies are scarce. There are mainly silage quality comparisons between round bale storage system and bunker silos, which are the most common storage systems in the Nordic part of Europe. These two storage systems are associated with two different mechanical treatments of forage prior to packing. Forage stored in bales is cut whereas forage ensiled in bunker silos is mostly precision chopped if not cut with a rotor cutter wagon. Precision chopping allows better forage compaction in comparison with cutting. This was shown in a study by Randby and Bakken [35], where ensiling of crops with DM contents of 18-20% resulted in much lower density in bales than in bunker (111 vs 164 kg DM/m^3). However, the sum of visible moulded losses as well as DM lost by crop respiration, effluent runoff, anaerobic fermentation, aerobic deterioration and gaseous losses was significantly higher from bunkers than bales (14.1 vs 7.2%) [35]. Randby et al. [36] reported that the packing density increased in bunker silo by replacing tractor with heavier wheal loader. Density was increased by 9%, however, in wetter and less fibrous crop silage losses were greater with wheel loader than with tractor compaction. In drier and more fibrous crops it was the opposite. Their observations suggest that packing pressure should be tuned to crop wetness and morphology and that a minimum wet weight crop density of 705 kg/m³ should be targeted as well as high filling rate, to avoid heating. They also observed density to be critical in surface layers, especially in silo shoulders where average spore concentration of C. tyrobutyricum was $> 5 \log cfu/g$. Similarly, Nadeau et al. [37] found elevated spore counts of C. tyrobutyricum near the edges of the bunker silos on Swedish dairy farms.

Müller et al. [38] studied the effect of dry matter and storage time on small bale silage and haylage quality and found silages to produce more fermentation products over time than haylage with rather stable formation of fermentation products. Müller and Johansen [39] also concluded that rebaling from large roundbales to small square bales can be done without large changes in chemical composition after three months of storage. However, yeast counts might increase in rebaled haylage and both yeast and mould counts may increase in silage.

The presence of toxins in silage is undesirable due to the health risk for animals. Their presence can be associated with inappropriate ensiling procedure (low packing density, delayed sealing, low ensiling hygiene, weak silo tightness) leading to mould growth in silages. Due to a lower packing density as well as a higher risk of cover damage, bale silages are more prone to mould occurrence than silage in bunker silos. Venslovas et al. [40] showed that aflatoxin B₁ prevailed in Lithuanian grass silages. Their comparison of silage storages revealed significantly higher concentrations of aflatoxin B₁ in trench silos than in clamps or bales. Coverage of silage bales is an important factor to achieve their proper tightness. A study by Spörndly and Nylund [41] compared a new 'mantelfilm' with traditional net for

round bales and showed that mantelfilm gave smaller bale volume and perimeter, larger proportion of CO_2 in the bale and greater seal integrity, resulting in less mould and lower ammonia-N concentration. The comparison of number of stretch film layers displayed that eight layers of stretch film gave the best results as far as the highest proportion of CO_2 , greater seal integrity, no mould presence, higher WSC and lower ethanol concentrations compared to four layers of stretch film. Six layers of stretch film also gave a larger proportion of CO_2 , greater seal integrity and less mould and ethanol compared to four layers.

3.1.6 Ensiling with additives – effects on silage fermentation and aerobic stability

Application of silage additives has become the conventional implement to control the ensiling process. The main objective in using silage additives is to ensure the fermentation process to produce well-preserved silages as well as to eliminate aerobic deteriorating processes in silages during the feed-out period. Silage additives can be classified into two main categories, biological and chemical additives. Biological additives are employed to encourage the lactic acid fermentation. Dosages from 1×10^5 to 1×10^6 are generally satisfactory to achieve the improvement of fermentation profile of silages. However, the efficiency of lactic acid bacteria to stabilize silages during the feed-out phase is limited, particularly of the homofermentative strains. This was shown by Saarisalo et al. [42] with an isolated strain of Lb. plantarum [43] and by Seppälä et al. [44], who showed that silages treated with a pure strain of Lb. plantarum can result in worse aerobic stability than untreated control silages. Therefore, the combination with heterofermentative lactic acid bacteria has been widely tested. In particular, the combination with Lb. buchneri, that possesses the ability to convert lactic acid to acetic acid, has been shown to be effective in eliminating yeast, thereby improving aerobic stability of silages during the feed-out phase. These results were observed in a number of studies [45, 46, 47, 48]. Furthermore, Seppälä et al. [44] observed that only lactic acid bacteria treatments including the heterofermentative strain Lb. brevis were able to improve aerobic stability of total mixed rations. Another way to improve the aerobic stability of lactic acid bacteria treated silages was to mix them with chemical additives. Saarisalo et al. [42] tested addition of sodium benzoate and formic acid to Lb. plantarum treated silages prior to ensiling as well as after storage prior to aerobic test and found that sodium benzoate addition improved silage aerobic stability. An improvement of silage aerobic stability by addition of sodium benzoate to a lactic acid bacteria mixture was also observed by Jatkauskas and Vrotniakiene [49]. However, Jatkauskas et al. [45] obtained no improvement from sodium benzoate addition to lactic acid bacteria mixture in comparison to a mixture containing Lb. buchneri.

The addition of enzymes is another way to improve the ensiling process. There is a particular interest in using fibrolytic enzymes, which break down cellulose and hemicellulose complexes, with the aim to provide more easily accessible substrate, such as sugars, for lactic acid bacteria as well as to improve plant fibre utilization. Rinne et al. [50] treated grass forage with three levels of cellulose/hemicellulose enzymes. Results showed a linear decrease in NDF concentration with increasing enzyme addition and the sugar increase was reflected in higher lactic acid formation. Furthermore, Dehghani et al. [51] showed that addition of glucanase, β glucanase and pectinase increased lactic acid and decreased butyric acid, ammonia and pH compared with the control in lucerne and grass-clover silages, and increased glucose concentration in lucerne silage. In a study by Feng et al. [31] where a mixture of a variety of enzymes and lactic acid bacteria were used, there was a positive effect on organic dry matter preservation. On the other hand, addition of molasses together with lactic acid bacteria and enzymes led to excessive production of ethanol [52]. Kasmaei et al. [53] tested the hypothesis that the inconsistent effect of Lb. buchneri LN 4017 on the digestibility of silage fibres is due to the catabolic repression of feruloyl esterase activity by substrates present in silage (e.g., glucose), however, without success.

Chemical additives partially restrict microbial growth, particularly silage undesirable microorganisms. A strong fermentation restrictive effect has been displayed by formic acid alone or in mixtures. The use of these mixtures showed a promising effect on reduction of proteolysis during the ensiling process. A study by Slottner and Bertilsson [32] showed that the application of 6 L/t of formic acid reduced proteolysis. Steinshamn et al. [19] tested the application of 2, 4 or 6 L/ton of formic acid-based additive and its mixture on herbage at DM contents of 25, 35 and 45% DM. The additive restricted the fermentation and conserved the WSC as well as improved the estimated protein value AAT20. Koivunen et al. [3] observed that formic acid addition during the ensiling of red clover also decreased lipolysis in silo. Furthermore, Sarelli et al. [5] studied the effect of silage additives on phytoestrogen content in red clover. Silage with an additive containing Lb. plantarum had higher concentrations of genistein and biochanin A than silage made with formic acid. Similarly, the study of Lindqvist et al. [4] showed that a LAB treatment of forage is better for preservation of a-tocopherol in clover-grass mixtures than a formic/propionic acid treatment.

Hetta et al. [52] found that the addition of formic acid resulted in silages with a good chemical composition and improved organic matter degradation in the rumen. Addition of 4 L of formic acid/ propionic acid mixture [52] resulted in higher density, reduced the proportion of non-protein N of total N, restricted silage fermentation to lactic and acetic acid, reduced NH₃-N values, increased ethanol fermentation and increased silage DM intake. There was only a weak effect on DM

losses reduction, aerobic stability was improved only in bales, and no effect on clostridia was observed. In another study, the addition of formic, propionic and benzoic acid [9] considerably improved silage quality from a crop contaminated with *A. vulgaris*. On the other hand, the addition of 4 L of formic acid in low dry matter clover-grass mixture resulted in insufficient clostridial activity reduction [55]. Disadvantages of using formic acid-based additives is their corrosive effect on machinery and harmful effects on persons handling the acid. Randby [56] showed that the easy-to-handle formic acid mixtures with ammonia or with acetic acid and wood pulp liquor have potential to result in a similar ensiling effect as formic acid, however, higher dosages were required: 4 and 7.3 L/ton of buffered additives would be equivalent to 3 L/ton of formic acid.

Another group of chemical additives with selective microbial inhibiting properties has been extensively tested. These are salt mixtures of organic acids such as benzoate, sorbate and propionate, often mixed with nitrite and/or hexamethylene tetramine. These mixtures eliminate undesirable clostridia and fungi activities during ensiling. A series of studies conducted by Knicky and Spörndly [57, 58] demonstrated at laboratory scale the efficiency of the mixture of sodium benzoate, potassium sorbate and sodium nitrite at a rate of 5 L/ton to improve silage quality by eliminating clostridial activity and extending aerobic stability in treated silages from a large variety of crops and DM contents ranging from 15 to 45%. It is known that the anticlostridial effect in this mixture is carried out by sodium nitrite, and its high anticlostridial properties were verified by König et al. [55] and Gismervik et al. [8]. Similar antifungal and anticlostridial properties to improve silage quality and aerobic stability were demonstrated by a mixture containing sodium nitrite, hexamethylene tetramine and potassium sorbate at rates of 2.0-2.5 L/ton and a mixture of sodium nitrite, sodium benzoate and sodium propionate at the rate of 2 L/t, when applied to grass at DM ranging from 23 to 40% [46]. The improved aerobic stability resulted in improved organic matter digestibility of the grass silage compared to the untreated control silage. Knicky and Lingvall [59] tested a variety of combinations of sodium benzoate, sodium nitrite, hexamethylene tetramine, sodium propionate, sodium bisulphite, and propionic acid at rates of 2-4.5 L/ton on a red clover/timothy mixture at 30 and 60% DM. Silage additives improved fermentation, reduced clostridial growth and DM losses, and improved aerobic stability, except for a mixture of 2 L/ton of sodium benzoate and sodium bisulphite.

The formation of a whole spectrum of gases occurs during the ensiling process. The formation of gases is undesirable, because it is often a sign of undesirable processes in silages, resulting in increased DM losses, and additionally, they cause concern about the impact on the global environment. The formation of CO_2 is the most abundant during the ensiling process. Processes where CO_2 occurs as a by-product are less effective in transformation of substrate to main fermentation

products, which results in higher ensiling losses. Besides CO₂, undesirable formation of volatile organic compounds (VOC) occurs during fermentation. Only one study has been conducted in this area. Knicky et al. [60] tried to estimate the total formation of VOC in Sweden from silage production. They found that ensiling of clover-grass forage at 32% DM content resulted in higher VOC emission than from silages at 53% DM content. Ethanol and methylacetate mainly contributed to VOC emission from both silages.

Proteolysis is an undesirable process during ensiling and its reduction is an important aspect of silage additive efficiency. There is, however, a scarcity of studies in the efficiency of different additives to reduce proteolysis during fermentation. Nadeau et al. [61] reported the improvement of the crude protein fractions of grass silages treated with acid-based additive (3 L/t), salt-based additive (2 L/t) and a mixture of homo- and heterofermentative lactic acid bacteria (2x10⁵ cfu/g). This study confirmed earlier findings of Slottner and Bertilsson [32], that both chemical and biological additives can reduce proteolysis. On the other hand, Sousa et al. [16] obtained improved protein quality of silages treated only with formic acid/propionic acid-based additive, but not with homofermentative lactic acid bacteria, when a lucerne/white clover mixture was ensiled.

3.1.7 Prediction models of silage quality

There has been certain uncertainty about the fate of some crop nutritional components, mainly carbohydrate fractions during ensiling process, which can affect the estimated feeding value of a silage. A better determination of recovery of a crop's components during ensiling process can help to improve the estimation of the silage feeding value as well as its prediction from composition of a fresh crop. Kasmaei et al. [62] evaluated a prediction model for silage quality based on available chemical and microbial characteristics of fresh crop variables, however, these had limited explanatory value. The best equations were obtained for acetic acid in perennial forage silages and for lactic acid and ethanol. The DM and CP concentrations of perennial forages had the strongest effects on fermentation products. Udén [63] investigated the fate of main crop components during silage fermentation to be able to use that to predict silage composition. He found that ash, aNDFom, crude fat and CP had close to 100% recoveries after ensiling and can be reasonably well predicted from initial crop levels. Others, such as starch (81% recovery), CPndf (62% recovery), organic acids (65% recovery) and pectin (64% recovery) indicate that not only WSC (29% recovery) as a main substrate are metabolized during ensiling, which makes them more difficult to use in prediction. In another study Udén [64] focused more closely on fate of individual plant organic acids in forages during silage fermentation and found their decrease by 38-56%,

except for oxalic acid, which was unaffected, and succinic acid, which increased >7-fold.

3.1.8 Ensiling with additives – effects on rumen fermentation and ruminant production

To evaluate economic benefits of using silage additives in addition to decreased proteolysis and DM losses during storage and feed out, a limited number of animal experiments have been carried out. Jaakkola et al. [65] studied the application rate of 100% formic acid (0, 2, 4 and 6 L/t) on fermentation quality of timothy/meadow fescue silage of 27% DM. They found that increased application rate of formic acid restricted silage fermentation in a curvilinear fashion, with decreased fermentation acids, increased WSC and decreased NH₃-N. These fermentation characteristics resulted in higher milk yield and protein yields of dairy cows fed silage treated with 6 L/t of formic acid. Thus, restrictively fermented silages are more limited in glucose precursors (propionic acid) and cows benefit more from propylene glycol than those having extensively fermented silages [65]. Furthermore, Jaakkola et al. [66] used the same treatment to direct-cut timothy/meadow fescue silage (18% DM) and observed an increased flow of total N at the duodenum with increased application rate of formic acid, resulting in increased net microbial protein synthesis in the rumen with increased dosage and a more efficient N-utilization in young bulls. Furthermore, increased rate of formic acid increased proportion of butyrate in the rumen [66]. Likewise, Nadeau et al. [67] showed higher yields of milk and energy-corrected milk (ECM), without any effect on DM intake, for diets containing grass silage treated with salt-based additive or an inoculant compared to the control diet, when the diets were formulated for a low proportion of rumen undegradable protein.

In a comparison between formic acid-based additive (5.4 L/t) and *Lb. plantarum* (10^6 cfu/g forage) to timothy/meadow fescue silage, Saarisalo et al. [68] reported that both additives improved silage fermentation compared to the control. Formic acid-treated silage had more WSC than the inoculant, giving a larger proportion of acetate in the rumen, which is needed for the milk fat synthesis. Higher lactic acid formation in the inoculant treated silage compared to the formic acid-treated silage resulted in a larger proportion of propionate in the rumen of dairy cows, which is a substrate for gluconeogenesis. No effect on DM intake, milk yield and ECM were reported by the additives compared to the untreated control. In contrast, Rinne et al. [69] found similar fermentation characteristics between formic acid-based (5 L/t) treated grass silage and inoculant treated silage gave higher DM intake compared to LAB-treated silage, which did not translate into higher milk yield. When the vitamin content in the milk was in focus, Shingfield et al. [21] found greater beta-

carotene content in milk from silage treated with an inoculant (5 x 105 lactic acid bacteria/g-enzyme [cellulase and hemicellulase]) preparation than with formic acid-based additive.

A few studies have investigated the effects of silage additives to growing cattle. Huuskonen et al. [70] did a continuous feeding trial with dairy bulls fed timothy silage (35-40% DM) with or without additives. The additives were 3.4 kg/t of a saltbased additive containing sodium benzoate (200 g/kg)/potassium sorbate (100 g/kg)/sodium nitrite (50 g/kg) and 5.8 kg/t of formic (589 g/kg)/propionic (199 g/kg)/ammonium formate (43 g/kg)/potassium sorbate (25 g/kg). The silages were fed in a total-mixed ration of 60% silage and 40% barley grain of DM intake. No differences in DM intake between control and additives were found but the control had greater intake at the early part when the primary growth of the timothy silage was fed. This translated into greater ME intake of the control silage, resulting in higher live-weight gain of the control compared to the treated silages during the first phase of the study and over the whole experiment. Bulls fed the treated silages had higher conformation score compared to the control (5.1 vs. 4.8). The control silage was of good fermentation quality. In another feeding experiment with bulls, Jatkauskas and Vrotniakiene [71, 72] compared control silage with inoculanttreated (Lb. plantarum and P. acidilatcicci and cellulase 10⁶ cfu/g) and formic acidtreated, 6 L/t, red clover/timothy/meadow fescue silage (22% DM). In the 126 day feeding experiment, using bulls of initial live weight of 312 kg, no effects of treatments on DM intake and live-weight gain were found. Both treatments decreased pH, butyric acid (0.2 vs 2.8 g/kg DM) and acetic acid (10 vs 25 g/kg DM) and increased lactic acid compared to the control silage, which had clostridial fermentation. Acid treated silage had more WSC than the control. Both treated silages increased the molar proportion of propionic acid of the total VFA in the rumen and decreased the acetate:propionate ratio compared to the control. In vivo digestibility using wethers showed greater DM (63.7 vs. 59.8%) and OM (66.1% vs. 62.2%) digestibility for the treated silages compared to the control silage [71, 72].

Grass/clover round bale silages treated with acid or salt-based additive were compared to untreated silage in a study with ewes and lambs [73]. There were no treatment effects on intake or body condition score of the ewes. Lambs born of ewes fed treated silage, however, had higher birth weight and live-weight gain to weaning than lambs whose mothers were fed untreated silage. After weaning, lambs fed treated silage had higher DM intake but no effect on live-weight gain was observed.

3.1.9 Biorefinery

This chapter gives a brief overview of the recovery of biorefined products. The DM and crude protein recovery into fibre pulp and press juice differs between plant

species, maturity stage of the plants at harvest and the DM content of the fresh or ensiled forage [74, 75, 76]. Also, the recovery of the crude protein fractions, that differ in solubility and digestibility, into concentrate and fibre pulp can differ between plant species [77]. Furthermore, Nadeau et al. [78] found larger differences in DM, NDF, CP and CP fractions between the fibre pulp and the raw material when silage instead of fresh grass/clover forage was biorefined. A meta-analysis based on 19 studies from Finland comprising 43 silages (grass, clover or a mix of them) revealed that silage DM concentration was the variable most highly correlated with the liquid yield and resulted in the best model predictions [79]. Experiments on the biorefinery at Aarhus University have resulted in 40% recovery of the fresh forage protein into a protein concentrate of 50% protein. Legumes give the highest protein yield in concentrate but this can depend on nitrogen fertilization of the grass species. White clover and lucerne give high crude protein yields in concentrate but red clover gives the highest protein yield per ha [75]. Despite low WSC in pulp, it seems to ensile well [80]. Press juice recovery could be enhanced by use of cellwall degrading enzymes as additives to the silage. Rinne et al. [50] showed a linear decrease in neutral-detergent fibre concentration with increasing addition of cellulase/hemicellulase at ensiling of timothy/meadow fescue, resulting in sugar production, which was further fermented to lactic acid. The decreased pH resulted in lower ammonia-N concentration in the silage. Press juice yield of DM, CP and WSC increased with increased enzyme application, which is beneficial for biorefinery applications. Biorefinery of forages is a relatively new research area with great potential, which need to be investigated in future scientific experiments, including the environmental and economical benefits for the producers.

3.1.10 Conclusions and knowledge gaps

There are both plant factors, such as forage species and maturity stage at harvest, and management factors, such as slurry application, wilting, harvest and storage methods, and use of right type of silage additive for the crop to be ensiled, that affect silage quality and animal performance. Therefore, it is important to do it right from the start, when choosing the seed mixture and at each following step until the feed bunk. This chapter has highlighted what research has been done so far and it is obvious that some parts have been researched more than other parts. When it comes to plant nutrients, it is important to emphasize that grass/legume mixtures are the main protein sources for ruminants. There is only limited research done on the effect of wilting method and use of silage additives to improve the protein quality of silage and animal performance, when formulating the diets for a high proportion of forage protein. The relatively new research area on green biorefinery of forages to produce press juice and protein concentrate from ley upgrades the protein content of the forages similar to the level of soybean meal. There is much more research to be done in this area in the use of ley as-is or as biorefined ley, as a climate-friendly protein source. Also, there is a lack of research on antinutritional components in different forage species, such as phytoestrogens, how these compounds are affected by wilting and ensiling, and their effects on ruminant performance.

As new techniques for mowing and harvest develop continuously, these techniques need to be researched in the future. Only limited research has been done in this area so far with focus on silage quality. Future research needs to include effects of techniques on animal performance. Likewise, effects of silage fermentation on rumen fermentation and ruminant performance need to be researched more extensively in the future as the research done so far has some conflicting results.

There is a lack of research on the use of ley crops that are resistant to flooding and drought periods, which is an increasing problem with the changing climate in the Nordic and Baltic countries. There is a need to evaluate the silage fermentation characteristics, aerobic stability, the nutritive value and animal performance of these ley crops. Moreover, research concerning formation of nitrogenous gases and volatile organic compounds during ensiling is needed as research so far is very limited.

3.1.11 References

- Arvidsson, K., A-M Gustavsson, K. Martinsson, Fatty acids in forages: A comparison of different pre-treatments prior to analysis. Animal Feed Science and Technology, 2009. 151: p. 143-152
- Arvidsson, K., K. Martinsson, and A-M. Gustavsson, Fatty acid concentrations in timothy (*Phleum pretense* L.) and meadow fescue (*Festuca pratensis* Huds.): Effects of seasonal variations atdifferent nitrogen fertilization levels. Acta Agriculturae Scandinavica, Section B – Soil & Plant Science, 2013. 63: p. 341-321.
- 3. Koivunen, E., et al., Effects of plant species, stage of maturity, and level of formic acid addition on lipolysis, lipid content, and fatty acid composition during ensiling. Journal of Animal Science, 2015. 93: p. 4408-4423.
- Lindqvist, H., E. Nadeau, and S.K. Jensen, Alpha-tocopherol and β-carotene in legume-grass mixtures as influenced by wilting, ensiling and type of silage additive. Grass and Forage Science, 2011. 67: p. 119-128.
- Sarelli, L., et al., Phytoestrogen content of birdsfoot trefoil and red clover: Effects of growth stage and ensiling method. Acta Agriculturae Scandinavica, Section A - Animal Science, 2003. 53: p. 58-63.
- 6. Müller, C.E., Influence of harvest date of primary growth on microbial flora of grass herbages and haylage, and on fermentation and aerobic stability of haylage conserved in laboratory silos. Grass and Forage Science, 2009. 64: p. 328-338.

- Schenk, J. and C.E. Müller, Microbial composition before and after conservation of grass-dominated haylage harvested early, middle, and late in the season. Journal of Equine Veterinary Science, 2014. 34: p. 593-601.
- 8. Gismervik, K., et al., Effect of invasive slug populations (*Arion vulgaris*) on grass silage. II: Microbiological quality and feed safety. Animal Feed Science and Technology, 2015. 199: p. 20-28.
- 9. Randby, A.T, et al., Effect of invasive slug populations (*Arion vulgaris*) on grass silage I. Fermentation quality, in-silo losses and aerobic stability. Animal Feed Science and Technology, 2015. 199: p. 10-19.
- Johansson, M., et al., Potential risks when spreading anaerobic digestion residues on grass silage crops – survival of bacteria, moulds and viruses. Grass and Forage Science, 2005. 60: p. 175-185.
- Müller, C.E., et al., Effect of anaerobic digestion residue vs. livestock manure and inorganic fertilizer on the hygienic quality of silage and haylage in bales. Grass and Forage Science, 2014. 69: p. 74-89.
- 12. Pauly, T.M and W.A. Tham, Survival of Listeria monocytogenes in wilted and additive-treated grass silage. Acta Veterinaria Scandinavica, 2003. 44: p. 73-86.
- 13. Gustavsson, S., Effekt av förtorkningsteknik och tillsatsmedel på ensilagekvalitet samt konsumtion och produktion hos mjölkkor. (Effects of pre-wilting technique and additive on silage quality, consumption and milk production in dairy cows.) Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Section of Production Systems. Student Report 140, 2008. 59 pages. In Swedish with English summary.
- Murphy, M., E. Nadeau, and S.Gustavsson, 2009.Effects of pre-wilting technique and additive on grass silage quality, intake and yield by dairy cows. In:
 Proceedings of XVth International Silage Conference, July 27-29 2009, Madison, WI., USA. pp. 423-424.
- 15. Spörndly, R., et al., Quality and economics of pre-wilted silage made by widespreading or by swathing. Grassland Science in Europe, 2008. 13: p. 645-647.
- Sousa, D., et al., Effects of wilting and ensiling with or without additive on protein quality and fermentation of a lucerne-white clover mixture. Animal Feed Science and Technology, 2019. 258: p. 114301.
- 17. Nadeau, E., et al., Protein quality dynamics during wilting and preservation of grass-legume forage. In: Proceedings of the XVI International Silage Conference, 2-4 July 2012, Hämeenlinna, Finland, p. 56-57.
- Bakken, A.K., et al., Protein characteristics in grass-clover silages according to wilting rate and fermentation pattern. Grass and Forage Science, 2016. 72: p. 626-639.
- Steinshamn, H., S. Krizsan, and L. Østrem, 2022. Effect of harvesting frequency and grassland species on silage protein value. Proceedings of the 11th Nordic Feed Science Conference, Uppsala, Sweden, August 22-23, p. 95-97.
- 20. Kokkonen, T., M. Tuori, and L. Syrjälä-Qvist, Effect of dry matter content and rapeseed meal supplementation on dairy cows. 2. Rumen fermentation and

digesta passage rate. Animal Feed Science and Technology, 2000. 84: p. 229-242.

- 21. Shingfield, K.J., et al., Effect of forage conservation method, concentrate level and propylene glycol on the fatty acid composition and vitamin content of cows' milk. Journal of Dairy Research, 2005. 72: p. 349-361.
- Halmemies-Beauchet-Filleau, A., et al., Effect of forage conservation method and ruminal lipid metabolism and microbial ecology in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. Journal of Dairy Science, 2013. 96: p. 2428-2447.
- Bernes, G., K. P. Waller, and S. K. Jensen. 2008. Hay and silage as vitamin sources in organic sheep production. Grassland Science in Europe, 2008. 13: p. 565-567.
- Nadeau, E., Hallin, O., Auerbach, H., Jakobsson, J. and Arnesson, A. 2013. Quality of baled grass-clover silage as affected by additives and harvest methods. In: Proceedings of the 22nd International Grassland Congress, Sydney, Australia, September 15-19, 2013, p. 744-745.
- 25. Nadeau, E., Arnesson, A. och Hallin, O. Vallskördeteknikens och tillsatsmedlens betydelse för ensilagets kvalitet. (Forage harvesting technique and additives effects on silage quality). Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Section of Production Systems. 2014. Report 37, 42 pages (In Swedish with English summary).
- Lingvall, P. and M. Knicky, Precision chopping or rotor cutting and its influence on ensiling capacity and silage fermentation. Grassland Science in Europe, 2008. 13: p. 666-668.
- 27. Nadeau, E., Arnesson, A. and Auerbach, H., 2012. Effects of additive and particle size on fermentation characteristics and aerobic stability of grass silage. In: Proceedings of the XVI International Silage Conference, Hämeenlinna, Finland, 2-4 July, 2012, p. 380-381.
- Nadeau, E. and Auerbach, H. Effects of particle size and chemical additives on fermentation and aerobic stability of grass-clover silageProceedings of the 5th Nordic Feed Science Conference, Uppsala, Sweden, 10-11 June, 2014, p. 19-24.
- 29. Hamberg, M., Silage quality of grass and red clover-dominated forages as affected by particle size and additive, when ensiled at different dry-matter concentrations. Swedish University of Agricultural Sciences, Faculty of Veterinary Medicine and Animal Science, Department of Animal Environment and Health, Section of Production Systems. Degree project in Animal Science, 2021. 51 pages.
- Müller, C.E. Long-stemmed vs. cut haylage in bales Effects on fermentation, aerobic storage stability, equine eating behavior and characteristics of equine faeces. Animal Feed Science and Technology, 2009. 152: p. 307-321.
- Feng, L., et al., Ensiling of tall fescue for biogas production: Effect of storage time, additives and mechanical pretreatment. Energy for Sustainable Development, 2018. 47: p. 143-148.

- Slottner, D. and J. Bertilsson, Effect of ensiling technology on protein degradation during ensilage. Animal Feed Science and Technology, 2006. 127: p. 101-111.
- 33. Hansen, N.P., et al., Shredding of grass-clover before ensiling: Effects on feed intake, digestibility, and methane production in dairy cows. Animal Feed Science and Technology, 2021. 282: p. 115124.
- 34. Samarasinghe, M.B., et al., Effects of shredding on silage density and fermentation quality. Grass and Forage Science, 2019. 74: p. 244-253.
- 35. Randby, A.T. and A.K. Bakken, Bunkers or round bales: Losses and silage quality with or without acid treatment of low dry matter grass crops. Animal Feed Science and Technology, 2021. 275: p. 114868.
- 36. Randby, A.T., H.N Halvorsen, and A.K. Bakken, Losses and grass silage quality in bunker silos compacted by tractor versus wheel loader. Animal Feed Science and Technology, 2020. 266: p. 114523.
- Nadeau, E., Arnesson, A. and Bengtsson, A. Investigation of clostridial spores in Swedish dairy herds. In: 14th International Symposium Forage Conservation, March 17-19 2010. Brno, Czech Republic, p. 80-82.
- Müller, C.E., T.M. Pauly, and P. Udén, 2007. Storage of small bale silage and haylage – influence of storage period on fermentation variables and microbial composition. Grass and Forage Science, 2007. 62(3): p. 274-283.
- Müller, C.E. and A. Johansen, Rebaling of silage and haylage and its effects on forage microbial and chemical composition-A pilot study. Grass and Forage Science, 2020. 75: p. 216-226.
- 40. Venslovas, E., et al., The prevalence of mycotoxins and their relation to nutrient composition of maize and grass silage. Zemdirbyste-Agriculture, 2021. 108: p. 147-152.
- 41. Spörndly, R. and R. Nylund, Net replacement film in round bale ensiling of ley crops. Grassland Science in Europe, 2016. 21: p. 284-286.
- 42. Saarisalo, E., et al., Effect of lactic acid bacteria inoculants, formic acid, potassium sorbate and sodium benzoate on fermentation quality and aerobic stability of wilted grass silage. Agricultural and Food Science, 2006. 15: p. 185-199.
- 43. Saarisalo, E., et al., Screening and selection of lactic acid bacteria strains suitable for ensiling grass. Journal of Applied Microbiology, 2007. 102: p. 327-336.
- Seppälä, A., et al., Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. Grass and Forage Science, 2016. 71: p. 458-471.
- 45. Jatkauskas, J., et al., The effects of three silage inoculants on aerobic stability in grass, clover-grass, lucerne and maize silages. Agricultural and Food Science, 2013. 22: p. 137-144.
- 46. Jatkauskas, J., V. Vrotniakiene, and A. Lanckriet, The effect of different types of inoculants on the characteristics of alfalfa, ryegrass and red clover/ryegrass/timothy silage. Zemdirbyste-Agriculture, 2015. 102: p. 95-102.

- 47. Stoškus, R., et al., The effect of mixed bacterial inoculant on the microbial population and aerobic stability of lucerne and maize balage. Journal of Animal and Feed Science, 2019. 28: p. 383-391.
- Auerbach, H., and E. Nadeau, Effects of additive type on fermentation and aerobic stability and its interaction with air exposure on silage nutritive value. Agronomy, 2020. 10: 1229; <u>http://dx.doi.org/10.3390/agronomy10091229</u>
- 49. Jatkauskas, J., and V. Vrotniakiene, Evaluation of fermentation parameters, microbiological composition and aerobic stability of grass and whole crop maize silages treated with microbial inoculants. Zemdirbyste-Agriculture, 2013. 100: p. 143-150.
- Rinne, M., et al., Fibrolytic enzyme treatments prior to ensiling increased pressjuice and crude protein yield from grass silage. Bioresource Technology, 2020. 299: p. 122572
- Dehghani, M.R., et al., Effect of enzyme addition to forage at ensiling on silage chemical composition and NDF degradation characteristics. Livestock Science, 2012. 150: p. 51-58.
- 52. Hetta, M., et al., The effect of additives in silages of pure timothy and timothy mixed with red clover on chemical composition and in vitro rumen fermentation characteristics. Grass and Forage Science, 2003. 58: p. 249-257.
- 53. Kasmaei, K.M., et al., Does glucose affect the de-esterification of methyl ferulate by Lactobacillus buchneri? Microbiologyopen, 2020. 9: p. e971.
- 54. Randby, A.T. and A.K. Bakken, Effect of acid based additive treatment of low dry matter grass crops on losses and silage quality in bunker silos. Animal Feed Science and Technology, 2021. 275: p. 114869.
- 55. König, W., et al., Effects of sodium nitrite treatment on the fermentation quality of red clover-grass silage harvested at two dry matter concentrations and inoculated with clostridia. Agricultural and Food Science, 2019. 28: p. 55-164.
- Randby, A.T., The effect of some acid-based additives applied to wet grass crops under various ensiling conditions. Grass and Forage Science, 2000. 55: p. 289-299.
- 57. Knicky, M. and R. Spörndly, Sodium benzoate, potassium sorbate and sodium nitrite as silage additives. Journal of the Science of Food and Agriculture, 2009.
 89: p. 2659-2667.
- Knicky, M. and Spörndly, Short communication: Use of a mixture of sodium nitrite, sodium benzoate, and potassium sorbate in aerobically challenged silages. Journal of Dairy Science, 2015. 98: p. 5729-5734.
- Knicky, M. and P. Lingvall, Ensiling of high wilted grass-clover mixture by use of different additives to improve quality. Acta Agriculturae Scandinavica Section A - Animal Science, 2004. 54: p. 197-205.
- Knicky, M., R. Spörndly, and H. Gonda, Estimation of VOC emission from silages in Sweden. In: Proceedings of the 10th Nordic Feed Science Conference, Uppsala, Sweden, 2019, p. 154-157.

- 61. Nadeau, E., Sousa, D.O. and Auerbach, H. Forage protein quality as affected by wilting, ensiling and the use of silage additives. Proceedings of the 10th Nordic Feed Science Conference, Uppsala, Sweden, 2019, p. 28-33.
- 62. Kasmaei, K.M., et al., Prediction models of silage fermentation products on crop composition under strict anaerobic conditions: A meta-analysis. Journal of Dairy Science, 2013. 96: p. 6644-6649.
- 63. Udén, P. Fresh and ensiled forage plants-total composition, silage losses and the prediction of silage composition from the crop. Grass and Forage Science, 2018.
 73: p. 420-431.
- 64. Udén, P. 2018. Plant organic acids in fresh and ensiled forage plants. Grass and Forage Science, 2018. 73: p. 583-587.
- 65. Jaakkola, S., et al., Effects of restriction of silage fermentation with formic acid on milk production. Agricultural and Food Science, 2006. 15: p. 200-218.
- 66. Jaakkola, S., V. Kaunisto, and P. Huhtanen, Volatile fatty acid proportions and microbial protein synthesis in the rumen of cattle receiving grass silage ensiled with different rates of formic acid. Grass and Forage Science, 2006. 61: p. 282-292.
- 67. Nadeau, E., et al., Protein quality of grass silage as affected by silage additives and its effects on dairy cow performance. Journal of Animal Science, 2015.
 93(Suppl. s3): p. 206
- 68. Saarisalo, E., E. Skyttä, and S. Jaakola, Effects of wilted grass silages varying in fermentation quality on rumen fermentation of dairy cows. Journal of Animal and Feed Sciences, 2004. 13(Suppl. 1): p. 199-202.
- 69. Rinne, M., et al., Effects of grass silage additive type and barley grain preservation method on rumen fermentation, microbial community and milk production of dairy cows. Agriculture, 2022. 12: p. 266.
- 70. Huuskonen, A., A. Seppälä, and M. Rinne, Effects of silage additives on intake, live-weight gain and carcass traits of growing and finishing dairy bulls fed prewilted grass silage and barley grain-based ration. Journal of Agricultural Science, 2017. 155: p. 1342-1352.
- 71. Jatkauskas, J. and V. Vrotniakiené, The effects of the forage preservation methods on fermentation parameters in ruminal fluid of fattening bulls. Journal of Animal and Feed Sciences, 2004. 13(Suppl. 1): p. 107-110.
- 72. Jatkauskas, J. and V. Vrotniakiené, Effects of silage fermentation quality on ruminal fluid parameters. Biologija, 2006. 4: p. 65-71.
- 73. Nadeau, E., and A. Arnesson, Intake and performance of ewes and lambs fed grass-clover silage treated with chemical additives. Grassland Science in Europe, 2016. 21: p. 479-481.
- 74. Santamaria-Fernandéz, M., et al., Demonstration-scale protein recovery by lactic acid fermentation from grass clover a single case of the production of protein concentrate and press cake silage for animal feeding trials. Biofuels, Bioproducts & Biorefining, 2019. 13: p. 502–513.
- 75. Santamaría-Fernandéz, M., N.K. Ytting, and M. Lübeck, Influence of the development stage of perennial forage crops for the recovery yields of

extractable proteins using lactic acid fermentation. Journal of Cleaner Production, 2019. 218: p. 1055-1064.

- 76. Jørgensen, U., S.K. Jensen, and M. Ambye-Jensen, Coupling the benefits of grassland crops and green biorefining to produce protein, materials and services for the green transition. Grass and Forage Science, 2022. 77: p. 295-306.
- 77. Thers, H., et al., Linking protein quality in biorefinery output to forage crop crude protein input via the Cornell Net Carbohydrate and Protein System. Applied Biochemistry and Biotechnology, 2021. 193: p. 2471-2482.
- 78. Nadeau, E., F. Dahlström, and D. Sousa, 2022. Feed value of pulp from fresh and ensiled grass-clover forage. Grassland Science in Europe, 2022. 27: p. 231-234.
- Franco, M., et al., Grass silage for biorefinery A meta-analysis of silage factors affecting liquid-solid separation. Grass and Forage Science, 2019. 74: p. 218-230.
- 80. Hansen N.P., et al., Effect of screw pressing and days of regrowth on grass silage characteristics and quality. Grassland Science in Europe, 2020. 25: 620-622.

4. Feed Value for Animals

4.1 Dairy Cattle

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4.1.1 Maturity stage at harvest

Grass silage is usually the main component of dairy cow diets in the Nordic countries because of its relatively low production cost and high nutritive and feeding value for dairy cows, especially when produced at an early growth stage. Forage maturity at harvest may be the major factor influencing the nutritive value of grass silage because of its effect on digestibility, as lignification of fibre increases with advanced maturity, leading to reduction in digestibility. Harvest of grass for silage at an early stage of maturity is expected to result in silage with a high concentration of energy and crude protein (CP), increasing energy intake and production. Feed intake depends on both physical characteristics and chemical composition of the ration and is, therefore, influenced both by digestibility and rate of passage from the rumen.

Rinne et al. [1] evaluated the effect of increased grass maturity on the performance of Finnish Ayrshire cows. Four silages were harvested at 1-week intervals from a primary growth of a timothy-meadow fescue sward on June 13, June 21, June 28, and July 4, 1994. Cows had ad libitum access to the silages and were supplemented with a fixed amount of concentrate per day. Contents of neutral detergent fibre (NDF) and indigestible NDF (iNDF) of the silages increased and DM intake, milk yield (MY) and energy-corrected milk (ECM) of the cows decreased as maturity progressed, specifically after the second harvest date. As diets were formulated to have the same amount of concentrate, regardless of the chemical composition of the silages, intake and milk production were driven by the composition of the diets. Diets containing silages with lower NDF contents (first and second harvests) resulted in cows with greater intake and milk production.

Similarly, Randby et al. [2] evaluated timothy-dominated sward harvest for silage at very early, early, and normal maturity stage in the primary growth. Silages were fed ad libitum with fixed amount of concentrate to Norwegian Red dairy cows and the authors observed that DM intake, MY and ECM decreased linearly as maturity progressed.

As maturity advances fibre components accumulate, affecting intake and performance negatively. However, Sousa et al. [3] evaluated the effect of early and very early maturity stages of tall fescue and timothy harvested at a 6-day interval (May 25 and May 31) on the performance of dairy cows. Diets were formulated to have the same forage-to-concentrate ratio (49:51) and were offered as total mixed ration (TMR) to Swedish Holstein and Red breed cows. The authors concluded that harvesting tall fescue or timothy at early or very early maturity stage did not affect DM intake, MY and ECM of dairy cows. Using the same grass species, Sousa et al. [4] evaluated the effect of regular and late harvest date of tall fescue and timothy in the first regrowth cycle on the performance of dairy cows. Diets were formulated to have the same forage-to-concentrate ratio (46:54) and were offered as TMR to Swedish Holstein and Red breed cows. The authors concluded that DM intake was greater in the diet that had the lowest content of NDF and maturity stage at harvest did not affect milk production.

Adler et al. [5] harvested grass-clover for silage at early and regular harvest date and fed it to Norwegian Red dairy cows in diets containing forage-to-concentrate ratios of 60:40 or 90:10. Authors observed that early harvest resulted in lower crop yields (2.17 t DM/ha) compared with regular (4.66 t DM/ha) and there was no difference between treatments on DM intake, MY and ECM. A common practice when evaluating forage for ruminants is to use the highest inclusion of forage in the diet as possible, believing that more forage in the diet will increase the chances of seeing a significant effect. However, high-forage diets will increase the rumen retention time and, consequently, increase the extent of digestion of feeds in the rumen. Thus, the high- and low-quality forages will likely be similar in digestion due to the longer time in the rumen. On the other hand, using the lowest possible inclusion of forage in the diet will increasing the chances of higher-quality forages to be more extensively digested than lowerquality forages due to the shorter rumen retention time.

The primary growth of timothy grass was harvested for silage at early and late maturity stages at 2-week intervals and fed to Swedish or Nordic Red dairy cows in two similar studies [6, 7]. Tahir et al. [6] harvested on 12 and 26 June in 2008, and Pang et al. [8] on June 17 and July 1 in 2015. Diets were formulated to have the same forage-to-concentrate ratio, regardless of the chemical composition of the forages. The content of NDF was greater in the late maturity silages and, consequently, authors observed that DM intake, MY and ECM were greater for cows fed early maturity grass silage-based diets compared to cows fed late maturity

grass silage-based diets. Similarly, Cabezas-Garcia et al. [9, 10] harvested timothyred clover for silage 2 weeks apart at early (June 10) and late (June 24, 2013) dates from one primary growth sward and fed to Swedish or Nordic Red dairy cows. The experimental diets were formulated to meet the energy and protein requirements but NDF content was greater for cows fed early maturity silage compared to late maturity. Due to the higher content of dietary NDF, DM intake was lower for cows fed early maturity silage compared to late maturity. However, as the diets contained the same concentrations of energy and protein there was no difference between treatments regarding MY and ECM. With that, the feed efficiency (ECM/DMI) was greater for cows fed early maturity silage.

Vanhatalo et al. [11] harvested primary growths of mixed timothy-meadow fescue and pure red clover swards for silage at early and late dates. Grass crops were harvested on June 17 (early heading) and June 26 (heading), and the red clover crops on July 2 (pre-flowering) and July 16 (early flowering), respectively. Silages were fed ad libitum with fixed amount of concentrate to Finnish Ayrshire cows. The authors observed that there was no effect of maturity stage on DM intake, MY and ECM in cows fed red clover or timothy-meadow fescue silages. However, [12] compared grass-clover silages harvested at early and late maturity stages during the spring and summer seasons. The four treatments were harvested as early first cut (May 9) in spring, a second cut with long regrowth (June 22, 42-d regrowth) in spring/early summer, an early fourth cut (August 7, 2009, 17-d regrowth) in summer, and a late fourth cut (August 23, 2009, 33-d regrowth) in summer. Diets were offered as TMR under two forage-to-concentrate ratios (80:20 or 50:50) to Holstein dairy cows. When the forage-to-concentrate ratio was 80:20, DM intake, MY and ECM were smaller only for cows fed late spring season grass-clover silages. However, when the forage-to-concentrate ratio was 50:50, cows fed early maturity silages showed greater DM intake, MY and ECM than cows fed late maturity silages, regardless of the season. According to the authors, the difference in DM intake, MY and ECM between maturity stages were related to differences in forage digestibility.

4.1.2 Harvest cut and maturity stage

Most studies investigating grass maturity effects on feed intake and milk production have been conducted using silages made from primary growth. However, a large amount of regrowth grass silages is used for dairy cows and few studies in Nordic conditions addressed this topic. First regrowth grass is typically leafier, contains less structural carbohydrates but is less digestible than the corresponding primary growth grass due to the higher proportion of indigestible NDF in the cell wall [13]. So, even though the regrowth is the same plant as the primary growth, it is a completely different material regarding its chemical composition and because of that the direct comparison between growths for animal feeding should not be done based on the amount of forage included in the diet but based on the chemical composition of the diet. The intake of ruminants is mainly regulated by the content of fibre (NDF), starch, and water-soluble carbohydrates (WSC). Thus, the comparison of forages should be done by formulating the diets according to the chemical composition of the forages, aiming to be iso-NDF and iso-starch in order to have the forages as the only difference between treatments.

Kuoppala et al. [13] compared silages made from different cuts and maturity stages on the performance of lactating Finnish Ayrshire cows. Silages were prepared from the primary growth of a timothy-meadow fescue sward at early and late stages of growth. Then, the regrowth of each primary growth (early and late) were harvested at early and late stages, resulting in six silages in total, as follows: Primary growth harvested early (1) and late (2), regrowth from early primary growth harvested early (3) and late (4), and regrowth from late primary growth harvested early (5) and late (6). The primary growth silages were harvested on 5 June at early (1) and on 17 June at late (2) stages of growth. The regrowth silages were harvested from the regrowth areas on 29 July at early (3 and 4) and on 12 August at late (5 and 6) stages of growth.

The authors observed that DM intake, MY and ECM were greater for cows fed primary growth silages than any regrowth. When comparing both regrowths, DM intake, MY and ECM were greater for cows fed late regrowth compared to early regrowth. The effect of maturity stage was more evident in the primary growth, where grass harvested at early maturity increased intake and milk production of dairy cows. However, silages were fed ad libitum with fixed amount of concentrate. As the regrowth silage had a greater content of NDF, the diets varied in filling effects, not giving the cows the same chance to eat the diets equally. Additionally, the greatest NDF content was observed in the early regrowth silage, which is also the silages that resulted in the lowest DM intake, MY and ECM observed in the cows.

Likewise, Kuoppala et al. [14] compared primary growth and regrowth timothymeadow fescue silages harvested at two maturity stages to lactating Finnish Ayrshire cows. Even though the silages varied in chemical composition, it was fed ad libitum with a fixed amount of concentrate. The authors observed that DM intake, MY and ECM were greater for cows receiving primary growth silage compared to cows fed regrowth silage. Regarding the maturity stage, cows fed silages harvested at early date showed greater DM intake, MY and ECM than cows fed silages harvested at late date.

Khalili et al. [15] and Pang et al. [16] evaluated the effects of feeding grass silages harvested at primary growth or its regrowth on the performance of dairy

cows. Both studies concluded that dairy cows receiving primary growth grass silages showed greater DM intake, MY and ECM compared to cows fed regrowth grass silages. On the other hand, Naadland et al. [17] evaluated the effects of gradually replacing organic grass-clover silage from primary growth with regrowth on the performance of Norwegian Red cows. Experimental treatments comprised diets with replacement of primary growth with regrowth silage in the proportions 0, 0.33, 0.67 and 1 on a DM basis. Authors observed that DM intake decreased linearly with the replacement of primary growth by regrowth but MY and ECM were greater when primary growth and regrowth silages were mixed (0.33 and 0.67) compared to primary growth or regrowth silages fed alone.

In order to evaluate the effect of a three-cut system on forage quality and milk production, Sairanen et al. [18] compared timothy-meadow fescue silages harvested at primary growth, first and second regrowth on the performance of dairy cows. The study was repeated three times, during different years, which differed markedly in weather conditions. Silages were fed ad libitum with fixed amount of concentrate. Chemical composition of the silages, intake and milk production varied greatly across the years, but, overall, cows fed the second regrowth (third cut) showed the highest feed efficiency.

Later, Pang et al. [7] compared only the regrowths (first and second regrowths) on milk production. Before harvesting the experimental silages, a sward containing primary growth of timothy-red clover (80:20) was cut at early and late date. Then, the regrowth from early and late primary growth were harvested at early and late date (making four regrowth silages). Additionally, one second regrowth was harvested from early regrowth from the early primary growth. The five silages were fed to lactating Nordic Red cows. The authors found that the highest feed efficiency was observed in cows fed second regrowth (third cut), followed by the first regrowth harvested early, and the lowest feed efficiency was in cows receiving silages from the first regrowth harvested late.

4.1.3 Ley species

Leys with high DM yields per hectare are important for a profitable milk production system but at the same time dairy cows require forages that are highly digestible and relatively high in protein to support lactation performance. Yield and quality of different grass and legume species are highly dependent on genetic and phenotypic differences, soil types, geographical location, weather conditions, and farming management. Therefore, selection of species is important for an optimal forage production. The experimental comparison of different grass and clover species in lactating dairy cows is very scarce due to its complexity. Thus, only a few studies comparing grass and clover species on dairy cow performance were conducted in Nordic conditions. Sousa et al. [4] compared two grass species (timothy and tall fescue) harvested at regular or late maturity stage in the first regrowth and observed that cows fed timothy silages showed greater MY and ECM than cows fed tall fescue silages, regardless of the maturity stage at harvest. However, when timothy and tall fescue were harvested at very early or early maturity stage in the primary growth [3], MY was similar between species and there was a tendency of greater ECM when cows received timothy compared to tall fescue.

Johansen et al. [19] examined how silages from the primary growth of different grass and clover species affect intake and milk production of Danish Holstein dairy cows. Eight treatments were evaluated (4 grasses, 2 clovers and 2 grass-clover combinations). The grasses grown in monocultures were: early perennial ryegrass, festulolium, tall fescue and late perennial ryegrass. Tall fescue, festulolium, and half of the perennial ryegrass (early perennial ryegrass) were mown May 21. The remaining perennial ryegrass (late perennial ryegrass), red clover, and white clover were all mown June 3. The developmental stage at harvest was elongation stage with 1, 2, and 3 nodes noticeable or visible for tall fescue, early perennial ryegrass, and festulolium, respectively, reproductive stage with visible spikelets for late perennial ryegrass, and late vegetative stage for red clover. White clover was in a vegetative stage without buds. The grass-clover combinations were: 50% red clover:50% late perennial ryegrass and 50% white clover:50 % late perennial ryegrass. Intake and milk production varied greatly, so feed efficiency that correlates intake to milk production is a better parameter to evaluate the effects of the treatments. The authors observed that the feed efficiency was greater for cows fed festulolium, late perennial ryegrass, 50% white clover:50% late perennial ryegrass, and white clover compared with cows that received red clover. Furthermore, the feed efficiency of cows receiving early perennial ryegrass, tall fescue, and 50% red clover:50% late perennial ryegrass did not differ among treatments.

However, it is important to state that diets were not adjusted to be iso-NDF in order to give the chance for the cows to eat equally. Diets were formulated to have the same inclusion of forage in the diet (70%), resulting in diets varying greatly in NDF concentration. With that intake and milk production of the cows were regulated based on the composition of the diets and not on the experimental forages. On the other hand, Steinshamn and Thuen [20] compared silages containing a mixture of timothy, meadow fescue and perennial ryegrass with white clover or red clover in iso-NDF diets on performance of Norwegian Red dairy cows. The authors observed that intake and milk production were not affected by treatments. Grass-clover proportion was on average 65:35, respectively; and silages were prepared from the second and third cut.

In a typical dairy cow diet based on grass-clover silage an imbalance between N and energy may exist, leading to low N efficiency. With that, Bertilsson et al. [21]

hypothesized that an increase in sugar content in grasses would be beneficial for N efficiency through increased microbial activity in the rumen, and could possibly lead to higher milk production. Thus, the authors compared two ryegrass cultivars (standard vs. high-sugar) that were mixed with red clover silage (75:25, DM basis) and fed ad libitum to Swedish Red breed cows. Authors concluded that neither DM intake, MY or ECM of the cows were affected by the experimental ryegrass cultivars.

4.1.4 Grass-to-legume ratio

Grass or grass-clover silage is the main ingredient in dairy cow diets. Due to its ability to fix atmospheric N, inclusion of clovers are of high value in leys and especially in organic and low input dairy production systems. Furthermore, due to the rising costs of inorganic N fertilizers and attempts to decrease the environmental footprint of ruminant livestock, clovers can be advantageous in conventional dairy production systems as well. This is confirmed in studies conducted in Nordic conditions, which evaluated the use of clovers as a sole forage and in mixture with grass silages, and showed a potential benefit on performance of dairy cows.

Vanhatalo et al. [22] evaluated the performance of Finnish Ayrshire dairy cows fed diets containing a forage-to-concentrate ratio of 60:40. Forage was composed by either grass or grass-red clover silages. The grass sward was composed of 94% meadow fescue and 6% timothy and the grass-red clover sward was composed of 60% grass and 40% red clover. The authors observed that there was no difference in DM intake between treatments, but MY (18.4 vs. 17.2 kg/d) and ECM (19.6 vs. 18.5 kg/d) were greater for cows fed grass-red clover silage compared to cows fed grass silage only, respectively. According to the authors, cows fed grass-red clover silage likely had an improved utilization of feed nutrients of the diet.

Halmemies-Beauchet-Filleau et al. [23] evaluated the gradual replacement of grass silage by red clover silage in the diet of Finnish Ayrshire cows. The diets had a fixed forage-to-concentrate ratio of 60:40 and red clover silage replaced grass silage at a gradual ratio of 0:100, 33:67, 67:33, and 100:0. The grass silage was composed by a mix of timothy and meadow fescue. The authors observed that DM intake, MY and ECM were greater for cows receiving a mixture of grass and red clover silages, regardless of the proportion (33:67 or 67:33) compared to either forage alone (grass silage or red clover silage).

Similarly, Gidlund et al. [24] compared two grass-red clover silage-based diets varying in grass to red clover proportions. The grass silage was a regrowth sward cut from a two-year-old ley of timothy and red clover (seed rate 80:20) harvested on 8 August in 2012. The red clover silage came from a primary growth and a regrowth of a one-year-old pure red clover ley, which was harvested on 6 July, while the regrowth was harvested on 15 August. Diets consisted of grass to red

clover ratio of 70:30 or 30:70, where the red clover proportion consisted of 50% from the primary growth and 50% from the regrowth on a DM basis. Diets were based on a forage-to-concentrate ratio of 60:40 and were fed to lactating Swedish Red cows. The authors observed that DM intake, MY and ECM were not affected by treatments. Diet digestibility was not measured in the present study, but the higher iNDF concentration in regrowth than primary growth red clover silage (155 compared with 106 g/kg DM) indicates lower digestibility of the former.

Red clover is a dominant grassland legume in cool temperate regions because of its generally higher yields compared with white clover, but under less favourable growth conditions red clover and white clover have a more similar yield potential and white clover-grass mixture tends to improve in yield over time compared to red clover-grass mixture [25], as white clover could be more persistent and better adapted to frequent defoliation than red clover. Thus, a few studies were performed in cool temperate regions in order to evaluate the performance of dairy cows fed white clover as sole forage and mixed with grasses.

Bertilsson and Murphy [26] evaluated the performance of Swedish Red Breed cows fed silages of perennial ryegrass, red clover, white clover or grass/red clover/white clover mixture (50% grass/50% clover) ad libitum and a fixed amount of concentrate (8 kg/d) for two consecutive years. Silages were made from monocultures of perennial ryegrass, red clover and white clover. The authors observed that DM intake, MY and ECM were similar among treatments during both years of the study. Steinshamn and Thuen [20] compared grass-clover silages on the performance of Norwegian Red dairy cows. The grass was composed by a mixture of timothy, meadow fescue, and perennial ryegrass. The grass was grown, harvested and ensiled with either white or red clover. The authors did not observe any difference between cows fed grass-white clover silage and grass-red clover silage regarding DM intake, MY and ECM. Eriksson et al. [27] compared two silage-based diets containing late harvested perennial ryegrass with either birdsfoot trefoil or white clover on the performance of Swedish Red Breed dairy cows. The authors concluded that there was no effect of experimental silages on DM intake and ECM, but there was a tendency for greater MY when cows received ryegrassbirdsfoot trefoil silage compared to ryegrass-white clover silage.

4.1.5 Forage-to-concentrate ratio

Swedish conventional dairy cows are fed diets with an average forage-toconcentrate proportion of 50:50 over a large portion of the lactation, and the use of feeds with a high concentration of metabolisable energy and protein, such as cereal grains and protein concentrate, is necessary in order to supply the energy and protein requirements, especially in early lactation. Prioritizing the use of grass and grass/clover silage over concentrate in rations for dairy cattle is important for several reasons. Grasslands have higher carbon sequestration [28], and more dry matter and protein yields per hectare than most annual crops. Further, grass is often cheap compared with other crops. Nevertheless, limiting the use of purchased concentrate for dairy cows and replacing it with home-grown high quality forage without compromising milk production can offer benefits in both organic and conventional dairy systems.

Patel et al. [29] evaluated three diets, all consisting of highly digestible silage (early first-cut ley of timothy and meadow fescue) and concentrate, but with different forage-to-concentrate ratios: 1) 50:50, 2) 70:30 and, 3) 90:10 g/kg DM. Total DM intake did not differ between treatments but MY (kg/d) was higher in the 50:50 diet compared to the 90:10 diet, with the 70:30 in between (21.4, 19.0 and 19.9, respectively). However, when calculated as kg ECM/d there were no differences between diets, 22.5, 22.2 and 21.4 for 50:50, 70:30 and 90:10 respectively.

Patel et al. [29] used Swedish Red Breed cows to evaluate three grass silagebased diets varying in forage-to-concentrate proportions. The three diets were composed of the same feeds: grass-clover silage and grain-based concentrate, but the forage-to-concentrate ratio differed (50:50, 70:30 or 85:15). The silage was made from leys dominated by timothy, meadow fescue and red clover. The authors observed that DM intake did not differ between treatments, but milk production was negatively affected as forage proportion increased, where milk yield was 31.1, 25.8 and 24.8 kg/d and ECM was 32.2, 28 and 26.6 kg/d for 50:50, 70:30 and 85:15 diets, respectively.

However, as shown above, a forage proportion in the diet greater than 70:30 seems to compromise milk production. In this regard, Patel et al. [30] conducted a full lactation trial comparing three diets differing in the mean forage-to-concentrate proportion over the lactation (50:50, 60:40 or 70:30). The diets were designed to represent a conventional dairy feeding, an organic production and a more extreme high-forage-based production, respectively. A high-quality grass-clover silage from leys of timothy, meadow fescue and red clover was harvested from primary growth at the end of May in both years, when the grass had reached the maturity stage of ear emergence. The authors observed that DM intake was similar between treatments (20.3, 20.4 and 19.9 kg in 50:50, 60:40 and 70:30, respectively) and ECM did not differ between cows fed diets containing 50:50 or 60:40 forage-toconcentrate ratio (31.3 vs. 31.1 kg ECM/d, respectively), but it was lower when 70:30 forage-to-concentrate ratio was fed (29.2 kg ECM/d). Increasing forage proportion from 50 to 60 % of DM intake did not have any adverse effects on milk production or DM intake. Thus, it is possible to produce the same quantity of milk with much less concentrate per cow on an annual basis. The lower use of concentrates in 60:40 compared with 50:50 provides benefits in terms of lower dependence on purchased feeds and decreases the sensitivity to price fluctuations.

The use of high-quality grass-clover silage in diets for dairy cows has significant potential regardless of production system, organic or conventional, which is crucial in future production systems to overcome the competition for land.

4.1.6 Replacing grass silage by whole-crop silage

Several studies performed in the United Kingdom have demonstrated that a partial replacement of grass silage with whole-crop cereals may not affect milk production in dairy cows [31-33]. In Nordic conditions, Ahvenjarvi et al. [34] examined the effects of gradually replacing grass silage with whole-crop barley silage on feed intake and milk yield in lactating Ayrshire dairy cows and observed similar results. In this study, grass silage was prepared from a primary growth of timothy and meadow fescue or whole-crop barley that was harvested 82 d after sowing and 27 d after the start of heading at the early dough stage. The diets were based on grass silage that was gradually replaced by whole-crop barley silage. The proportion of barley silage in the forage was adjusted to 0, 0.20, 0.40, and 0.60 kg/kg of DM replacing grass silage. The authors concluded that DM intake did not differ between treatments but milk yield and fat corrected milk (FCM) decreased linearly as grass silage was replaced by whole-crop barley silage. Milk yield was 33.2, 32.2, 31.4, and 30.9; FCM was 33.1, 33.2, 32.8, and 31 for cows receiving whole-crop barley silage at 0, 0.20, 0.40, and 0.60 kg/kg of DM of replacement rate, respectively.

Alternatively to whole-crop cereals, whole-crop faba bean was evaluated in attempt to mix with grass silage to feed dairy cows. Faba bean is an annual legume and its whole-crop digestibility and, subsequently, energy value is typically lower than a high-quality grass silage, which would be expected to decrease energy intake and subsequently milk yield of dairy cows. However, including faba bean silage at a correct proportion has resulted in positive associative effects in feed intake and milk production. Lamminen et al. [35] compared the effects of grass silage and grass-faba bean silage (50:50 ratio) on the performance of dairy cows and the authors observed that DM intake, MY and ECM were not affected by treatments. On the other hand, when grass silage was mixed with whole crop faba bean silage at a proportion of 75:25 [36], cows fed grass-whole crop faba bean silage showed greater DM intake, MY and ECM than cows fed grass silage only.

4.1.7 Silage versus hay

Milk fat is highly dependent on the breed of the cows. It is well known that the Swedish Red breed (SRB) and Finnish Ayrshire have higher milk fat contents when compared to the American Holstein breed. However, management practices such

as forage conservation method and diet formulation can also affect the milk fat yield. Murphy et al. [36] using SRB dairy cows compared diets consisted of either hay or silage with a forage-to-concentrate ratio of either 30:70 or 50:50. Both hay and silage, were harvested from the same field and composed by late first-cut mixtures of timothy and meadow fescue. The hay was harvested 5 d after the silage. The authors found that DMI was higher for cows fed 50:50 forage-to-concentrate ratio diet, and specifically higher when cows received hay compared to silage. However, milk, fat corrected milk (FCM) and fat yields were generally greater when cows received silage instead of hay as the forage source, regardless of the forage-to-concentrate ratio. Cows fed silage-based diets produced 0.8 kg/d more milk, 0.9 kg/d more FCM, and 0.04 kg/d more milk fat than when hay-based diets were fed.

Halmemies-Beauchet-Filleau et al. [37] [38] used lactating Finnish Ayrshire cows to compare diets varying in forage conservation method, such as hay- or silage-based diets when studying the effect on intake and milk production. Forages were prepared from the same primary growths of timothy and meadow fescue on June 15, 2005. Grass used for the production of hay was wilted extensively in the field over a 4-d period to a final DM content of 750 g/kg and baled and grass used for the production of silage was cut using a mower-conditioner, wilted in the field for 3 to 4 h to a DM content of 235 g/kg, before being collected with a precision-chop harvester and ensiled in bunker silos. The authors observed that cows receiving silage-based diet showed greater DM intake (+1.2 kg/d), MY (+2.7 kg/d), ECM (+2.6 kg/d), and milk fat (+0.13 kg/d) compared to cows fed a hay-based diet.

4.1.8 N fertilization

Dairy farming based on leys is dependent on the use of nitrogen (N) fertilizer in order to provide sufficient herbage mass and protein yields to sustain milk production at economically attractive levels. Fertilizers have traditionally been applied at rates corresponding to optimal economic forage performance rather than on the basis of the efficiency of animal utilization. Ensiling heavily fertilized swards may further exacerbate N emissions, since part of the protein contained in grass silage is rapidly and extensively degraded in the rumen and excreted primarily through the urine but also in the faeces, resulting in environmental concerns. One option to increase the efficiency of N utilization in grass silage-based diets is to reduce the protein content of ensiled herbage, either by delaying harvest or restricting the use of fertilizer N. However, ensiling grass at an advanced stage of maturity is inappropriate for dairy cows due to concomitant decreases in digestible energy content caused by the lignification process. In contrast, reductions in herbage protein content by decreasing the use of N fertilizer may improve efficiency and N utilization without compromising the performance of dairy cows. Two studies evaluated the effects of N fertilization on grass swards used for silage production on dairy cow intake, milk production and N excretion. In the first study, Shingfield et al. [39] compared two silages prepared from primary growth of timothy and meadow fescue swards fertilized with either 52 or 104 kg N/ha. The grass silages were fed to Finnish Ayrshire cows and the authors concluded that there was no difference between treatments on DM intake, MY and ECM but cows fed grass silage fertilized with 104 kg N/ha excreted more N in the milk, urine and faeces than cows fed grass silage fertilized with 52 kg N/ha. In the second study, Arvidsson et al. [40] evaluated grass silages made from first year timothy leys and subjected to three N fertilization doses (30, 90 and 120 kg N/ha). The grass silages were fed to Swedish Red breed cows and the authors observed a positive effect on DM intake as N fertilization increased but no effect on MY or ECM, so the feed efficiency was negatively related to the N fertilization dose.

4.1.9 Wilting prior to ensiling

Wilting of leys prior to ensiling has been widely adopted as a means to improve silage fermentation and quality, and also to reduce the production of effluents. However, the effects of wilted forages on intake and performance of dairy cows have been variable. Kokkonen et al. [41] evaluated the effect of wilting on the performance of Ayrshire and Friesian dairy cows. Grass silages were made of second cut timothy-fescue swards and harvested with a precision chopper either directly after cutting or after wilting overnight (for 16-20 h). The weather was sunny and windy without any rain. Both silages were ensiled in plastic-covered clamp silos using formic acid as a preservative. The DM content for direct-cut and wilted silage were 186 and 288 g/kg, respectively. The authors observed that DM intake was greater and MY was lower for cows fed wilted silage compared to cows fed direct cut silage, but ECM was similar between treatments.

Due to the great benefits to silage quality and hygiene, moderate wilting is established as a standard procedure of silage making and no other dairy cow experiments has been conducted to evaluate if wilting should be done or not. Later, a study were performed in Nordic conditions, which evaluated the degree of wilting and results showed that, besides the fermentation parameters, wilting degrees did not affect the performance of dairy cows. Johansen et al. [42] evaluated two degrees of wilting on grass-clover silages containing perennial ryegrass, hybrid ryegrass, white clover and red clover. Grass-clover leys were wilted to reach approximately 350 or 700 g/kg DM. Silages were fed to Danish Holstein cows and authors observed that there was no effect on intake and milk production.

4.1.10 Ley mechanical processing

Leys can be cultivated and mechanically processed through a biorefinery system in order to locally produce feedstuff, energy and fertilizers. After screw pressing, grassland plants are separated into a protein-rich juice and a fibrous pulp. The liquid phase can be used as protein supplement for livestock and the fibrous pulp can be suitable as forage source for ruminants. Likewise, both liquid and solid phases can be used to produce bioenergy in the form of methane. The concept of green biorefinery refers to the conversion of fresh biomass into value-added products that leads to local grassland development resulting in more carbon sequestration in grain crop intensive areas; less dependency on protein-rich feed import; and further synergies between agricultural sectors, such as crop and livestock farming. The use of home-grown protein feed is especially important in organic farming, where circular bioeconomy is essential.

The fibrous pulp can be produced from fresh forage directly after harvesting or from ensiled forage. However, there are logistical benefits in producing pulp from silage instead of from fresh forage as smaller amounts of silage can be processed regularly on a dairy farm. Alternatively, pulp from fresh forage can be ensiled in round bales, which can be transported and used over a long period. The mechanical pressing removes moisture and soluble nutrients, increasing fibre content and decreasing the nutritional value of the pulp compared with the original forage. Those differences have to be considered when formulating diets, especially when feeding high-producing dairy cows, because dietary forage NDF content is the primary factor limiting intake and performance when high-producing dairy cows in early-to-mid lactation are fed forage-based diets.

Sousa et al. [43] evaluated the complete substitution of dietary grass-clover silage for its silage pulp (SP) after screw pressing on milk production of dairy cows. The authors expected that the mechanical process of pressing the silage would increase fibre digestibility of SP compared to the original silage. Also, the greater fibre digestibility could compensate for the increased fibre content of the SP-based diet, resulting in similar milk yield of dairy cows fed the silage-based diet. However, the authors concluded that the mechanical pressing process did not increase fibre digestibility in SP, thus, the complete substitution of silage for SP reduced the milk production of dairy cows over time.

Another alternative to use SP in the diet of dairy cows is to partially include it by mixing with silage. Savonen et al. [44] compared diets that substituted 0.25 or 0.50 (DM basis) of the silage with SP to a diet containing only silage as forage. The authors observed that the substitution of 50% of the silage for SP did not affect DM intake or MY, but there was a tendency of lower ECM when compared to the silagebased diet. However, when 25% of the silage was substituted by SP, no difference was reported on MY or ECM but intake was greater, reducing its feed efficiency when compared to the silage-based diet. The authors concluded that the reduction in performance was considered mild and SP can be partially included in the diet of dairy cows if there is a consequent reduced feed cost.

Damborg et al. [45] compared the partial inclusion of ensiled grass-clover pulp or grass-clover silage in the diet of lactating Danish Holstein cows on intake and milk production. Grass-clover containing 45% grass and 55% clover was harvested and either directly ensiled or screw pressed to produce pulp before ensiling. The grass–clover and the pulp were ensiled in bales without additives. The authors observed that intake was not affected by treatments but cows fed pulp-based diets showed greater milk and ECM yields and improved feed efficiency compared to cows fed silage-based diets. However, it is important to highlight that the diets were not iso-NDF and the grass-clover used for ensiling was harvested 6 days after the material used to produce pulp.

Hansen et al. [46] harvested a grass-clover mixture containing perennial ryegrass, festulolium, white clover, and red clover at early and late maturity stages. Grass-clover of each stage was either ensiled or shredded before ensiling. The four treatments were given to primiparous Danish Holstein cows as the sole diet. The authors observed that there was no effect of mechanical processing on DM intake, MY or ECM. Similarly, Rustas et al. [47] also evaluated the effect of maturity stage but the grass silage was either fed directly to the cows or screw pressed right before feeding the cows. Authors observed that the mechanical processing increased DM intake and MY in Swedish Red dairy cows.

4.1.11 Conclusion and knowledge gaps

Most of the studies comparing ley-based diets for dairy cows in Nordic conditions were performed using diets containing the same forage-to-concentrate ratio, regardless of the chemical composition of the forages. How one formulates the diet depends on the aim of the experiment. However, in order to proper evaluate the ley itself, diets have to be formulated based on the chemical composition of the forages to give cows the same chance of eating the diets. Thus, future research or forage comparison at farm level should formulate diets according to the chemical composition of each forage, aiming to have similar chemical composition between diets.

When comparing different ley species it is important to keep in mind that the comparison cannot only be on the performance of the cows but also on the performance of the whole operation. Species with high yields and low nutritive quality will be included in cows' diets at lower levels, requiring more concentrate but on the other hand will be possible to feed more cows per hectare of ley. However, when a low yield and likely high nutritive quality species is used, the dependence of concentrate feeds are reduced but the number of cows fed per hectare of ley will be lower. So, future research should focus on the performance evaluation

of the whole production system and not only the individual performance of the cows. Additionally, due to climate change and drastic variations related to weather conditions, ley production has been, and most likely will continue to be, compromised. Thus, future research could focus on the comparison of ley species that are more adapted to periods of drought and heavy rainfall on the performance of dairy cattle.

The forage-to-concentrate ratio was evaluated by only one group in Nordic conditions and it was stated that a ratio equal or greater than 70:30 compromises performance, however forage-to-concentrate ratios of 50:50 or 60:40 lead to similar performance results, indicating that it is possible to produce the same but using less concentrate, which could impact the profitability of the production system. However, the effects of forage-to-concentrate ratio on the performance of dairy cows will vary according to the quality of the leys, so future research could test the effects of forage-to-concentrate ratio on dairy cows fed leys varying in quality such as different species, maturities and cuts.

Ley biorefinery is a new concept that aims to expand the use of leys and few studies have been recently conducted, but there is a lack of information regarding the order of processing before feeding the cows. Leys can be harvested, ensiled and processed through a biorefinery before feeding or rebaled for later feeding of the cows or it can be harvested, processed through a biorefinery and ensiled/stored to be fed later. There is an operational advantage in ensiling before processing; on the other hand the quality of the liquid component can be increased if the fresh ley is screw pressed or shredded before storing. Thus, future research could focus on unveiling the effects of feeding biorefined fresh or ensiled leys to dairy cows.

4.1.12 References

- Rinne, M., P. Huhtanen, and S. Jaakkola, Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. Journal of Animal Science, 2002. 80(7): p. 1986-1998.
- Randby, A.T., et al., Early lactation feed intake and milk yield responses of dairy cows offered grass silages harvested at early maturity stages. Journal of Dairy Science, 2012. 95(1): p. 304-317.
- 3. Sousa, D.O., et al. Effect of early or very early harvest date of tall fescue and timothy on performance of dairy cows. in EGF. 2020. Helsinki, Finland: Natural Resources Institute Finland (Luke).
- 4. Sousa, D.O., et al., Effects of harvest date and grass species on silage cell wall components and lactation performance of dairy cows. Journal of Dairy Science, 2021. 104(5): p. 5391-5404.
- 5. Adler, S.A. and A.T. Randby. Early or normal cut grass silage for dairy cows in organic farming. In 12th International Symposium of Forage Conservation, Brno,

Czech Republic, 3-5 April, 2006. 2006. Brno, Czech Republic: University of Veterinary and Pharmaceutical Sciences.

- 6. Tahir, M.N., P. Lund, and M. Hetta, The effects of and interactions between the maturity of grass silage and concentrate starch source when offered as total mixed rations on the performance of dairy cows. Animal, 2013. 7(4): p. 580-590.
- Pang, D.G., T.H. Yan, and S. Krizsan, Effect of strategy for harvesting regrowth grass silage on performance in dairy cows. Journal of Dairy Science, 2021. 104(1): p. 367-380.
- Pang, D., et al., Effect of grain- or by-product-based concentrate fed with earlyor late-harvested first-cut grass silage on dairy cow performance. Journal of Dairy Science, 2018. 101(8): p. 7133-7145.
- 9. Cabezas-Garcia, E.H., et al., Effects of replacement of late-harvested grass silage and barley with early-harvested silage on milk production and methane emissions. Journal of Dairy Science, 2017. 100(7): p. 5228-5240.
- 10. Cabezas-Garcia, E.H., et al., Effects of replacement of late-harvested grass silage and barley with early-harvested silage on ruminal digestion efficiency in lactating dairy cows. Journal of Dairy Science, 2018. 101(2): p. 1177-1189.
- Vanhatalo, A., et al., Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. Journal of Dairy Science, 2009. 92(11): p. 5620-5633.
- 12. Alstrup, L., K. Soegaard, and M.R. Weisbjerg, Effects of maturity and harvest season of grass-clover silage and of forage-to-concentrate ratio on milk production of dairy cows. Journal of Dairy Science, 2016. 99(1): p. 328-340.
- 13. Kuoppala, K., et al., The effect of cutting time of grass silage in primary growth and regrowth and the interactions between silage quality and concentrate level on milk production of dairy cows. Livestock Science, 2008. 116(1-3): p. 171-182.
- Kuoppala, K., et al., The effect of harvesting strategy of grass silage on digestion and nutrient supply in dairy cows. Journal of Dairy Science, 2010. 93(7): p. 3253-3263.
- 15. Khalili, H., et al., Effects of silage made from primary or regrowth grass and protein supplementation on dairy cow performance. Livestock Production Science, 2005. 96(2-3): p. 269-278.
- Pang, D.G., et al., Modelling feed intake and milk yield responses to different grass ley harvesting strategies. Grass and Forage Science, 2019. 74(3): p. 509-524.
- 17. Naadland, S.S., et al., Effect of replacing organic grass-clover silage from primary growth with regrowth on N digestion in dairy cows. Animal Feed Science and Technology, 2016. 220: p. 57-66.
- Sairanen, A., et al., A comparison of grass silages harvested at first, second and third cut on feed intake and milk production of dairy cows. Agricultural and Food Science, 2021. 30(2): p. 74-84.
- Johansen, M., et al., Digestibility and clover proportion determine milk production when silages of different grass and clover species are fed to dairy cows. Journal of Dairy Science, 2017. 100(11): p. 8861-8880.

- 20. Steinshamn, H. and E. Thuen, White or red clover-grass silage in organic dairy milk production: Grassland productivity and milk production responses with different levels of concentrate. Livestock Science, 2008. 119(1-3): p. 202-215.
- Bertilsson, J., M. Akerlind, and T. Eriksson, The effects of high-sugar ryegrass/red clover silage diets on intake, production, digestibility, and N utilization in dairy cows, as measured in vivo and predicted by the NorFor model. J Dairy Sci, 2017. 100(10): p. 7990-8003.
- 22. Vanhatalo, A., T. Gaddnas, and T. Heikkila, Microbial protein synthesis, digestion and lactation responses of cows to grass or grass-red clover silage diet supplemented with barley or oats. Agricultural and Food Science, 2006. 15(3): p. 252-267.
- 23. Halmemies-Beauchet-Filleau, A., et al., Effect of replacing grass silage with red clover silage on nutrient digestion, nitrogen metabolism, and milk fat composition in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. Journal of Dairy Science, 2014. 97(6): p. 3761-3776.
- 24. Gidlund, H., M. Hetta, and P. Huhtanen, Milk production and methane emissions from dairy cows fed a low or high proportion of red clover silage and an incremental level of rapeseed expeller. Livestock Science, 2017. 197: p. 73-81.
- Halling, M.A., C.F.E. Topp, and C.J. Doyle, Aspects of the productivity of forage legumes in Northern Europe. Grass and Forage Science, 2004. 59: p. 331-344.
- Bertilsson, J. and M. Murphy, Effects of feeding clover silages on feed intake, milk production and digestion in dairy cows. Grass and Forage Science, 2003. 58(3): p. 309-322.
- Eriksson, T., L. Norell, and N. Nilsdotter-Linde, Nitrogen metabolism and milk production in dairy cows fed semi-restricted amounts of ryegrass-legume silage with birdsfoot trefoil (*Lotus corniculatus* L.) or white clover (*Trifolium repens* L.). Grass and Forage Science, 2012. 67(4): p. 546-558.
- 28. Soussana, J.F., et al., Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use and Management, 2004. 20: p. 219-230.
- Patel, M., et al., Enteric methane emissions from dairy cows fed different proportions of highly digestible grass silage. Acta Agriculturae Scandinavica, 2011. 61: p. 128-136.
- 30. Patel, M., et al., Whole lactation production responses in high-yielding dairy cows using high-quality grass/clover silage. Journal of the Science of Food and Agriculture, 2017. 97(9): p. 2883-2890.
- 31. Phipps, R.H., J.D. Sutton, and B.A. Jones, Forage mixtures for dairy-cows the effect on dry-matter intake and milk-production of incorporating either fermented or urea-treated wheat, brewers grains, fodder beet or maize silage into on grass-silage. Animal Science, 1995. 61: p. 491-496.
- 32. Sinclair, L.A., R.G. Wilkinson, and D.M.R. Ferguson, Effects of crop maturity and cutting height on the nutritive value of fermented whole crop wheat and milk production in dairy cows. Livestock Production Science, 2003. 81: p. 257-269.

- Abdalla, A.L., et al., Digestion in the rumen of lactating dairy cows given mixtures of urea-treated whole-crop wheat and grass silage. Animal Science, 1999. 69: p. 203-212.
- 34. Ahvenjarvi, S., et al., Effects of replacing grass silage with barley silage in dairy cow diets. Journal of Dairy Science, 2006. 89(5): p. 1678-1687.
- 35. Lamminen, M., et al. Partial replacement of grass silage with faba bean wholecrop silage in the diet of dairy cows. in EGF. 2015. Wageningen, Netherlands: Wageningen Academic Publishers.
- 36. Murphy, M., M. Åkerlind, and K. Holtenius, Rumen fermentation in lactating cows selected for milk fat content fed two forage to concentrate ratios with hay or silage. Journal of Dairy Science, 2000. 83(4): p. 756-764.
- 37. Halmemies-Beauchet-Filleau, A., et al., Effect of forage conservation method on plasma lipids, mammary lipogenesis, and milk fatty acid composition in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. Journal of Dairy Science, 2013. 96(8): p. 5267-5289.
- Halmemies-Beauchet-Filleau, A., et al., Effect of forage conservation method on ruminal lipid metabolism and microbial ecology in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. Journal of Dairy Science, 2013.
 96: p. 2428-2447.
- 39. Shingfield, K.J., S. Jaalkola, and P. Huhtanen, Effects of level of nitrogen fertilizer application and various nitrogenous supplements on milk production and nitrogen utilization of dairy cows given grass silage-based diets. Animal Science, 2001. 73: p. 541-554.
- 40. Arvidsson, K., et al., The effect of N-fertilisation rate or inclusion of red clover to timothy leys on fatty acid composition in milk of dairy cows fed a commercial silage: concentrate ratio. Animal, 2012. 6(7): p. 1178-1186.
- Kokkonen, T., et al., Effect of silage dry matter content and rapeseed meal supplementation on dairy cows. 1. Milk production and feed utilisation. Animal Feed Science and Technology, 2000. 84(3-4): p. 213-228.
- 42. Johansen, M., et al., Metabolisable protein supply to lactating dairy cows increased with increasing dry matter concentration in grass-clover silage. Animal Feed Science and Technology, 2017. 227: p. 95-106.
- 43. Sousa, D., M. Larsson, and E. Nadeau, Milk Production of Dairy Cows Fed Grass-Clover Silage Pulp. Agriculture-Basel, 2022. 12(1): p. 11.
- 44. Savonen, O., et al., Grass silage pulp as a dietary component for high-yielding dairy cows. Animal, 2020. 14(7): p. 1472-1480.
- Damborg, V.K., et al., Ensiled pulp from biorefining increased milk production in dairy cows compared with grass-clover silage. Journal of Dairy Science, 2019. 102(10): p. 8883-8897.
- 46. Hansen, N.P., et al., Shredding of grass-clover before ensiling: Effects on feed intake, digestibility, and methane production in dairy cows. Animal Feed Science and Technology, 2021. 282: p. 14.

 Rustas, B.O., M. Managos, and T. Eriksson, Extrusion of Grass Silage Increase Intake and Milk Yield in Dairy Cows. Journal of Animal Science, 2021. 99: p. 176-176.

4.2 Beef Cattle

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4.2.1 Maturity stage at harvest

Yield and quality are major factors to consider when growing leys for animal production systems, and maturity stage at harvest can affect both factors comprehensively. Yield increases and quality decreases with advanced maturity. In grass swards that are mown for silage, an early stage of maturity at harvest is a prerequisite for obtaining high energy and protein in the silage, leading to high energy intake and performance. Digestibility is a key to achieving high intakes and improved animal performance, but cutting silage more frequently at an early maturity stage means lower forage yields, higher cutting costs, reduced life of the stand, and more acreages needed. On the other hand, less frequent cutting means lower quality grass, longer feeding time and greater dependency on concentrate feeds to finish the animals in a reasonable time. It is an important decision, which has to be done individually, based on the conditions of each production system, including different types of breeds. However, two studies were conducted in Nordic conditions to evaluate the effects of maturity stage at harvest on performance of growing beef cattle.

Randby et al. [1] harvested grass-clover swards at three early stages of maturity in the primary growth stages 1 and 2, which were dominated by tillers in stem elongation with two and three visible nodes, respectively, and stage 3, which was dominated by tillers at early heading, with visible heads but without head stems. The harvest at each stage occurred with one week apart from the previous one. Grass-red clover swards were composed of timothy (50%), meadow fescue (35%) and red clover (15%). Silages were fed ad libitum to Norwegian Red bulls with an initial age of 7 months and live weight (LW) of 288 kg at start of the experiment. Generally, there was no difference regarding the performance of bulls fed silages from stages 1 and 2. However, bulls fed silage from stage 3 showed lower intake, live weight gain (LWG; 936 vs. 1342 g/d) and carcass weight gain (465 vs 702 g/d) compared to bulls fed silages 1 and 2. Consequently, the rearing time until slaughter was similar between bulls fed silages 1 and 2 (458 days) but longer for bulls fed silage 3 (543 days). Later, Manninen et al. [2] evaluated the effect of grass-red clover maturity stage on the performance of Hereford bulls with an initial age of 7.5 months and LW of 289 kg at start of the experiment. Second year timothy (65%), meadow fescue (30%) and red clover (5%) swards were harvested for silage

at early (17-18 June) or late (31 June-1 July) maturity stages. The authors observed that bulls fed grass-red clover silage harvested at the early maturity stage showed no difference in intake, but greater LWG (1799 vs. 1609 g/d) and, consequently, shorter rearing time until slaughter (179 vs. 197 days) compared with bulls fed late maturity stage silage. Carcass traits were not affected by treatment.

4.2.2 Harvest cut

In general, early-first cut produces highly digestible grass for silage, but the digestibility of the second cut tends to be lower. However, in Nordic conditions the third cut can have high digestibility as a consequence of low amounts of fibre due to low average temperatures and low radiation during the late-summer period. Furthermore, a three-cut strategy might provide better utilisation of the entire growing season than a two-cut strategy [3, 4]. Two studies were conducted to evaluate the effect of harvest cut on the performance of beef cattle bulls.

Huskonen and Pesonen [3] studied the effects of first-year timothy stands harvested for silage at first-, second-, or third-cut on intake, performance and carcass characteristics of Simmental finishing bulls. Animals were 328 (±13.9) days old and with an initial LW of 475 (\pm 36.8) kg. The experimental diets included either first-, second- or third-cut grass silage mixed with rolled barley in a forage-toconcentrate ratio of 55:45 and fed ad libitum to Simmental bulls. The authors observed that DM intake (11.5 vs. 10.4 kg/d), LWG (2089 vs. 1883 g/d) and carcass weight gain (1301 vs. 1169 g/d) were greater for bulls fed first and third cut grass silage compared with bulls fed second cut silage. As a consequence of the greater weight gain, the slaughter age in days was 449, 463 and 457 for bulls fed first, second and third cut silages, respectively. Huuskonen et al. [5] evaluated the same first- and second-cut silages used in the previous study on the performance of Hereford bulls. The two dietary treatments included either first or second cut grass silage (55% DM basis) supplemented with rolled barley (43.5% DM basis) and a mineral-vitamin mixture (1.5% DM basis). The authors observed that bulls fed firstcut grass silage showed greater DMI (9.93 vs. 9.17 kg/d) and LW gain (1717 vs. 1543 g/d) than bulls fed grass silage harvested at second cut. Consequently, the rearing time to finish bulls fed first-cut grass silage was shorter than when secondcut grass silage was fed (167 vs. 180 d). Carcass traits were not affected by treatment.

4.2.3 Ley species

Over-conditioning prepartum beef cows is expensive and increases the risks of parturition-related problems. On the other hand, negative energy status prepartum

and poor body condition at parturition may have negative effects on cow reproductive performance and on calf weight gain and weaning weight. Therefore, it is important to offer the cows a diet that will neither over- nor under-feed. In the Nordic countries, timothy and meadow fescue are frequently used in mixed grass silages but their suitability in diets to mature beef cows has been questioned due to their relatively high digestibility, which may result in unnecessary large feed consumption that leads to gain in body weight (BW) and body condition score (BCS).

Delaying the harvest of common grass mixtures for silage is a strategy to increase yield and to avoid overfeeding of pregnant suckler cows fed ad libitum. Additionally, festulolium and reed canarygrass are possible alternatives to the traditional timothy-meadow fescue based forage due to the often greater concentrations of NDF and lower digestibility. However, the use of festulolium and reed canarygrass has been studied in dairy cows and sheep, but only two studies were conducted in Nordic conditions where these forages were fed to prepartum suckler cows studying the performance of the cows.

Jardstedt et al. [6] compared three diets based on traditional timothy-meadow fescue silage, festulolium silage plus urea and reed canarygrass silage on the performance of prepartum Hereford cows. After calving, all the cows were fed timothy silage. The authors observed that cows fed timothy-meadow fescue silage and festulolium silage showed greater DM intake (12.9 and 13.9 vs. 9.17 kg/d), BW change (99 and 127 vs. 43 kg) and BCS change (0.32 and 0.77 vs. -0.35) compared to cows fed reed canarygrass silage in the prepartum phase. However, there was no effect of treatments on performance during the early postpartum phase, where cows were fed the same forage. Calves were also evaluated but there was no effect of treatments on calf birth weight, daily growth rate until weaning or adjusted 200-d weaning weight. Similarly, Jardstedt et al. [7] fed the same silages to Hereford pregnant suckler cows and observed that cows fed timothy-meadow fescue silage and festulolium silage showed greater DM intake, but there was no difference regarding final BW compared to cows fed reed canarygrass silage. Reed canarygrass silage appears to be a good alternative to timothy-meadow fescue silage when feeding prepartum beef cows ad libitum, as intake and, consequently, conditioning are lower than when timothy-meadow fescue silage or festulolium silage is fed.

4.2.4 Grass or grass-clover

Although timothy and meadow fescue grasses are predominantly used in grassland farming in the Nordic countries for beef cattle feeding, clovers may play an increasingly significant role in future silage production because of their nitrogen fixation ability. Usually, clovers are grown as a mixture with grass species.

However, different to what is observed for dairy cows, there are only two studies comparing clover silage or mixed grass–clover silage to grass silage in the diet of growing and finishing cattle. Pesonen et al. [8] evaluated silages that were harvested from either monoculture of timothy or mixed timothy and red clover stands. Timothy was harvested at early heading stage (on 30 June and 19 August, primary and regrowth, respectively) and timothy and red clover mixed stands at early flowering of red clover (on 4 July and 24 August). According to botanical determinations, timothy and red clover stands contained timothy (42%), red clover (56%) and other plants (2%). Diets were formulated to have a forage-to-concentrate ratio of 67:33 or 34:66 and fed ad libitum to crossbred bulls (Angus × Nordic Red). At the beginning of the experiment the bulls had an average LW of 322 kg and 195 days of age. The authors concluded that there was no difference between silages on the performance (including DM intake, LWG, carcass WG (CWG), and carcass characteristics) of crossbred bulls.

Similarly, Huuskonen et al. [9] evaluated the effect of replacing timothy by alsike clover silage on the performance of Aberdeen Angus and Nordic Red bulls. Aberdeen Angus animals started the study with an initial LW of 479 and 335 days old and Nordic Red bulls were with an initial LW of 366 and 303 days old. Experimental silages were harvested from either timothy at heading stage or alsike clover at flowering stage. Treatments were composed of 1) timothy silage (65%) and barley (35%); 2) timothy silage (32.5%), alsike clover silage (32.5%) and barley (35%); and 3) alsike clover (65%) and barley (35%). Authors concluded that replacing timothy silage by alsike clover silage did not affect DM intake, growth, carcass conformation or meat quality traits of the bulls.

4.2.5 Grass replacement

Forage-based cattle production is an important component of sustainable agriculture because of its ability to transform feed not suitable for humans into highquality food. Most of the forages fed to growing cattle in Nordic countries are based on ensiled mixtures of different leys. The nutritive value of forages alone is usually insufficient to meet target growth rates, and diets are typically supplemented with concentrate feeds to improve performance of growing cattle. However, high-quality silages can support high levels of performance of growing cattle with moderate or even with no concentrate supplementation [1].

Huuskonen et al. [10] fed growing bulls of dairy breed a total mixed ration (TMR) consisting of silage made from a primary growth from a timothy and meadow fescue ley and three levels of barley based concentrate: 300 (L), 500 (M) and 700 (H) g/kg DM. Increasing the proportion of concentrate from 300 to 500 g/kg DM led to an improvement in daily LWG (1090 vs.1205 g/d) but no further improvement was seen for the H diet (1196 g/d). There were no significant treatment differences in the DM intake (8.72, 9.03 and 8.24 for L, M and H,

respectively) which lead to a decreasing feed conversion ratio with increasing concentrate proportion (8.02, 7.53 and 6.90 kg DM/ kg LWG for L, M and H, respectively).

Pesonen et al. [11] studied the effects of increasing the proportion of concentrate in the diet on performance and carcass traits. Hereford and Charolais bulls were offered a TMR consisting of grass silage (timothy and meadow fescue) with either 200 (L) or 500 (M) g barley-based concentrate/kg DM. Increasing the level of concentrate resulted in higher DM intake (8.93 vs. 9.68 kg/d L and M, respectively) and average daily gain (ADG; mean 1158 and 2087 g/d for L and M, respectively). Increasing the proportion of concentrate also improved carcass conformation.

Manni et al. [12] evaluated four strategies of replacing grass silages by concentrate on the performance of Holstein and Nordic Red bulls with an initial LW of 230 (\pm 36.9) kg and 200 (\pm 24.9) days of age. High-quality silage was made of the primary growth of timothy stands harvested at early heading stage and concentrate made of rolled barley grain. Four treatments were evaluated: 1) grass silage alone during the whole experimental period; 2) TMR contained grass silage-to-barley ratio of 70:30; 3) grass silage alone during the first half of the experiment and then TMR containing grass silage-to-barley ratio of 40:60 during the second half; and 4) TMR containing grass silage alone during the second half. The amount of concentrate throughout the year was the same for treatments 2, 3 and 4.

Authors observed that bulls fed the strategy of silage alone during the first half and 40:60 grass silage-to-barley ratio during the second half showed greater DM intake (8.90 vs. 7.97 kg/d), LWG (1314 vs. 1119 g/d) and carcass weight (696 vs. 580 g/d) compared to silage alone during the whole period. However, bulls fed the silage alone during the whole period showed similar results compared with bulls fed the strategies 2 and 4. This study indicates that dairy bulls can achieve moderate to high performance when high-digestible grass silage is used as a sole feed. However, including barley in the diet further improved growth performance.

Johansson et al. [13] evaluated three different forage-to-concentrate proportions on seventy-nine Swedish Red and seventy-five Swedish Holstein dairy bull calves. Calves were fed the same feeds in three different forage-to-concentrate proportions, with clover/grass silage (70% red clover and 30% grass) constituting 40, 50 or 60% of DM in the TMR. Authors observed that calves receiving diets with 40 or 50% silage showed similar intake (5.6 kg DM/d), LWG (1.38 kg/d) and total weight gained (194 kg), and calves fed the diet containing 60% silage showed the lowest intake (5.2 kg DM/d), LWG (1.22 kg DM/d) and total weight gained (172 kg). However, feed efficiency (g gain/MJ ME) was similar among all treatments.

Huuskonen et al. [14] compared grass silage fed alone or mixed with triticale or whole crop barley on the performance of Hereford and Charolais bulls. Grass silage was prepared from a primary growth of timothy and spring-sown barley and springsown triticale were used as whole crop silages. Diets were formulated to have a forage-to-concentrate ratio of 60:40. The experimental diets were composed by: 1) grass silage (60%) and concentrate (40%); 2) grass silage (30%), triticale silage (30%) and concentrate (40%); 3) grass silage (30%), barley silage (30%) and concentrate (40%). The authors observed that bulls fed grass silage alone showed lower DM intake but similar LWG, carcass gain, final LW and carcass weight compared with bulls fed grass mixed with whole crop silages. Thus, the feed efficiency of the bulls fed grass alone was greater than when grass was mixed with whole crop silages (barley or triticale).

High-quality grass silage can be fed alone to growing bulls and get similar results compared to when silage is mixed with concentrate feeds. Also, in TMR containing concentrate feeds, grass silage can be fed alone or mixed with clovers without affecting performance of finishing bulls. As discussed before, high-quality grass can be produced by adjusting the harvest time in order to reach a proper maturity stage according to the goals of the production system, additionally by choosing the right cut to feed animals with greater requirement, or by choosing the forage species that shows the best results on animal performance.

4.2.6 Conclusions and knowledge gaps

Harvesting leys at early maturity stage affects beef bulls performance positively, however, the decision of harvesting earlier and likely more frequent in the growing season has to be evaluated carefully. Research evaluating the economics of the systems is uncommon due to high variability of the costs that are specific for the location and time of each production system. Therefore, the economic viability of this practice has to be evaluated locally. Only two studies were conducted in Nordic conditions comparing the performance of bulls fed leys at early or late maturity stage, and, as expected, animals fed early maturity stage grass-clover silages showed greater daily live weight gain and shorter feeding time until slaughter. However, future research could focus on the evaluation of the whole production system. Thus, a more comprehensive analysis of the production system is needed to support decision making by producers.

Regarding the performance of beef cattle bulls the three-cut strategy appears advantageous. Harvesting each cut earlier to be able to harvest three cuts within the same growing season will enhance the quality of the material and, consequently, increase LW and carcass weight gain, reducing the rearing time required to finish the animals. By harvesting more frequently than three times a year, which, to a certain extent is practiced by dairy farmers and some beef farmers in Sweden, ley quality can be improved further and this might enhance animal performance even more. More frequent cuttings will likely increase as the growing season will become longer because of climate change. However, as discussed previously, the economic viability of the system has to be evaluated locally to reach the best yieldquality balance.

The only comparison of ley species for beef cattle was performed by the same group in two studies feeding pre- and post-partum cows, where the goal was to prevent under- or over-conditioning of cows. However, there is a lack of studies in Nordic conditions comparing ley species for other categories of beef cattle such as finishing bulls or growing steers and heifers. Additionally, due to climate change and drastic variations related to weather conditions, ley production has been, and most likely will continue to be, compromised. Thus, future research could focus on the comparison of ley species focused on improving nutritive quality to increase energy intake and performance, but also on investigating the performance of beef cattle fed ley species that are more adapted to periods of drought and heavy rainfalls.

Harvest cut was evaluated in two studies by the same group where three- and two-cut systems were investigated. In the three-cut study bulls performed better when the first and third cuts were fed compared to the second cut. Similarly, in the two-cut study bulls fed the first cut performed better than bulls fed the second cut. However, it is important to highlight that the diets were formulated to have the same forage-to-concentrate ratio, regardless of the chemical composition of the forages. As the silages from the second cut showed greater concentration of fibre components, intake was limited by rumen fill, which compromised performance. So, the performance results were more related to the composition of the diet than to differences between leys. It is recommended for future research when comparing different leys for beef cattle to formulate diets based on the chemical composition of the forages, aiming to have similar nutrient composition between treatments.

Biorefinery of leys is a growing technology that aims to improve the utilisation of leys by screw pressing grassland plants in order to produce a protein-rich juice and a fibrous pulp. The juice can be used as protein supplement for livestock and the fibrous pulp is a suitable forage source for ruminants, especially for low-energy demanding animals such as lactating and non-lactating beef cows. However, all studies performed in Nordic conditions so far were conducted feeding the fibrous pulp to high-producing dairy cows. Thus, future research could focus on evaluating the performance of different categories of beef cattle receiving the fibrous pulp from ley biorefinery as a forage source.

In conclusion, it is important to state that no single strategy is always the best option for all production systems. The most profitable approach depends on the relationship between available land area and other variables that are highly dependable on the fluctuations of the market, such as input costs and product revenue.

4.2.7 References

- Randby, A.T., P. Norgaard, and M.R. Weisbjerg, Effect of increasing plant maturity in timothy-dominated grass silage on the performance of growing/finishing Norwegian Red bulls. Grass and Forage Science, 2010. 65(3): p. 273-286.
- 2. Manninen, M., et al., Effects of grass-red clover silage digestibility and concentrate protein concentration on performance, carcass value, eating quality and economy of finishing Hereford bulls reared in cold conditions. Agricultural and Food Science, 2011. 20(2): p. 151-168.
- Huuskonen, A. and M. Pesonen, A comparison of first-, second- and third-cut timothy silages in the diets of finishing beef bulls. Agricultural and Food Science, 2017. 26(1): p. 16-24.
- 4. Hyrkäs, M., et al. EGF. in EGF. 2015. Wageningen, Netherlands Wageningen Academic Publishing.
- 5. Huuskonen, A., S. Ramo, and M. Pesonen, Effects of primary growth compared to regrowth grass silage on feed intake, growth performance and carcass traits of growing bulls. Agricultural and Food Science, 2018. 27(4): p. 232-242.
- 6. Jardstedt, M., et al., The effect of feeding roughages of varying digestibility prepartum on energy status and metabolic profiles in beef cows around parturition. Animals, 2020. 10(3).
- Jardstedt, M., et al., Feed intake and urinary excretion of nitrogen and purine derivatives in pregnant suckler cows fed alternative roughage-based diets. Livestock Science, 2017. 202: p. 82-88.
- 8. Pesonen, M., E. Joki-Tokola, and A. Huuskonen, The effect of silage plant species, concentrate proportion and sugar beet pulp supplementation on the performance of growing and finishing crossbred bulls. Animal Production Science, 2014. 54(10): p. 1703-1708.
- Huuskonen, A., M. Pesonen, and M. Honkavaara, Effects of replacing timothy silage by alsike clover silage on performance, carcass traits and meat quality of finishing Aberdeen Angus and Nordic Red bulls. Grass and Forage Science, 2017. 72(2): p. 220-233.
- Huuskonen, A., K. H., and E. Joki-Tokola, Effects of three different concentrate proportions and rapeseed meal supplement to grass silage on animal performance of dairy-breed bulls with TMR feeding. Livestock Science, 2007. 110: p. 154-165.
- Pesonen, M., et al., Effects of concentrate level and rapeseed meal supplementation on performance, carcass characteristics, meat quality and valuable cuts of Hereford and Charolais bulls offered grass silage-barley-based rations. Agricultural and Food Science, 2013. 22: p. 151-167.
- 12. Manni, K., M. Rinne, and A. Huuskonen, Effects of barley intake and allocation regime on performance of growing dairy bulls offered highly digestible grass silage. Livestock Science, 2016. 191: p. 72-79.
- 13. Johansson, B., A. Hessle, and K.I. Kumm, Using clover/grass silage as a protein feed for dairy bull calves. Organic Agriculture, 2016. 6(1): p. 57-63.

 Huuskonen, A., S. Jaakkola, and K. Manni, Intake, gain and carcass traits of Hereford and Charolais bulls offered diets based on triticale, barley and grass silages. Agricultural and Food Science, 2020. 29(4): p. 318-330.

4.3 Sheep

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4.3.1 Selective behaviour

Sheep are in general ten times smaller than cattle, but their maintenance requirement per kg of live weight is higher. Sheep compensate mainly through selection, avoiding more fibrous plants or plant parts. This was demonstrated in a feeding study with lambs offered grass silage harvested at different plant maturity in the primary growth. The results showed that lambs were able to select the more nutritious components from all silages offered, and that their selection was based more on the concentration of neutral detergent fibre (NDF) and estimated metabolizable energy (ME) than on crude protein (CP) [1]. The same tendency was seen in an experiment by Kischel et al. [2], where wastage from ewes fed round bale haylage of lower quality (later harvest) differed more in NDF content from the fed forage, compared to when feeding less mature haylage. A study with ewes fed grass silage harvested at early (leaf stage) or at medium maturity (early heading stage) in the first cut showed a greater sorting behaviour with the more mature forage [3]. A similar selective behaviour was found with wethers fed grass silage at leafy-to-early heading stage compared to wethers fed grass silage at medium-to-late heading stage [4]. Intake in kg dry matter (DM) per day did however not differ between the groups. Other factors than maturity also can affect selection. Helander et al. [5] noted that the content of NDF was higher in the refusals than in the feed when feeding un-chopped grass silage compared to chopped silage to pregnant and lactating ewes.

4.3.2 Feeding forage in different production stages

Swedish sheep breeds are in general more prolific than sheep in many other countries, which affects their nutrient requirements. Energy and protein requirements of ewes double from maintenance and early pregnancy to late pregnancy and early lactation, when ewes often expend more energy than they consume. Their feed intake capacity in late pregnancy is also affected, as more foetuses reduce the space for the rumen. This leads to a negative energy balance and hence a mobilization of body reserves. Feeding only forage during this period is challenging, since its nutritional value can vary considerably. In a study by Bernes and Stengärde [6], dry-matter (DM) intake was similar between the two experimental years in early pregnancy although silage quality differed, e.g. with a higher NDF content the second year. Later in pregnancy and during lactation, there were large differences in intake between the years. The lower nutrient consumption in the second year had obvious consequences, with lower body condition score (BCS) of ewes with many lambs, higher concentrations of nonesterified fatty acids in the blood, higher lamb mortality and lower live-weight gain (LWG) of the lambs. The largest effects of low nutrient intake on blood metabolites of the ewes were observed 2 weeks after lambing. The authors concluded that it may be difficult to maintain a good nutritional status in ewes with more than one lamb, feeding only forage. In that case, the forage needs to have very high nutritional quality. Somewhat contrary to this, silage of medium quality was sufficient to cover the nutrient requirements during pregnancy, according to a study with twin-bearing ewes fed silage of different quality and a fixed amount of concentrate [3]. This was partly because of the ewes' selection behaviour against fibrous particles in favour of more digestible plant parts. Feed intake was relatively constant until two days before lambing and started to increase directly after lambing. Furthermore, [7] found a significantly lower intake of low-quality forage the last five days prepartum in ewes bearing three, compared with two foetuses. There was a significant increase in DM intake from pre- to post-partum. The authors concluded that ewes have the capacity to sustain growth and nutrition of multiple foetuses during severe energy restriction in late pregnancy, but the capacity to produce milk for the lambs after birth may be compromised.

In a study by Sormunen-Cristian and Jauhiainen [8], DM intake was similar in pregnant ewes fed either hay or silage. Intake decreased for both forages during the last 8 weeks of pregnancy, but to a greater extent in the hay group. Barley supplementation during the final 6 weeks of pregnancy did not appear to depress forage intake. The highest forage intake occurred 6 weeks after lambing. Due to the higher nutritive value, ewes fed silage were closer to energy and protein requirements during late pregnancy and early lactation. Intakes of DM and NDF were around 45% higher in ewes during early lactation than during late gestation in a study with *ad libitum* feeding of grass silage and a fixed amount of concentrate [5]. The same research group [3] presented a mean total NDF intake of 20 g/kg body weight for twin-bearing lactating ewes, where 90% of the NDF originated from the grass silage. This is higher than in many international references.

4.3.3 Effect of harvest date

Later harvest dates decreased voluntary intake of silage and, thereby, LWG and feed conversion efficiency in lambs in a feeding study with grass silage harvested at three different maturity stages in the primary growth [1]. Soybean meal at a maximum of 120 g DM per day was used to equate the protein concentration of the diets. Concentration of NDF was shown to be the most important variable in explaining the variation in silage intake between the diets. Together with live weight (LW), NDF concentration explained more than 75% of the variation in the lambs' intake of silage. The impact of fibre content on rumen degradation rate is one possible explanation. Although the lambs were fed in excess and thus were able to select more nutritional fractions of their feed, this could not fully compensate for the differences in nutritive value between the silages. Somewhat

contrary to this, lambs fed grass hay harvested at two occasions in the first cut with 25 days in between did not differ significantly in DM intake [9]. The lambs, however, also were fed 400 g of concentrates per day. Total energy intake differed between the groups, and the lambs fed earlier harvested hay grew faster and were heavier at slaughter.

Nadeau et al. [3] showed that ewes fed early harvested grass silage (leaf stage) showed only a marginal loss of body condition and started to recover their body condition after three weeks of lactation, whereas ewes fed medium (early heading stage) or late harvested (late heading stage) grass silage continued to lose condition during their whole lactation period. The negative effect of later harvest on silage intake was larger in early lactation than in late pregnancy. Stage of maturity of grass silage did not affect mean eating, ruminating and chewing time per kg of NDF intake in twin-bearing ewes [10]. However, feeding early harvested grass with a low lignification of NDF resulted in a higher DM intake and shorter chewing time per kg of DM intake. The early harvested silage also resulted in a higher BCS.

A diet with only silage was compared to one with silage plus concentrates in a two-year study with ewes and lambs [11]. The silage fed the second year had lower crude protein content, contained more indigestible NDF and had a higher concentration of fermentation acids. The DM intake of the lambs, particularly those fed only silage was generally higher the first year compared to the second year. The intake of NDF was also relatively high, 16 g/kg LW, indicating that the fibres in the silage had a high degradability. Lambs who had only silage before weaning the first year had higher total DM intake per kg LW in the period after weaning, compared to lambs fed concentrates before weaning. The study indicated that having free access to good-quality silage from an early age seems to promote high feed intake later on. In another study, where grass silages of differing maturity were fed to lambs, the highest intake was 14 g NDF/kg LW [1].

A lower concentrate intake was observed in lambs born to ewes fed early harvested grass silage, compared to later harvest. This indicates a higher milk production of ewes fed early harvested silage due to higher energy intake [3]. The lambs born by the ewes fed early harvested silage had a higher LWG and LW at weaning than lambs raised with low quality silage, despite the latter's higher intake of concentrate.

4.3.4 Effects of plant species and phyto-oestrogens

When silages were fed *ad libitum* to rams in a small-scale experiment, they preferred silage with timothy/meadow fescue to silage made by tall fescue [12]. The silages, however, also differed in digestibility with timothy/meadow fescue silage being more digestible.

Fields with timothy and 0, 20 or 40% red clover were cut simultaneously and later fed as silage to lambs for winter fattening [13]. Soybean meal was fed to level the CP percentage of the diet between the groups. Silage DM intake increased with increased clover content, and thereby silage protein and energy intake. The

differences between the groups decreased when looking at the total diet, but still the highest DM intake and LWG was in the group with the highest red clover intake. The NDF intake per kg LW was around 12 g in all groups.

Anti-nutritional factors often affect sheep to a greater extent than they do cattle. One example is phyto-oestrogens that may have an effect on reproduction. A Finnish study aimed to determine the effects of red clover silage with high phytooestrogen content on ewes during their first breeding season [14]. The red clover silage appeared to be more palatable than the grass silage used as control diet and, although the diets were planned to be iso-energetic, the ewes fed on red clover silage gained weight faster. The ewes were slaughtered after five months of experimental feeding, six weeks after the end of the breeding season. The total mass of the uterus with its contents was significantly greater in red clover fed ewes, compared with those of the control group. This was mainly explained by a greater volume of foetal fluids. There was no effect on the weight of the foetuses. Serum progesterone concentration in the red clover group was significantly lower than in the control group, but the fecundity of the ewes was not reduced. The larger volume of foetal fluids could, however, increase the risk for vaginal prolapse before lambing.

4.3.5 Effects of mixed feeding and silage vs hay

Is it recommendable to feed only forage to sheep? As stated above, it may be difficult to sustain ewes with more than one lamb only on forage [6]. In addition, the nutritional requirements for optimal growth of lambs can be difficult to meet even with high-nutrient-quality silage [11].

Feeding a mixed ration of silage and concentrate gives a more even distribution of energy from carbohydrate metabolism to be used for microbial protein synthesis in the rumen, compared to separate feeding, according to [15]. Increased microbial protein synthesis results in more amino acids being absorbed in the small intestine, to be used for milk production. Feed intake of the ewes fed total mixed ration increased from day 11 to day 42 in lactation, which resulted in increased feed intake during the whole lactation period, compared to separately fed ewes. An increased LWG from birth to weaning of lambs fed the mixed diet as compared with separate feeding of silage and concentrate was probably related to the increased intake by the ewes, generating higher milk yield. Feed intake in pregnancy was not affected by mixing silage and concentrate, compared to separate feeding [5].

In a Finnish study, ewes were fed either hay or silage or both [8]. The forages were harvested at different occasions and the hay contained more fibrous constituents, less protein and had a lower digestibility than the silage. Intake of hay or silage did not differ, but silage feeding resulted in higher LWG of the ewes during pregnancy. Ewes fed silage were also able to suckle more triplets than those

fed hay, and their lambs grew better. Feeding both silage and hay did not give any advantages in animal performance during late pregnancy and early lactation. The intake of hay was larger than that of silage harvested at the same time, in a follow-up study with Finnsheep lambs [9]. In addition, performance was better, with higher growth rate, and carcass weight for the hay-fed lambs. Type of forage had no effect on carcass fatness or conformation score. The OMD of silage was somewhat higher than that of hay and the silage was well preserved, but the DM content of the silage was only 23%, which probably explains the lower feed intake.

A Norwegian study showed a greater level of competition when hay was fed, compared with silage, probably because the structure of hay makes it easier to distinguish between palatable leaves and fibrous stalks. This suggests that even if there are plenty of refusals of hay in the feed rack, the sheep may prefer to wait for new feed to be offered [16].

In a comparison between silage and hay, type of forage had no effect on the time spent eating, but the DM intake was higher with silage [17]. The nutrient content of the two forages was not reported, making it somewhat difficult to evaluate the results. The forages were fed in 100% (semi-restricted) or 120% amounts (*ad libitum*), counted on previous intake. In the groups fed silage, the number of displacements at the feeding rack increased when feeding level was changed from *ad libitum* to semi-restricted. The authors concluded that even a minor restriction in feeding level of roughage decreased eating time and resulted in more queuing.

Three types of roughage (silage, hay and ammonia-treated straw) were compared to evaluate the effect of restricted roughage allowance to ewes [18]. The silage had better nutrient quality but its DM content was only 20%. There were also groups of ewes that had restricted amounts of concentrates and free access to roughage. The DM intake of hay was higher than that of silage in the groups that had free access but there was no difference in weight gain of the lambs between these groups. Wool and wood eating was observed in ewes fed silage and large amounts of concentrate, but not in the corresponding hay or straw groups or in those with free access to roughage.

A Norwegian survey of factors affecting performance in sheep flocks showed that one of the factors positively affecting the number of lambs born per ewe was percentage grass silage of total roughage [19]. However, the authors also concluded that all the studied factors appeared to explain only a small part of the total variation in performance between farms. A similar survey concerning management practices associated with neonatal lamb mortality in Norwegian sheep flocks [20] showed that feeding a combination of grass silage and hay might stimulate the appetite compared to when providing only one type of roughage.

4.3.6 Effects of chopping length and pelleting

Feed intake was not clearly affected by chopping silage in experiments with ewes in late pregnancy and early lactation [5], contrary to previous studies. Forage in other studies has been chopped before ensiling/storing, whereas those used in the experiments by [5] and [15] were chopped after opening the bales prior to feeding. Chopping herbage before ensiling improves the fermentation process [21], which has a direct effect on feed intake in ruminants, and increases silage digestibility, which has been clearly shown to increase feed intake in ewes [3]. Consequently, the effect on intake due to chopping in previous studies cannot be completely separated from the effect of the silage fermentation procedure [5]. Both pregnant and lactating ewes fed chopped silage in general spent less time eating and more time ruminating, compared to ewes fed un-chopped silage. The silage had a high nutrient quality, which may have decreased the effect of chopping. However, when the highly digestible silage was fed to the weaned lambs, the LWG of the lambs increased (444 g/day vs. 373 g/day) without affecting intake, which indicates improved feed utilization [15].

In an Icelandic study, pelleting hay resulted in an increased intake and decreased *in vivo* digestibility in castrated lambs. The effects increased with decreasing quality of the hay [22]. The authors hypothesize that passage rate is higher, which increases intake when the hay is pelleted, which in turn counterbalances the decreased digestibility. There was also a positive effect of pelleting on carcass weight.

4.3.7 Effects of feeding system, frozen silage and fertilization

Feeding forage in round-bale feeders is a cheap and work-reducing system. One disadvantage is, however, the high feed wastage. In a Norwegian study, ewes spent more time eating with their heads inside the feeder when they were fed half round-bales, dropping more of the potential wastage inside the feeder [2]. Wastage was almost double when feeding whole bales. The design of the feeder is also important to make the ewes keep their heads inside the feeder while eating. Type of roughage was, however, the most important factor, with higher wastage of more mature and fibrous silage. The ewes pulled out the long fibrous stems from the round bale feeders and left them as wastage on the floor. Time spent eating from the floor was very low, i.e. dropped feed is a true waste.

Supplying roughage to ewes at least twice per day versus only once gave lower odds of neonatal lamb mortality, according to a Norwegian survey [20]. The authors speculate that frequent feeding stimulates appetite and thus increases feed intake, compared to feeding only once a day. Frequent supply of roughage may also reduce competition between ewes during feeding.

Feed intake in the first 4 h after feeding was lower in a group of pregnant ewes fed frozen silage, compared to those in the control group with unfrozen silage [23]. Frozen silage, however, had only a moderate effect on the total daily feed intake. Time spent tearing off feed was higher in the group fed frozen silage but the total eating time was only moderately increased. Partly thawing improved the intake of frozen silage. DM content also had an impact, as frozen silage with 45% DM was eaten faster than silage with 22% DM.

The effect of different fertilization strategies on forage intake was investigated in experiments with Finnsheep wethers [24]. The ley was fertilised after the first cut and mown 36-40 days later. In one study, cattle slurry application on the surface decreased intake of silage from that area, compared to where the slurry had been injected or mineral fertiliser had been used. Rainfall may have affected the results in later experiments. Clostridia spores increased with surface application of slurry. The authors also concluded that chemical composition or fermentation quality do not always indicate palatability differences of silage made from slurry-treated grass.

4.3.8 Effect of silage intake on product quality

It is well known that fatty acid (FA) composition in meat and milk is affected by the animal's diet. This was confirmed in a Swedish study, where the level of n-3 FA in milk increased when ewes were fed silage only, compared to silage plus concentrates [11]. The FA profile of the lamb meat also differed due to the ewe's milk FA profile influencing the offspring. As long as lambs are suckling, their tissue FA composition is largely dependent on their mother's diet. A higher proportion of n-3 FA is said to be beneficial for human health.

In a comparison of different rearing systems for lambs [25], three systems included pasture and one was based on indoor feeding of silage *ad libitum* plus some concentrates. All lambs were meant to be slaughtered at a similar LW. The indoor group had the highest LWG and lowest age at slaughter. There were no differences in meat quality attributes between the groups, showing that intact ram lambs can be reared under different conditions without any effects on meat quality. FA were not analysed in this study.

4.3.9 Conclusions and knowledge gaps

As the sources for this synthesis are limited in timespan and geography there are subject areas that are not covered, like the effect of DM content on forage intake, or where there are just one or a few studies, e.g. the effect of chopping length. Other areas have been more investigated, such as the effect of harvest date.

Forage intake of sheep is largely affected by harvest date / plant maturity / concentration of NDF. Sheep have a selective feeding behaviour. This is more obvious when feeding more fibrous forage. Ewe nutrient requirement and forage

intake vary a lot over the production year. Intake ability is reduced the last days before lambing and increases rapidly thereafter.

One area for future research should be to evaluate new forage crops suitable for Nordic sheep, partly due to changes in cropping conditions as a consequence of climate change. Studies of production performance should be done on iso-NDF diets to give the sheep the possibility to have the same intakes. The effects of feeding existing or new forage species on meat quality is also of interest.

Different methods to increase forage quality and intake are important to study, with the aim to reduce the use of concentrates containing human edible ingredients as much as possible. The effect of different fermentation products on palatability and thus silage intake is also worth more investigation, as well as the impact of mycotoxins in silage on intake and sheep health. The effect of forage particle length on silage intake of lambs is another factor where more research could be needed.

Lamb meat production is hard to make economically profitable. Therefore, economic comparisons of the effect of different forage feeding systems would be of interest, i.e. number of feedings per day, the impact of different equipment on amount of feed losses and need for working time, etc. As the use of round bales is the predominant system on Nordic sheep farms, optimal round bale systems from field to sheep is of great interest.

The genetics of sheep is also of interest for future studies, e.g. to investigate if there are differences between sheep breeds in forage utilization efficiency, and to investigate the possibility to breed for increased forage utilization efficiency.

4.3.10 References

- 1. Bernes, G., M. Hetta, and K. Martinsson, Effects of harvest date of timothy (Phleum pratense) on its nutritive value, and on the voluntary silage intake and liveweight gain of lambs. Grass and Forage Science, 2008. 63(2): p. 212-220.
- Kischel, S.G., I. Donnem, and K.E. Boe, The effect of round-bale feeder design and roughage type on feed wastage in sheep feeding. Animal, 2019. 13(10): p. 2388-2396.
- Nadeau, E., A. Arnesson, and C. Helander, Effects of grass silage feed value on feed intake and performance of pregnant and lactating ewes and their lambs. Grass and Forage Science, 2016. 71(3): p. 448-457.
- 4. Nadeau, E., et al., Digestibility and protein utilization in wethers fed whole-crop barley or grass silages harvested at different maturity stages, with or without protein supplementation. Journal of Animal Science, 2019. 97(5): p. 2188-2201.
- Helander, C., et al., Effects of chopping grass silage and mixing silage with concentrate on feed intake, dietary selection, chewing activity and faecal particle size of ewes in late pregnancy and early lactation. Livestock Science, 2014. 163: p. 69-79.
- Bernes, G. and L. Stengarde, Sheep fed only silage or silage supplemented with concentrates. 1. Effects on ewe performance and blood metabolites. Small Ruminant Research, 2012. 102(2-3): p. 108-113.

- Norgaard, P., et al., Metabolic challenges in late pregnancy in multiparous ewes fed silage or hay. Acta Veterinaria Scandinavica, Supplementum, 2003 (supp. 98): p. 248-249.
- Sormunen-Cristian, R. and L. Jauhiainen, Comparison of hay and silage for pregnant and lactating Finnish Landrace ewes. Small Ruminant Research, 2001. 39(1): p. 47-57.
- 9. Sormunen-Cristian, R. and L. Jauhiainen, Comparison of hay and silage for growing Finnish Landrace lambs. Multi-function grasslands: quality forages, animal products and landscapes. Proceedings of the 19th General Meeting of the European Grassland Federation, La Rochelle, France, 27-30 May 2002, ed. J.L. Durand, et al. 2002, Versailles cedex, France: Organizing Committee of the European Grassland Federation. 230-231.
- Jalali, A.R., et al., Effect of stage of maturity of grass at harvest on intake, chewing activity and distribution of particle size in faeces from pregnant ewes. Animal, 2012. 6(11): p. 1774-1783.
- Bernes, G., T. Turner, and J. Pickova, Sheep fed only silage or silage supplemented with concentrates 2. Effects on lamb performance and fatty acid profile of ewe milk and lamb meat. Small Ruminant Research, 2012. 102(2-3): p. 114-124.
- Sarkijarvi, S., et al., Effect of grass species and cutting time on in vivo digestibility of silage by horses and sheep. Livestock Science, 2012. 144(3): p. 230-239.
- Bernes, G., M. Hetta, and K. Martinsson, Vallfodrets kvalitet påverkar lammens konsumtionsförmåga, in Nytt från institutionen för norrländsk jordbruksvetenskap. 2006, Swedish University of Agricultural Sciences: Umeå.
- Mustonen, E., et al., Fertility and growth of nulliparous ewes after feeding red clover silage with high phyto-oestrogen concentrations. Animal, 2014. 8(10): p. 1699-1705.
- 15. Helander, C., et al., Effects of chopping grass silage and of mixing silage with concentrate on feed intake and performance in pregnant and lactating ewes and in growing lambs. Small Ruminant Research, 2014. 116(2-3): p. 78-87.
- Boe, K.E. and I.L. Andersen, Competition, activity budget and feed intake of ewes when reducing the feeding space. Applied Animal Behaviour Science, 2010(125): p. 109-114.
- Boe, K.E., A.M. Ottosen, and I.L. Andersen, Feed intake and competition in ewes on ad libitum or semi-restricted feeding of grass silage and hay. Journal of Animal Science, 2012. 90(11): p. 4014-4019.
- Lind, V., Effects of feeding limited roughage to sheep on ewe performance, economic aspects and behaviour. Acta Agriculturae Scandinavica Section a-Animal Science, 2002. 52(2): p. 65-71.
- Simensen, E., P.S. Valle, and S. Vatn, A mail survey of factors affecting performance in 627 selected sheep flocks in south-eastern Norway. Acta Agriculturae Scandinavica Section a-Animal Science, 2010. 60(3): p. 194-201.

- 20. Holmoy, I.H., et al., Housing conditions and management practices associated with neonatal lamb mortality in sheep flocks in Norway. Preventive Veterinary Medicine, 2012. 107(3-4): p. 231-241.
- 21. Nadeau, E. and H. Auerbach. Effects of particle size and chemical additives on fermentation and aerobic stability of grass-clover silage. in 5th Nordic Feed Science Conference. 2014. Uppsala, Sweden Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences.
- 22. Larusson, T. and J. Sveinbjornsson, The effect of pelleting hay upon feed intake, digestibility, growth rate and energy utilization of Icelandic lambs. Proceedings of the 7th Nordic Feed Science Conference, Uppsala, Sweden, 14-15 June 2016, 2016: p. 67-72.
- Boe, K.E. and I. Donnem, The effect of frozen grass silage on the feed intake and feeding behavior of pregnant ewes. Journal of Animal Science, 2015. 93(10): p. 4819-4825.
- 24. Heikkila, T., et al., Effect of slurry application on grass and silage quality and intake in sheep. Land use systems in grassland dominated regions. Proceedings of the 20th General Meeting of the European Grassland Federation, Luzern, Switzerland, 21-24 June 2004, ed. A. Luscher, et al. 2004, Zurich, Switzerland: vdf Hochschulverlag AG an der ETH Zurich. 1119-1121.
- 25. Stenberg, E., et al., Carcass characteristics and meat quality attributes in lambs reared indoors, on cultivated pasture, or on semi-natural pasture. Agricultural and Food Science, 2020. 29(5): p. 432-441.

4.4 Reindeer

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4.4.1 Research results

Although partly domesticated and owned by herders, the majority of reindeer in Fennoscandia are free-ranging and dependent on natural pastures all year around [1-3]. Nevertheless supplementary feeding is sometimes needed because of lack of pastures or unfavourable weather conditions (primarily hard snow or ice covering the pastures). Loss of land to competing activities and a changing climate has increased the need for feeding [4]. Reindeer are usually fed grain-based pellets combined with grass silage or hay and sometimes limited amount of reindeer lichens. Many herders in Sweden and Norway, prefer to feed only grain-based feed due to negative experiences of feeding hay or silage [2]. The use of hay or silage is more common in Finland where supplementary feeding of reindeer has been commonly practiced for a longer time and where many herders have land to grow their own hay/silage [3].

Negative effects from feeding hay/silage to reindeer on their health have been summarised by Josefsen and Sundset [5] and Åhman et al [1], specifically sections 4.1.3.1; 4.2.1; 4.2.5, the most common problem being grass accumulation in the rumen (see below).

Most experiments on the use of hay and grass silage to reindeer have been done by a few research groups (Syrjälä-Qvist at the University of Helsiki, Aagnes, Mathiesen and co-workers at the University of Tromsø, and Nilsson and co-workers at the Swedish University of Agricultural Sciences).

Liisa Syrjäla-Qvist [6, 7] did an experiment investigating the utilization of grass silage in reindeer compared to sheep of similar age and sex (three animals each, all fed all the diets). The silage was a third growth of mainly cocksfoot (*Dactylis glomerata*) (harvested on three occasions, with one week in between). The silage contained 24.5-25.2% DM, 14.5-15.9% crude protein, 27.2-28.0% crude fibre, and *in vitro* digestibility of organic matter was 69.6-70.2%). Feed consumption was considerably lower in reindeer (16 g DM/kg live weight per day) than in sheep (27 g), and the apparent digestibility of protein was also lower in reindeer (69%) compared to sheep (75%). The rumen function (measured by rumen pH, VFA and relative volume of ciliates and bacteria) strongly indicates that reindeer do not utilize grass silage as well as sheep do.

Early studies on providing different diets to reindeer (mixed herd with females and calves) during the whole winter period were made by Nieminen et al. [8]. Reindeer fed only lichens consumed more dry matter (2.4 kg/d) than those fed lichens and hay in equal amounts (1.7 kg/d). Reindeer fed only hay consumed even

less (1.2 kg/d). In spite of *ad libitum* feeding most reindeer did not gain weight during the feeding period.

Aagnes, Mathiesen and co-worker did several experiments testing different qualities of grass silage to reindeer adults and calves [9-12]. The used silages (made either from timothy or mixed grasses) varied in chemical composition (cellulose was in the range 18.7-30.4% and water-soluble carbohydrates/WSC ranged from 10 to 30%) but all seemed to be highly palatable. Digestibility varied between the different qualities of silage (organic matter from 63.4%, for silage with 10% WSC, up to 86.6% on the silage with most WSC). No digestion disorders were observed. Adult animals generally increased in BM during the first week of feeding, seemingly an effect of increased rumen/reticulum fill, but did not put on any significant weight after this. Reindeer calves fed leafy silage (re-growth) kept their body mass, while those fed first cut silage lost substantial body mass, when the effect of increased digesta was corrected for. In an additional experiment [13] reindeer calves were fed first cut timothy leafy silage (97% leaves, 21% cellulose and 28% WSC). Feed intake was up to about 1.2 kg DM per day and the animals generally gained some weight. In another experiment [14] it was shown that mixing silage with molasses (increased WSC from 8.2 to 16.0% of the diet) increased feed intake significantly, but that feed intake was highest in a group fed only hay made from the same grass as the silage. Results on *in vivo* and *in vitro* experiments differed, as the in vivo experiments showed lowest digestibility for hay and no significant difference depending on addition of molasses or not, while the DM digestion of cellulose in the in vitro experiment was significantly higher for reindeer fed the mixed diet compared to those fed only silage, and intermediate in those fed hay. Total VFA was highest for reindeer fed hay. The authors conclude that the leafy timothy preserved as hay was more suitable for reindeer than silage made from the same grass.

More detailed studies on rumen function were made as part of the above experiments by Aagnes and co-workers. The morphology of rumen papilla differed depending on diet. Reindeer fed silage with a low content of cellulose (19%) showed a higher ruminal surface enlargement factor than those fed silage with more cellulose (30%) [15]. Pathological lesions in the rumen mucosa were found in reindeer fed silage or grain-based pellets (more in those fed silage, while lesions were uncommon in free-ranging or lichen-fed reindeer [16]. The lesion did however not seem to affect the health of the animals. The number of leukocytes ($y\partial$ T cells) showed to be linked to the diet-induced lesions [17].

[18] reports on failure of cellulolysis in the rumen of one of three reindeer calves fed timothy silage (76% leaves, 25% cellulose, 20% WSC) in metabolism cages for 48 days. These reindeer had a large amount of rumen content, high rumen pH and low VFA. [19] found no difference in viable populations of rumen bacteria

depending on quality of timothy silage (first cut, 27% leaves, 6.2% WSC, or regrowth with 89% leaves, 30% WSC) [20].

[21] fed two qualities of silage, one dry and one wet (39% and 53% DM, respectively; 7.6% WSC, 57% NDF, 31% ADF and 13.5% WSC, 54% NDF, 29% ADF), to yearling reindeer (17 months at the start of the experiment) for three or five months. The quality of the silage did not affect feed intake or BM. The reindeer spent most of their time around the feeding site, seemingly hungry, although there was still silage left. Several reindeer showed signs of illness and two even died. In other experiments by the same group reindeer calves were fed grass silage combined with either 30% or 60% barley [22], or combined with commercial grainbased reindeer feed, 60% or 80% [23]. In the first experiment health problems (rumen indigestion, emaciation and infections) affecting most of the reindeer started after 4-7 weeks in both groups and nine (out of totally 75) reindeer died or had to be slaughtered. Reindeer that adjusted to the high barley diet seemed to manage well and gained BM, but those on the low barley diet consumed considerably less feed and gained less BM. In the second experiment the reindeer remained healthy. Both diets resulted in a considerable gain of BM and did not differ between the diets (even after correcting for some difference in rumen content) although DM, crude protein and energy intake was higher on the HC diet.

An experiment was made by Nilsson and co-workers [24, 25] with the purpose of evaluating the difference between feeding strategies on reindeer adaptation to new diets. After feeding a "natural" diet (reindeer lichens combined with shrubs and leaves), and then exposed to a 9-day period of feed shortage, the reindeer were either fed a full ration of the natural diet, commercial grain based feed (pellets) combined with either 20% lichens or 20% grass silage, or initially only silage (2% WSC, 52% NDF, 31% ADF, 4% lignin) and after five days, a gradual increase of pellets up to 80%. Some of the pellet-fed reindeer had some diarrhoea but recovered. Several reindeer in the silage group initially had severe health problems (malnutrition and so-called wet belly), which led to loss of animals, and this group was then excluded from the experiment. The combined 20% silage 80% pellets diet worked well and resulted in increased BM and body fat. Reindeer fed pellets and lichens gained significantly more rumen-free body mass than those fed pellets and silage [24]. Rumen DM, pH and total VFA, individual VFAs, and count of protozoa per g did not differ depending on whether pellets had been combined with lichens or silage [25]. Total count of culturable bacteria was significantly higher in reindeer that received some lichens and the count of lichen utilizing bacteria was considerably higher (almost as high as in reindeer fed 80% lichens).

[26] investigated the *in vivo* and *in vitro* digestibilities of different combinations of grass silage (two qualities, 0.2% WSC, 51% NDF, 33% ADF, 6% lignin, and 1.6% WSC, 42% NDF, 30% ADF, 7% lignin, respectively) and reindeer lichens using 9-month-old reindeer and rumen fluid from both reindeer and cattle. All

reindeer consumed almost all the lichens, while the consumption of silage varied between individuals. Most reindeer gained some body mass during the experiment. Rumen fill was higher in reindeer fed 80% silage than in those fed 80% lichens. *In vivo* and *in vitro* digestibility agreed very well for the silages (around 75%), while the digestibility of lichens was a bit higher *in vivo* than *in vitro* (67%), indicating that lichens need more time for complete digestion. *In vitro* digestibility in rumen fluid from cattle was similar to reindeer rumen fluid for silage, but differed substantially for lichens (15% digestibility in rumen fluid from cattle)

There has been concerns regarding possible negative effects on reindeer's natural pastures from feeding free-ranging reindeer. Effects on vegetation was investigated [27], and found an increase in coverage and height of *Deschampsia flexuosa* on plots where reindeer had been fed during previous winters. They also found lower coverage of *Dicranum* sp., *Pleurozium schreberi* and some dwarf shrubs on plots where reindeer had been fed with grass silage and leftovers had not been cleared, compared to controls. Some dwarf shrubs, e.g., *Calluna vulgaris*, showed a similar response after the second winter. The N content also increased in some shrubs as an effect of spreading hay or silage on the ground. The authors concluded that feeding on natural pastures in the forest may lead to gradual shifts towards more nutrient-rich forest types.

4.4.2 Conclusions and knowledge gaps

Reindeer are usually provided supplementary feed to secure animal health and survival when natural pastures are insufficient or inaccessible [1]. Maximizing growth or production is seldom the primary goal. Moreover, reindeer may be in rather poor condition when feeding starts which may aggravate the adaption to a novel diet. The most important features of a supplementary feed are therefore not to cause digestive problems and to provide enough energy and nutrients to maintain body condition.

High enough digestibility seems to be a key factor when it comes to hay or silage. This is mainly secured by high content of leaves and water-soluble carbohydrates and low content of fibre. It seems to be difficult to produce hay or silage with high enough digestibility for giving to reindeer as their only feed. The exceptions are silages produced in a northern cool climate with a lot of sunlight in summer (like the Tromsø area). Feed accumulation in the rumen, when the reindeer is not able to digest the feed fast enough, is a recurring and serious problem that may even result in death of animals. The hygienic quality is evidently also a key factor for securing good animal health.

In some of the experiments, timothy grass preserved as hay seemed to be more suitable for reindeer than silage made from the same grass. It is however unclear why this would be the case, and seems unlikely that this should be a general rule. In spite of a high interest concerning ley feeds to reindeer, problems often occur and there are still rather large knowledge gaps. Herders have expressed a need for more hands-on recommendations regarding how to judge the nutritional and hygienic quality of silage and the suitability for reindeer [2].

It would also be valuable to test combinations of grasses and other plants in the ley to fit the requirements of reindeer, and also how to combine hay/silage with other feeds to create a suitable diet. Another topic for investigation would be the effect of pre-feeding preparation of hay or silage, e.g. cutting it into smaller pieces (a practice used by some herders, mainly in Finland), on the ability of reindeer to digest the feed.

4.4.3 References

- Åhman, B., G.L. Finstad, and T.D. Josefsen, Feeding and associated health problems, in Reindeer and caribou: health and disease, M. Tryland and S.J. Kutz, Editors. 2018, Taylor & Francis: Boka Raton. p. 135-156.
- 2. Horstkotte, T., et al., Supplementary feeding in reindeer husbandry Results from a workshop with reindeer herders and researchers from Norway, Sweden and Finland. 2020: Umeå. p. 36.
- Turunen, M. and T. Vuojala-Magga, Past and Present Winter Feeding of Reindeer in Finland: Herders' Adaptive Learning of Feeding Practices. Arctic, 2014. 67(2): p. 173-188.
- Åhman, B., et al., Role of supplementary feeding in reindeer husbandry, in Reindeer Husbandry and Global Environmental Change: Pastoralism in Fennoscandia Earthscan Studies in Natural Resource Management, T. Horstkotte, et al., Editors. 2022, Routledge: New York. p. 232-248.
- 5. Josefsen, T.D. and M.A. Sundset, Feeding of reindeer and diseases associated with feeding. Foring og foringsbetingede sjukdommer hos rein. Norsk Veterinartidsskrift, 2014. 126(2): p. 162-171.
- Syrjala-Qvist, L., Comparison of grass silage utilization by reindeer and sheep. 1. Palatability, feeding values and nutrient supply. Journal of the Scientific Agricultural Society of Finland, 1982. 54(2): p. 119-126.
- Syrjala-Qvist, L., Comparison of grass silage utilization by reindeer and sheep. 2. Rumen fermentation and rumen microbiota. Journal of the Scientific Agricultural Society of Finland, 1982. 54(2): p. 127-135.
- Nieminen, M., A.S. Pokka, and U. Heiskari, Artificial feeding and nutritional status of semi-domesticated reindeer during winter. Rangifer, 1987. 7(2): p. 51-58.
- Aagnes, T.H., A.S. Blix, and S.D. Mathiesen, Food intake, digestibility and rumen fermentation in reindeer fed baled timothy silage in summer and winter. Journal of Agricultural Science, 1996. 127: p. 517-523.
- 10. Aagnes, T.H. and S.D. Mathiesen, Round baled grass silage as food for reindeer in winter. Rangifer, 1995. 15(1): p. 27-35.

- Aagnes, T.H. and S.D. Mathiesen, Gross anatomy of the gastrointestinal tract in reindeer, free-living and fed baled timothy silage in summer and winter. Rangifer, 1996. 16(1): p. 31-39.
- Sara, E., et al., Gras som krisefor til rein, in Forsknngsinformasjon om rein og reindrift. 1997, Reindriftens Fagråd, Forskningsformidlingen, Universitetet i Tromsø, 9037 Tromsø.
- Norberg, H.J. and S.D. Mathiesen, Feed intake, gastrointestinal system and body composition in reindeer calves fed early harvested first cut timothy silage (*Phleum pratense*). Rangifer, 1998. 18(2): p. 65-72.
- Moen, R., et al., Digestion of timothy silage and hay in reindeer. Rangifer, 1998.18(1): p. 35-45.
- Josefsen, T.D., T.H. Aagnes, and S.D. Mathiesen, Influence of diet on the morphology of the ruminal papillae in reindeer calves (*Rangifer tarandus tarandus* L.). Rangifer, 1996. 16(3): p. 119-128.
- Josefsen, T.D., T.H. Aagnes, and S.D. Mathiesen, Influence of diet on the occurrence of intraepithelial microabscesses and foreign bodies in the ruminal mucosa of reindeer calves (*Rangifer tarandus tarandus*). Zentralblatt fur Veterinarmedizin. Reihe A, 1997. 44(5): p. 249-57.
- Josefsen, T.D. and T. Landsverk, Increase in gamma delta T cells in the ruminal mucosa of reindeer calves (*Rangifer tarandus tarandus* L.) induced by baled grass silage. Veterinary immunology and immunopathology, 1997. 60(1-2): p. 197-202.
- 18. Olsen, M.A., T.H. Aagnes, and S.D. Mathiesen, Failure of cellulolysis in the rumen of reindeer fed timothy silage. Rangifer, 1995. 15(2): p. 79-86.
- Olsen, M.A., T.H. Aagnes, and S.D. Mathiesen, The effect of timothy silage on the bacterial population in rumen fluid of reindeer (*Rangifer tarandus tarandus*) from natural summer and winter pasture. Fems Microbiology Ecology, 1997. 24(2): p. 127-136.
- Olsen, M.A. and S.D. Mathiesen, The bacterial population adherent to plant particles in the rumen of reindeer fed lichen, timothy hay or silage. Rangifer, 1998. 18(2): p. 55-64.
- Nilsson, A., I. Olsson, and P. Lingvall, Comparison between grass-silages of different dry matter content fed to reindeer during winter. Rangifer, 1996. 16(1): p. 21-30.
- Nilsson, A., I. Olsson, and P. Lingvall, Evaluation of silage diets offered to reindeer calves intended for slaughter. I. Feeding of silage and barley from September to March. Rangifer, 1996. 16(3): p. 129-138.
- Nilsson, A., I. Olsson, and P. Lingvall, Evaluation of silage diets offered to reindeer calves intended for slaughter. II. Feeding of silage and concentrate from January to March. Rangifer, 1996. 16(3): p. 139-146.
- Nilsson, A., et al., Health, body condition and blood metabolites in reindeer after submaintenance feed intake and subsequent feeding. Rangifer, 2000. 20(4): p. 187-200.

- Nilsson, A., et al., Rumen function in reindeer (Rangifer tarandus tarandus) after sub-maintenance feed intake and subsequent feeding. Rangifer, 2006. 26(2): p. 73-83.
- Wallsten, J., In vivo and in vitro digestibility of lichens and silage for reindeer, in Institutionen för husdjursgenetik, Department of Animal Breeding and Genetics.
 2003, Sveriges lantbruksuniversitet, Swedish University of Agricultural Sciences: Uppsala. p. 26.
- 27. Turunen, M., et al., Impacts of winter feeding of reindeer on vegetation and soil in the sub-Arctic: insights from a feeding experiment. Polar Research, 2013. 32: p. 12.

4.5 Pigs

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4.5.1 Feed value and potential to use ley crops as feed ingredient to pigs

Ley crops are traditionally used as forage to horses and ruminants but to a lesser extent to non-ruminant animals. Due to a favourable protein and amino acid composition, ley crops may serve as a locally produced and highly valuable protein and energy ingredient also for monogastric animals. Research has shown that ley crop silage can be included with the potential to replace parts of the diet to sows [1] and growing/fattening pigs [2, 3, 4, 5, 6, 7]. For pigs, the high plant fibre content limits the protein utilisation. Early cut harvest can benefit the protein digestibility as the leaf:stem ratio is higher in less mature plants. However, a crucial factor when optimizing pig diets is the quality of the protein, i.e., composition of specific amino acids and their digestibility. Research has emphasized that silage, in addition to straw, increases the possibilities for pigs to consume, forage, root and to chew [8, 9]. Moreover, forage diets induce longer periods of satiety and reduce the risk of gastric ulcerations, both when fed as complete silage in e.g. feeding racks and supplementary to straw [10] and with a smaller particle size included in the pigs' feed ration [6].

Although pigs only use forages in limited amounts, forage-based biorefined protein feed can potentially increase the use of forage to pigs and other monogastrics with limited ability to digest fibre. Extraction of the proteins into a liquid fraction through bio refining, increases the bioavailability of the soluble proteins remaining in the juice fraction and might therefore be a better option for pigs. From the juice fraction, proteins can be separated into a paste through heating and centrifugation, and with further drying, a dry protein concentrate can also be recovered [11, 12]. The protein concentrate has a high protein content and a balanced amino acid composition, comparable to soybean meal and its potential to be able to replace imported protein for pigs is therefore of interest.

4.5.2 Digestibility of ley crops

A crucial factor when optimizing pig diets is the quality of the protein, i.e., composition of specific amino acids and their digestibility. Forages are expected to reduce nutrient and energy digestibility. Due to a lower utilisation of energy from volatile fatty acids absorbed in the hindgut than from nutrients absorbed in the ileum, inclusion of dietary fibre content would have a negative effect on the metabolizable and net energy utilisation. The apparent crude nutrient digestibility of lucerne and red clover products (i.e. lucerne leaf silage, lucerne whole plant

silage, and red clover whole plant silage) were estimated to be 53.6, 69.9 and 63.0% for organic matter (OM) and 56.3, 69.3 and 58.0% for CP [13]. Wüstholz et al. [5] suggested better nutrient digestibility by destroying the cell structure, e.g. by extrusion of silage. However, Presto Åkerfeldt et al. [6] could not confirm any significant difference in the apparent total tract digestibility (ATTD) of OM, energy, CP and neutral detergent fibre, neither between diets nor between grass/clover silage of smaller particle size (<5 mm) and chopped silage (5-20 mm). When comparing the ATTD for the silages, a large numerical variation (although not statistically significant) in the digestibility of OM, energy and CP was found with generally higher digestibility in the silage with particle size <5 mm (31.3; 35.6 and 16.8% vs. 23.6; 24.0 and 18.1%). The true faecal digestibility of protein in protein concentrates from white clover plant and leaf showed digestibility values varying from 0.81-0.88 [14]. According to Stodkilde et al. [15] the total tract digestibility of DM in the protein concentrates from white and red clover, lucerne and perennial ryegrass ranged from 60.8% to 76.5% and the standardised N digestibility from 75.4% to 85.0%.

4.5.3 Silage consumption and effect on production

The amount of fresh silage that can be consumed by pigs depends on the age (size) of the pig, the physical structure and chemical composition of the silage, e.g. dry matter (DM) content, fibre content and particle size, as well as the palatability of the silage. Sows have greater capacity to consume and utilize nutrients from fibre rich feed sources than younger pigs. Especially during pregnancy, ley crops have the potential for a high contribution to the sows' requirements for energy, amino acids, minerals and vitamins. In a study by Fernandez et al. [1] the daily intake of energy from grass clover silage, calculated on the basis of the growth of pregnant sows, was on average 1.17 FEs (feed units, equals to 7.9 MJ NE) corresponding to 42% of their daily energy intake. The authors concluded that sows fed moderate amounts of silage are able to perform well in terms of growth, body condition and feed conversion and that silage can contribute with sufficient levels of calcium, phosphorus and zinc during pregnancy.

For growing/fattening pigs, the proportion of silage in the diet is often lower. Fattening pigs (25-100 kg live weight) fed grass silage in combination with a concentrate feed at "high" (100%) level, consumed silage, on a net energy (MJ NE) basis, corresponding to 4% of their total energy intake. When the concentrate fed was restricted to a "low" (70%) level, the silage consumption increased to 5-6% of their total NE intake [16]. The performance of the pigs was in general good, however the "low" concentrate feed reduced the daily gain of the pigs although the feed conversion ratio increased and with a corresponding increase in lean meat content. Wallenbeck et al. [4] found poorer growth among growing/fattening pigs that were fed diets where 20% (energy basis) was replaced by a grass/clover silage,

either fed as intact silage in silage racks or chopped silage mixed with cereal based feed. In that study, the pigs did not consume the total amount of silage ration, and silage refusals were estimated to correspond to approximately 5-10% of the fed silage. Although pigs in the silage treatments were fed 20% less commercial feed on an energy basis compared with control pigs, their weight gain was only 5%–15% lower. This indicates that grass/clover silage can contribute with parts of the nutrient supply to pigs, but the form in which the silage is fed is important for consumption, nutrient utilisation and for the ability of pigs to consume the silage. Also, when growing/fattening pigs were served ad libitum supply of whole-crop (stem, leaf and flower) chicory or red clover silage in the silage rack in combination with 80% (energy basis) of the commercial feed, the growth was diminished by 15 and 10%, respectively [17].

Several studies have included grass/clover silage up to 19% of growing/fattening pigs DM intake [3, 5, 6] with different effects on growth performance and carcass characteristics. Bikker et al. [3] concluded that daily gain, energy utilisation for gain and dressing percentage were lower in silage fed pigs and that the silage intake in the grower period may be too low for optimal feeding of grass silage. In the fattening period however, a higher proportion of grass silage, close to 20%, can be included in the ration. In the study by Presto Åkerfeldt et al. [6], silage was included in the diet at 20% of DM, fed as a total mixed ration (TMR). Silage with smaller particle size (1-3 mm) was consumed to 96% by the pigs, whereas the pigs fed TMR with a chopped silage (5-20 mm) had a lower proportion consumed and left 23% of the fed silage uneaten. A reduction of the particle size by e.g. extrusion could improve the nutrient availability and it was concluded that restricted concentrate feeding enables consumption of high amounts of lucerne silage [5]. On the other hand, a high proportion of fibre in the feed to pigs was related to poor growth performance. Still, lucerne silage can make a relevant contribution to the protein supply of the animals in organic pig fattening [5]. In a study by Friman et al. [7], a grass/clover silage accounted for an estimated 20.5% of the pigs' total DM intake/day and the pigs that received diets with silage inclusion had similar feed conversion ratio (FCR) as pigs with control diets without silage inclusion. Friman et al. [7] suggest that freshly processed silage with a finer structure and fed as a complete feed can supply nutrients to growing pigs.

Johansson et al. [18] studied the effect of red clover silage on the intra-muscular fat content and the fatty acid composition of loin (*M. longissimus dorsi*), and fat content and fatty acid composition in meat after cooking. The pigs consumed 1.25 kg silage/day, equal to 10% of their total energy intake. The results showed lower relative levels of saturated and monounsaturated fatty acids and higher relative levels of polyunsaturated fatty acids and omega 6 fatty acids in raw loins from silage-fed pigs compared with those from pigs on conventional feed. Even though there was a difference in the concentrations of saturated, monounsaturated,

polyunsaturated and omega 6 fatty acids between the raw and the cooked loins, the ratio of omega 6:omega 3 fatty acids remained low in loins from silage-fed pigs after cooking, and thus more beneficial for human nutrition. The sensory quality and cooking loss of frozen stored pork loins from pigs fed conventional feed with or without an admixture of red clover silage showed that pork from pigs fed silage had a more acidulous taste and a greater off-flavour intensity than pork from pigs that did not receive any silage [19]. Silage inclusion had no effect on tenderness, juiciness, off-odour or odour intensity and no differences were found for cooking loss, which were on average 18% in meat from pigs fed conventional feed and 19% for pigs fed additional silage.

4.5.4 Total mixed ration (TMR) with silage to pigs

Including silage in a total mixed ration (TMR) can be beneficial for silage consumption and nutrient utilization as it prevents pigs from sorting out palatable parts of the diet, resulting in less silage refusals. According to Friman et al. [7], growing/finishing pigs fed TMR with either chopped silage (4-15 mm stem length) or intensively manipulated silage (<5 mm stem length), consumed all the silage in the diet. The feeding strategy showed satisfactory growth of all pigs, and pigs fed TMR including silage with the shorter stem length (<5 mm) grew similarly as the pigs on a 100% commercial control diet, whereas those pigs with chopped silage (4-15 mm stem length) required a longer period to reach slaughter weight. Feed conversion ratio and lean meat content was unaffected by silage inclusion, although protein efficiency and lean meat growth were lower among the pigs with TMR with silage compared to those without silage inclusion.

4.5.5 Fractions from biorefined fresh and ensiled ley crops

Circular bioeconomy and biorefining of green biomasses for the production of bioenergy have received much attention in recent years [20]. The fractionation of the green biomass through juicing results in a fibrous press cake and a juice that contains soluble proteins, sugar and certain minerals and could be used for the production of protein feed for farm animals. The content of crude protein and amino acids in the refined fractions depend on plant species and harvest time as well as separation technique. In recent years, research has focused on the value chain for the refining process, including the choice of different raw materials to produce a protein concentrate [15, 11, 21].

4.5.6 Juice

When juice from fresh ley (80% timothy and meadow fescue, 20% red clover) was included at 10% (DM basis) in liquid diets to pigs from 43 kg to slaughter, the pigs performed similarly to those that received a control diet with only water added

[22]. Pig growth was in the same range, except for a slightly reduced growth in pigs with ley crop juice between days 18-58 of the study period, but over the whole study period daily weight gain did not differ. Feed conversion ratio and meat percentage were also unaffected by the silage juice inclusion. The results also showed that the juice did not affect meat and fat quality negatively and had a more favourable omega 6: omega 3 ratio in the subcutaneous fat. The authors also concluded a good stomach health in the pigs fed silage juice [22].

Using fresh harvest of grasses and legumes limits the possibilities to use it during winter seasons, but harvested and stored as silage, it can be used as a local all year-round feed ingredient. The feed value of silage feed juice, from a mixed timothy and meadow fescue sward, in diets to pigs was studied by Keto et al. [23]. No difference was found between pigs fed the diets with silage juice compared to pigs on a control diet, in terms of average daily growth and feed conversion ratio. Moreover, meat quality (pH, colour, drip loss and sensory quality) were unaffected by the juice intake and no differences in gut microbiota could be found. Juice from fresh ley in the study by Adler et al. [22] showed a crude protein (CP) content of 204 g/kg DM and a lysine content of 50 g lysine/kg CP. In juice fractions from white clover, red clover, lucerne and perennial ryegrass in the study by Damborg et al. [11] the CP and lysine values were 282, 238, 323 and 151 g/kg DM and 42.7, 40.2, 48.8 and 41.1 g/kg CP, respectively. According to Keto et al. [23] corresponding values in the silage juice contained 279 g CP/kg DM and 48 g lysine/kg CP.

4.5.7 Protein concentrate

From the juice fraction, the proteins can be precipitated by heat treatment, acid precipitation and centrifugation into a green semi-dry paste, which can be further dried into a protein concentrate [14, 15, 11, 12]. The interest for the dried protein concentrate has been large in pig nutrition, as it contains high levels of crude protein (up to 50% of DM) and a good amino acid composition, comparable to e.g. soybean meal. Examples of contents of crude protein and amino acids in concentrates from white clover, red clover, lucerne and perennial ryegrass are shown in Table 3. A feeding trial in which pigs were fed protein concentrate extracted from ryegrass showed that the pigs maintained good growth and feed conversion ability [23]. Correspondingly, starter, grower and finisher pigs did not differ in terms of performance parameters such as feed intake, growth and slaughter weight [24]. With increasing level of inclusion of grass/clover protein concentrate, the meat percentage in the carcasses increased linearly. The addition of grass/clover protein concentrate also increased the content of omega 3 fatty acids and lowered the ratio of omega 6: omega 3 fatty acids in adipose tissue, liver and Longissimus dorsi.

	Protein concentrate					
	White	Red clover	Lucerne	Perennial	Grass/clover	
	clover			ryegrass	mix ¹	Reference
СР	450.6 (plant)	-	-	_	-	[14]
	530.0 (leaf)	-	-	-	-	[14]
	347	343	388	240	-	[15]
	404	346	405	245	-	[11]
				339	-	[12]
	-	-	-	-	458	[24]
Ly	55.7 (plant)	-	-	-	-	[14]
	51.8 (leaf)	-	-	-	-	[14]
	62.6	66.7	66.2	55.5	-	[15]
	54.2	54.4	56.7	48.9		[14]
				53.4	-	[12]
	-	-	-	-	57.6	[24]
Met	16.4 (plant)	-	-	-	-	[14]
	16.3 (leaf)	-	-	-	-	[14]
	18.3	18.6	19.4	20.9	-	[15]
	16.2	15.7	17.4	18.3		[14]
	-	-	-	19.2	-	[12]
	-	-	-	-	22.7	[24]
Thr	45.4 (plant)	-	-	-	-	[14]
	43.3 (leaf)	-	-	-	-	[14]
	49.5	50.4	49.9	47.6	-	[15]
	45.3	43.8	44.6	42.8		[14]
	-	-	-	44.2	-	[12]
	-	-	-	-	50.2	[24]

Table 3 Contents of crude protein (CP; g/kg DM) and amino acids (g/kg DP) lysine (Lys), methionine (Met) and threonine (Thr) in protein concentrate from white clover (plant and leaves), red clover, lucerne, perennial ryegrass and a grass/clover mix.

¹25 % red clover (*Trifolium pratense*; Callisto), 5 % white clover (*Trifolium repens*; Silvester), 33 % hybrid ryegrass (Italian Ryegrass x meadow fescue) (*Festulolium*; Perseus), 8 % perennial ryegrass (*Lolium perenne*; Abosan 1), 8 % perennial ryegrass (*Lolium perenne*; Calvano 1), 16 % perennial ryegrass (*Lolium perenne*; Humbi 1), and 5 % red fescue (*Festuca rubra*; Gondolin)

4.5.8 Conclusions and knowledge gaps

Ley crops are an interesting option to be included in feed rations to pigs as they can serve as a locally, sustainably produced and highly valuable protein and energy ingredient. Harvested and stored as silage, it can be used as a local all year-round feed ingredient. Although the silage dry matter intake depends on the age (size) of the pig, the physical structure and chemical composition of the silage, e.g. dry matter (DM) content, fibre content and particle size, as well as the palatability of the silage, research indicates that ley crop silages have the potential to contribute with up to around 40% of pregnant sows' daily energy intake. For growing pigs however, the plant fibre content is a limiting factor, which impedes the protein utilisation. Early cut harvest can benefit the protein digestibility as the leaf:stem ratio is higher in less mature plants. Extraction of plant proteins into a liquid fraction through bio refining, increases the bioavailability of the soluble proteins remaining in the juice fraction. Followed by separation and drying, a dry protein concentrate can also be recovered that might be a better option for pigs. However, a crucial

factor when optimizing pig diets is the quality of the protein, i.e., composition of specific amino acids and their digestibility. The content of crude protein and amino acids in silage and the refined fractions depend on plant species, harvest time and fractionation technique and show a large variation in different raw materials and fractions. The nutrient digestibility in pigs is also not very well studied. Research indicates that pigs can perform well in terms of growth and carcass quality as well as get positive behaviour effects by inclusion of ley crop silage in their feed rations. However, it can be concluded that more information on nutrient quality of silage, contents of anti-nutritional substances in some forages and how well the nutrients can be used by the pigs is needed. Moreover, the physical form and structure of the ley crops and silage as well as feeding techniques need to be paid attention for future improvements.

4.5.9 References

- Fernandez, J. A., V. Danielsen, K. Soegaard, H. D. Poulsen, and S. K. Jensen. 2006. "Clover/grass mixtures - grazed or silage - can cover at least half the feeding requirements of pregnant sows. Klovergras - afgrasset eller ensileret - kan dakke mindst halvdelen af dragtige soers naringsbehov." *DJF Rapport, Husdyrbrug* (72):27 pp.
- Danielsen, V., L. L. Hansen, F. Moller, C. Bejerholm, and S. Nielsen. 2000. Production results and sensory meat quality of pigs fed different amounts of concentrate and ad lib. Clover grass or clover grass silage. Edited by J. E. Hermansen, V. Lund and E. Thuen, Ecological animal husbandry in the Nordic countries. Proceedings from NJF-seminar No. 303, Horsens, Denmark, 16-17 September 1999. Tjele, Denmark: Danish Research Centre for Organic Farming (DARCOF).
- 3. Bikker P, Binnendijk G, Vermeer H, van der Peet C 2014. "Grass silage in diets for organic growingfinishing pigs." 4th ISOFAR Scientific Conference 'Building Organic Bridges', at the Organic World Congress, Istanbul, Turkey., 2014, 13-15 Oct.
- 4. Wallenbeck, A., M. Rundgren, and M. Presto. 2014. "Inclusion of grass/clover silage in diets to growing/finishing pigs Influence on performance and carcass quality." *Acta Agriculturae Scandinavica A: Animal Sciences* 64 (3):145-153. doi: 10.1080/09064702.2015.1006668.
- 5. Wüstholz, Jessica, Salomé Carrasco, Ulrich Berger, Albert Sundrum, and Gerhard Bellof. 2017. "Fattening and slaughtering performance of growing pigs consuming high levels of alfalfa silage (Medicago sativa) in organic pig production." *Livestock Science* 200:46-52.
- Presto Åkerfeldt, M., S. Holmström, A. Wallenbeck, and E. Ivarsson. 2018.
 "Inclusion of intensively manipulated silage in total mixed ration to growing pigsinfluence on silage consumption, nutrient digestibility and pig behaviour." *Acta*

Agriculturae Scandinavica A: Animal Sciences 68 (4):190-201. doi: 10.1080/09064702.2020.1725104.

- Friman, J., T. Lundh, and M. Presto Åkerfeldt. 2021. "Grass/clover silage for growing/finishing pigs-effect of silage pre-treatment and feeding strategy on growth performance and carcass traits." *Acta Agriculturae Scandinavica A: Animal Sciences* 70 (3-4):151-160. doi: 10.1080/09064702.2021.1993319.
- 8. Olsen, AW, E-M Vestergaard, and L Dybkjaer. 2000. "Roughage as additional rooting substrates for pigs." *Animal Science* 70 (3):451-456.
- 9. Presto, M Høøk, B Algers, E Persson, and HK Andersson. 2009. "Different roughages to organic growing/finishing pigs—Influence on activity behaviour and social interactions." *Livestock Science* 123 (1):55-62.
- Holinger, Mirjam, Barbara Früh, Peter Stoll, Michael Kreuzer, and Edna Hillmann.
 2018. "Grass silage for growing-finishing pigs in addition to straw bedding: Effects on behaviour and gastric health." *Livestock Science* 218:50-57.
- Damborg, Vinni Kragbæk, Søren Krogh Jensen, Martin R Weisbjerg, Anders Peter Adamsen, and Lene Stødkilde. 2020. "Screw-pressed fractions from green forages as animal feed: chemical composition and mass balances." *Animal Feed Science and Technology* 261:114401.
- 12. Ravindran, Rajeev, Sybrandus Koopmans, Johan PM Sanders, Helena McMahon, and James Gaffey. 2021. "Production of Green Biorefinery Protein Concentrate Derived from Perennial Ryegrass as an Alternative Feed for Pigs." *Clean Technologies* 3 (3):656-669.
- Messinger, D., P. A. Weindl, K. Aulrich, L. Pleger, P. N. Weindl, and G. Bellof. 2021. "Determination of apparent total tract digestibility of nutrients of lucerne (Medicago sativa) and red clover (Trifolium pratense) products in growing pigs." *Zuchtungskunde* 93 (5):389-405.
- 14. Stødkilde, Lene, Vinni K Damborg, Henry Jørgensen, Helle N Lærke, and Søren K Jensen. 2018. "White clover fractions as protein source for monogastrics: dry matter digestibility and protein digestibility-corrected amino acid scores." *Journal of the Science of Food and Agriculture* 98 (7):2557-2563.
- Stodkilde, L., V. K. Damborg, H. Jorgensen, H. N. Laerke, and S. K. Jensen. 2019.
 "Digestibility of fractionated green biomass as protein source for monogastric animals." *Animal* 13 (9):1817-1825. doi: 10.1017/s1751731119000156.
- Hansen, L. L., C. Claudi-Magnussen, S. K. Jensen, and H. J. Andersen. 2006. "Effect of organic pig production systems on performance and meat quality." *Meat Science* 74 (4):605-615. doi: 10.1016/j.meatsci.2006.02.014.
- Presto Åkerfeldt, M., J. Nihlstrand, M. Neil, N. Lundeheim, H. K. Andersson, and A. Wallenbeck. 2019. "Chicory and red clover silage in diets to finishing pigs—influence on performance, time budgets and social interactions." *Organic Agriculture* 9 (1):127-138. doi: 10.1007/s13165-018-0216-z.
- Johansson, L., K. Lundstrom, and A. Jonsall. 2002. "Effects of RN genotype and silage feed on fat content and fatty acid composition of fresh and cooked pork loin." *Meat Science* 60 (1):17-24. doi: 10.1016/s0309-1740(01)00100-0.

- Jonsall, A., L. Johansson, and K. Lundstrom. 2000. "Effects of red clover silage and RN genotype on sensory quality of prolonged frozen stored pork (M. Longissimus dorsi)." *Food Quality and Preference* 11 (5):371-376. doi: 10.1016/s0950-3293(00)00010-0.
- 20. Corona, Andrea, Morten Ambye-Jensen, Giovanna Croxatto Vega, Michael Zwicky Hauschild, and Morten Birkved. 2018. "Techno-environmental assessment of the green biorefinery concept: Combining process simulation and life cycle assessment at an early design stage." *Science of the Total Environment* 635:100-111.
- Stødkilde, Lene, Saman Lashkari, Jørgen Eriksen, and Søren Krogh Jensen. 2021.
 "Enhancing protein recovery in green biorefineries through selection of plant species and time of harvest." *Animal Feed Science and Technology* 278:115016.
- Adler, SA, A Johansen, AK Ingvoldstad, R Eltun, and EJ Gjerlaug-Enger. 2018.
 "Forages-a local protein source for growing pigs." Proceedings of the 9th Nordic Feed Science Conference, Uppsala, Sweden, 12-13 June 2018.
- Keto, L., I. Tsitko, S. Perttila, S. Sarkijarvi, N. Immonen, K. Kytola, H. L. Alakomi, T. Hyytiainen-Pabst, M. Saarela, and M. Rinne. 2021. "Effect of silage juice feeding on pig production performance, meat quality and gut microbiome." *Livestock Science* 254:9. doi: 10.1016/j.livsci.2021.104728.
- Stødkilde, Lene, Morten Ambye-Jensen, and Søren Krogh Jensen. 2021. "Biorefined organic grass-clover protein concentrate for growing pigs: Effect on growth performance and meat fatty acid profile." *Animal Feed Science and Technology* 276:114943.

5. Ley economy

5.1 Ley economics

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5.1.1 Introduction

Innovation, value creation, new product development and other forms of entrepreneurial activity are viewed across the EU as vital pathways to increase agricultural competitiveness and farm survival (see e.g., CAP Rural development programs, EIP-Agri⁴; LRF, 2011⁵; EU, 2020⁶). The agricultural sector is undergoing a protracted state of structural change and rationalization where farms are decreasing in number and becoming larger. This is also the case in the sectors that produces field forage in systems with arable farmland that temporarily is converted into grassland also known as leys. These production systems are complex and contain a wide range of parameters recognized to optimize the system and enable fulfillment of production goals. There is a wide span in the economic result of different choices of production processes and between different farms depending on the circumstances in place and the management.

Despite a lot of effort in trying to develop models and methods in the economy of forage and ley production, there is a discussion among researchers, advisors, and practitioners on how to analyze and describe the economy in forage and ley production, and ultimately put this part of the production into the perspective of the whole operation of the business [1]. One key problem is the collection of data, in the different parts of the process. This along with the structuring, synthesis and analysis is essential for more efficient forage and ley management. Furthermore, digitalization is introduced at a fast pace in all parts of the production chain and

⁴ <u>https://ec.europa.eu/eip/agriculture/en/node.html</u>

⁵ <u>http://www.publishingfarm.com/uploads/Livsmedelsstrategin.pdf</u>

⁶ <u>https://food.ec.europa.eu/system/files/2020-05/f2f_action-plan_2020_strategy-info_en.pdf</u>

gives new opportunities to measure and control the process. In turn, this provides opportunities to evaluate the economy in the production chain.

The aim of this part of the project is to identify knowledge gaps in the production chain, especially focusing on production economics, modelling, methods for collecting data, evaluation of data and farm management.

A literature review was conducted on the forage and ley production system economics. Results were discussed in focus groups related to research, advisory services, and farmers.

5.1.2 Literature review

The aim of the literature review was, as stated in the proposal, to identify current knowledge and gaps in literature. A geographic restriction was set to the Nordic and Baltic countries. From these searches 379 articles were retrieved. After analyzing the abstracts of these, looking for relevance according to the scope, 16 articles remained.

These articles are arranged in groups as follows; production and feed costs, policy and resource optimizing and management.

As a part of the project work, grey literature and other activities connected directly or indirectly to ley production were analyzed. The search for grey literature in relevant Nordic institutions turned out to be challenging and it turned out that surprisingly few relevant works, connected to the questions for this work package were found. To further exemplify, the most relevant work addressing the focus on production economics, modelling, methods for collecting data, evaluation of data and farm management in the valuechain of ley production are connected to advisory tools like Grovfoderverktyget, which is described further below.

5.1.3 Production costs and feed cost

An interesting observation is that most of the articles that are identified in this area have their origin in the field of research on bioenergy. This indicates that the interest in production costs is essential in the bioenergy sector as a foundation for decision making on e.g. investments.

Gissén et al (2014) studied energy inputs and costs in the production of crops as feedstock for biogas production [2] focusing on performance regarding methane yield per hectare and energy input and costs in the production and supply of crops as biogas feedstock. This perspective is not applicable to ley production in practice. Furthermore, calculations are made based upon assumptions, e.g. "nomalized yields". Still the process perspective and ambition to discuss the problem in terms of system and model is interesting.

A similar approach analysed the economic profitability of producing energygrass fuels on marginal agricultural land in Sweden [3]. The authors used a model and assumption costs are calculated based on field shapes and assumed machinery costs. Furthermore, Nilsson and Rosenqvist (2021) studied the impact of field conditions (e.g., field size and shape) and payments (subsidies) for environmental benefits on profitability [4]. The focus on factors like field shape is interesting in practice. The study suggests that field shape is one important factor that leads to higher costs. The study built its results on models which is relevant for the purpose of research in investigating differences in costs of different crops. However, the method is less relevant in the context of the on farm real cost.

Gunnarsson et al (2008) built a model to evaluate the handling system comprising harvest, transport and ensiling of forage for production of biogas [1]. The costs of different transport and harvesting systems were compared in a structured model based on the specification for the specific plant. This gives interesting views on how to construct a model on e.g., a specific farm. This type of method, modelling to structure different types of costs is also used in other studies [5, 6]. Here they used models to examine different aspects of costs in silage production like the effects of cutting frequencies, and also present dataset for decision support. The model is of a realistic farm that gives results that are easy to adapt in real life farms. The calculations are made based on a model and with assumed inputs from available software to optimize feed. The findings come closer to the context of real farms, but still lack on-field measures, and point out concrete favourable measures. These could be leading in the construction of management practices on the specific farm.

Latvietis et al (2008) discussed the role of feed costs in the building of competitive advantages in milk production in Latvia [7]. This approach had its starting point at a time when the industry was struggling with low competitiveness, causing a decline in milk production. The solution in this case was to have a higher grass forage content in total forage content in mixed leys [7].

Grovfoderverktyget⁷ is a web-based tool that was launched in February 2014. In contact with stakeholders, it turns out that the tool is well known among advisors but not so frequently used. One obstacle is the models for calculation, that is often based on generalizations and data that tend to be outdated. This raises questions about current relevance and how the tool is maintained and developed as well as how it is implemented in the advisory services capacity building and education.

Agriwise⁸ is a web-based tool where one can experiment, plan, and compare different production possibilities. The data used in the calculations are taken from organizations within the industry and the tool is updated annually. The tool can serve as a base for calculation on farm level but not really addresses the on-farm situation and its complexity.

⁷ www.grovfoderverktyget.se/?p=31048&m=4436

⁸ www.agriwise.se/web

The project "Räkna med vall" (Count on ley) has its aim to shed light on how harvest levels, economics and the environment are affected when two-year mixed ley are introduced in crop rotations with one-year crops. Specific objectives were to show how production costs and profitability of energy and protein production change when ley for silage is introduced into crop rotations. The project contributes to the understanding of complexity when taking into account crop rotation as a part of the total economy on the farm [8].

5.1.4 Policy and resource optimizing

Policy has an important role in economy and optimization of resources. It affects profitability of different land-use options. This is an important factor in the perspective of ley economics [9, 10]. However, there was little support for promotion through premium payments to increase clover-grass production in forage production in Finland [9, 10]. The authors also found that the effect is dependent on the product prices for milk and meat.

Trubins (2013) highlight a policy of transformation of land use, in this case 2003 CAP reform [9]. In the region that was studied the change of policy led to an abundance of temporary grass areas, compared to the estimated use for forage in the area. This in turn points out the possibility that forage from temporary ley, results in more extensive use of permanent grasslands and fallows. This indicates that, given economic rational, policy can have impact on the choice of crops and use of forage.

5.1.5 Management

The articles categorized as "Management" deal with a wide spectrum of issues.

Lehtonen et al (2018) contributes with views on ley production yields from farmers and other stakeholders views in Finland [11]. The project has a clear focus on management method. Important means for higher crop yields, indicated by workshop with stakeholders, were improved soil conditions with drainage and liming, in addition to improved crop rotations, better sowing techniques, careful selection of cultivars and forage grass mixtures. Stakeholders also suggested solutions for improving both crop yields and farm income, including optimized use of inputs, focusing production at the most productive fields and actively developed farming skills and knowledge sharing. A similar approach to stakeholder involvement [12] studied methods to apply and analyse research results in discussion groups of farmers and advisers. The project resulted in a significant rise of average silage yield for the group, which in turn had a big impact on silage production costs and on the economy of the whole farm. Some topics, which the group had experienced to be particularly important to their development were the effect of grass establishment on grass density, diverse grass mixtures and the discussion group membership. This gives implications on how to develop efficient methods to develop and transfer knowledge. A similar approach to study knowledge transfer was used by Puumala (2004), in a project to improve margins by mixing feed on farm [13]. Building on most common feeding principles, ensure sufficient good quality grass forage, the project studied the different ways and techniques of distributing feed and concentrated feed alternatives.

On farm management focusing on the entrepreneurs' awareness of their own resources and their development is a key for competitiveness [14]. In addition to efficient use of labour, dairy farms with low production costs also shared other characteristics, like the use of more home-grown grass silage per cow than other groups. Furthermore, operational managerial practices can contribute to improved farm level efficiency on dairy farms [15]. The main contribution of the project was investigating aspects that can be adjusted in the everyday management to improve farm efficiency [15]. The results showed that changes in breeding and feeding practices can affect efficiency substantially, which in turn emphasises the management perspective as a crucial part in farm business.

5.1.6 Conclusions and knowledge gaps

With the described approach to the literature review, it can be concluded that just a few articles were found to be relevant to the theme economy of forage and ley production. It is also interesting to find that there is a close relationship to the field of bioenergy.

The management in relation to the challenges in farm businesses is studied in a few articles. This is somewhat surprising since ley and production based on ley production, is of such great economic importance in the geographic area of the study.

5.1.7 Stakeholder workshop

Building on the literature review and observations of "grey" literature it is argued that the theme of economy of forage and ley production, is not very well established in literature. On the background of the challenges described in the project proposal one important part of the process was a workshop with stakeholders. The workshop, that was held as digital workshop February 8, 2022, gave some important insights from different stakeholder on actual problems. More detailed notes are in Appendix 2.

The ley production in Swedish context is multi-facetted. Production systems vary from north to south, and even from east to west. Also, ley production systems vary depending on type of production with differences between dairy, beef, lamb, pigs and reindeers. In the perspective of economy and management, this puts focus on the need of versatile systems and models that can handle calculations, measurements and analyses in different systems.

Furthermore, different aspects on farm level and inter-farm level problems have been raised in the discussions with stakeholders.

- The economy of scale and diminishing returns is a real problem at farm level. Often connected to farm to field logistics, field logistics, field form and type of production systems.
- The diversification at farm level, between different crops and crop rotations, irrigation and drainage challenges the current models for economic calculation and analysis.
- Management systems are also challenged by different system approaches on choice of machinery, harvest methods and feeding systems.
- Pricing models for ley as feed is raised as a pivotal part of the discussion about the evaluation of economy in both arable and animal husbandry parts of farming.

This leads to several opportunities for future research.

- Linear programming (LP) can help in determining the optimal crop pattern and production-planning pattern for feed crops. This will fuel the discussion on how to maximize the profits on the farm business level.
- Machine Learning (ML) algorithms, have emerged as promising alternative and complimentary tools to the commonly used modelling approaches in agriculture and allied sciences. ML algorithms have gained popularity in crop production and yield prediction. There are still few examples of actual implementation in "real" farming.
- Digitalization and precision farming, connected to ley production, is still to be implemented in a broader perspective.

5.1.8 References

- Gunnarsson, C., L. Vågström, and P.A. Hansson, Logistics for forage harvest to biogas production-Timeliness, capacities and costs in a Swedish case study. Biomass and Bioenergy, 2008. 32(12): p. 1263-1273.
- Gissen, C., et al., Comparing energy crops for biogas production yields, energy input and costs in cultivation using digestate and mineral fertilisation. Biomass & Bioenergy, 2014. 64: p. 199-210.
- Nilsson, D., H. Rosenqvist, and S. Bernesson, Profitability of the production of energy grasses on marginal agricultural land in Sweden. Biomass and Bioenergy, 2015. 83: p. 159-168.
- Nilsson, D. and H. Rosenqvist, Profitability of crop cultivation in small arable fields when taking economic values of ecosystem services into account. Sustainability, 2021. 13(23): p. 20.
- Flaten, O., et al., Forage production strategies for improved profitability in organic dairy production at high latitudes. Livestock Science, 2019. 223: p. 97-107.

- 6. Flaten, O., D.M. Atsbeha, and T. Lunnan, Data to estimate costs of producing grass-clover silages. Data in brief, 2020. 31: p. 106003.
- Latvietis, J., et al., Characteristics of forage from the aspect of milk costs. Latvijas Lauksaimniecibas Universitate - Raksti, 2008(21): p. 51-56.
- 8. Tidåker, P., et al., Räkna med vall: Hur påverkas ekonomi och miljö när vall införs i spannmålsdominerade växtföljder? 2016.
- 9. Trubins, R., Land-use change in southern Sweden: Before and after decoupling. Land Use Policy, 2013. 33: p. 161-169.
- 10. Lehtonen, H. and O. Niskanen, Promoting clover-grass: Implications for agricultural land use in Finland. Land Use Policy, 2016. 59: p. 310-319.
- Lehtonen, H., et al., Higher crop yield levels in the North Savo region means and challenges indicated by farmers and their close stakeholders. Agriculture, 2018. 8(7): p. 93.
- Makila, R., A. Ella, and J. Storberg. Sharing best practice in discussion groups encourages farmers to achieve top results. in EGF. 2018. Fermoy, Irish Republic: Teagasc, Animal & Grassland Research and Innovation Centre.
- Puumala, L., Economical feeding planning and alternatives. Teho, 2004(5): p. 35-36.
- 14. Lampinen, K., Workload and profitability on dairy farms. Teho, 2006(5): p. 9-10, 37.
- Hansson, H. and B. Ohlmer, The effect of operational managerial practices on economic, technical and allocative efficiency at Swedish dairy farms. Livestock Science, 2008. 118(1-2): p. 34-43.

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Appendix 1. Search terms

Crop Production

((TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian) OR AD= (Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian)) and TS= ((ley* or forage* or silage* or hay or haylage or grassland or meadow or grass or legume) and (timothy or fescue or clover* or lucerne or alfalfa or ryegrass* or "birdsfoot trefoil" or "goat's rue" or "common vetch" or sainfoin or chicory or caraway or "ribwort plantain" or "salad burnet" or cocksfoot or "meadow grass" or "smooth brome" or "reed canary-grass" or lotus or trifolium or medicago or vicia or lolium or festuca or "poa pratensis" or "phleum pratense" or "galega orientalis" or "dactylis glomerata" or "plantago lanceolata" or "bromus inermis" or "onobrychis viciifolia" or "cichorium intybus" or "sanguisorba minor" or "carum carvi" or "phalaris arundinacea") and (breeding or genetics or cultivar* or variet* or "forage mixture" or "seed mixture" or drain* or fertili* or lime or liming or (sowing near/2 (date* or rate*)) or "remote sensing" or satellite or spectr* or "precision agriculture" or "digital tool*" or harvest* or pest* or fungicide* or herbicide* or cut* or manure or slurry or urine or irrigation or "variable rate*" or (spatial near/1 (variability or variation)) or till* or drought or stress or toleran* or weed* or competit* or temperature or "crop rotation" or "root rot" or "clover rot" or scleroti* or inoculat*) and (yield* or qualit* or "winter survival" or toleran* or overwintering or "winter hardening" or harden or "dry matter" or carbohydrate* or NDF or ADF or "crude protein" or longevity or producti* or "nutrient value*" or "nutritive value" or resistan* or "growth rate" or sward or "nitrogen fixation" or "botanical composition" or digesti* or metaboli* or sustainab* or tannin* or establish* or competit* or persisten* or proportion or canopy or height* or stolon* or shoot or development* or pheno* or stem or lea* or length* or germinat*)))

Search string categories:

Geographic restriction:

TS,AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian)

Subject area:

TS=(ley* or forage* or silage* or hay or haylage or grassland or meadow or grass or legume)

Species:

TS=(timothy or fescue or clover* or lucerne or alfalfa or ryegrass* or "birdsfoot trefoil" or "goat's rue" or "common vetch" or sainfoin or chicory or caraway or "ribwort plantain" or "salad burnet" or cocksfoot or "meadow grass" or "smooth brome" or "reed canary-grass" or lotus or trifolium or medicago or vicia or lolium or festuca or "poa pratensis" or "phleum pratense" or "galega orientalis" or "dactylis glomerata" or "plantago lanceolata" or "bromus inermis" or "onobrychis viciifolia" or "cichorium intybus" or "sanguisorba minor" or "carum carvi" or "phleuri arundinacea")

Management:

TS=(breeding or genetics or cultivar* or variet* or "forage mixture*" or "seed mixture*" or drain* or fertili* or lime or liming or (sowing near/2 (date* or rate*)) or "remote sensing" or satellite or spectr* or "precision agriculture" or "digital tool*" or harvest* or pest* or fungicide* or herbicide* or cut* or manure or slurry or urine or irrigation or "variable rate*" or (spatial near/1 (variability or variation)) or till* or drought or stress or toleran* or weed* or competit* or temperature or "crop rotation" or "root rot" or "clover rot" or scleroti* or inoculat*)

Outcome:

TS=(yield* or qualit* or "winter survival" or toleran* or overwintering or "winter hardening" or harden or "dry matter" or carbohydrate* or NDF or ADF or "crude protein" or longevity or producti* or "nutrient value*" or "nutritive value" or resistan* or "growth rate" or sward or "nitrogen fixation" or "botanical composition" or digesti* or metaboli* or sustainab* or tannin* or establish* or competit* or persisten* or proportion or canopy or height* or stolon* or shoot or development* or pheno* or stem or lea* or length* or germinat*)

Web of Science Core Collection, 2000-2022 => 1832 poster CAB Abstracts 2000-2022 => 2963 poster Medline 2000-2022 => 387 poster Scopus:

(TITLE-ABS-KEY (ley* OR forage* OR silage* OR hay OR haylage OR grassland OR meadow OR grass OR legume)) AND (TITLE-ABS-KEY (timothy OR fescue OR clover* OR lucerne OR alfalfa OR ryegrass* OR "birdsfoot trefoil" OR "goat's rue" OR "common vetch" OR sainfoin OR chicory OR caraway OR "ribwort plantain" OR "salad burnet" OR cocksfoot OR "meadow grass" OR "smooth brome" OR "reed canary-grass" OR lotus OR trifolium OR medicago OR vicia OR lolium OR festuca OR "poa OR "phleum pratense" OR "galega orientalis" OR "dactylis pratensis" glomerata" OR "plantago lanceolata" OR "bromus inermis" OR "onobrychis viciifolia" OR "cichorium intybus" OR "sanguisorba minor" OR "carum carvi" OR "phalaris arundinacea")) AND (TITLE-ABS-KEY (breeding OR genetics OR cultivar* OR variet* OR "forage mixture*" OR "seed mixture*" OR drain* OR fertili* OR lime OR liming OR (sowing AND near/2 (date* OR rate*)) OR "remote sensing" OR satellite OR spectr* OR "precision agriculture" OR "digital tool*" OR harvest* OR pest* OR fungicide* OR herbicide* OR cut* OR manure OR slurry OR urine OR irrigation OR "variable rate*" OR (spatial W/1 (variability OR variation)) OR till* OR drought OR stress OR toleran* OR weed* OR competit* OR temperature OR "crop rotation" OR "root rot" OR "clover rot" OR scleroti* OR inoculat*)) AND (TITLE-ABS-KEY (yield* OR qualit* OR "winter survival" OR toleran* OR overwintering OR "winter hardening" OR harden OR "dry matter" OR carbohydrate* OR ndf OR adf OR "crude protein" OR longevity OR producti* OR "nutrient value*" OR "nutritive value" OR resistan* OR "growth rate" OR sward OR "nitrogen fixation" OR "botanical composition" OR digesti* OR metaboli* OR sustainab* OR tannin* OR establish* OR competit* OR persisten* OR proportion OR canopy OR height* OR stolon* OR shoot OR development* OR pheno* OR stem OR lea* OR length* OR germinat*)) AND (TITLE-ABS-KEY (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvian OR lithuania OR lithuanian OR estonia OR estonian) OR AFFIL (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvian OR lithuania OR lithuanian OR estonia OR estonian))

Begränsat till år 2000-2022: 1703 poster Sammanlagt: 6885 Efter automatisk dublettsortering: 4043 Efter ytterligare manuell dublettsortering: 3925

Harvest and conservation

Web of Science Core Collection

TS=(ley* or forage* or silage* or hay or haylage)

AND

TS=((harvest* near/2 (method or technique)) or mowing or "pre wilt*" or wilting or shredd* or "dry matter content" or (bunker or tower or clap or trench or laboratory or mini) near/1 (silo or silage) or "glass jars" or bags or "round bale" or bale or tubes or conservation or biorefinery or chopping or proteoly* or "particle size" or "precision choppe*" or "cutting wagon" or "self-loading wagon" or baler or "plastic film" or "silage additive*" or ((addition or inoculation or inoculant or additive) near/1 (enzyme or acid* or salt or microb* or silage)))

AND

TS=("harvest* loss*" or "storage loss*" or "dry matter loss*" or ferment* or proteolys* or "aerobic stability" or "green protein" or "fiber pulp" or "protein quality" or clostridia or "silage quality" or antioxidant or "nutrient composition" or intake or "milk yield" or "average daily gain" or "animal performance")

AND

TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or lativian or Lithuania or lithuanian or Estonia or estonian) OR AD= (Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or lativian or Lithuania or lithuanian or Estonia or estonian)

Limit to publication year 2000 - 2022-03-24

 \Rightarrow 303 hits

CAB Abstracts

Same search query as for Web of Science CC ⇒ 500 hits

Medline Same search query as for Web of Science CC ⇒ 61 hits

Scopus

TITLE-ABS-KEY (ley* OR forage* OR silage* OR hay OR haylage) AND

TITLE-ABS-KEY ((harvest* W/2 (method OR technique)) OR mowing OR "pre wilt*" OR wilting OR shredd* OR "dry matter content" OR ((bunker OR tower OR clap OR trench OR laboratory OR mini) W/1 (silo OR silage)) OR "glass jars" OR bags OR "round bale" OR bale OR tubes OR conservation OR biorefinery OR chopping OR proteoly* OR "particle size" OR "precision choppe*" OR "cutting wagon" OR "self-loading wagon" OR baler OR "plastic film" OR "silage additive*" OR ((addition OR inoculation OR inoculant OR additive) W/1 (enzyme OR acid* OR salt OR microb* OR silage)))

AND

TITLE-ABS-KEY ("harvest* loss*" OR "storage loss*" OR "dry matter loss*" OR ferment* OR proteolys* OR "aerobic stability" OR "green protein" OR "fiber pulp" OR "protein quality" OR clostridia OR "silage quality" OR antioxidant OR "nutrient composition" OR intake OR "milk yield" OR "average daily gain" OR "animal performance")

AND

TITLE-ABS-KEY (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR lativian OR lithuania OR lithuanian OR estonia OR estonian) OR AFFIL (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR lativian OR lithuania OR estonia OR estonian)

Limit to publication date 2000 - 2022-03-24 $\Rightarrow 238$ hits

All together; =>1102

After deduplication by Endnote: 678

After manual deduplication: 657

After sorting after titles 374

Preliminary

After sorting after abstracts Ensiling 66 Feeding 17 Biorefinery 13 Forage species 3 Harvest 5 Wilting 2 Storage 4 Haylage 1

Feed value for animals

Dairy cattle

Ley WP4 Dairy Cattle 2022-03-30

Web of Science Core Collection:

(TS=("ley" or "leys" or "hay" or haylage or ((forage* or silage* or roughage) near/3 (grass* or legume* or timothy or fescue* or clover* or lucerne or alfalfa or perennial* or lolium or phleum or dactylis or festuca or bromus or trifolium or medicago or "lotus corniculatus" or "onobrychis viciifolia")))) AND (TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or latvian or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or Islandic or "Faroe islands" or Baltic or Latvia or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or Itelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian))

AND

TI=("dairy cow*" or "dairy cattle" or "cow") or ab=("dairy cow*" or "dairy cattle" or "cow") or ak=("dairy cow*" or "dairy cattle" or "cow")

Limit to publication year 2000-2022

 \Rightarrow 444 results

CAB Abstracts, Medline

(TS=("ley" or "leys" or "hay" or haylage or ((forage* or silage* or roughage) near/3 (grass* or legume* or timothy or fescue* or clover* or lucerne or alfalfa or perennial* or lolium or phleum or dactylis or festuca or bromus or trifolium or medicago or "lotus corniculatus" or "onobrychis viciifolia")))) AND (TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or latvian or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi* or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuanian or Estonia or estonian))

AND

TS=("dairy cow*" or "dairy cattle" or "cow") Limit to publication year 2000-2022

CAB => 580 results Medline => 166 results

Scopus

(TITLE-ABS-KEY ("dairy cow*" OR "dairy cattle" OR "cow")) AND ((TITLE-ABS-KEY ("ley" OR "leys" OR "hay" OR haylage OR ((forage* OR silage* OR roughage) W/3 (grass* OR legume* OR timothy OR fescue* OR clover* OR lucerne OR alfalfa OR perennial* OR lolium OR phleum OR dactylis OR festuca OR bromus OR trifolium OR medicago OR "lotus corniculatus" OR "onobrychis viciifolia")))) AND (TITLE-ABS-KEY (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvian OR lithuania OR lithuanian OR estonia OR estonian) OR AFFIL (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvian OR lithuania OR lithuanian OR estonia OR estonian)))

Limit to publication year 2000-2022 ⇒ 453 results

All together: => 1643 results After automatic deduplication => 809 results After further manual deduplication => 778 results

Beef cattle

Beef cattle 2022-03-30

Web of Science Core Collection

TI=("beef cattle" or "growing cattle" or "finishing cattle" or bull or bulls or steer or steers or heifer or heifers or "suckler cow*") or AB= ("beef cattle" or "growing cattle" or "finishing cattle" or bull or bulls or steer or steers or heifer or heifers or "suckler cow*") or AK=("beef cattle" or "growing cattle" or "finishing cattle" or bull or bulls or steer or steers or heifer or heifers or

AND

(TS=("ley" or "leys" or "hay" or haylage or ((forage* or silage* or roughage) near/3 (grass* or legume* or timothy or fescue* or clover* or lucerne or alfalfa or perennial* or lolium or phleum or dactylis or festuca or bromus or trifolium or medicago or "lotus corniculatus" or "onobrychis viciifolia")))) AND (TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or latvian or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or Latvia or latvian or Lithuania or Bithuanian or Estonia or Baltic or Latvia or latvian or Lithuania or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuanian or Estonia or estonian))

Limit to publication year 2000 to 2022

 \Rightarrow 158 results

CAB Abstracts and Medline

TS=("beef cattle" or "growing cattle" or "finishing cattle" or bull or bulls or steer or steers or heifer or heifers or "suckler cow*")

AND

(TS=("ley" or "leys" or "hay" or haylage or ((forage* or silage* or roughage) near/3 (grass* or legume* or timothy or fescue* or clover* or lucerne or alfalfa or

perennial* or lolium or phleum or dactylis or festuca or bromus or trifolium or medicago or "lotus corniculatus" or "onobrychis viciifolia")))) AND (TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi*

Limit to publication year 2000-2022

CAB => 178 results Medline => 42 results

Scopus

((TITLE-ABS-KEY ("ley" OR "leys" OR "hay" OR haylage OR ((forage* OR silage* OR roughage) W/3 (grass* OR legume* OR timothy OR fescue* OR clover* OR lucerne OR alfalfa OR perennial* OR lolium OR phleum OR dactylis OR festuca OR bromus OR trifolium OR medicago OR "lotus corniculatus" OR "onobrychis viciifolia"))) AND (TITLE-ABS-KEY (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvian OR lithuania OR lithuanian OR estonia OR estonian) OR AFFIL (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelands" OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvian OR lithuania OR baltic OR latvia OR latvian OR lithuania OR stonian))

Limit to publication year 2000-2022 ⇒ 139 results

All together: => 517 results After automatic deduplication => 259 results After manual deduplication => 238 results

Sheep

Web of Science Core Collection:

TS=("ley" or "leys" or "hay" or haylage or ((forage* or silage* or roughage) near/3 (grass* or legume* or timothy or fescue* or clover* or lucerne or alfalfa or perennial* or lolium or phleum or dactylis or festuca or bromus or trifolium or medicago or "lotus corniculatus" or "onobrychis viciifolia")))

AND

TI=(sheep or lamb* or "ewe" or "ewes" or hogget* or wether or wethers or "ram" or "rams") or AB=(sheep or lamb* or "ewe" or "ewes" or hogget* or wether or

wethers or "ram" or "rams") or AK=(sheep or lamb* or "ewe" or "ewes" or hogget* or wether or wethers or "ram" or "rams")

AND

TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian)

Limited to publication date 2000-01-01 to 2022-03-30

 \Rightarrow 127 results

CAB Abstracts och Medline

TS=("ley" or "leys" or "hay" or haylage or ((forage* or silage* or roughage) near/3 (grass* or legume* or timothy or fescue* or clover* or lucerne or alfalfa or perennial* or lolium or phleum or dactylis or festuca or bromus or trifolium or medicago or "lotus corniculatus" or "onobrychis viciifolia")))

AND

TS=(sheep or lamb* or "ewe" or "ewes" or hogget* or wether or wethers or "ram" or "rams")

AND

TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian)

Limited to publication date 2000-01-01 to 2022-03-30

CAB: \Rightarrow 145 results

Medline => 35 results

Scopus:

(TITLE-ABS-KEY (sheep OR lamb* OR "ewe" OR "ewes" OR hogget* OR wether OR wethers OR "ram" OR "rams")) AND ((TITLE-ABS-KEY ("ley" OR "leys" OR "hay" OR haylage OR ((forage* OR silage* OR roughage) W/3 (grass* OR legume* OR timothy OR fescue* OR clover* OR lucerne OR alfalfa OR perennial* OR lolium OR phleum OR dactylis OR festuca OR bromus OR trifolium OR medicago OR "lotus corniculatus" OR "onobrychis viciifolia")))) AND (TITLE-ABS-KEY (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvian OR lithuania OR lithuanian OR estonia OR estonian) OR AFFIL (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvia OR latvian OR lithuania OR lithuanian OR estonia OR estonian)))

Limited to publication year 2000-2022 \Rightarrow 131 results

All together: => 438 results After automatic deduplication: => 228 results After further manual deduplication => 220 results

Reindeer

Web of Science Core Collection

TI=(reindeer or "rangifer tarandus") or AB= (reindeer or "rangifer tarandus") or AK=(reindeer or "rangifer tarandus")

AND

(TS=("ley" or "leys" or "hay" or haylage or ((forage* or silage* or roughage) near/3 (grass* or legume* or timothy or fescue* or clover* or lucerne or alfalfa or perennial* or lolium or phleum or dactylis or festuca or bromus or trifolium or medicago or "lotus corniculatus" or "onobrychis viciifolia")))) AND (TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or Iithuanian or Estonia or estonian) or AD=(Nordic or scandi* or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Iithuanian or Estonia or estonian))

 \Rightarrow 16 results

CAB Abstracts and Medline

TS=(reindeer or "rangifer tarandus")

AND

(TS=("ley" or "leys" or "hay" or haylage or ((forage* or silage* or roughage) near/3 (grass* or legume* or timothy or fescue* or clover* or lucerne or alfalfa or perennial* or lolium or phleum or dactylis or festuca or bromus or trifolium or medicago or "lotus corniculatus" or "onobrychis viciifolia")))) AND (TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi* or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or lithuanian or Estonia or estonian))

 $CAB \Rightarrow 47$ results

Medline \Rightarrow 8 results

Scopus

TITLE-ABS-KEY ("ley" OR "leys" OR "hay" OR haylage OR ((forage* OR silage* OR roughage) W/3 (grass* OR legume* OR timothy OR fescue* OR clover* OR lucerne OR alfalfa OR perennial* OR lolium OR phleum OR dactylis OR festuca OR bromus OR trifolium OR medicago OR "lotus corniculatus" OR "onobrychis viciifolia"))) AND (TITLE-ABS-KEY (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvian OR lithuania OR lithuanian OR estonia OR estonian) OR AFFIL (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR lithuania OR lithuania OR lithuania OR finland OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR lithuania OR lithuania OR lithuania OR finland OR lithuania OR lithuania OR lithuania OR finland OR lithuania OR lithuania OR estonia OR estonian) AND TITLE-ABS-KEY (reindeer or "rangifer tarandus")

Scopus \Rightarrow 20 results

All => 91 results Automatic deduplication => 65 Further manual deduplication => 61

Pigs

Web of Science Core Collection

TI=(pig or pigs or swine or "hog" or "hogs" or "sow" or "sows" or piglet*) or AB= (pig or pigs or swine or "hog" or "hogs" or "sow" or "sows" or piglet*) or AK=(pig or pigs or swine or "hog" or "hogs" or "sow" or "sows" or piglet*)

AND

(TS=("ley" or "leys" or "hay" or haylage or ((forage* or silage* or roughage) near/3 (grass* or legume* or timothy or fescue* or clover* or lucerne or alfalfa or perennial* or lolium or phleum or dactylis or festuca or bromus or trifolium or medicago or "lotus corniculatus" or "onobrychis viciifolia")))) AND (TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or lithuanian or Estonia or estonian) or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or Bittic or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Icelandic or "Faroe islands" or Baltic or Latvia or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or Itelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands" or Baltic or Latvia or Itelandic or "Faroe islands"

Limit to publication year 2000-2022

WoSCC \Rightarrow 60 results

CAB Abstracts, Medline

TS=(pig or pigs or swine or "hog" or "hogs" or "sow" or "sows" or piglet*) AND

(TS=("ley" or "leys" or "hay" or haylage or ((forage* or silage* or roughage) near/3 (grass* or legume* or timothy or fescue* or clover* or lucerne or alfalfa or perennial* or lolium or phleum or dactylis or festuca or bromus or trifolium or medicago or "lotus corniculatus" or "onobrychis viciifolia")))) AND (TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or finland or finnish or Iceland or icelandic or estonian) or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or AD=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or latvian or Lithuania or Islandic or "Faroe islands" or Baltic or Latvia or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or Ithuanian or Estonia or Baltic or Latvia or Ithuanian or Estonia or Icelands" or Baltic or Latvia or Ithuanian or Estonia or Ithuanian or Icelandic or "Faroe islands" or Baltic or Latvia or Ithuanian or I

Limit to publication year 2000-2022

CAB Abstracts => 73 results

Medline \Rightarrow 11 results

Scopus

TITLE-ABS-KEY ("ley" OR "leys" OR "hay" OR haylage OR ((forage* OR silage* OR roughage) W/3 (grass* OR legume* OR timothy OR fescue* OR clover* OR lucerne OR alfalfa OR perennial* OR lolium OR phleum OR dactylis OR festuca OR bromus OR trifolium OR medicago OR "lotus corniculatus" OR "onobrychis viciifolia"))) AND (TITLE-ABS-KEY (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvian OR lithuania OR lithuanian OR estonia OR estonian) OR AFFIL (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvian OR lithuania OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR latvian OR lithuania OR lithuanian OR estonia OR estonian)) AND TITLE-ABS-KEY (pig OR pigs OR swine OR "hog" OR "hogs" OR "sow" OR "sows" OR piglet*)

Limit to publication year 2000-2022 ⇒ 56 results

All: 200 Automatic deduplication => 122 Further manual deduplication => 103

Ley economy

Web of science

TS=(profitab* or econom* or business or "cost efficien*" or "cost effect*" or "agronomic efficiency" or "economic performance" or "least cost" or "income over feed cost" or iofc or "cost benefi*" or "return rate" or financ* or investm* or "earning capacit*" or financ*) AND TS=("ley" or "leys" or "hay" or haylage or ((forage* or silage*) near/3 (grass* or legume* or timothy or fescue* or clover* or lucerne or alfalfa or perennial* or lolium or phleum or dactylis or festuca or bromus or trifolium or medicago or "lotus corniculatus" or "onobrychis viciifolia"))) and (TS=(Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or lativian or Lithuania or lithuanian or Estonia or estonian) OR AD= (Nordic or scandi* or Sweden or swedish or Norway or norwegian or Denmark or danish or Finland or finnish or Iceland or icelandic or "Faroe islands" or Baltic or Latvia or lativian or Lithuania or lithuanian or Estonia or estonian)) and 2022 or 2021 or 2020 or 2019 or 2018 or 2017 or 2016 or 2015 or 2014 or 2013 or 2012 or 2011 or 2010 or 2009 or 2008 or 2007 or 2006 or 2005 or 2004 or 2003 or 2002 or 2001 or 2000 (Publication Years)

Web of Science Core Collection =>160 CAB Abstracts =>196 Medline =>37

Scopus:

TITLE-ABS-KEY (profitab* OR econom* OR business OR "cost efficien*" OR "cost effect*" OR "agronomic efficiency" OR "economic performance" OR "least cost" OR "income over feed cost" OR iofc OR "cost benefi*" OR "return rate" OR financ* OR investm* OR "earning capacit*" OR financ*) AND TITLE-ABS-KEY ("ley" or "leys" OR "hay" OR haylage OR ((forage* OR silage*) W/3 (grass* OR legume* OR timothy OR fescue* OR clover* OR lucerne OR alfalfa OR perennial* OR lolium OR phleum OR dactylis OR festuca OR bromus OR trifolium OR medicago OR "lotus corniculatus" OR "onobrychis viciifolia"))) AND (TITLE-ABS-KEY (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR lativian OR lithuania OR lithuanian OR estonia OR estonian) OR AFFIL (nordic OR scandi* OR sweden OR swedish OR norway OR norwegian OR denmark OR danish OR finland OR finnish OR iceland OR icelandic OR "Faroe islands" OR baltic OR latvia OR lativian OR lithuania OR lithuanian OR estonia OR estonian))

Tid 2000 till 2022-03-28

⇒ 222

Tillsammans: => 615 Efter automatisk deduplicering => 398 Efter manuell deduplicering => 379

Appendix 2. Stakeholder workshop

The workshop, was held as a digital workshop on February 8, 2022, and gave some important insights from different stakeholder on actual problems.

Theme	Comments
Value of feed	 Shall we harvest more energy-dense forage, or shall we harvest less frequently when the fertilizer and fuel prices are high? Protein feed is also expensive. Shall we use the forage as a protein source more intensively? Count on the digestibility and not just on protein content and quality from forage. It is more relevant to evaluate the ley costs per unit of energy instead of per unit of dry matter as forages can differ much in quality. Set a price of the ley based on its nutrient content Different prices for different silages Values for protein and digestibility etc. Costs of concentrates when feeding different qualities of forages.
Strategies in use of inputs	 Economical outcome from fertilization of ley. Is it economical to fertilize the ley to get a better forage quality for the sheep or shall we buy pelleted forage? Optimal nitrogen application rate in relation to the forage feed value
Evaluating costs of production	 Costs of the ley on farm level Forage costs per kg of milk have a wide range and the need for further research Difficult to estimate dry-matter losses during harvest, ensiling and feeding. Transportation costs to fields and storage system Measure and weigh to get the harvested forage mass. Digitalization Outcomes of different harvesting systems – Effects of cutting frequency on economy and environment. Economy for the use of silage additives and plastic film, harvest, ensiling and dry-matter losses. A challenge to do economy on farm level and how to gain profit. On-farm studies are needed to get relevant reference values Need to compile different forage costs Are there experiences compiled of high yields in organic production? Analysis for making a ley high yielding on energy, protein, and dry matter during a long time Costs vary quickly depending on new harvest capacities and price lists of raw materials Expensive to establish ley. How much can we save by having the ley for

Climate and economy	 Climate effects and economy Energy losses in all steps in forage management. 	
Optimizing resources	 Alternative costs for ley Costs for forages that do not survive because of disease or hard winter, e.g. red clover. Need better genetic material. Ongoing SLU Grogrund projects on red clover and timothy breeding. Increase or decrease the acreages of ley on farm level? Alternatively 	
Management and	grow maize. Collaboration between farmers when using biorefinery of the ley. Models for collaboration between different types of farms: cattle, pigs, crops, biogas.	
collaboration	 Collaboration between farmers when harvesting ley. Plan the machine collaboration so the harvesting strategies become optimal. Collaboration between farmers in ley cropping, i.e. a crop farm and an animal farm. How to evaluate the effect of ley on the organic matter content, decreased need of chemicals, increased yield in the following crop in rotation etc. Use the long-term experiments in Sweden. 	