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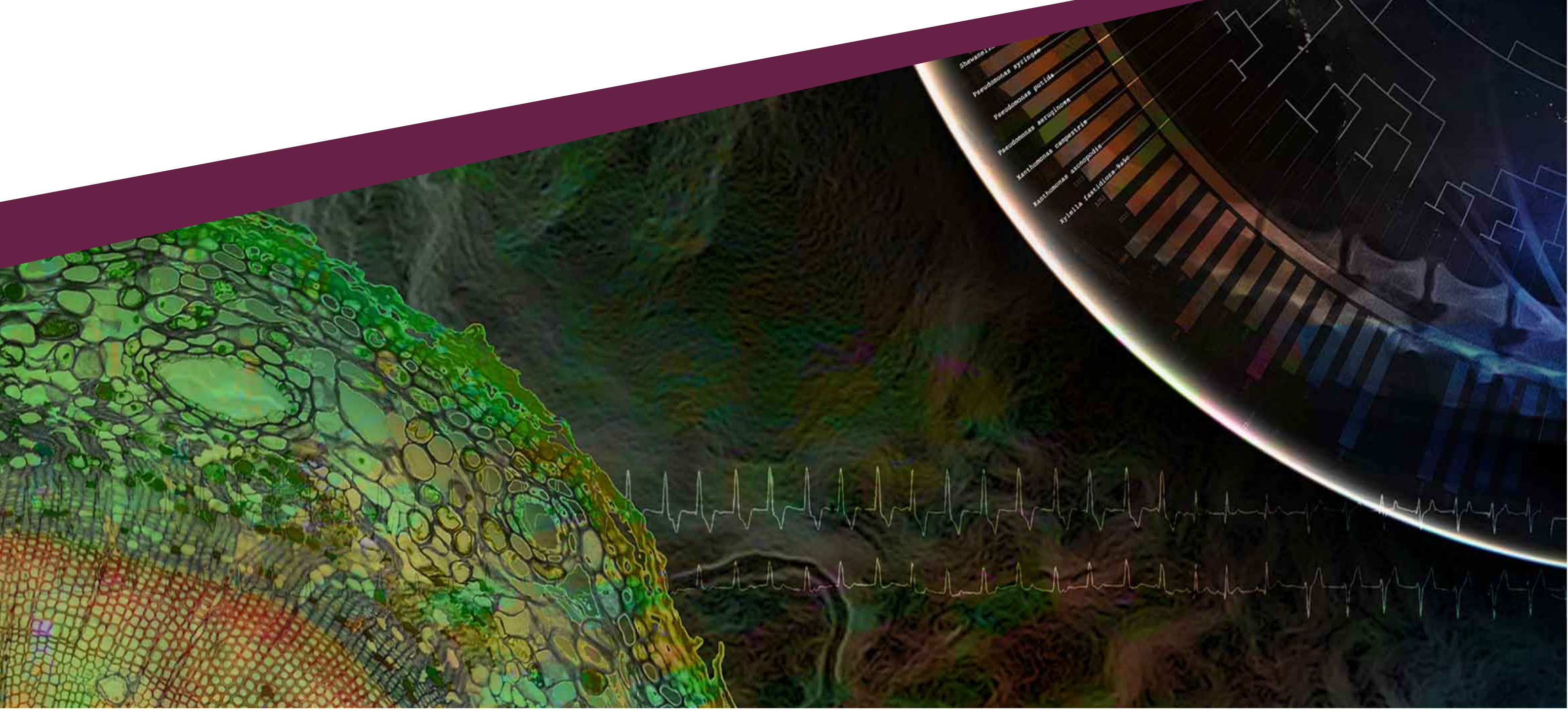

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Proceedings of the 12th Nordic Feed Science Conference, Uppsala, Sweden

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Foreword

We are proud of the fact that this year, we can get together for the 12th conference in a row with only one year with Corona when we had to cancel. The future of the conference is, however, uncertain as the organizers are getting older and even retired. You are welcome to contact the organizers with ideas of how to proceed in coming years.

We have received a total of 34 written contributions this year with the following distribution: 10 on Ruminant Nutrition, 6 on Conservation, 5 on Methods and Miscellaneous, 10 on Methane and 3 on plants.

Climate change is serious threat to human and animal life and will also affect plant distribution and plant survival. Farmers will need to adapt and cultivate less familiar plant species to secure feed to their livestock in coming years. Three of the invited speakers to this conference, that we have been lucky to engage, will address climate change effects on future plant cultivation for ruminant feeding. Professor Édith Charbonneau from Université Laval, Québec, Canada will present consequences of climate change on milk production in Canada. Professor Karl-Heinz Südekum from the University of Bonn, Germany will talk about possible future forage production and livestock feeding in N. Europe. Swedish perspectives on forage plant resilience will be presented by Professor David Parsons from the Swedish University of Agricultural Sciences. A somewhat different talk in this conference will be given by author and Senior Consultant Gunnar Rundgren, Garden Earth, Sweden. The presentation will end this conference and compares the feed use and production of human edible food among Swedish livestock systems.

We also want to take the opportunity to thank the main sponsor of the conference, Stiftelsen Seydlitz MP bolagen.

You are all most welcome to the conference! For downloading proceedings of earlier conferences, please go to our homepage: https://www.slu.se/en/departments/department-of-applied-animal-science-and-welfare/conferences/nordic-feed-science-conference-2024/proceedings/

Uppsala 2024-05-30

Peter Udén

Contents

Forage plants

Methane emission

Ruminant nutrition

Conservation

Miscellaneous

Utilizing forage crops more resistant to extreme weathers and an overall warmer climate – nutritional perspectives for the Northern Europe ruminant livestock sector

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Introduction

Climate refers to the long-term (decades) pattern of several weather events or parameters that may be described on a regional basis. Weather is the present (daily, hourly or instantaneous) components of climate, including temperature, all forms of precipitation, relative humidity, wind and solar radiation. Forage species and plant populations can adjust or adapt physiologically and morphologically over a certain range of climatic variability (e.g. temperature and rainfall) to produce and persist (Baron and Bélanger, 2020). Thus, climate change has long-term effects, whereas extreme weathers have short-term effects and require short-term adaptation. Craine *et al.* (2013) studied global diversity of drought tolerance and grassland climate-change resilience of 426 grass species and concluded that "our findings suggest that diverse grasslands throughout the globe have the potential to be resilient to drought in the face of climate change through the local expansion of drought-tolerant species". In line with this statement, model-based estimates for median seasonal above-ground timothy (*Phleum pratense*) grass yield on dairy farms in four regions of Norway (south-east, south-west, central and north) varied between 11,000 kg and 16,000 kg dry matter per hectare and was higher in all projected future (2046-2065) climate conditions than in the baseline (1961-1990) (Özkan Gülzari *et al.*, 2017). The authors also emphasized that the uncertainty associated with future climate and decision making at farm level reflect that the implications of the future climate projections will vary from farm to farm. The uncertainty is further increased based on climate model indications that global warming through stimualion of atmospheric exchange processes will increase rainfall variability, leading to longer dry periods and more intense rainfall events (Walter *et al.*, 2012). Recent studies suggest that both the magnitude of the rainfall events and their frequency may be as important for temperate grassland productivity as the annual sum. These phenomena may be of particular importance for the Nordic countries with their strong gradient in annual precipitation from the wetter (south-)west to the drier (north-)east (Skjelvåg, 1998).

Most research on adjustment or adaptation of forage species or plant communities to climate change and extreme weathers has focused on (warm) arid and semi-arid regions and less on cold-humid areas. In the following passage, an attempt is made to briefly delineate nutritional perspectives for the Northern Europe ruminant sector in utilizing forage crops more resistant to extreme weathers and climate change with some general considerations, after which forage grass and legume species are separately addressed.

General Considerations

It is generally assumed that increasing temperature and elevated carbon dioxide (eCO_2) have positive effects on forage yield in northern Europe (Liu *et al.*, 2023). Although this sounds promising, a more specific look at boreal regions generates a complex picture. Ergon *et al.* (2018) have reviewed climate change and its effects on grassland productivity across Europe and shown that the Nordic regions are facing increasingly warmer and wetter winters, such that – at a first glance – warming and eCO_2 may boost forage production in the Nordic region due to an extended growing season. These effects may however be balanced or even overcompensated by more winter thaws and less snow cover along with more summer drought events, which may reduce winter survival and summer regrowth, thus potentially reducing the persistence of perennial forages over time (Thivierge *et al.* 2023). It also appears that benefits of an extended growing season can only be utilized when impacts on forage quality and the altered annual productivity cycles are counterbalanced with adequate adaptations in defoliation and fertilization strategies. The identity of species and mixtures with optimal performance is likely to shift somewhat in response to altered climate and management systems. It is argued that breeding of grassland species should aim: (i) improve plant strategies to cope with relevant abiotic stresses and (ii) optimize growth and phenology to new seasonal variation, and that plant diversity at all levels is a good adaptation strategy (Ergon *et al.*, 2018).

Based on nearly two decades of research on the effects of climate change on forages in cold and humid areas of northeastern North America, Thivierge *et al.* (2023) have evaluated and summarized the expected effects of climate change on forage production in terms of yield, nutritive value, and winter survival. The authors have also proposed a set of 12 adaptation and resilience-building strategies for forage systems that can be implemented at the field and farm levels to which the interested reader is referred. Selected aspects from this comprehensive review, particularly those related to changes in forage yield and quality, are briefly summarized in the following paragraph as they address both, grass and legume species:

"*The positive yield response to atmospheric eCO$_2$ concentration is generally greater in legumes than in grasses, due to the N-fixing ability of legumes. The increased plant demand for N under eCO$_2$ often limits growth while biological N fixation in legumes, is enhanced at eCO$_2$ as a response to low soil-N availability. Without any adaptation to the harvest schedule, climate change is then expected to reduce forage nutritive value, although this effect will depend on the species. Drought will also affect plant species differently. As a drought sensitive species, timothy (Phleum pratense L.) is expected to suffer from more frequent and intense water stress events, with reduced summer regrowth. Without any adjustment to the harvest schedule of timothy, its nutritive value (averaged across five sites in eastern Canada) is expected to decrease through increased neutral detergent fibre (NDF) concentration and decreased in vitro NDF digestibility (dNDF) for the first and second harvests, respectively. This projected negative effect on the nutritive value of timothy is in line with experimental results that showed a reduction in dNDF of timothy with increasing air temperatures. Alfalfa (Medicago sativa L., also called lucerne) is not only expected to have a greater positive yield response to atmospheric eCO$_2$ concentration than most grasses but is likely also less affected than timothy by water stress under future climate conditions in eastern Canada. When climate conditions are less suitable for alfalfa growth, a yield increase was projected by mid-century for another legume–grass mixture composed of white clover, red clover and timothy.*"

Grass Species (C$_3$ grasses)

Grass species in natural or sown pastures in boreal regions are all C$_3$ grasses and, although the general expectation is that they will benefit in terms of biomass yield from a longer vegetation period caused by climate change, the observed or projected effects of increased inter-annual precipitation, *i.e.*, rainfall, variability on forage yield and quality are variable and sometimes contradictory. Of the two extreme rainfall variabilities, drought has been studied more often in terms of forage yield and quality than excessive rainfall with long-lasting wet conditions and the former aspect will thus be addressed in the following. However, heavy rainfall, often accompanied by low temperatures, during the growing season also affects plant biomass accumulation and composition but, more severly, may not allow crops to be harvested, demanding that alternative feed resources, sometimes called ermergency feeds, such as wood products, are considered and evaluated. This aspect has been addressed, e.g., at the 10th Nordic Feed Science Conference (Prestløkken & Harstad, 2019; Rinne & Kuoppala, 2019) and, notwithstanding its periodic relevance, will not be covered here.

Bērziņš *et al*. (2019) assessed the total dry matter yield and regrowth capacity of seven grass species in 2018, the driest year in the history of Latvian weather observations with extreme drought durig the summer. The grass species in the field experiment ranked in the following descending drought resistance sequence: Tall fescue (*Lolium arundinaceum* (Schreb.) Darbysh., formerly *Festuca arundinacea* Schreb.) > red fescue (*F. rubra*); > orchard grass (also called cocksfoot, *Dactylis glomerata*) > timothy (*P. pratense* > meadow fescue (*F. pratensis*) > festulolium (*xFestulolium*) > perennial ryegrass (*Lolium perenne*). Fariaszewska *et al*. (2020) examined yield and quality traits of nine forage grass varieties belonging to *Festuca*, *Lolium* and *Festulolium* under mild drought stress conditions in a semi-controlled field experiment. Dry matter yield was significantly lower in drought stress than under control conditions and the physiological parameters reacted within 2 weeks after start of the drought treatment in all species. In contrast, drought stress significantly increased water use efficiency, the content of proline, phenolic acids, flavonoids, water soluble carbohydrates and decreased neutral and acid detergent fibre on both years. Based on total dry matter yield, Italian rygrass (*L. multiflorum)* ranked highest in the first and tall fescue in the second investigated year.

Grant *et al*. (2014) presented data from a field experiment in which a temperate European grassland was subjected to altered intra-annual precipitation variability (low, medium, high) in interaction with management strategies namely fertilization and alteration of harvest date (delay by 10 days). Increased intra-annual precipitation variability decreased forage yield of the grassland. Furthermore, proportion of functional groups was altered toward less grass and more forb biomass with amplified precipitation variability. Increased crude protein content and reduced fibre content with increasing precipitation variability improved relative feed values. On the contrary, McGranahan & Yurkonis (2018), in a study conducted in North Dakota, USA, found that, although grasses (C_3 and C_4) increased forage quality and quantity under eCO_2 concentrations, forage quality and quantity of C_3 grasses declined under simulated drought. Crude protein content was enhanced by fertilization during drought but was reduced by delayed harvest after the drought period. Similarly, Carlsson *et al*. (2017) showed that sward resistance and resilience to drought stress were increased by nitrogen fertilization.

Because morphological and nutritional adjustments within plants in response to warming and drought vary among species and less is known how these relate to production and forage quality, Catunda *et al*. (2022) grew two common pasture species, tall fescue and alfalfa in a climate-controlled facility, under different temperatures (ambient and elevated) and watering regimes (well-watered and droughted). They found that drought had a strong negative impact on biomass production, morphology and nutritional quality while warming only significantly affected both species when response metrics were considered in a multivariate principal component analysis, although to a lesser degree than the drought. Catunda *et al*. (2022)

concluded that in future climate scenarios, drought may be a stronger driver of changes in the morphology and nutritional composition of pasture grasses and legumes, compared to modest levels of warming.

The potential of orchard grass performance and in particular, nutritive value, under a combined climate-change (increased temperature and eCO2 concentration) and water stress (drought) scenario was studied by Küsters *et al*. (2021a, b) in an alpine environment in Austria. The combination of increased temperature and eCO_2 concentrations lead to an increased and accelerated conversion of non-protein-N compounds into complex protein compounds. Simultaneously, it accounted for increased proportions of water-soluble carbohydrates (WSC), and higher WSC is connected to increased digestibility and metabolizable energy contents. Height and weight of plants decreased under elevated temperature and eCO_2 in the second and third cuts. Under increased temperature and eCO_2,orchard grass accumulates certain nutrients while a decline in tiller growth in the second and
third growth is likely. This observation on orchard grass is not at odds with reports from the in northeastern United States (reviewed by Thivierge *et al*., 2023) that yield is expected to remain stable or to decrease.

In the seond study, Küsters *et al*. (2021b) observed that, besides elevated temperature and eCO2, impact of water stress can be severe on the performance of orchard grass and its nutritive value. Water stress has improved valuable nutritive characteristics like WSC, crude protein and ME. Consequently, restrictive water supply is leading to an accumulation of specific nutrients, hence improving the nutritive value but with a prospective decline in biomass production. The authors concluded that consequences of drought conditions are mostly overcome in orchard grass as soon as the precipitation achieves its normal expected values for the specific season.

Legume species

Already more than 30 years ago, Peterson *et al*. (1992) determined effects of drought on herbage yield and quality and stand persistence of birdsfoot trefoil *(Lotus comiculatus* L.), cicer milkvetch *(Astragalus cicer* L.), red clover *(Trifolium pratense* L.) and alfalfa. Legumes were subjected to two soil water regimes promoting 'droughted' and 'well-watered' plant growth. Average herbage yield of droughted alfalfa was 120% greater than yields of birdsfoot trefoil and cicer milkvetch, and 165% greater than red clover yield. Droughted alternative legumes produced herbage with lower fibre concentrations than alfalfa. Improved quality in droughted legumes was related to greater leaf:stem weight ratio, delayed maturity, and often higher quality in both the leaf and stem fractions compared to the control treatment. Although drought reduced herbage yield of all legumes, alfalfa had the greatest yield potential under drought, albeit at a lower nutritive value.

About 20 years later, an experiment on the effect of water shortage on the nutritive value of legume species was conducted by Küchenmeister *et al*. (2013). They examined effects of drought on crude protein fibre and WSC concentration of six legumes, namely birdsfoot trefoil, marsh birdsfoot trefoil (*L. uliginosus* Schkuhr); black medic (*M. lupulina* L.); yellow alfalfa (*M. falcata* L.); sainfoin (*Onobrychis viciifolia* Scop.) and white clover (*T. repens* L.) in monoculture and in mixture with perennial ryegrass in a container experiment in a vegetation hall. Moderate and strong drought stress was applied during three periods in two years. Effect of drought on nutritive values was considerably less pronounced than on yield.

While the impact of moderate stress on nutritive quality was negligible, Küchenmeister *et al.* (2013) found a decrease in crude protein and fibre fractions and increased WSC under strong stress. This may indicate that water scarcity could even increase forage quality and digestibility. However, the choice of legume species and stand (monoculture or mixture) had stronger effects on nutritive values than drought. Küchenmeister *et al.* (2013) concluded that the influence of temporary drought on nutritive value characterstics seems to be less important for the selection of suitable forage legumes species than other agronomic properties under conditions of climate change.

An interesting facet to alfalfa quality was reported by Pecetti *et al*. (2017) who investigated three alfalfa populations divergently selected for higher leaf-to-stem ratio, multifoliolate leaves or short-internode stems in four managed environments. Their results suggested that environmental effects might have greater impact on quality than genetic effects, even for a population set including material selected for quality-driven morphology. This can be taken as a subtle hint that adaptation measures in forage production and management in response to climate change and weather extremes are at least as important as selection of appropriate varieties within a given species.

Maize

Maize (*Zea mays* L.) is a C_4 grass which is typically grown in warmer climates but also has a long history even in Nordic countries. Already Virtanen (1938) has documented research interest towards maize production in Finland, but the field area used for forage maize production in boreal European regions has remained low (according to official statistics from, e.g., Sweden and Estonia). Both climate change and more droughts during the vegetation period have revived efforts to cultivate forage maize in boreal regions.

These efforts may receive additional incentive from a recent study conducted in northern Germany (Taube *et al*., 2020). Based on reports that yield increases in forage maize in northwestern Europe over time are well documented, they stated that the driving causes for these, however, remain unclear as there is little information available regarding the role of plant traits triggering this yield progress. Ten different hybrids from the same maturity group, which have typically been cultivated in Northwest Germany from 1970 to recent and are thus representing breeding progress, over four decades, were then selected for a 2-year field study in northern Germany. Based on the results of their study, Taube *et al.* (2020) concluded that the observed increase in silage yield in northwestern Europe can largely be explained through the increased temperature sum during the vegetation period of maize crops and the resulting earlier maturity in the last four decades. These increased temperatures have a direct effect on yield, as shown by results from a simulation model (MaisProg). Apart from this direct effect, the increased temperature also indirectly contributed to the higher yield through the selection or breeding of maize varieties with an increased leaf area index (higher number of leaves and longer leaves), a higher radiation use efficiency and a generally lower leaf angle. The study showed an annual progress, mainly driven by these plant traits, of about 130 kg DM/ha. The N efficiency of newer hybrids was also higher compared with older ones while overall forage quality was not affected. Future selection and adaptation of maize hybrids to changing environmental conditions are likely to be the key for high productivity and quality and for the economic viability of maize growing and expansion in Northern Europe.

Lehtilä *et al.* (2024) stated that the cultivation of whole crop forage maize for cattle feed has a potential for increased forage yield while reducing N fertilization compared to perennial grass-based systems. The aim of their study was to compare the environmental impact of forage maize with more widely cultivated forage crops in Finland that included perennial silage grass mixtures and whole crop spring cereal harvested as silage. The use of plastic mulch film in forage maize cultivation was included in the assessment as well. A life cycle assessment was conducted and the overall conclusion was that forage maize could be used to supplement perennial grass cultivation without major associated environmental risks. Future research shall be conducted on the effect of forage choices on the environmental impact of boreal dairy milk production and on decreasing the current high uncertainty associated with nitrous oxide emission factors and soil organic carbon stock modelling choices.

Conclusions

Climate change has long-term effects, allowing plant communities and individual species to adapt in different ways. Extreme weathers have short-term effects and require short-term adaptation. Of overriding importance is that plant species must (1) survive and (2) perform under more extreme stress situations. Only if these two aspects are secured, forage quality can be addressed and modifications may come into effect through adapted and optimized forage management ranging from species selection (if applicable) and cultivation to harvest and preservation.

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Resilience of forages to drought in Nordic countries

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Climate change and forages

Global average temperatures have increased 1.4°C above the average of 1850-1900 (World Meteorological Organization, 2024) with the recent period between 2015-2023 having the warmest 9 years on record. The WMO also expects an increased frequency of extreme weather events such as drought and extreme heat.

In Northern Europe, climate change is increasing the frequency and intensity of both droughts and excessive rainfall (IPCC, 2012, Strandberg 2015), which challenges forage production and the industries that rely on it. Various studies have examined the potential effects of climate change on forages. Climate change scenarios for Nordic and Baltic countries 2040-2065 (Höglind *et al.*, 2013) predict increased growing season temperatures, increased evapotranspiration (ET), and increased precipitation for most sites. Compared to the reference period (1960-1990) the projected future dry matter yields of timothy (*Phleum pratense*) were 11% greater, largely due to an increase in the length of the growing season. The optimal time for the first harvest was 8-20 days earlier than the reference period and an additional harvest could be taken at all sites. A study in Norway using climate projections (until 2099) combined with a simulation model (Persson and Höglind, 2014) found that timothy yields will increase at sites all over Norway. This was caused by increasing temperature, 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. Grusson *et al.* (2021) specifically examined the potential effect on agriculture in southern Sweden using different climate models and the SWAT soil water tool. The results suggest that although climate models may project increased rainfall, this will not lead to an increase in soil water content, firstly because increased rainfall is associated with heavy rainfall events that lead to increased runoff, and secondly because increased temperature increases transpiration.

Regardless of whether water deficits will occur more frequently in the future, they are a part of the current reality. The year 2018 in Northern Europe was one of the harshest and widespread droughts in recent memory and caused yield losses far above normal levels (Beillouin *et al.*, 2020) with dire consequences. In Sweden, the drought caused historic cereal yield losses and livestock number reductions due to lack of feed (Statistiska Meddelanden, 2018). Cattle farmers resorted to using a variety of alternative unconventional feed sources (Bergkvist and Spörndly, 2019) and some farmers were forced to emergency slaughter cattle, leading to widespread mainstream news coverage. This event substantially increased awareness of the devastating effects that droughts can have, particularly for producers of ruminants, and provoked a widespread debate about climate change and the potential effect on agricultural production in the future.

While climate change might bring some benefits to forage producers in the Nordic countries, it comes with risks of future droughts. To adapt and thrive during the climate of the future, it is crucial to have access to forage crops which can both produce well in typical years (stability) and under water limitations (resilience).

Defining drought

The World meteorological organization (World Meteorological Organization, 2010) acknowledges that what might be drought in one area may not be drought in another, because drought is relative to the average conditions in that area. There are many ways to define meteorological drought, all with their strengths and weaknesses. For example, Mooley and Parthasarathy (1982) defined the severity of a drought through dividing actual rainfall by average rainfall and dividing the result by the standard variation. The standardized precipitation index (SPI) (Mckee *et al*., 1993) is based on precipitation only, which makes it easily usable from widely available weather data. It is a continuous statistical method which compares rainfall variability to historic rainfall. The Standardized Precipitation Evapotranspiration Index (SPEI) (Vicente-Serrano *et al*., 2010) takes both precipitation and evapotranspiration (ET) into account, which leads to a more robust measurement, but requires a method to estimate ET, either directly or through a model.

An important distinction is between a drought and water limitations. For agricultural purposes, a shortage of water for plant growth doesn't necessarily mean that there is a drought, however in this paper we will use the word drought more generally.

Defining terms

There are many terms related to agroecosystem performance, and they are used inconsistently, often causing confusion. Grimm and Wissel (1997), Death (2024), and Picasso *et al*., (2019) have good summaries of the terms, and their potential meanings. For the purpose of this paper, we will use terms in the following way, according to Sanford *et al*., (2021):

> Resistance – the ability to stay unchanged in the presence of a disturbance.
>
> Recovery – the ability to return to normal after a disturbance.
>
> Resilience – the combination of both resistance and recover.
>
> Stability – the ability to remain unchanged in the face of normal variability.

Cropping systems with stability reduce uncertainty and risk due to less variability in yield from one year to the next. Resilient cropping systems remain relatively productive through a climatic crisis (*e.g*., drought), providing a buffer in the face of extreme weather (Sanford *et al*., 2021; Urruty *et al*., 2016).

Definitions are also complicated and inconsistent when discussing the ability of plants to respond to water limitations. We will use the following terms:

> Drought resistance – the ability of plants to maintain normal physiological functions and growth, even when exposed to water limitations.
>
> Drought avoidance – the ability of plants to avoid water scarcity through adjusting their growth and development.

> Drought recovery – the ability of plants to recover normal function after a period of water scarcity.
>
> Drought resilience – a more general term including resistance, avoidance, and recovery.

The mechanisms for drought resistance are complex and vary between species but can include osmotic adjustment, plant growth regulation, stress responsive proteins and antioxidant scavenging defence systems (Aslam *et al*., 2015). For forages in Northern Europe, the most important mechanisms of drought avoidance are variations in root morphology and depth. Deep roots enable plants to access water from lower in the soil profile, potentially avoiding dehydration. Alternatively, a spreading root system can explore more of the soil volume. The mechanisms of drought recovery are less clear. Plant breeding has traditionally focused on mostly yield, while drought resilience breeding has been less common (Tardieu *et al*., 2018).

Drought effect on forage quality

A review by Wang and Frei (2011) summarised that for forage crops there is a general pattern of increased protein concentration and digestibility during drought. In a meta-analysis of nineteen studies (Dumont *et al*., 2015) water stress led to an average increase of 5% crude protein, a decrease of 3% in neutral detergent fibre concentration, and more variable effects on digestibility.

There are various potential reasons for a potential positive effect of drought on forage quality. Drought stressed plants may have higher leaf to stem ratios (Peterson *et al*., 1992) and reduced lignin concentration (Peterson *et al*., 1992; Petit *et al*., 1992). However, this can depends on the extent of the water stress, which in extreme situations can reduce leaf mass through accelerated senescence of older leaves (Buxton and Fales, 2015).

Another reason for potential improved forage quality is that water stress delays the maturation of forages (Halim *et al*., 1989). This means that if the water stress is not too severe then the forage quality may be better in comparison with unstressed forage of the same age (Buxton, 1996). For example, a glasshouse study (Staniak, 2016) found that after a prolonged period of low soil moisture the maturity of the forage grasses were delayed by the drought stress, leading to higher protein concentrations and lower concentrations of crude fibre, but at the expense of decreased yield (31%) compared with the control. Such results are not consistent. For example, Küchenmeister *et al*. (2014) found no significant effect on any nutritive quality, including protein, between control and drought conditions, as long as the botanical composition was consistent. In a separate study, Küchenmeister *et al*. (2013) artificially induced drought stress in a range of perennial forage legumes. The results were variable, and the most significant finding was that drought stress has a stronger effect on yield than on nutritive value.

A suggested reason for a potential increase in protein concentration during moderate drought is an increasing legume content of the sward, for reasons described below. The results are variable and depend on the extent of the water limitation and the drought resilience of the legume species.

For forages, which have experienced moderate to severe drought, it is mainly the yield that is of concern, as most studies agree that quality factors such as protein and digestibility either are comparatively better or similar if the harvest date is kept the same. However, if farmers delay harvesting after drought to aim to achieve a typical level of yield, it is possible that they will end up harvesting forage of lower quality.

Drought recovery

The recovery phase of forage plants can be slow, and quite different between species. For example, in a study in Switzerland of simulated summer drought (Deléglise *et al*., 2015), the plants did not recover fully until the following spring.

Post-drought stressed mixed grass and legume swards in Switzerland showed that grasses over-yielded by 52% in the regrowth compared to the control, whereas the legume did not respond in the same way (Hofer *et al*., 2017). In a pot experiment study (Staniak, 2016) forage grasses were grown in monocultures and either subjected to a simulated summer drought or were grown in optimal conditions. During drought conditions, the yields were reduced by an average of 31% compared to the irrigated control, however during the next year the grasses managed to overcompensate and had yields that exceeded the control group by an average of 7.5%.

A suggested reason for post-drought over-yielding is the “Birch effect” (Birch, 1964). The Birch effect is the rapid mineralization of nitrogen when a dry soil becomes wet. The Birch effect may in part be responsible for the over-yielding of forage swards following a drought. The Birch effect may also explain why forage grasses, and not legumes, have a strong post-drought growth response, because grasses do not fix atmospheric nitrogen and are thus more sensitive to soil nitrogen availability and responsive to nitrogen fertilization. This hypothesis could be tested by supplying both drought-recovering and control plants with optimal nitrogen levels.

Beyond the differences between grasses and legumes, there is little information on differences among or within species focusing specifically on drought recovery.

Grass species comparisons

In many studies comparing forage species for drought resilience there is no distinction between drought resistance and drought avoidance, and usually the methods used do not enable a distinction. In practice, it may not matter whether plants are more resilient because of resistance or avoidance – resilience is likely to be a function of multiple traits. The following section will summarise some research comparing the drought resilience of different forage species. There are fewer studies on drought tolerance in the Nordic countries in comparison with other stresses, such as cold and ice damage.

The main perennial forage grasses used in Sweden are timothy, meadow fescue (*Festuca pratensis*), tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*), typically grown in polyculture with legumes. Of these species, only tall fescue is recognised as drought tolerant (Cherney *et al*., 2020).

In a Danish field experiment (Kørup *et al*., 2018) different grass species and cultivars were grown in lysimeters in order to evaluate their biomass production and water use efficiency during and after drought stress. One cultivar of Cocksfoot (*Dactylis glomerata* cv. ‘Sevenop’) and two varieties of tall fescue (cvs. ‘Jordane’ and ‘Kora’) gave the greatest yields during both control conditions and during drought. A different cultivar of Cocksfoot (cv. “Amba”) and a cultivar of festulolium (x *Festulolium* cv. Hykor) had the lowest yield reduction (i.e. the yield in relation to the control yield). A lower yield reduction is likely due to lower predrought yields leading to greater water availability at the onset of drought. Not all festuloliums were drought tolerant. Two Festulolium cultivars originating from crosses between meadow fescue and perennial ryegrass were sensitive to drought. The study also found that some commercial cultivars which have not been specifically bred to be drought tolerant still exhibit drought resilience traits. The authors theorized that this has occurred during selection for yield in outdoor trials during drought years which will then select for drought resilience indirectly.

Some grass species have different ploidy levels, which can have various effects on the phenotypic response. One study (Akinroluyo *et al*., 2020) researched the effect of ploidy level in Westerwolds ryegrass (*Lolium multiflorum ssp. multiflorum*) on drought resilience. Diploid cultivars and induced autotetraploids were compared in mild drought conditions. While autotetraploids produced more biomass during non-drought conditions the difference was significantly greater during drought conditions because they produced more phenolic compounds and had significantly higher antiradical activity. An experiment (Kemesyte *et al*., 2017) with perennial ryegrass showed similar results – the tetraploid perennial ryegrass outperformed the diploid variant under drought stress conditions.

An early study with tall fescue (Burch and Johns, 1978) found that it has characteristics of drought avoidance, through a deep root system, and drought resistance, through stomatal control and dehydration tolerance. Tall fescue also has potential for its drought tolerance to be improved even further (Waldron *et al*., 2021). Resilience to drought had a moderately high heritability and the authors predict that it can be increased by 2.7% to 3.1% per selection.

During the drought year 2018, the average of total seasonal dry matter yield over three sites from pure stand of species in forage variety trials in South and Middle Sweden were compared with year 2015, representing good rainfall (Halling, 2020). For first year leys, tall fescue had 60% of the 2015 yield, compared with 57% for meadow fescue, 66% for perennial ryegrass, and 54% for timothy. However, for second year leys, tall fescue had 72% of the 2015 yield, compared with 59% for meadow fescue, 59% for perennial ryegrass, and 65% for timothy. This confirms the drought resilience of tall fescue, but also that it is a slow species to establish, and its drought resilience is not fully developed in the first production year.

Legume species comparisons

In the Halling (2020) study, first year lucerne (*Medicago sativa*) had a relatively low percentage yield (45%), higher than white clover (*Trifolium repens*) (40%) but lower than red clover (*Trifolium pratense*) (64%). However, by the second year lucerne had a very high percentage yield (85%), compared to red clover (57%) and white clover (22%). The results confirm that white clover is drought sensitive and that lucerne is resilient once it has had sufficient time to become established.

Küchenmeister *et al*. (2013) compared drought resilience of a range of legume species and found that yield reduction depended on the strength and duration of stress. However, the study focused on forage quality and there was no statistical analysis of yield data.

Komainda *et al*. (2019) compared drought resilience of white clover to birdsfoot trefoil (*Lotus corniculatus*), yellow lucerne (*Medicago falcata*), black medic (*Medicago lupulina*), and common sainfon (*Onobrychis viciifolia*) monocultures in controlled environment conditions. Black medic and birdsfoot trefoil had the lowest relative reduction in yield, whereas white clover had the greatest. However, white clover still had a similar level to black medic and birdsfoot trefoil under drought stress. Because this was a controlled environment experiment with limited space for roots to explore, it is likely that the species had similar yields under drought conditions because all species used up the limited available water. It is unclear how these results would transfer to field conditions.

Deep rooting is one of the major mechanisms of drought avoidance. In a set of experiments in Switzerland and Ireland, Hofer *et al.* (2016) compared monocultures of white and red clover in a simulated summer drought experiment. Red clover was consistently higher yielding than white clover in both control and drought conditions. The yield reduction due to drought was similar for the two species. The authors conclude that the 'deep rooting' trait might contribute to drought resistance, but that the effect could be small and might become important only under extreme drought conditions. Another study in Switzerland (Prechsl *et al*., 2014) found that contrary to expectation, grasslands under drought conditions did not exhibit a strong increase in water uptake from deep in the soil. This was observed in mixed swards and it is unclear if these results can be generalized.

Lucerne is well-known to be a drought resilient species, but few published articles in Northern Europe have compared its performance in detail with other species. In a pot experiment, Norton *et al.* (2021) compared water use of white clover and lucerne during a drying cycle. They found that white clover used a greater proportion of the soil water, likely due to its more branched and finer root system. Lucerne used water more conservatively, allowing it to survive longer during the drying cycle. Lucerne also partitioned more of its energy into root growth, maintained a higher relative water content, and had higher water use efficiency. The experiment did not address the potential for lucerne to access deep water, due to the limitations of the pot volume.

Even within the species, there are differences between lucerne cultivars. Picasso *et al*., (2019) found that US cultivars of lucerne differ in resilience to drought, stability, and productivity. Cultivar stability was not associated with productivity, and it was negatively associated with disease resistance. Cultivar resilience was negatively associated with productivity, and not associated with other traits. With increasing year of release of cultivar, cultivar productivity increased, stability was not changed, and resilience decreased. In the Czech Republic, Hakl *et al*., (2019) also found that stability and productivity of lucerne cultivars were not related. These studies highlight the importance of maintaining resilience in new cultivars, and not having productivity as the only breeding goal.

Sward mixtures and diversity

Küchenmeister *et al.* (2014) compared the response of monocultures and mixtures to drought, in a controlled environment. On average, yield was reduced by 12% with moderate drought stress, with the worst performing sward reduced by 22% compared to the control, demonstrating that sward composition had a significant effect. Importantly, functional group diversity (legumes, grasses and forbs) was more important than the number of species. The importance of functional groups was confirmed by Komainda *et al.* (2020). They found that swards performing at the maximum level could be achieved with as few as 3 species (although a maximum of 5 species were used and the options were limited), as long as functional diversity was high. This is likely because functional group diversity is the surest way of increasing functional trait diversity, where traits include factors such as rooting depth, nitrogen fixation and speed of phenological development. It is important not to generalise the "optimal" number of species, because this may depend on the conditions. The Komainda *et* *al*. (2020) study only included one type of legume (white clover). Most studies do not include multiple sites, years, stress events, soil types, etc., and we hypothesize that swards with greater species diversity have potential to be more resilient across diverse environments.

A specific reason to combine legumes and grasses in swards is that legumes can fix atmospheric nitrogen. This gives legumes an advantage over grasses in low nitrogen conditions, such as occur during drought where lack of water also makes nitrogen less accessible. In practice, this can lead to greater water use efficiency for legumes than grasses, as demonstrated with lucerne in New Zealand (Moot *et al*., 2008). Hofer *et al*. (2017) found that legumes had an advantage over grasses during drought, due to their nitrogen fixation, whereas grasses were able to overcompensate during post-drought recovery. This confirms that mixtures of legumes and grasses can provide a more stable yield during and after drought.

Underused species

Brome species are known for their drought tolerance, but are not commonly used in Nordic countries, partly due to a lack of seed supply. Smooth brome (*Bromus inermis*) has been tested in the past, whereas less is known about Alaska brome (*Bromus sitchensis*). Preliminary research in Estonia (Tamm *et al*., 2020) compared the two species and found that Alaska brome can be higher yielding than smooth brome (Tamm *et al*., 2020) and tall fescue (Tamm *et al*., 2018) and is a good companion species to lucerne.

A related species of timothy is the lesser known Boehmer's cat's-tail (*Phleum phleoides*). In a set of studies, 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.

Another perennial grass known to be drought resilient is intermediate wheatgrass (IWG) (*Thinopyrum intermedium*). Studies comparing the response of several perennial grasses to deficit irrigation show that intermediate wheatgrass responded better than tall fescue to partial water deficits in dry environments (Lauriault *et al*., 2005; Orloff *et al*., 2016; Smeal *et al*., 2005). The drought resilience of IWG is confirmed by trials in South and Middle Sweden, which were assessed during the drought year of 2018 (Mårtensson *et al*., 2022). IWG is able to avoid drought through its deep root system that reaches to at least a depth of one meter (Pugliese *et al*., 2019; Sakiroglu *et al*., 2020).

In addition to those already discussed, there are a range of other perennial legumes that may potentially have a high level of drought resilience. However, for these species to be useful they also need to be suitable in other aspects, not least of which is their winter hardiness, which means that the pool of possible species reduces as the area of focus moves north. Even if a potential species is able to survive the winter, they must also be able to compete in mixed swards, be resistant to local diseases, and have suitable forage quality. Species known to have drought tolerance and which can potentially be cultivated in the Nordic countries include birdsfoot trefoil (*Lotus corniculatus*), kura clover (*Trifolium ambiguum*), zig-zag clover (*Trifolium medium*), sainfoin (*Onobrychis viciifolia*), liquorice milkvetch (*Astragalus glycyphyllos*), cicer milkvetch (*Astragalus cicer*), black medic (*Medicago lupulina*), strawberry clover (*Trifolium fragiferum*) and Talish clover (*Trifolium tumens*) (Bender *et al*., 2017; Butkute *et al*., 2018; Elgersma *et al*., 2014; Parsons, 2020). Forb species of interest include salad burnet (*Sanguisorba minor*), caraway (*Carum carvi*), ribwort plantain (*Plantago lanceolata*), and chicory (*Cichorium intybus*) (Elgersma *et al*., 2014). These species are either not widely used in the Nordic countries, or little is known about their drought resilience, or both.

Irrigation

Irrigation is one way to compensate for a lack of water, however it is uncommon in the Nordic countries and mostly used for high value crops such as potato. The irrigable land, as a fraction of total utilized agricultural area ranges from 2.4% in Finland to 8.4% in Denmark (Eurostat, 2016). Despite the lack of irrigation in Sweden, there is abundant surface water in the form of lakes and rivers, and there is also potential to collect water on-farm through strategically located dams. For most locations in Sweden, it is likely that irrigation will remain a risk management strategy to cope with water deficits rather than a strategy used through the growing season to boost overall production. Despite the cost of irrigation in terms of investment, time, and operational expenses, more widespread use could help ruminant producers to become more resistant to drought conditions and be able to protect their valuable breeding animals. However, irrigation comes with a number of challenges, in addition to the cost. Irrigation design and management are crucial. Farmers must have access to good information about how to plan irrigation in different soil types, understand crop water requirements, and schedule irrigation. Besides the issues associated with large scale irrigation projects, even small-scale irrigation can come with potential environmental challenges, including leaching of nutrients, salinization, waterlogging, runoff reducing downstream water quality, effects on aquatic ecosystems, and impact on groundwater, including leaching of nutrients (Stockle, 2002).

Recommendations

Even though there are many unknowns regarding drought resilience of forages in the Nordic countries, there are practices that can help farms to become more resilient. We do not know the extent or all of the mechanisms of drought resilience in Nordic forages. However, there is ample experiential evidence that certain species such as tall fescue, cocksfoot, and lucerne are more drought resilient. In locations where rainfall is comparatively lower and drought is common, these species should be the main components of forage mixtures. In locations where drought is less common, these species should be included at a lower rate in mixtures or in selected fields. Diversity is a major source of ecological stability. In leys, diversity can be achieved by having multiple species in a sward, or by having multiple fields with different species combinations.

The combination of red clover and timothy is an important mix that represents large areas of ley crops in Nordic countries. Consequently, much of the knowledge about ley management is adapted to these two species. Using the same management principles for other species will likely not lead to the same forage quality or level of persistence. For example, tall fescue may need to be harvested more frequently than timothy to achieve the same level of digestibility. Lucerne needs careful management in autumn to ensure appropriate winter hardening if it is to be persistent.

Future research

Although much is known about the relative differences in drought resilience of forage species, more knowledge is needed on how these species perform in Nordic conditions with different average rainfalls and soil types. The approach of Halling (2020), which used multiple variety trial sites, can be further explored, including longer-term comparisons and other metrics of drought resilience.

Drought recovery of forages is an area where there are limited field studies. It may be possible to address recovery through long term datasets, however it would also benefit from specially designed studies with specific measurements.

To better understand the drought avoidance characteristics of different species, *in situ* rooting depth studies are an option. This can be done with great difficulty through direct observance of roots, or indirectly through soil water monitoring equipment. The capacity to measure soil water content and crop water use will become a more important technique, particularly if irrigation becomes more widespread.

Forage breeding in Nordic countries has traditionally focused on yield and winter survival. Developing robust ley production systems for the future necessitates species that can resist and recover from stresses such as cold, ice, waterlogging, and drought. Breeding for these characteristics requires the development of efficient methods for screening plants for resilience to multiple abiotic stresses. It is important that new cultivars bred for resilience to certain stresses are not inadvertently more susceptible to other stresses. The potential of new and alternative species should also not be forgotten in the quest for more resilient forages systems.

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Forage nutritive value of dual-use populations of Kernza Intermediate wheatgrass

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Introduction

Intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & Dewey; IWG) is a perennial cool-season grass native of Europe, used for forage, which has been recently bred for grain and marketed as Kernza in North America (Lanker *et al*., 2019). It is adapted to dry regions, with optimal rainfall between 300 to 750 mm, and produces well in regions up to 1150 mm of rainfall. It grows on a wide range of soils (coarse, medium, and fine-textured, with pH ranging from 5.6 to 8.4), and prefers well-drained soils, loamy to clay-textured. It is cold-tolerant and not susceptible to spring and fall freezing. It can also withstand moderate periodic flooding in the spring (Hybner & Jacobs, 2012). It can also provide multiple ecosystem services: protect soil from erosion, annual weed control (Zimbric *et al*., 2020), reduce nutrient leaching (Jungers *et al*., 2019), increase soil organic carbon and improve biota linked to high soil quality (Culman *et al*., 2013). Furthermore, this crop is very drought tolerant due to its deep root systems at a depth of one meter and thus contribute to increased carbon storage in the soil (Sakiroglu *et al*., 2020).

The IWG has been introduced to Sweden and tested in several locations (Alnarp, Rådde, Lundsbrunn, Uppsala, Umeå). Preliminary forage yield from a pilot study at Rådde (Nadeau *et al*., 2023) showed an increase from 3100 kg DM per ha on 8 June to 7400 kg DM per ha three weeks later at one harvest. It can be 1.70 m tall at maturity in September, and the straw yield reached over 8,000 kg DM per ha at SLU in Alnarp (Dimitrova Mårtensson *et al*., 2022). It is also being tested in other countries in Europe (France, Finland, Norway, Lithuania, Estonia, Denmark, Poland, Belgium, Germany, and Ukraine).

Growing IWG as a dual-use perennial crop provides two sources of income to farmers: forage and grain. First year forage harvest yields in the US range from 4.5 to 11.7 tons per ha in summer, and up to 3.9 tons per ha in spring and autumn (Franco *et al*., 2021). The forage nutritive value of IWG is high in spring and autumn harvests and are suitable for lactating beef cows, dairy cows, and growing livestock (Favre *et al*., 2019). Farmers let animals graze the crop in early spring, harvest it as forage early in the summer or harvest grain and use the straw at grain harvest as bedding or mixed in feed rations (Lanker *et al*., 2019). Intercropping legumes with IWG in mixtures increases forage quality (Pinto *et al*., 2023).

Intermediate wheatgrass breeding programs have improved agronomic performance of the crop in monoculture systems, but no systematic evaluations of breeding lines have been done in intercropping with legumes. The objective of this study was to compare dual use IWG populations intercropped with lucerne on forage yield and quality.

Materials and Methods

Breeding populations from The Land Institute (KS, USA), University of Minnesota (MN, USA), and University of Manitoba, Canada, were evaluated at Arlington, Wisconsin, USA (43°18’9.47″N, 89°20’43.32″W). Intermediate wheatgrass was seeded in the autumn in a mixture with lucerne, harvested the next summer for grain and in the subsequent autumn for forage, over two years. The treatment design was a single-factor experiment with 11 breeding populations (2 from Canada, 4 from KS, 5 from MN). The field plot design was a completly randomized block design with 4 replications. Seeding rate was 13.5 kg ha^{-1} of pure live seeds (PLS) with 30.5 cm row-spacings. The plot sizes was 1.2 by 3 m. Lucerne was frost seeded at 16.8 kg ha^{-1} in the spring after seeding intermediate wheatgrass. Soils were Plano silt loam with 2 to 6% slope, and no fertilizer was applied. Forage was hand-harvested at the soil level with sickles, using one 50 x 50 cm quadrat to include two intermediate wheatgrass rows. Forage samples were then placed in a forced-air dryer at 52°C for at least five days. Forage quality was analyzed with wet NIRS in the UW-Madison forage quality lab.

Results and Discussion

The total summer forage biomass (IWG, lucerne and weeds) in the first year was not separated and there were no differences among populations (Figure 1). Mean summer forage biomass was 8565 kg ha^{-1} and 6650 kg ha^{-1} for the first and second year, respectively. Mean summer forage yield in the second year for IWG was 4554 kg ha^{-1} and for lucerne 1852 kg ha^{-1}. Forage yield in the autumn harvest of the first year was 576 kg ha^{-1}. The populations from KS had lower weed biomass in the second summer than other populations (Figure 1).

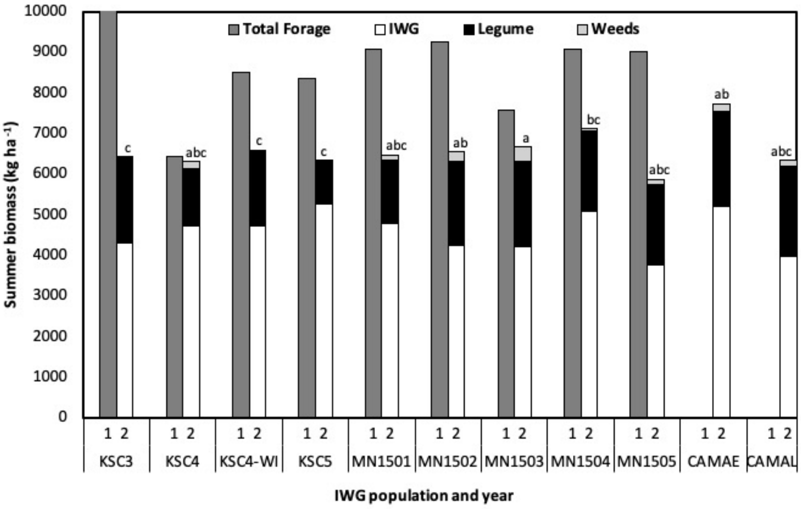

Figure 1 Summer forage biomass per IWG population and harvest year. First year total forage biomass and second year biomass for IWG, Lucerne, and weeds is reported. No differences were found for total forage, IWG, or legume forage biomass. Weed biomass values with the same letter denote no statistical difference ($p<0.05$).

The forage nutritive value of IWG in the summer after grain harvest was relatively low, comparable to wheat straw (Table 1). It must be mixed with higher quality forage for beef or dairy cattle diets. No differences were found for CP or ADF among populations, but populations differed in NDF. The quality of the mixture was similar, given that both species were past their maturity stage; no differences were found between populations. The percent of legume in the mixture in the summer averaged 29.5%. The autumn forage quality of the mixture was high (Table 1), and populations differed in CP (MN1505 was highest).

Table 1 Mean (and range) of forage quality parameters (g/kg DM) for 11 Kernza intermediate wheatgrass populations intercropped with lucerne in Wisconsin (second year for summer and first year for autumn)

	CP	ADF	NDF
IWG summer straw	27 (22-32)	459 (445-478)	682 (656-707)
IWG+Lucerne summer	65 (52-76)	467 (456-479)	656 (634-678)
IWG+Lucerne autumn	188 (178-206)	272 (243-315)	404 (355-491)

Conclusions

The different populations of Kernza intermediate wheatgrass had similar forage production and quality. Summer forage from IWG harvested after grain maturity was of high productivity but limited feed value for cattle. In contrast, the forage quality of the autumn regrowth was of adequate quality for beef and dairy. This highlights the benefits of dual use Kernza for perennial grain and forage systems.

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Effects of two novel feed additives on enteric methane production of Nordic Red dairy cows

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Introduction

Ruminants with their unique ability to convert feedstuffs into high-quality products as milk and meat contribute to the food production systems. However, due to the ruminal fermentation of feed, they contribute to climate change, mainly by emitting methane (CH_4), which is a potent greenhouse gas. Therefore, practical solusions need to be sought to mitigate CH_4 emissions from ruminants. DiGestoChar is a commercial product based on biochar and fibrolytic enzymes that has indicated positive effects on CH_4 mitigation in vitro and milk production in vivo (pers. comm; Branko Petrujkic, GoBioFarm, Iisalmi, Finland). It has been hypothesized to improve digestibility of feed via enzymes and provide optimal conditions for development of rumen anaerobic bacteria, protozoa and fungi. Calcium peroxide (CaO_2) has indicated positive effects on reducing CH_4 in beef cattle (Roskam at al., 2023). Oxydising agents like CaO_2 can release O_2 and subsequently reactive oxygen species when decomposed in the rumen which negatively affect methanogens. The release of extra O_2 in such an anaerobic environment has the potential to directly inhibit methanogens. Therefore, the objective of this study was to evaluate dietary supplementation with DiGestoChar and two levels of calcium peroxide on enteric CH_4 emissions, performance, and rumen fermentation of dairy cows.

Materials and Methods

Four multiparous Nordic Red lactating dairy cows (days in milk 58 ± 9.2, body weight 637 ± 59.3 kg, milk yield 38.4 ± 2.6 kg/day) were used in a 4 × 4 Latin square experiment, which consisted of four 28-day periods. The first 24 days of each period were used for adaptation when the cows were housed in free-stall pens with controlled individual feed bins. The cows were kept in respiration chambers for sample collection and gas exchange measurements during the four last days (Days 24-28) of each period. Experimental treatments comprised: 1) CON, a control diet based on grass silage (timothy-meadow fescue) supplemented with dietary concentrates, 2) DC, the CON diet supplemented with 0.2% DiGestoChar (GoBioFarm, Iisalmi, Finland), 3) CaPe1, the CON diet supplemented with 0.75% calcium peroxide, and 4) CaPe2, the CON diet supplemented with 1.5% calcium peroxide. The diets were balanced based on Luke (2024) recommendations and were fed as total mixed rations with forage to concentrate ratio of 65:35 for CON and DC diets. However, to balance Ca:P ratio in CaPe1 and CaPe2 diets, forage to cocentrate ratios were marginally compromised. Sugar beet pulp and rapeseed meal were kept constant in the diets to avoid any confounding effect from palatability and crude protein concentration with the treatment effect, respectively.

The cows were fed four times a day at 7:00, 13:00, 17:00 and 19:00 h. Milking was done twice a day at 07:00 and 17:00 h. Silage and concentrate samples were taken on Days 24 and 26 during each sampling period. Daily feed intake and milk yield recorded between Days 24 and 28 of each period were used for statistical analysis. Milk was sampled in 6 consequative milkings and the samples were analyzed separately for fat, protein, lactose, urea and somatic cells (MilkoScan FT6000, Foss Electric, Hillerød, Denmark). Energy corrected milk (ECM) yield was calculated according to Sjaunja et al. (1990).

Four open-circuit respiratory chambers were used to measure CH_4 production of individual cows over 4 days (Days 24 to 28), with the first day as acclimatization. On Day 28 of each experimental period, at 10:00 h and after respiratory chamber measurements, samples of rumen liquid (0.5 L) were collected by stomach tubing using a Ruminator apparatus. Immediately after collection, pH was measured using a portable pH meter and two subsamples were taken for volatile fatty acid (VFA) and ammonia-N determination. Obtained data was analyzed using PROC GLIMMIX of SAS 9.4. (SAS institute Inc., Cary, NC, USA), using period and treatment as fixed effects and cow as random effect in the model. The LSD test was used to compare the means and effects were considered as statistically significant when $P<0.05$.

Results and Discussion

Dry matter (DM) and organic matter (OM) intakes were reduced ($P<0.05$) by the CaPe treatments on average by 13.8 and 15.5%, respectively. Milk yield was lower ($P<0.05$) for CaPe2 compared with the control and DC. Yields of ECM, fat and protein were lower ($P<0.05$) for CaPe1 and CaPe2 compared with DC but only the values for CaPe2 were lower than CON. Lactose yield was lower ($P<0.05$) for both CaPe diets compared with CON and DC. Feed efficiency expressed as milk/OM intake and ECM/OM intake were not affected by the treatments.

Table 1 Feed intake, milk yield and feed efficiency of dairy cows fed the experimental diets

	Treatment[1]				SEM	*P*-value
	CON	DC	CaPe1	CaPe2		
DM intake (kg/d)	24.2^{ab}	24.7^{a}	21.3^{bc}	20.4^{c}	1.06	0.025
Organic matter (kg/d)	21.7^{a}	22.2^{a}	19.1^{b}	18.0^{b}	0.94	0.017
Yield (kg/d)						
Milk	33.9^{a}	33.9^{a}	30.6^{ab}	28.2^{b}	1.87	0.015
Energy corrected milk (ECM)	37.8^{ab}	38.6^{a}	33.8^{bc}	30.8^{c}	1.44	0.020
Fat	1.62^{ab}	1.67^{a}	1.44^{bc}	1.31^{c}	0.066	0.026
Protein	1.26^{ab}	1.28^{a}	1.12^{bc}	1.01^{c}	0.059	0.015
Lactose	1.55^{a}	1.54^{a}	1.39^{b}	1.29^{b}	0.103	0.013
Milk/OM intake	1.56	1.54	1.60	1.56	0.052	0.82
ECM/OM intake	1.75	1.75	1.77	1.72	0.053	0.89

[1] CON, control diet; DC, DigestoChar; CaPe1, calcium peroxide 0.75% DM; CaPe2, calcium peroxide 1.5% DM.

Daily CH_4 production was on average 13.6% lower for CaPe diets compared with CON and DC, whereas CH_4 yield (g/kg OM intake) or intensity (g/kg milk or ECM) were not different among treatments. Similarly, CO_2 production was lower for CaPe diets compared with CON and DC while CO_2 yield and intensities were not different among treatments. Hydrogen production was lower ($P<0.01$) for CaPe diets compared with CON and DC. Rumen pH, ammonia-N and total VFA concentrations were not affected by treatment (results of rumen fermentation are not presented). Ruminal molar proportion of acetate was not affected by treatment but propionate tended ($P=0.06$) to be lower for CaPe2 than DC and CaPe1 diets

(176 vs. 189 mmol/mol, on average). Ruminal acetate to propionate ratio tended (P=0.07) to be higher for CaPe2 compared with CaPe1.

Table 2 Methane, carbon dioxide and hydrogen emissions of dairy cows fed the experimental diets

	Treatment[1]				SEM	*P*-value
	CON	DC	CaPe1	CaPe2		
Methane						
g/d	492^a	489^a	431^b	419^b	19.7	0.018
g/kg OM intake	22.7	22.3	22.6	23.3	0.78	0.40
g/kg Milk	14.6	14.5	14.2	15.0	0.69	0.24
g/kg ECM	13.1	13.1	12.8	13.6	0.55	0.14
Carbon dioxide						
kg/d	14.9^a	15.0^a	13.3^b	12.7^b	0.54	0.006
g/kg OM intake	687	679	698	711	16.8	0.38
g/kg Milk	443	442	438	457	17.0	0.60
g/kg ECM	396	389	395	414	15.0	0.43
Hydrogen (L/d)	8.13^a	8.76^a	2.87^b	2.19^b	0.79	0.001

[1] CON, control diet; DC, DigestoChar; CaPe1, calcium peroxide 0.75% DM; CaPe2, calcium peroxide 1.5% DM.

Conclusions

DiGestoChar did not influence feed intake, milk and ECM yields, feed efficiency or CH_4 and CO_2 emissions. Calcium peroxide, especially when used at a high level (1.5% DM), reduced feed intake and milk yield without affecting feed efficiency. Daily CH_4 and CO_2 emissions were reduced by both levels of calcium perioxide but CH_4 and CO_2 yield or intensities were not diffent from the control indicating that the reduction in intake was the main reason for reduced CH_4 and CO_2 levels.

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The effect of the methane inhibitors nitrate and 3-NOP on enteric methane in dairy cow

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Introduction

Methane (CH_4), one of the major greenhouse gases, is emitted from ruminant enteric fermentation. Danish dairy farmers need to reduce enteric CH_4 by 2030 by approximately 40%, which relies on the use of CH_4 reducing additives. Two of the major additives allowed for use today are nitrate (NO_3; SilvAir®, Cargill Inc., Minneapolis, United States) and 3-nitrooxypropanol (3-NOP; Bovaer®, DSM-Firmenich AG, Kaiseraugst, Switzerland). There is a need for reliable and easy-to-use predictions of the effect of these additives on commercial farms for climate reporting protocols. Therefore, we have modelled the effect of NO_3 and 3-NOP on enteric CH_4 in dairy cows for use in the NorFor model.

Materials and Methods

The models were developed using information of CH_4 production, feed intake, diet composition, and dose of 3-NOP and NO_3, respectively, from published feeding experiments on dairy cows. For 3-NOP, 11 papers (Hristov et al., 2015; Lopes et al., 2016; Haisan et al., 2017; Melgar et al., 2020a, 2020b, 2020c; Van Gastelsen et al., 2020, 2022; Schilde et al., 2021; Nielsen et al., 2022; Maigaard et al., 2023) and 32 treatments, and for NO_3, 8 papers (van Zijderveld et al., 2011; Lund et al., 2014; Veneman et al., 2015; Guyander et al., 2015a, 20615b, 2016; Klop et al., 2016; Olijhoek et al., 2016) and 15 treatments were included in the model development dataset. The models were created to describe the percentual reduction in CH_4 production.

CH_4 reduction was predicted by fitting mixed models to the lmer procedure of R statistical language (R Core Team 2022; version 4.2.2). All parameters included in the developed models presented were significant at $p < 0.05$. Models were evaluated on root square mean error (RMSE), RMSE%, and Bayesian information criterion (BIC).

Results and Discussion

The models developed for predicting the CH_4 reducing effect of 3-NOP are in Table 1. The model with lowest RMSE and RMSE% was Model 5, and models with lowest BIC were Models 1, 2 and 4. However, Model 4 had low values for RMSE and RMSE% along with the lowest BIC. That is why Model 4 was implemented in the NorFor feed evaluation system.

Table 1 Evaluation of models predicting the CH_4 reduction from 3-NOP in percent compared to control

Model no.	Model	RMSE	RMSE%	BIC
1	-18.88 – 0.195 × dose	2.20	7.41	160
2	-54.48 – 0.175 × dose + 0.107 × NDF	1.18	3.96	160
3	-56.00 – 0.175 × dose + 0.111 × NDF + 0.001 × ST	1.16	3.92	168
4	-40.51 – 0.199 × dose + 0.097 × NDFr	1.10	3.69	160
5	-50.84 – 0.177 × dose + 0.104 × NDFr + 0.027 × ST	1.07	3.60	166

Dose – concentration of 3-NOP in total diet (mg/kg DM). NDF – Neutral detergent fiber in total diet (g/kg DM). ST – Starch in total diet (g/kg DM). NDFr – roughage NDF in total diet (g/kg DM).

The models developed for predicting the CH_4 reducing effect of NO_3 are in Table 2. Model C had the lowest BIC while Model B had the lowest RMSE. When considering both RMSE and BIC at the same time, Models A and E showed similar performance. Model A, with dose of NO_3 in total diet, was the most simple model and yet it performed quite well when considering RMSE, RMSE% and BIC. That is why Model A was implemented in the NorFor feed evaluation system.

Table 2 Evaluation of models predicting the CH_4 reduction from NO_3 in percent compared to control

Model no.	Model	RMSE	RMSE%	BIC
A	-1.026 × dose	0.28	1.20	111
B	-0.883 × dose – 0.215 × CFat	0.11	0.49	109
C	-0.544 × dose – 0.100 × CFat – 0.013 (dose × CFat)	1.25	5.39	98
D	-0.945 × dose – 0.017 × NDF	0.25	1.07	116
E	-0.912 × dose – 0.032 × ST	0.32	1.36	110

Dose – concentration of NO_3 in total diet (g/kg DM). CFat – Crude fat in total diet (g/kg DM). NDF – Netural detergent fiber in total diet (g/kg DM). ST – Starch in total diet (g/kg DM).

Conclusions

Several models for predicting the reduction on enteric CH_4 emissions from 3-NOP and NO_3 were developed and evaluated. NorFor has implemented models where the CH_4 reducing effect of 3-NOP includes the dose of 3-NOP and NDF in roughage and for NO_3 the model includes the dose of NO_3.

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Effect of dairy cows' yield index on the effect of enteric methane reducing dietary treatments

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Introduction

The Yield index (Y index) is an index for milk production value based on genetic and phenotypic data, and is used for genetic selection in dairy herds across Scandinavia (NAV, 2016). While productivity is important, use of dietary treatments that reduce enteric methane (CH_4) emission from dairy production is increasing in relevance. Previous studies have found that high CH_4 emitting cows are less responsive to such dietary treatments compared to low CH_4 emitting cows (Giagnoni *et al.*, 2022).

The current study investigated the effect of the Y index on the response of cows to dietary treatments aiming at a reduced enteric CH_4 emission. It is hypothesised that the Y index is positively correlated with CH_4 production (g CH_4/d), and negatively correlated with CH_4 yield (g CH_4/kg DMI) and intensity (CH_4/kg ECM). Cows with high Y index are assumed to be low in CH_4 emission metrics. Furthermore, it is hypothesised that there is an interaction between Y index and response to CH_4 reducing dietary treatments, so that high Y index cows have a greater response to CH_4 reducing treatments.

Materials and Methods

A dataset was computed from three experiments and included 716 observations from 141 individual cows (Giagnoni *et al.*, 2022), where all experiments used Latin squares design, 21 d periods, and half primiparous and half multiparous cows. The Y index used for the cows were estimated in February 2024 on population average, and recentred within the dataset. A total of 20 dietary treatments were used, balanced for first order carry-over effects within experiment, and including methane mitigating additives such as 3-nitrooxypropanol (3-NOP) and nitrate, as well as different levels and sources of dietary fat and crude protein. The cows were fed partial mixed rations and individual intake of dry matter (DMI) was recorded using automatic feeding bins (Hokofarm group, the Netherlands). Additionally, cows could receive up to 1 kg of DM as pelleted concentrate from the GreenFeed units (C-Lock, USA), which were used to measure gas emissions. Milk yield was measured in the milking parlour during the two daily milkings. Milk composition for energy corrected milk (ECM) yield calculation was obtained over the last six milkings of each period using a MilkoScanTM 7RM FT+ infrared analyzer (Foss, Denmark). All measures were averaged over the last week of each period.

Cow estimates for the methane metrics (CH_4 production, CH_4 yield, and CH_4 intensity) were obtained using a linear model with a fixed intercept for treatment times period and a random intercept for the individual cow (the cow estimates). The Y index was correlated to cow estimates for the CH_4 metrics using a linear model (Y index as Y, and CH_4 as X). The interaction between the Y index and the CH_4 yield mitigation effect of the treatments was tested on the variables recorded (DMI, ECM yield, CH_4 production, CH_4 yield, and CH_4 intensity) with a linear mixed model as follow:

$$Y_{ipqke} = \mu + Yindex \cdot X_{1k} + Trteff \cdot X_{2i} + YindexTrteff \cdot X_{3ki} + DIM \cdot X_{4ke} + Parity_p + Period_{qe} + Cow_{ke} + \varepsilon_{ipqke}$$

where: Y_{ijkp} is the dependent variable, μ is the mean intercept, *Yindex* is the fixed slope coefficient for the cow specific Y index X_{1k} in cow *k*; *Trteff* is the fixed slope coefficient for the mean treatment effect X_{2i} in treatment i (mean CH_4 yield within treatment, centred on zero within experiment); *YindexTrteff* is the fixed slope coefficient for the interaction between the Y index and the mean treatment effect in cow *k* and treatment *i*; DIM is the fixed slope for DIM for the cow within experiment X_{4ie}, $Parity_p$ is the fixed change in intercept for parity p (primiparous or multiparous), $Period_{qe}$ is the fixed change in intercept for the experimental period q within experiment *e* (16 in total). Cow_k is the random intercept of cow *k* in experiment *e*. P-values for fixed effects were obtained using ANOVA type two.

Results and Discussion

Descriptive statistics of the data are in Table 1. The correlation coefficient for Y index and CH_4 metrics (production, yield, and intensity) are in Figure 1. The correlation between CH_4 production and intensity was weak, but significant (r = 0.24 and −0.21, respectively), with the Y index. For CH_4 production this can be explained by the fact that high Y index cows should be cows with high feed intake, and therefore a high daily CH_4 production. For CH_4 intensity, high Y index cows should be cows with high ECM and, the negative relationship should therefore be explained by a greater dilution of the CH_4 emission.

Model estimates and P-value for the mitigation effect and the Y index are in Table 2. The interaction between CH_4 mitigation dietary treatment effect (treatment effect) and the Y index was not significant for any of the metrics considered. This suggest that an interaction between treatment effect and Y index is not relevant. However, previously findings by Giagnoni *et al.* (2022) showed that low CH_4 yield phenotypes were associated with high response to dietary treatments, and vice versa. Therefore, the second hypothesis, which expected greater response to treatment effect in high Y index cows, could not be verified, and the current study suggest the Y index does not affect individual responses to CH_4 mitigation diets.

Table 1 Descriptive statistics of the dataset

	Descriptive statistics					
Variable	n	Mean	SD	CV	Min.	Max.
DMI, kg/d	716	20.8	3.20	15.4	11.7	30.0
ECM production, kg/d	716	32.6	6.46	19.8	16.1	54.3
CH_4 production, g/d	716	318	79.0	24.8	91.4	590
CH_4 yield, g CH_4/kg of DMI	716	15.3	3.08	20.1	5.14	24.8
CH_4 intensity, g CH_4/kg of ECM	716	9.90	2.24	22.6	3.56	18.0
Y index	122	95.0	9.50	9.99	75.0	123

CV = coefficient of variation (mean/SD) expressed in percentage.

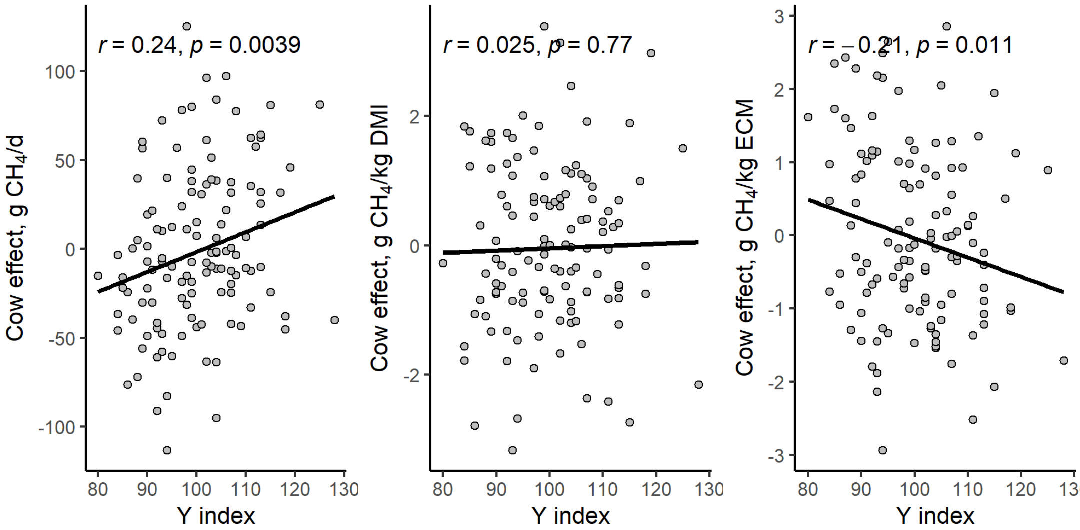

Figure 1 Correlation plots between the Y index (x axis, centred on 100) and the random cow estimates for CH4 production (centred on zero), yield and intensity (CH_4 g/d, g CH_4/kg DMI, and g CH_4/kg ECM, respectively).

Table 2 Estimated mean intercept, slope for the treatment effect (the CH_4 yield mitigation effect, expressed as the mean value for CH_4 yield in the treatment), slope for Y index, and related P-values

Variable	Intercept	Slope			P-value		
		Treatment effect	Y index	Treatment effect × Y index	Treatment effect	Y index	Treatment × Y index
DMI, kg/d	14.6	0.23	0.076	0.0042	0.66	<0.001	0.42
ECM, kg/d	13.9	0.046	0.21	0.0064	0.95	<0.001	0.42
CH_4 production	230	25.7	1.07	0.056	0.007	0.001	0.55
CH_4 yield	15.7	1.44	−0.0051	−0.0043	<0.001	0.73	0.34
CH_4 intensity	13.3	1.22	−0.036	−0.0046	<0.001	0.003	0.19

DMI = dry matter intake; ECM = energy corrected milk yield; CH_4 production = g CH_4 /d; CH_4 yield = g CH_4/kg of DMI; CH_4 intensity = g CH_4/kg of ECM.

Conclusions

The Y index correlated positively to DMI, ECM, CH_4 production, and CH_4 intensity, but it did not correlate to CH_4 yield. The Y index did not affect response to the CH_4 mitigation dietary treatments.

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Thirty years of intensive research to reduce methane emissions – what has been achieved?

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Introduction

Estimates of the contribution of enteric methane (CH_4) production is 27% of anthropogenic emissions and 16% of total emissions including natural sources. As a result, campaigns promoting for plant-based diets cite solving climate warming as one of the primary reasons to decline consumption of ruminant products. However, these opinions fail to distinguish differences between biogenic and fossil fuel carbon. When the CH_4 emissions from ruminants are constant the warming effect is neutral (Liu et al., 2021). Methane is a short-lived climate pollutant with atmospheric lifetime of 10 to12 years. Decreasing CH_4 emissions from ruminant production has therefore a potential to decrease warming potential. However, the potential is not very large as the warming caused by atmospheric CH_4 will drop to near zero in a decade when its emission becomes zero. It could be estimated that total amount of greenhouse gasses (GHG) would be about 3% lower after one decade if ruminant food production were immediately finished ($100 \times 0.20 \times 0.16 = 3.2\%$; 0.20 = proportion of CH_4 of total GFH; 0.16 = ruminants' share of total CH_4 emissions)

Intensive research has been conducted during the last years to explore strategies to mitigate CH_4 emissions from ruminants. Huge investments have been made to measure CH_4 emissions in research facilities, even in practical farms. Effects of diet composition and different additives have been intensively studies. Breeding low-emitting animals has been suggested as a strategy to reduce emissions. Studies to link rumen microbiome and CH_4 emission has placed rumen microbiome studies in the forefront of animal agricultural research. The objective of this paper is to discuss practical achievements of methane research and potential of different mitigation strategies.

Diet Manipulation

Researchers are often citing Johnson and Johnson (1995) that CH_4 energy varies between 2 and 12% of GE intake. This highly cited paper (>3 700 citations) gives wrong impressions that reducing CH_4 has a great potential to spare feed energy for productive purposes. This range represents the extremes; the lowest values observed with 90-95% concentrate diets fed to feed-lot cattle and the highest with sheep fed high quality forages with some concentrates at maintenance level of feeding. With typical dairy cow diets, the proportion of CH_4-E of gross energy (GE) intake ranges from 5 to 7%. In respiration chamber data of between-diet CV was 6.3% (Guinguina et al., 2020). One SD unit difference in CH4 corresponds to 1.4 MJ increase in ME intake at DMI of 20 kg/d, provided that digestibility does not decrease. There is very little evidence in the literature that energy spared from reduced CH_4 production is used for production.

Increased concentrate supplementation has been suggested as a strategy to mitigate CH_4 emissions. However, within the typical range of concentrate proportion in dairy cow diets, expected effects are rather small. The model of Sauvant and Giger-Reverdin (2009) predicted maximum CH_4 yield (g CH_4/kg DM) at 35% concentrate on a DM basis and moderate decreases of between 35 and 60% of concentrate. Effects of concentrate proportion on rumen fermentation pattern and CH_4 production are not linear with greater changes taking place above 70 to 80% of concentrates in the diet. Overall effects on GHG emissions should holistically be evaluated on whole farm basis. Compared with grass, the total GHG emissions (CO_2-ekv per kg DM) of grain are higher, especially when differences in soil carbon balance are taken into account. Sustainability of using grain to reduce CH_4 emission is questionable, since grain can be used directly as human food or much more efficiently for swine and poultry.

The effects of fats supplements on CH_4 emissions were already shown by Czerkawski et al. (1966). Fat supplements are not fermented in the rumen, decrease feed intake, and can modify rumen fermentation pattern, thereby decreasing CH_4 emissions. Using fat supplements for mitigating CH_4 emission has several drawbacks. High levels of fat supplementation can decrease cell wall digestibility and feed intake and has consistently decreased milk protein concentration. Carbon footprints are greater for fat supplements and is greater than that of grain, at least partly compensating for the effects of lower CH_4 emission in total CO_2-ekv/kg ECM. The sustainability of using human edible fat to decrease CH_4 emissions can also be questioned.

Overall, potential of diet manipulation to specifically reduce CH_4 emissions is rather limited beyond feeding high quality balanced diets. In ration formulation program, optimizing feed income over feed cost, it is possible to set a constraint for CH_4 intensity (CH_4 g/kg ECM) and thereby evaluate costs of feeding CH_4 mitigating diets.

Breeding for Reduced Methane

Genetic selection has been suggested as an alternative to reduce CH_4 emissions from dairy cows. Effects of genetic selections are permanent and cumulative and breeding for lower emissions is also considered to be inexpensive (Hayes et al., 2013). It has been shown that CH_4 emission is under some genetic control by the host animal (Lassen and Løvendahl, 2016). To reduce CH_4 emissions by animal breeding emissions should be measured accurately and between-cow and variability should be sufficiently large. When CH_4 estimated from CH_4 concentration or CH_4/CO_2 ratio measured by "Sniffer" methods under farm conditions, between-cow variability (CV) has been 15 to 20% suggesting good potential for genetic selection. However, the CV was only 6.6% in CH_4 yield (CH_4-E/GE) when measured in respiration chambers (Guinguina et al., 2020a). True variability may even be less, since CH_4 yield (g CH_4/kg DMI) is related to feeding level, *i.e.,* the values should be adjusted for feeding level effects. Variability in CH_4/CO_2 measured in respiration chambers of by the GreenFeed system have been 5 to 6%. When the GreenFeed system was run "in Sniffer mode", CH_4 concentration explained only 10% of the variation in CH_4 flux measured in normal GreenFeed mode (Huhtanen et al., 2015). Between-animal CV measured with the laser technique was 50% (Lanzoni et al., 2022). Much higher CV observed with the Sniffer and laser methods compared with the CV measured in chambers is most likely related to large random measurement errors.

Calculating total CH_4 production from CH_4/CO_2 ratio measured by the Sniffer system, using predicted CO_2 production (Madsen et al. 2010), is even a greater problem, since it favours inefficient cows (Huhtanen et al., 2021). CO_2 is predicted from ECM yield and metabolic body weight (MBW). Efficient cows produce more milk and less CO_2 than average cows with the same intake, but their predicted CO_2 is higher due to higher yield (and *vice versa* for inefficient cows). This problem cannot be solved as both ECM yield and MBW are positively correlated to heat and consequently CO_2 production.

Differences in CH_4 emissions between cows is related to a smaller rumen size and faster digesta passage rate. Therefore, lowering CH_4 emissions by genetic selection will reduce diet digestibility (see Løvendahl et al., 2018), especially cell wall digestibility. A digestibility (D) is a function of digestion and passage rate [$D = k_d /(k_d + k_p)$], to compensate effects of increased k_p, also digestion rate k_d must increase. This increases the amount of substrate available for CH_4 production. Methane emissions can still decrease, since faster digesta passage rate increases efficiency of microbial synthesis. As a result, more fermented carbon is partitioned towards cells instead of gasses and VFA. Low-emitting sheep had a smaller rumen volume (Goopy et al., 2014) that can become a limiting factor of intake potential of dairy cows fed high-forage diets. Selection of dairy cows for reduced CH_4 emission will decrease ruminants' most important character in human food production: ability to utilize non-human edible resources (forages, fibrous by-products) to produce high quality human food. Methane is produced by the microbes that could set the limits of how much CH_4 can be decreased by animal breeding. Therefore, will the cow eventually become a simple-stomached animal?

Another alternative to reduce CH_4 emission indirectly is breeding cows for improved feed efficiency. In Finland, total enteric CH_4 emissions and CH_4 intensity (g CH_4/kg ECM) decreased 57% and 37% from 1960 to 2020, respectively (Huhtanen et al., 2022). This was partly due to decreased number of cows and partly due to increased efficiency from genetic improvements, better nutrition, and management. Until now, improved efficiency is derived from the dilution of maintenance requirement to a greater volume of milk. From current production levels in the Nordic countries, the potential to improve feed conversion efficiency by increasing milk yield is rather limited, especially if associated with increased BW. However, there is variation in digestion and metabolic efficiency between cows. When cows (n=840) from respiration chamber studies were divided in low, medium, and high efficiency groups, according to residual feed intake (RFI) or residual ECM yield (RECM), the low efficiency groups produced 13 and 32% more CH_4 per kg ECM, respectively (Guinguina et al., 2020b). An advantage of breeding for improved efficiency is that also the profit will increase either because of reduced feed cost (RFI) or increased yield (RECM). Breeding for reduced CH_4 emissions has no effect on profit, in contrast to breeding for reduced CH_4. In addition, improved efficiency decreases other environmental impacts (e.g., N emissions, fossil fuel) of dairy production per kg ECM. Lack of reliable and cost-effective on-farm methods of measuring DMI has limited introducing efficiency traits in breeding indexes of dairy cows. Good agreement between RFI and residual CO_2 (RCO_2) production in respiration chamber data studies (Huhtanen et al., 2021) suggested that RCO_2 could be used as a proxy of feed efficiency if CO_2 production can be accurately measured in on-farm conditions. In a recent study (32 cows) at Luke, there was a good relationship in O_2 consumption and CO_2 production determined in respiration chambers and by the GreenFeed system and also in feed metrics based on measured intake and gas production (unpubl. data).

Rumen Microbiome

Over the recent years, there has been a growing body of evidence demonstrating the link between the microbiome and its effect on productivity of the host animals and the environment (Mizrahi and Jami, 2018). Several statistical associations between rumen microbiome *vs*. CH_4 emissions, production characteristics and feed efficiency has been reported (for review, Mizrahi and Rahi, 2018). Whether these are causal relationships or just statistical relationship is not known. For example, are changes in microbial populations related to increased digesta passage rate that can be the primary reason for lower CH_4 emissions. Modulation of the microbiome toward a more optimized phenotype remains one of the main challenges in the field. Manipulation of rumen microbiome has been attempted in numerous studies with limited success (Mizrahi and Rahi, 2018). Most attempts at introducing bacteria from other animals have failed, even when rumen contents of one cow replaced completely with the rumen fluid from another cow. Just within a few weeks, rumen microbiome content reverted to a content closely resembling its original composition (Weimer et al., 2017). This research has produced much data, but it is questionable if it will lead to any practical applications of mitigating CH_4 emissions. In meta-analysis (Cabezas-Garcia et al., 2017), CV of stoichiometric CH_4 production per mole VFA was only 1.1% in cows fed the same diet. This suggests that despite large between-animal variability in rumen microbiome the profile of end-products of rumen fermentation is very constant. Greater variability in the concentrations of individual VFA indicates that differences in passage rate can contribute more to between-animal differences in CH_4 emissions than microbiome. In line with this, no differences were observed CH_4 production *in vitro* when rumen fluid was taken from low- and high-emitting cows (Cabezas-Garcia et al., 2021).

Feed additives

Mitigation of CH_4 has been a subject of intensive research. Only a few examples will be discussed here. The potential to decrease global CH_4 emissions from ruminants is limited for several reasons. 3-NOP has constantly decreased CH_4 emissions about 30% and the potential to decrease emissions globally is rather limited. It needs to be delivered frequently to reach full mitigation potential, preferentially by including it in total mixed ration. Intensive total mixed ration-based systems cover only a small fraction of world's ruminant population. In these systems, CH_4 intensity is well below average. If slow-release products were developed, the use of 3-NOP could be expanded, but still cover only a minor fraction of the world's cattle population.

Asparagopsis taxiformis has efficiently decreased CH_4 emissions but it will never be practical solution for at least two reasons. Its current price is about 120 €/kg (Wittington, 2022), *i.e.*, with 0.5% of daily dose about 12 €/cow/day, which is more than the value of the milk (Willington, 2022). It was estimated that even when the price decreases 3 €/kg, it would still increase feed cost by 20% under Australian conditions. To produce enough *Asparagopsis* even for Australian dairy cows (1.5 million) and feedlot cattle (1 million) would require 20000 hectare of aquaculture farms (20 t dry matter per hectare).

Nitrate has shown to decrease CH_4 emission, but a potential for toxicity exists. Nitrate could be used to replace urea when supply of rumen degradable N is limited. However, price per unit of N is higher for Ca-nitrate than for urea. When the supply of rumen degradable N is sufficient using nitrate as an additive to mitigate CH_4, emissions will increase urinary N excretion, and consequently increase the risk for higher ammonia emissions and nitrate leaching.

Modelling

Several models, mainly empirical, predicting CH_4 from different animal and dietary characteristics have been developed. Prediction errors have been variable, probably reflecting more the quality of data than the model structure. Feed intake is the main driver of CH_4 production explaining at least 90% of the variance explained by the best model including dietary variables such as digestibility, fat and NDF concentrations. The problem of linear models is that they can only be within the limits of data from which the models were developed. For example, the intercept of DM intake models depends very much on range of DMI in the dataset. Forcing intercept to zero can lead to serious underestimation of CH_4 emissions at low DM intake and overestimation at high DM intakes. Non-linear models can solve this problem. Axelsson (1949) published more than 70 years power function model ($CH4 = a \times DMI^b$) using data from respiration chamber studies conducted in early 1900's and 1930's. The Axelsson (1947) model worked surprisingly well (unpubl.), better than many recent models using the data of Ramin and Huhtanen (2013).

The mechanistic and dynamic Nordic dairy cow model Karoline performed well in two evaluations (Ramin and Huhtanen, 2015; Kass et al., 2021). Root mean squared error within study was low (6-7% of mean). This was only marginally greater than residual SD (about 5%) in carefully conducted experiments. Part of the model errors are related to inaccuracies in the values of some key parameter, e.g., indigestible NDF concentration and digestion rate of potentially degradable NDF. When these parameters were available, prediction errors were smaller (unpubl. data). A number of feeding experiments have been conducted exploring dietary effects on CH_4 emissions as the main hypothesis. Rather than making animal experiments, modelling and *in vitro* analyses can be as good as animal studies.

Many resources are used for national CH_4 inventories. However, it is questionable if the accuracy is improved compared to simple model based on ME requirement and dietary dietary ME concentration to calculate average DM intake that is then multiplied CH4 intensity factor (g/kg DM) and number of cows. Errors of this model is likely to be < 5% and improvements compared with more complicated models are likely to be small and cannot be validated. Attempts to measure CH_4 emissions from the barn will certainly result in greater errors than the simple model described above.

Conclusion

Intensive research for 30 years to develop CH_4 mitigations has not produced practical applications of any quantitative impact. In Western countries improved production efficiency together with reduced number of animals has decreased total emissions and emissions per unit of product. We should also question if CH_4 produced by ruminants is a real problem because it is biogenic, *i.e.,* recyclable carbon in contrast to fossil CH_4 released to atmosphere from gas and oil production. The potential of feed additives to mitigate CH_4 emissions globally will be marginal since most of world's ruminant population is in developing countries where the use of additives is not feasible economically and technically. Breeding for improved feed efficiency is a more sustainable strategy to reduce CH_4 emissions per unit product than selecting for low-emitting animals. Farmers do not get any befits of breeding low-emitting animals but eventually pay the costs of equipment and breeding programs breeding organisations. Farmers will benefit economically from improved feed efficiency, CH_4 emissions decrease as much as with direct selection, and total N and CO_2 emission decrease as less input is required per unit of product. Improving production efficiency globally and decreasing number of animals while maintaining total production is the only sustainable strategy to reduce CH_4 emissions. As a scientific community we should try convincing decision makers that investing a large proportion of research funding to methane research is not the most efficient strategy, not even to reduce CH_4 emission. During the last 30 years CH_4 emission per unit of product has gradually decreased, but it is because of improvement in genetics, nutrition, and management – not because of achievements in methane research. A large proportion of methane research is repeating experiments without any real novelty. The title of Formas project of the late Professor Jan Bertilsson in 2011 "Mitigating methane emissions from dairy cows - a mission impossible?" is still appropriate and valid. Most of world's ruminants are in developing countries and where the CH_4 intensity is poor. The greatest potential to decrease global CH_4 emissions is to improve production efficiency and decrease number of animals in developing countries.

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Harvesting frequency, grassland species and silage additive affects *in vitro* methane production from silage

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Introduction

Grass silage is the main ingredient in the diet of dairy cows in the Nordic countries, and silage feed and fermentation quality have a strong impact on feed intake and milk production. The quality of silages is affected by management factors like plant maturity stage at harvest, harvesting frequency, crop botanical composition and silage fermentation pattern. Studies have shown that silage feed quality also affects enteric methane production *in vivo* (Brask *et al*., 2013) and *in vitro* (Navarro-Villa *et al*., 2011), and silage fermentation quality affects enteric CH_4 production *in vitro* (Navarro-Villa *et al*., 2011; Weiby *et al*., 2022). However, there is less knowledge of combined effects of management factors on CH_4 production. The objective of the current study was therefore to test the effect of harvesting frequency, crop type, wilting and use of a formic acid-based additive on *in vivo* predicted CH_4 production.

Materials and Methods

Silages were prepared from three crop types harvested from a replicated field experiment at Fureneset research station (61°17.6′ N, 5°2.9′ E; elevation 30 m a.s.l.), Norway, with Timothy (T) (*Phleum pratense L.*); Timothy-red clover (RC) (*Trifolium pratense* L.) mixture and perennial ryegrass (RG) (*Lolium perenne* L.) cut two or three times per season. The herbage was wilted to two dry matter (DM) levels, aiming at 22.5 or 37.5% DM, chopped, and ensiled without or with a formic acid-based additive (equivalent to 4 L/Mg fresh grass material with GrasAAT® Lacto, ADDCON Nordic AS, Porsgrunn) in evacuated sealed polyethylene bags. After 3 months of fermentation, the silages were stored frozen and thereafter samples were prepared for analysis of feed quality and fermentation products and for *in vitro* gas testing as described by Weiby *et al*. (2023). In short, the samples for *in vitro* gas testing were freeze dried and milled using a 1-mm mesh screen. They were then incubated in 60 mL buffered rumen fluid in glass bottles placed in a water bath at 39°C for 48 h in a fully automated gas *in vitro* system at the Swedish University of Agricultural Sciences in Umeå. The total gas volume produced was automatically recorded. Gas samples for CH_4 determination were collected at 2, 4, 8, 24, 32 and 48 h and analysed using a gas chromatograph. The data were analysed using the procedure GLIMMIX in SAS (Version 9.4, SAS Institute Inc, Cary, NC, USA).

Results and Discussion

The effect of cutting frequency and crop type on silage feed and fermentation products are presented as averages across cuts, wilting rate, and additive treatment in Table 1. Three cuts rather than two cuts per season resulted in 10 g/kg DM lower organic matter (OM) content, 7.5% units higher organic matter digestibility (OMD), 29 g/kg DM higher crude protein (CP) content, 102 g/kg DM lower NDF content and 52 g/kg DM lower iNDFom content. Silages prepared from T had on average 9 and 38 g/kg DM higher CP and NDF content, respectively, than RG. On average, OMD was 1.1 % unit lower, CP content was 3 g/kg DM lower, and NDFom and iNDFom contents were 37 and 6 g/kg DM higher, respectively, in T than in the T + RC mixture. Silage pH and fermentation products was not affected by crop type, but pH was higher, and the sum of fermentation acids were 30 g/kg DM lower in silage cut two compared to three per season.

Table 1 Effect of cutting frequency (C: 3 or 2 cuts per season) and crop type (M: T=timothy, T+RC=timothy+red clover, RG=ryegrass) on silage feed quality parameters (OM=organic matter, OMD=organic matter digestibility, CP=crude protein, and NDFom=neutral detergent fibre in g/kg DM), pH and sum of fermentation acids (TA in g/kg DM) averaged across DM and silage additive treatments, n=12 for 3 cuts and n=8 for 2 cuts

Item	T		T+RC		RG		SEM	*P*-values					
	3cut	2cut	3cut	2cut	3cut	2cut		C	M	C×M	C1	C2	C3
DM, g/kg	311	352	323	350	308	346	46.7	ns	ns	ns	ns	ns	ns
OM	930	941	922	937	921	925	47	***	***	***	***	***	***
OMD, %	74.8	67.0	75.8	68.1	74.4	67.5	0.46	***	***	***	***	ns	***
CP	134	105	142	99	119	104	1.7	***	***	***	***	***	**
NDFom	482	569	433	550	438	539	9.0	***	***	***	***	***	***
iNDFom	67	124	63	116	75	122	3.4	***	***	***	***	ns	*
pH	4.18	4.51	4.30	4.69	4.15	4.76	0.193	***	ns	ns	***	ns	ns
TA	58	33	72	36	66	38	15.0	**	ns	ns	***	ns	ns

SEM is standard error of the mean; P-values: C1 = 3 *vs.* 2 cuts per season, C2= timothy *vs.* perennial ryegrass, C3 is timothy *vs.* timothy red clover mixture. Significance ns: P>0.05, *: P<0.05, **: P<0.01, ***: P<0.001, respectively.

Wilting rate and additive had no effect on silage feed quality (results not presented in table), except that wilting from 22.5 to 37.5% DM increased the NDFom content by 12 g/kg DM (482 *vs.* 494 g/kg DM, P<0.05). Both stronger wilting rate and the use of additive reduced (P<0.001) the total content of fermentation acids, from 74 to 35 g/kg DM at 22.5 % and 37.5 % DM, respectively, and from 67 without to 42 g/kg DM with formic acid. The effect of the additive was stronger at low (-32 g/kg DM) than high (-16 g/kg DM) wilting rate, *i.e.* a wilting by additive interaction effect (P<0.01). The use of additive increased (P<0.001) the content of formic acid in silages from on average 0.7 to 12 g/kg DM at low and from 0.3 to 5.2 g/kg DM at high wilting rate.

On average, the *in vitro* CH_4 production was 7.5% higher with three than two cuts per season (Figure 1, 31.5 *vs.* 29.3 ml/g DM, p<0.001). The higher CH_4 production in silages prepared from three as opposed to two cuts are due to lower NDFom and INDFom concentrations, and thereby more digestible feed available for fermentation. Silages prepared from the first cut of RG produced on average 11% (35.8 *vs.* 32.2 ml/g DM) and 8% (30.7 *vs.* 28.2 ml/kg DM) more CH_4 than silages prepared from T from the three and two cut systems, respectively. The reason for higher CH_4 production from RG than T is probably due to a lower NDF content and greater substrate fermentability. The average OMD across cuts was not different between RG and T, but in the first cut of RG cut three times, OMD was on average 2% units higher than T. The use of the formic acid-based additive increased CH_4 production on average by 5% (30.3 *vs.* 31.9 ml/kg DM), but the effect was mainly observed in the three-cut system (three-way interaction between cut, witing rate and additive, P<0.05). Silages without additive had higher concentration of fermentation acid, particularly lactic acid, which is less methanogenic as it is fermented to propionic acid in the rumen. Besides, residual formic acid in the additive treated silages were likely converted to CH_4 *in vitro*.

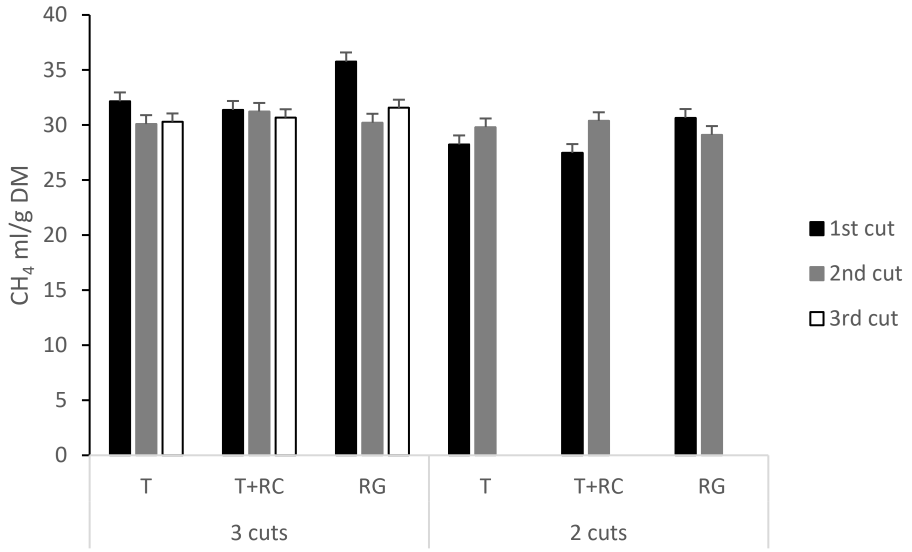

Figure 1 Effect of cut (3 or 2 cuts per season) and crop type (T=timothy, T+RC=timothy+red clover, RG=ryegrass) on silage in vitro CH_4 production (mL/g DM), average across wilting rate and additive treatment. Bars represent standard error of mean.

Conclusions

Silage prepared from two instead of three cuts per season, extensive silage fermentation without formic acid based additive and use of T compared to RG reduced in vivo predicted CH_4 production. These findings need to be confirmed *in vivo* and in relation to animal production responses.

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Milk and methane production from dairy cows fed grass silage of different grassland species and harvest frequencies

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Introduction

Methane emission from ruminant production systems account for 5% of global greenhouse gas emissions, and forage management can have a significant impact (Beauchemin *et al.*, 2020). Feeding silages produced in a three-cut system (i.e., plants harvested at early growth stage) compared to silages from a two-cut system (i.e., plants harvested at more mature growth stage) may increase CH_4 production (g/day), but reduce CH_4 yield (g/kg dry matter intake (DMI)) and intensity (g/kg ECM) if DMI and milk production increase (Warner *et al.*, 2016). Different grassland species differs in organic matter digestibility (OMD), and will affect feed intake and milk production (Johansen *et al.*, 2018), which may result in altered CH_4 yield and intensity. The aim of this study was to quantify the effect of grassland species and harvest frequency (two *vs*. three cuts per season) on feed intake, milk production, digestibility, and methane emission in dairy cows. We hypothesized that more frequent harvesting, use of grass species with greater organic matter digestibility and legumes with lower NDF concentration would increase silage dry matter intake and milk yield and thereby decrease CH_4 yield and intensity.

Materials and Methods

Silages produced in 2021 and cows kept at Livestock Production Research Centre at the Norwegian University of Life Sciences the following winter were used. Forty Norwegian Red cows (15 primiparous and 25 multiparous) in early- to mid-lactation (102 ± 21.2 DIM; mean ± SD), weighing 584 ± 79 kg and yielding 30.2 ± 6.0 kg of milk/d were blocked according to parity, days in milk and body weight and within block randomly allocated to five treatments in a cyclic changeover design comprised of four 21-d periods (14 d of adaptation, 7 d of recording and sampling). The five treatment diets evaluated silages produced from timothy (*Phleum pratense L.*) in a two-cut system (T2), timothy in a three-cut system (T3), perennial ryegrass (*Lolium perenne L.*) in a three-cut system (PR3), red clover (*Trifolium pratense L.*) in a three-cut system (RC3) and a mix of T3 and RC3 (50:50 on DM basis) (T3/RC3). The proportion of DM from spring growth, first regrowth and second regrowth in the different treatment diets was based on each cut's share of the total DM yield over the season.

Gas emissions were measured using two Greenfeed units, and the cows were offered the experimental silages *ad libitum* in forty electronic feeding bins designed to measure feed intake of each cow. The same level of concentrate was offered across treatments (6 kg/d to primiparous and 9 kg/d to multiparous cows). Milk yield was recorded in the milking robot at each visit, and cows had access every 6 h with a maximum of 4 milkings every 24 h. Three separate milk samples were collected from each cow at three consecutive milkings during 48 hours in the last week of each period. Cows were weighed after each milking, and total tract digestibility of each diet was estimated using acid insoluble ash as internal marker in fecal grab samples, collected at 6:00 am and 3:30 pm on three consecutive days during the last week of each period. The data were analysed using the MIXED procedure of SAS with block, period and treatment as fixed effects and animal within block as random effect. The contrast function was used to test the effect of cutting system of timothy (T2 *vs* T3) and the effect of grassland species, where the comparisons were timothy *vs*. perennial ryegrass (T3 vs PR3), and linear and quadratic effects of increasing the proportion of red clover, using T3 as the treatment without red clover. Statistical significance between treatments was declared at $P \leq 0.05$.

Results and Discussion

The description of experimental silages and concentrate are in Table 1. All silages were well preserved.

Table 1 Description of the experimental diets[1] and concentrate (g/kg DM if nothing else is given)

	T2		T3			PR3			T3/RC3 50/50			RC3			Conc-entrate
Cut number	1	2	1	2	3	1	2	3	1	2	3	1	2	3	
Proportion, %[2]	64	36	45	30	25	47	29	24	22/24	14/16	12/12	46	31	23	
DM, %	45		32			33			30			28			86
Crude protein	132		158			146			180			200			189
NDF	591		513			450			401			299			175
iNDF	141		76			72			82			87			
WSC[3]	98		58			124			45			33			69
NEL_{20}, MJ/kg[4]	5.3		6.2			5.9			6.0			5.9			6.6
AAT[5]	75		79			76			78			77			124
PBV[6]	-9		35			27			56			75			14

[1]T2 = Timothy 2 cut system, T3 = Timothy 3 cut system, PR3 = Perennial ryegrass 3 cut system, T3/RC3 = Timothy 3 cut system/red clover 3 cut system, RC3 = Red clover 3 cut system, [2]Proportion (%) from spring growth, first regrowth and second regrowth used in the experimental diets, [3]Water soluble carbohydrates, [4]NEL20 reference: net energy of lactation at 20 kg of DMI/d, [5]Amino acids absorbed from small intestine, [6]Protein Balance in the rumen.

Total DMI varied from 20.0 to 23.2 kg/d and did not differ between T2 and T3 diets, but was 1.8 kg DM higher ($P < 0.001$) for T3 than for PR3. There was a quadratic effect ($P < 0.001$) of increased proportion of red clover, with maximum intake for the T3/RC3 diet and higher intakes of T3 than of RC3. Energy corrected milk (ECM) yield was 2.4 kg/d lower ($P < 0.001$) for T2 than T3, and 1.9 kg/d lower for PR3 compared with T3 ($P < 0.001$). There was a quadratic effect ($P = 0.002$) of increased proportion of red clover, with 2.0 and 2.4 kg/d higher ECM in T3 and T3/RC3 than in RC3, respectively. Organic matter digestibility was lower ($P < 0.001$) for T2 compared with T3, but it did not differ between T3 and PR3. Including red clover in the diet linearly ($P = 0.001$) decreased organic matter digestibility.

Methane production (g/d) did not differ between T2 and T3, but CH_4 intensity was 1.2 g/kg ECM greater ($P = 0.003$) for T2 than for T3. There was no difference between T3 and PR3 for CH_4 production but CH_4 yield and CH_4 intensity were 1.3 g/kg DMI and 0.9 g/kg ECM greater ($P \leq 0.05$) for PR3 than T3. Including red clover in the diet linearly increased ($P \leq 0.05$) CH_4 production, CH_4 yield and CH_4 intensity with 19 g/d, 2.7 g/kg DMI and 1.8 g/kg ECM greater amount in the 100% red clover diet (RC3) than in T3.

Despite no differences in feed intake, the higher NDF concentration and lower OMD resulted as expected in lower ECM production and hence a higher CH_4 intensity for T2 compared to T3. Also, as DMI and ECM production was higher in the T3 treatment compared to the PR3 treatment, the CH_4 yield and CH_4 intensity were lower for T3 than for PR3. The positive effect on both DMI and ECM production when including 50% red clover in the diet disappeared when exceeding this level of inclusion. The increased CH_4 yield and CH_4 intensity observed in the RC3 diet was probably related to low digestibility of both NDF and OM, which may have led to unfavourable conditions for rumen fermentation and microbial synthesis, and hence lowered DMI and ECM yield.

Conclusions

This study showed that increasing harvesting frequency for timothy from two to three harvests per season reduced CH_4 intensity in dairy cows. Replacing timothy with perennial ryegrass and increased inclusion rate of red clover both increased CH4 yield and intensity.

Acknowledgements

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3-NOP reduces methane emissions more when used in total mixed ration than in separate feeding

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Introduction

Finland has set a target to reach carbon neutrality in 2035 (Huttunen et al., 2022). To reach this target, it is important to reduce both the fossil fuel as well as agricultural greenhouse gas emissions. In 2021, agriculture contributed 13% of Finland's greenhouse gas emissions of which 36% originated from enteric fermentation. Since cattle produce approximately 90% of Finland's enteric fermentation (Forsell et al., 2023), it is important that every possible solution to reduce methane emissions from cattle is examined. One of the most promising additives to reduce ruminal methane emissions is 3-nitrooxypropanol (3-NOP), which is accepted as safe to use in dairy cow diets (Bampidis et al., 2021). In various studies, 3-NOP was able to reduce dairy cow methane emissions on average by 30% (Kebreab et al., 2023). The aim of the experiment was to examine the efficacy of 3-NOP when supplemented in separate feeding (SEP) as a common practice in Finland versus total mixed ration (TMR) which experiences a growing trend in the country, and to study the diurnal variation of methane emissions with 3-NOP when used under SEP or TMR feeding.

Materials and Methods

A dairy cow experiment was conducted during spring 2023 in Luke's research barn in Jokioinen, Finland.

The experimental design was 4 × 4 Latin square with 2 × 2 factorial arrangement of diets: separate feeding without or with 3-NOP (SEP-O and SEP-NOP) and TMR feeding without or with 3-NOP (TMR-O and TMR-NOP). The experiment was conducted with 4 multiparous Nordic Red dairy cows (days in milk 120 ± 21.8, milk yield 40 ± 4.1 kg/d, body weight 635 ± 37.8 kg), using respiratory chambers. The experiment consisted of four 21-day periods when the first 17 days of each period were used for adaptation and during the last four days (days 18-21), the cows were kept in metabolic chambers for sample collection and gas exchange measurements.

Experimental diets consisted of grass silage and concentrate in the ratio of 65:35 on dry matter (DM) basis. The targeted amount of 3-NOP was 65 mg/kg diet DM and it was added as powder directly into concentrates or TMR mixer. TMR was mixed three times a week. Feed samples were taken twice a week to measure the DM content of silage to adjust the forage to concentrate ratio. Cows were fed four times a day at 7:00, 13:00, 17:00 and 19:00. For SEP-NOP cows, 3-NOP was mixed in the concentrates offered at 07:00 and 19:00. At 13:00 and 17:00, SEP-NOP cows were offered concentrates without 3-NOP. For TMR-NOP cows, 3-NOP was included at the stage of TMR mixing. Milking was done twice a day at 07:00 and 17:00. Feed intake and milk production of the cows were measured every day, but data of the last 4 days was used for calculations.

Rumen samples were taken at the end of each period using a stomach tube after the cows were taken out of the chambers.

Results were analysed with PROC Mixed of SAS® 9.4. (SAS institute Inc. Cary. NC. USA), using period, 3-NOP, feeding method and 3-NOP × feeding method interaction as fixed effects and cow as a random effect in the model.

Results and Discussion

All diets had similar chemical composition. On average, the diets had DM content of 475 g/kg as fed, gross energy of 18.1 MJ/kg DM while organic matter, crude protein, ether extract, NDF, and starch were 927, 153, 38, 431 and 124 g/kg DM, respectively. Dry matter intake was reduced by 3-NOP (on average 5.4%; P=0.05) and the cows fed SEP diets had lower DM intake compared with those fed TMR (on average 5.4%; P=0.03). Feeding method or 3-NOP did not, however, affect milk yield, milk composition, energy corrected milk yield (ECM), and feed efficiency calculated as milk or ECM yield divided by DMI.

3-NOP reduced methane production (g/d) by 24.4% and 10.7%, methane yield (g/kg DMI) by 16.9% and 9.4% and methane intensity (g/kg ECM) by 21.4% and 9.0% by (Figure 1), when used in TMR or SEP diets, respectively ($P < 0.01$ for the interaction of feeding method and 3-NOP).

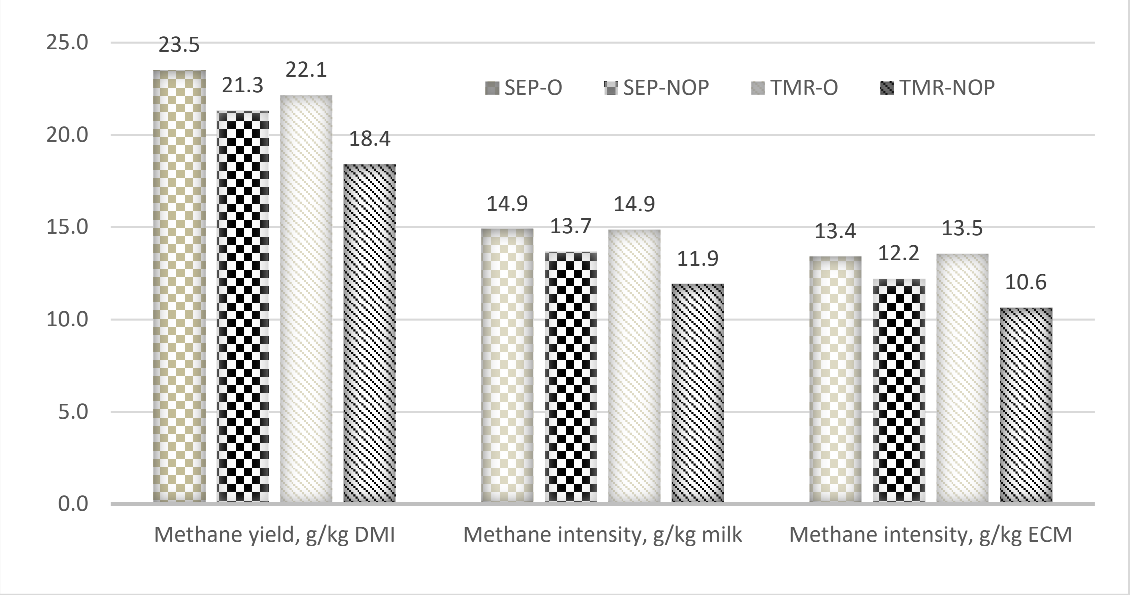

Figure 1. Methane yield (g/kg DMI) and intensity (g/kg milk and g/kg ECM) of dairy cows fed separate or TMR diets supplemented with 0 or 65 mg 3-NOP /kg DM.

3-NOP decreased ($P < 0.01$) ruminal molar ratio of acetate, and increased propionate, butyrate and isobutyrate. Consequently, 3-NOP reduced ($P < 0.01$) ruminal acetate to propionate ratio.

The effect of 3-NOP on the diurnal variation of methane production is presented in Figure 2. There was a noticeable drop in methane production when cows were fed SEP-NOP at 7:00 and 19:00, but the drop was followed by a peak on the methane production shortly after depletion of 3-NOP in the rumen. When cows were fed TMR-NOP, the methane production stayed consistently lower compared to TMR-O throughout the day.

Conclusions

3-NOP was effective on reducing ruminal methane emissions, decreasing methane intensity (ECM) by 21.4% when fed in TMR and 9.0% in separate diet. The greater intensity reduction with TMR diet was in line with previous studies. The reduction with the TMR diet was, however, somewhat lower than observed in previous international studies (21.4 vs 30 % reduction in methane intensity), which may be due to the relatively high NDF content of the grass silage-based diet used in this study. The clear effect of feeding method on the extent of methane mitigation was anticipated, and based on the current results, only 42% of the full potential of 3-NOP in methane intensity reduction can be achieved if twice a day 3-NOP administration compared to TMR feeding is used.

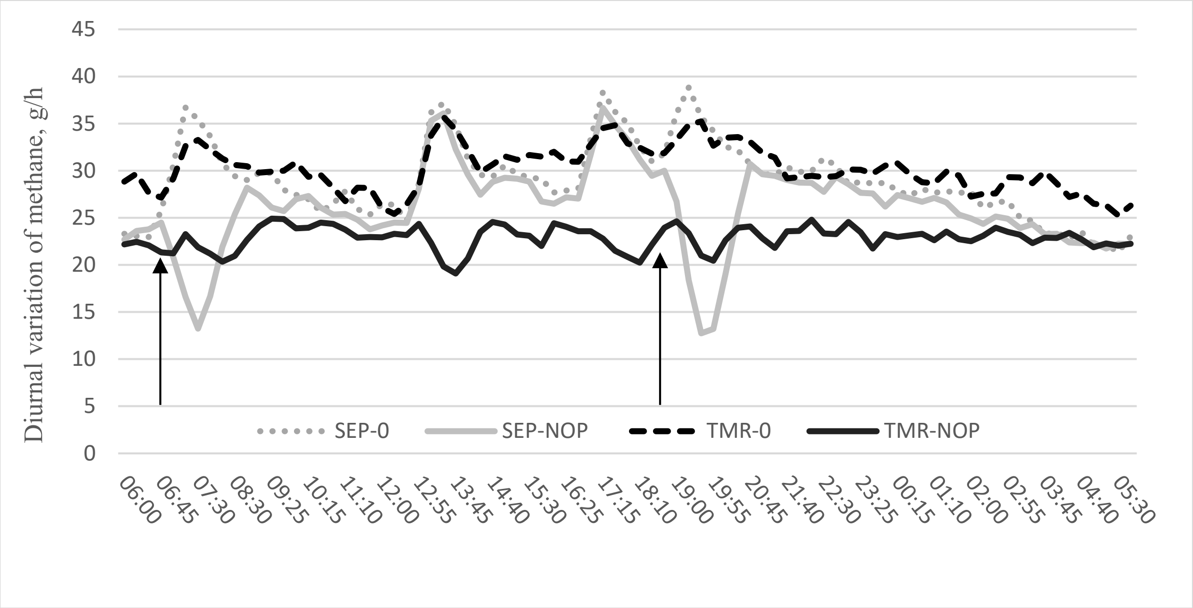

Figure 2. Diurnal variation of methane (g/h) of dairy cows fed separate or TMR diets supplemented with 0 or 65 mg 3-NOP /kg DM. Arrows indicate feeding times, when cows fed with SEP-NOP got concentrate with the supplementation of 3-NOP.

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The effect of pelleted concentrate containing 3-NOP on methane emissions of dairy cows using separate vs. total mixed ration feeding

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Introduction

The need to mitigate methane emissions from livestock has resulted in various nutritional manipulations. One of the most promising feed additives to this date is 3-nitrooxypropanol (3-NOP, commercialized as Bovaer® by DSM), as several international studies have proven its effect to be around 30% when added to the diet of dairy cows (Kebreab et al., 2023). Most trials have used total mixed ration (TMR) or partial mixed ration (PMR) diets due to the fast degradation of 3-NOP in rumen leading to only a short-term methane mitigation when administered with discrete concentrate meals (Vattulainen et al., 2024). In typical TMR-based 3-NOP feeding, the active ingredient is provided in the mineral mixture, which is not suitable for separate feeding of concentrate and forages. In this experiment, the aim was to compare the effect of pelleted 3-NOP concentrate on dairy cow methane emissions when mixed into TMR or offered separately from automated concentrate feeders and from milking robot.

Materials and methods

The experiment was conducted in Luke research barn in Maaninka, Finland, during autumn 2023. The experimental design was a switch-back with four Periods: 1) control Period with TMR, 2) TMR feeding with 3-NOP (TMR+NOP1), 3) separate feeding with 3-NOP (SEP+NOP) and 4) again TMR with 3-NOP (TMR+NOP2). The aim was to compare the effect of 3-NOP when fed in pelleted concentrate either in separate feeding or in TMR. The concentrate pellets were prepared at the feed mill of A-Rehu (Varkaus, Finland) after careful optimization of the pelleting process and temperature to maintain the activity of 3-NOP without compromising feed hygiene. The experiment was conducted with 25 multiparous dairy cows (days in milk 147 ± 79.4 days). Gas exchange was measured with two GreenFeed Systems (C-Lock Inc., Rapid City, South Dakota, USA). Cows had an *ad libitum* access to TMR or silage, which was offered from automated feed bins (BioControl AS, Rakkestad, Norway). Cows were milked by a milking robot on average of 2.3 times a day. Experimental diets consisted of grass silage and a pelleted concentrate feed. During the second, third and fourth experimental period, cows received 3-NOP, which was included into the concentrate prior to pelleting, the targeted amount of active 3-NOP in the diet being 75 mg/kg dry matter (DM). During separate feeding Period 3, the concentrate was administered from concentrate feeders and from milking robots. Cows also received concentrate from GreenFeed systems on average 1.2 kg DM per day. The targeted forage to concentrate ratio was 60:40 on DM basis. On TMR Periods, cows got 25% of their concentrate from TMR and the rest of the concentrates were given from the milking robot and from the GreenFeed systems. The visits of cows to the GreenFeed, milking robot, and during the SEP+NOP also to the concentrate feeders were monitored daily. Milk samples were taken at the end of each Period from four consecutive milkings.

Results were analysed with SAS® 9.4. PROC Mixed- procedure (SAS, 2013), using contrasts. The effect of 3-NOP was calculated comparing Periods 1 (TMR) and 2

(TMR+NOP1). The effect of lactation stage and 3-NOP were confounded in the model between two first Periods. The effect of feeding methods (TMR vs separate feeding) was calculated comparing Periods 2 and 4 (TMR+NOP) to Period 3 (SEP+NOP).

Results and discussion

The cows visited the GreenFeeds on average 2.9 times a day during the experiment (±1.03). During SEP+NOP, cows received the concentrate from automatic feeders or from the milking robot on average of 4.3 times a day (±1.05). The targeted amount of 3-NOP in the diet was reached during the TMR+NOP Periods, but during the SEP+NOP Period, the amount was slightly lower (Table 1). This was due to the unexpected increase in the silage intake by the cows, which decreased the proportion of concentrate in the diet. There were some palatability issues of the 3-NOP containing concentrate that may be unrelated to the active ingredient itself, which affected the DMI during TMR+NOP Periods. 3-NOP decreased TMR intake, when the control Period (TMR) and first treatment Period (TMR+NOP1) were compared ($P<0.01$).

Table 1 Intake of silage, concentrate, total dry matter (DM) and 3-NOP, and milk and energy corrected milk (ECM) yield and milk composition of dairy cows fed separate or TMR diets with supplementation of 0 or 75 mg/kg DM of 3-NOP

	Period (Treatment)					Statistical significance	
	TMR	TMR+NOP1	Sep+NOP	TMR+NOP2	SEM	TMR - TMR+NOP1	SEP+NOP – TMR+NOP
Silage intake, kg DM/d	13.8	12.7	15.3	13.4	0.39	<0.01	<0.001
Concentrate intake, kg DM/d	8.96	8.64	8.69	8.53	0.21	<0.01	0.24
Total feed intake, kg DM/d	22.7	21.4	24.0	21.9	0.57	<0.001	<0.001
3- NOP, g/d	0	1.62	1.56	1.67	0.04	<0.001	0.02
3- NOP, mg/kg DM	0	76	65	76	0.50	<0.001	<0.001
Milk, kg/d	31.0	29.5	29.2	28.6	1.5	<0.001	0.60
ECM, kg/d	34.1	33.2	33.6	33.3	1.26	0.02	0.29
Milk fat, g/kg	48.2	48.7	50.3	51.4	0.12	0.40	0.61
Milk protein, g/kg	36.5	38.9	40.0	39.9	0.07	<.0001	0.00
Milk urea, mg/dl	10.6	12.1	10.7	8.98	0.71	<0.01	0.77

Milk yield (kg/d) decreased when TMR+NOP1 was compared to TMR ($P <0.001$), but not when SEP+NOP was compared to TMR+NOP ($P=0.60$), as shown in Table 1. Energy corrected milk yield (ECM, kg/d) decreased slightly with TMR+NOP1 treatment compared to TMR ($P=0.02$), but there was no effect when TMR+NOP and SEP+NOP were compared ($P=0.29$). 3-NOP reduced methane production by 22.4% (g/d), yield by 17.5% (g/kg DMI), and intensity by 19.2% (g/kg milk) and 20.2% (g/kg ECM), when TMR+NOP1 (Period 2) was compared to TMR ($P <0.001$). When SEP+NOP and TMR+NOP (Period 3 vs 2 and 4) were compared, TMR+NOP reduced methane production by 15.6% (g/d), and intensity by 14.9% (g/kg milk) and by 14.3% (g/kg ECM) ($P<0.001$). The results were similar to Vattulainen et al. (2024), TMR+NOP having greater mitigation potential than SEP+NOP, but the difference was slightly smaller in this experiment probably due to greater number of 3-NOP containing meals per day. The reduction of methane (g/d and g/kg DM) was greater during TMR+NOP1 than TMR+NOP2 (Figure 1). The reduction of daily methane production was 18.4% (g/d), yield 14.3% (g/kg DMI), and intensity 12.0% (g/kg milk) and 16.1% (g/kg ECM), when TMR and TMR+NOP2 were compared.

The 3-NOP in pelleted concentrate was effective in decreasing ruminal methane production as indicated by the 20.2% methane intensity (ECM) decline when the TMR diet without and with 3-NOP was compared (Period 1 vs. Period 2). This provides opportunities to farms without TMR system to use 3-NOP as well, as the efficacy of 3-NOP can be maintained in the industrial production of pelleted concentrate. Still, 3-NOP was more effective when added on the TMR than in separate diet, mostly because 3-NOP is quickly degraded in the rumen, and the number of meals per day is less than in TMR feeding and, thus, the full potential of 3-NOP in methane mitigation is not achieved. Under the current conditions, 3-NOP with separate feeding reduced methane intensity only 6.43% (g/kg ECM) (Period 1 vs Period 3). During Period 3, cows had more concentrate feeding times than in Vattulainen et al. (2024) (4.4 vs 2), so the reduction was expected to be greater. The methane intensity reduction on TMR feeding was within the same range as in Vattulainen et al. (2024), i.e., somewhat lower than the generally observed 30% in response to 3-NOP administration (Kebreab et al. 2023). The reason of the lower efficacy may be the high fibre content of the grass silage based diets used in the Finnish studies.

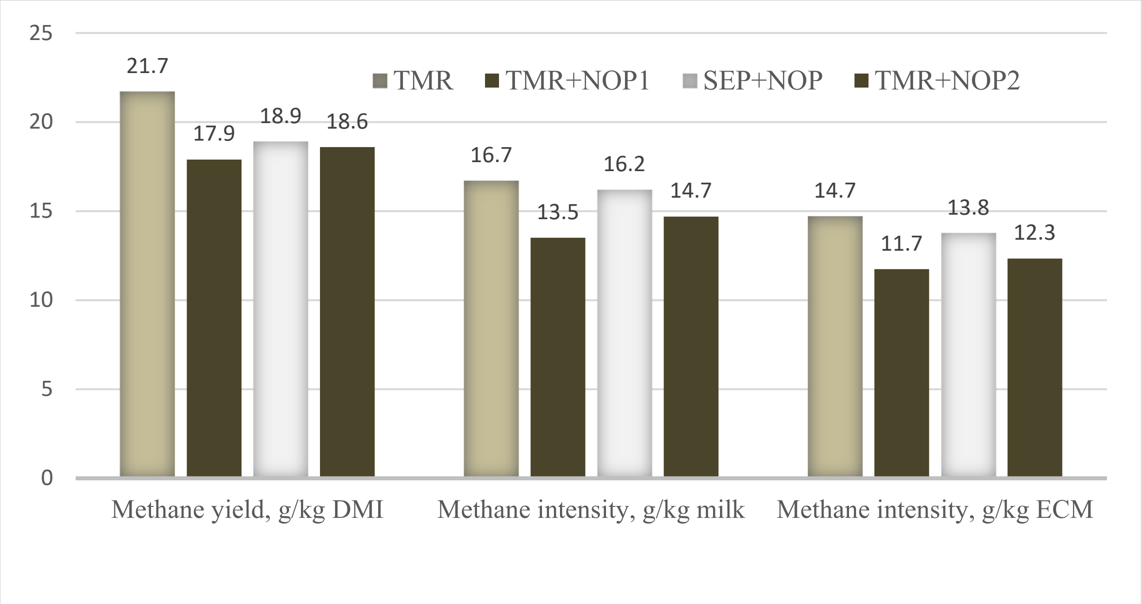

Figure 1 Methane yield (g/kg DMI) and intensity (g/kg milk and g/kg energy corrected milk, ECM) or dairy cows fed total mixed ration (TMR) or separate (SEP) diets, supplemented with 0 or 75mg 3-NOP /kg DM.

Conclusions

The pelleted concentrate was effective in reducing methane emissions when added into the diet of dairy cows showing that the activity of 3-NOP can be maintained in the industrial production of pelleted concentrate. The methane reduction potential was greater with TMR+NOP treatment than with SEP+NOP treatment due to more constant administration of the active ingredient.

Acknowledgements

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Enteric methane emissions from Norwegian Red dairy cows fed compound feeds differing in the level of local ingredients

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Introduction

Cereal grains are desired energy sources in Norwegian dairy cow diets in combination with grass silage. However, a lower protein content of typical Norwegian grains and varying grass silage quality necessitate inclusion of protein ingredients to cover protein requirements of high-yielding cows. A large part of these protein ingredients is often imported. Secondly, the high starch content of cereals challenges the rumen environment, especially at high inclusion levels, for dairy cows with consequences on rumen health and animal performance.

Ammoniation of cereal grains using modern alkalization methods is expected to increase the utilizable protein level in the diet through addition of non-protein nitrogen (NPN). Furthermore, alkalization is presumed to modulate rumen pH as an effect of increased buffering capacity, consequently improving fiber digestibility. Altered rumen fermentation pattern due to fermented substrate source and extent of fiber degradation affects rumen fermentation products including enteric methane (CH_4). As such, CH_4 emissions are highly dependent on diet composition. Here we evaluated CH_4 emissions from Norwegian Red dairy cows (NRF) fed soya-based *vs.* alkaline barley-based concentrates. Secondly, we assessed possible interactive effects of soya-based and alkaline barley-based concentrates with grass silages differing in organic matter digestibility (OMD).

Materials and Methods

Enteric CH_4 emissions were measured from NRF dairy cows fed compound feeds differing in level of local ingredients in two separate experiments. The first experiment (Exp.1) evaluated effects of two slow-releasing ammonia (Home n' Dry, Dugdale Nutrition Ltd, Lancashire, England) treated concentrates. Both contained a higher proportion local ingredients but differed in pre-pelleting particle size (*i.e.*, Alka mjolk-fine = Alka-F, and Alka mjolk-coarse = Alka-C) compared against a negative control prepared using the same ingredients as for the two formerly described diets, but combined with urea as an NPN-source (Urea-F) and a positive control, based on soya meal (Soya-F).

The experiment involved eight multiparous early lactation NRF dairy cows in a 4×4 duplicated Latin square design experiment, including the four previously described diets tested in four periods of 35 days (9 d adaptation and 26 d data recording period). The cows were kept in tie-stalls with *ad libitum* access to grass silage and water. Concentrate allowance was optimized with Soya-F for the first period using TINE Optifôr (NorFor) based on individual cow requirement. Concentrate allowance was reduced by 10% for each of the following periods considering the progression of lactation. The remaining concentrates replaced Soya-F on a weight by weight (w/w) basis.

Data was recorded daily for feed intake and milk yield. Feed and milk samples were taken weekly. Energy corrected milk yield (ECM) was calculated based on milk composition according to Sjaunja et al. (1991). Methane emissions were measured over 120 h in 24-h cycles using the sulfur hexafluoride (SF_6) tracer technique, as described by Johnson et al. (1994). Gas samples were analyzed for CH_4 by gas chromatography (GC, Model 7890A Agilen, Santa Clara, CA, USA).

For the second experiment (Exp.2) Soya-F and Alka-F were, based on overall performance, selected from Exp.1 to be assessed for interactive effects with grass silage differing in OMD levels. Forty early lactation NRF cows of mixed parity, housed in a free stall system with free access to grass silage and water, were blocked by parity and initial milk yield into one of four groups. The study lasted 63 days (21 d adaptation and 42 d of measurements) and followed a 2×2 factorial design with the two selected concentrate types tested against two qualities of grass silage differing in estimated OMD (early cut = High-OMD [75.5%], late cut = Low-OMD [70.2%]). Concentrate allowance was optimized based on individual requirements according to the allocated grass silage quality combined with Soya-F in similar fashion as in Exp.1. Alka-F replaced Soya-F on w/w basis. Feed intake and milk yield were recorded daily with weekly milk and feed samples. Energy corrected milk yield was calculated as for Exp.1. Methane data were collected daily in two GreenFeed units (C-Lock Inc., Rapid City, SD, USA).

Data from both experiments were subjected to statistical analysis using a linear mixed model in R (Version 4.2). For Exp.1 the model included treatment, period, day within period, parity, and days in milk (DIM) as fixed effects with individual cow as random factor. For Exp.2, all data were averaged per week, and treatment, measurement week, parity and DIM were included as fixed effects in addition to covariate data, when available. Individual cows were included as random effects. For all models, covariance structure was selected based on Bayesian information criterion.

Results and Discussion

In Exp.1, both dry matter intake (DMI) and ECM (Table 1) tended to differ between the treatments ($0.05 \leq P \leq 0.1$). For enteric CH_4 (g/d), cows in the Alka-C diet produced the lowest, while those fed Urea-F produced the highest ($P < 0.01$). Methane yield (g CH_4/kg DMI) was lower ($P = 0.04$) for Alka-C compared with Soya-F and Urea-F, whereas Alka-F was intermediate. Finally, CH_4 intensity (g CH_4/kg ECM) followed a similar pattern to daily CH_4 production. Notably, a difference ($P < 0.001$) was found between Alka-C and Urea-F with Soya-F and Alka-F serving as intermediates. The somewhat lower CH_4 production of Alka-C compared to Urea-F is believed to be a result of a lower starch digestibility (~2%, unpublished data) compared to the fine-structure diets, which again resulted in a lower CH_4 yield compared to Soya-F due to the similar DMI. Furthermore, the difference in CH_4 intensity between Alka-C and Urea-F was likely a result of a lower feed-to-milk conversion from the Urea-F diet.

In Exp.2, no differences were seen between concentrate types, consistent with the results of Exp.1 (Table 2). However, silage quality did impact all CH_4 parameters, with the High-OMD grass silage consistently resulting in lower CH_4 production, yield and intensity ($P < 0.01$). These findings align with existing knowledge where fiber content is positively correlated with enteric CH_4 emissions (Storlien *et al*., 2014), explained by a greater acetate and lower propionate production due to higher content of complex fibers and lower content of readily fermented carbohydrates (Janssen, 2010).

Table 1 Dry matter intake (DMI), energy corrected milk yield (ECM), and enteric CH4 emission by NRF dairy cows fed either soya-, alka-, or urea-based concentrates.

	Treatment					
	Soya-F	Alka-C	Alka-F	Urea-F	SE	P-Value
DMI, kg/d	24.4	24.6	25.1	25.3	0.44	0.09
ECM, kg/d	33.7	34.3	34.3	32.5	0.93	0.05
CH_4, g/d	631^{ab}	577^{b}	631^{ab}	664^{a}	20.4	<0.01
CH_4:DMI, g/kg	26.2^{a}	23.4^{b}	25.4^{ab}	26.2^{a}	0.99	0.04
CH_4:ECM, g/kg	19.5^{ab}	17.1^{b}	18.9^{ab}	21.2^{a}	1.04	<0.001

Different superscripts within a row indicate significance at $P \leq 0.05$.

Table 2 Dry matter intake (DMI), energy corrected milk yield (ECM) and enteric CH4 emissions as g/d, g/kg DMI, and g/kg ECM by NRF dairy cows fed soya- or alka-based concentrates silage of varying quality

	Concentrate			Silage			P-value		
	Soya-F	Alka-F	SE	High-OMD	LowOMD	SE	Con	Sil	Con×Sil
DMI, kg/d	20.2	19.8	0.36	19.8	20.1	0.36	ns	ns	ns
ECM, kg/d	29.6	29.6	0.35	29.8	29.3	0.37	ns	ns	ns
CH_4, g/d	460	457	10.7	432	486	11.0	ns	<0.01	ns
CH_4:DMI, g/kg	23.2	23.3	0.44	22.1	24.3	0.45	ns	<0.001	ns
CH_4:ECM, g/kg	16.1	15.4	0.58	14.4	17.0	0.59	ns	<0.01	ns

Con = Concentrate; Sil = Silage; Con×Sil = Concentrate×Silage; ns = not significant ($P > 0.05$).

Conclusions

When comparing ammoniated barley-based diets to soya-based diets with similar pre-pelleting structure, no differences were observed on enteric CH_4 emissions, regardless of silage quality. However, the results indicated the feasibility of reducing enteric CH_4 emissions through modulating growth stage at harvest of grass for silage.

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Methane emissions in Icelandic dairy herds. Improved prediction of methane by utilizing available farm management data

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Introduction

In ruminants enteric CH_4 is a side-product from rumen microbial fermentation of feed to volatile fatty acids (VFAs). Ruminal fermentation processes generate an excess of hydrogen that is reduced in the rumen by methanogens with reduction of CO_2 to CH_4. Many factors affect the amount of enteric CH_4 produced. Among them are dry matter intake, diet composition, rumen microbial population, and animal physiology and genetics (Shibata & Terada, 2010). Until now, enteric CH_4 production of Icelandic dairy cows has not been directly measured. It has been assessed by the Environment Agency of Iceland (Umhverfisstofnun) with a generic method for estimating enteric CH_4 from cattle in the the National Inventory Report to the United Nations Framework Convention on Climate Change (UNFCCC) and Kyoto Protocol/Paris Agreement. The method involves calculating Gross Energy (GE) of annual feed intake values (Keller et al., 2023). The GE intake is then multiplied by a methane conversion rate (Y_m) of 6.5 % indicating that 6.5% of GE in the feed is converted to CH_4.

Due to conversion of all feed values to GE and using a fixed CH_4 conversion rate, the method does not take into account how a difference in feed composition could affect enteric CH_4 emissions. Models for enteric CH_4 emission based on feed intake and feed composition have been developed to predict CH_4 production more accurately. Nielsen *et al.* (2013) published a basic model for the prediction of enteric CH_4 emission from dairy cows based on 12 studies carried out in Norway, Sweden and Denmark. This equation is used in the Nordic Feed Evaluation System -NorFor (Norfor, 2022). In Iceland, facilities for feeding experiments are limited. However, information about milk yield, and daily concentrate feeding are easily available in herd management systems and can be used to predict daily intake of roughage.

Materials and Methods

Three Icelandic dairy farms collaborated in the study that took place from September 2022-June 2023. On all three farms, cows of the Icelandic breed were housed in free stall barns with one milking robot. The herd size ranged from 50 to 70 lactating cows. Roughage/PMR were offered *ad lib* on a feed alley and concentrates were rationed in milking robots and additional concentrate feeders based on milk yield of individual cows. One GreenFeed unit (C-Lock Inc., Rapid City, SD) was placed in the dairy barn according to manufacturer's instructions. All cows in the herd had access to the unit during an adaptation time that was a minimum of 2 weeks.

After the adaptation time, approximately 40 lactating cows with good attendance to the unit were selected for a 3-week data collection period. The aim of the selection was that this group represented the herd lactation's number, milk yield and days after calving. Samples were taken from roughages on each farm, representing daily feed offered *ad lib*. Information of nutrient composition of purchased feed products in PMR and concentrate blends was found in Tine Optifor feed table. Apart from CO_2 and CH_4 measurements, all data was collected from the herd management systems on the farms: age, parity, days in milk, daily milk yield (kg/day) and information about concentrates fed (kg/day). On Farm 1, body weight and milk composition during the data collection period was also collected. Available individual cow data was averaged representing an average day during the data collection period. Seven cows, 1-3 per farm, with fewer than 14 visits to the GreenFeed unit were excluded from the dataset. The removal of these cows did not have any considerable effect on how representative the cows in the final dataset were for the farms. The data set included CH_4 and CO_2 production (g/day), amount of different concentrates mixtures fed (kg/day), days from parturition, parity and milk yield (kg/day).

Table 1 Number of dairy cows included in the database and their mean values of days in milk, feed intake, milk yield, feed intake, CH4 emissions and yield

	Farm 1	**Farm 2**	**Farm 3**
Number of animals	39	36	36
Days in milk	189	161	201
Milk yield, kg/day	19.1	22.3	25.3
Roughage/PMR (predicted), kg DM per animal/day	7.9	9.5	9.2
Concentrate, kg DM per animal/day	6.8	6.9	6.9
Observed CH_4 production, g/day	332	371	354
CH_4 yield (Y_m), % of GEI	6.9%	6.9%	6.6%

Voluntary intake of feed offered *ad lib* was estimated on an individual level by the prediction equations of NorFor (Volden *et al.*, 2011). The estimations were based on information in the database about lactation number, estimated live weight, milk yield & days in milk. Where live weight was missing, it was estimated 465, 495 and 525 kg for first, second and ≥ third parity cows, respectively. Where milk composition was missing, it was estimated 4.00 % fat, 3.40 % protein and 4.53 % lactose. Information about concentrates fed and estimated voluntary feed intake was used to calculate chemical composition of the diet dry matter (DM), crude fat, fatty acids (FAs), neutral detergent fibre (NDF), starch (ST), gross energy (GE) and DMI. Then, prediction models for enteric methane production developed in Nordic countries were found that fitted input variables in the database. The ability of the chosen models to predict CH_4 production was compared, using mean squared prediction error (MSPE) and concordance correlation coefficient (CCC) (Tedeschi, 2006). CH_4 measurements of the GreenFeed unit were expressed as grams per day. The following factor was used in converting prediction models CH_4 to g/d: 1 g CH4 = 0.0565 MJ.

Results and Discussion

Of the models accessed, the 5 best performing models, judged by CCC with addition of the model by (Keller *et al.*, 2023) are in Table 2. Judging from RMSPE and CCC, the model from Storlien *et al.* (2014) with only DMI (kg/d), and FAs (g/kg DM) as input variables predicted enteric CH_4 emissions most accurately of the models accessed judged by CCC (0.535), and RMSPE (13.4%). The model of Nielsen *et al.* (2013) with DMI (kg/d), NDF (g/kg DM) and FAs (g/kg DM) followed closely according to CCC (0.529), and RMSPE (13.8%). Both models predict enteric CH_4 emissions considerably better than the Keller *et al.* (2023) model with by CCC (0.474), and RMSPE (16.9%).

Table 2 Evaluation of prediction models ordered by decreasing CCC

Source	n	Prediction Equation	RMSPE, %	CCC
(Storlien et al, 2014)	111	CH_4 = (6.8+1.09*DMI-0.15*FAs)	13.4%	0.535
(Nielsen et al., 2013)	111	CH_4 = (1.23*DMI-0.145*FAs+0.012*NDF)	13.8%	0.529
(Niu et al., 2021) Model 1	111	CH_4 = (4.92+1.13*DMI-0.118*FAs)	14.4%	0.516
(Niu et al., 2021) Model 2	111	CH_4 = (-3.01+1.19*DMI-0.103*FAs+0.017*NDF)	14.3%	0.495
(Niu et al., 2021) Model 3	111	CH_4 = (1.13*DMI-0.114*FAs+0.012*NDF)	14.2%	0.493
(Keller et al., 2023)	111	CH_4 = (GE*(0.065))	16.9%	0.474

n, number of treatment means; CH_4, methane (MJ/day); DMI, dry matter intake (kg/day); FAs, fatty acid content (g/kg DM); NDF, neutral detergent fiber content (g/kg DM) ; $RMSPE$, root mean squared prediction error expressed as a percentage of the observed mean; CCC, concordance correlation coefficient.

Conclusions

The Nielsen *et al.* (2013) model is the one used in the NorFor feed evaluation system to predict enteric methane emissions. The NorFor feed evaluation system (Volden, 2011) is well established in Iceland and could be used to predict enteric methane emissions from dairy cows on farm level with good accuracy.

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Climate adaptation of dairy farms in northern climate: a Canadian perspective

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Context

Some summers are more difficult than others in agriculture. Medium- and long-term conclusions cannot be drawn from a single year, but the grouping of a significant period of time, thirty years for example, allows to better understand the evolution of the climate. Thus, it is this long-term view, over a hundred years, which allows climatologists to affirm that there is currently climate change. Climate projections for 2020-2080 predict an increase in winter temperatures of 1.0 to 8.2°C, and in summer temperatures of 1.0 to 7.2°C. The precipitation in winter should remain stable or increase by 36% and variable changes are expected in summer precipitation in Quebec, Canada (Ouranos, 2015). While this evolution will have mostly negative repercussions on agricultural performance on a global scale, in northern climates, it will be positive in some cases. Indeed, an increase in the length of the growing season and temperatures is expected, allowing yield increase for crops such as corn, soybeans or certain forage species (Charbonneau *et al*., 2013). On the other hand, certain diseases or insects may become more prevalent (Gagnon *et al*., 2013). Precipitation may not be distributed as well as today during summer and more intense weather events are anticipated (*e.g.,* storms and brief periods of heavy rainfall; Ouranos, 2015). Currently, for perennial forages, alfalfa and timothy are the dominant species used across Canada, but other species could also be advantageous under future climatic conditions. Winter survival of certain perennial species, such as alfalfa, will be more difficult due to an increase in winter thaws combined with a decrease in snow cover (Quian *et al*., 2024) and timothy summer regrowth is also projected to decrease. Hence, it is imperative to address the challenges associated with climate change, not only to anticipate impacts but also to enhance farmer's capacity for effective adaptation strategies. With Canada's supply management system for milk, stability in milk production is a priority for Canadian dairy producers. Consequently, alterations in both the quality and quantity of forage have a substantial impact on diet formulation, as concentrate utilization is adjusted to sustain production levels based on available forages. The objective of this presentation is to better understand the impact of climate change on dairy farms in northern regions, using results from Canada, and to explore adaptation strategies. Although mostly forage utilization will be discussed, a whole-farm perspective will be used.

Projection of Climate Indices

Six global climate models (GCM) and three socioeconomic pathways of atmospheric CO_2 concentration (ssp126, ssp370 and ssp585) were used to project different possible climatic conditions of 4 distinct Canadian regions for the 1981-2100 period. These climatic projections were grouped following similitude of their effect on climate change in 3 different periods. Projections at the level of agroclimatic index, calculated from projected climatic conditions, show a probable impact of climate change on future performance of plants grown on dairy farms (Table 1). In the future, the increase in growing degree-days (GDD) may lead to favourable conditions in certain regions that currently cannot support growth of grain corn and soybeans. It has already been observed as corn and soybeans are currently cultivated in regions where it was not feasible twenty years ago.

In case of perennial forage crops, knowing that an accumulation of 500 to 700 GDD is necessary between two cuts (Bélanger *et al*., 2016), the increase in the accumulation of GDD during the growing season could allow 1 or 2 additional cuts according to certain climate simulations (Jing *et al*., 2014; Thivierge *et al*., 2016). Projected temperature increases are likely to facilitate additional cutting in future growing seasons, resulting in increased yields. Of course, forage species differ in their resistance to heat and drought during summer. Our team has conducted several studies exploring this variability (Thivierge *et al*., 2016, Payant *et al*., 2021, Thivierge *et al*., 2023). Furthermore, this increase in temperature can also lead to changes in forage quality and composition.

Table 1 Average climatic conditions and agro-climatic indexes under different global climatic models for 4 distinct Canadian regions

Period[a]	Cluster	Mean Temp (°C)	Growth season (day)	GDD[b]	Precipitation growth season (mm)	Mean Temp (°C)	Growth season (day)	GDD	Precipitation growth season (mm)
		Halifax (NS)				Québec (QC)			
Reference	Reference	6.9	126	1486	449	4.1	114	1374	481
Near future	Moderate	9.2	149	1974	523	6.9	139	1879	585
Near future	Intermediate	11.6	175	2574	740	10.2	164	2586	703
Distant future	Moderate	8.6	142	1827	513	6.0	132	1699	561
Distant future	Intermediate	11.4	171	2476	603	9.0	156	2279	654
Distant future	Intense	15.1	211	3538	891	13.2	191	3357	786
		Ottawa (ON)				Red Deer (AB)			
Reference	Reference	6.6	139	1878	416	3.9	106	1122	256
Near future	Moderate	9.0	158	2361	471	5.8	126	1491	276
Near future	Intermediate	11.2	177	2870	520	7.0	144	1843	333
Distant future	Moderate	9.3	159	2396	476	7.1	137	1806	256
Distant future	Intermediate	11.6	178	2921	555	7.4	144	1903	240
Distant future	Intense	15.3	208	3964	607	12.4	190	3128	343

a: Reference = 1981-2015, Near future =2040-2069, Distant future= 2070-2099; b=Growing degree day base 5.

Impact on Timothy, Alfalfa and Other Forage Mixtures

A few important factors influence projected performance when assessing impact of climate change on forage plants. First, a concentration of atmospheric CO_2 is expected to continue to increase. In addition, longer growing season will be accompanied by an increase in temperature (Bertrand *et al*., 2008; Piva *et al*., 2013; Jing *et al*., 2014). Studies have shown that these conditions would have negligible to negative impacts on the growth of timothy, whereas they would significantly benefit alfalfa (Bertrand *et al*., 2007; Messerli *et al*., 2015; Thivierge *et al*., 2016). Thus, since these two species are often grown in a mixture, survival of timothy could be compromised, not because of a reduction in its productivity, but rather because of the improvement in productivity of other forage species. However, a possibly less abundant and more variable snow cover in the future climate could counterbalance the positive effects of temperature and CO_2 on alfalfa by increasing the risk of winter damage (Qian *et al*., 2024). Without adaptation and maintaining current cutting management, simulations showed that climate change would have little impact on annual yield of timothy and could increase alfalfa yields but their nutritional value would be reduced (Jing *et al*., 2014; Thivierge *et al*., 2016). However, by adjusting cutting management based on cumulative GDD between cut (450 GDD) annual yields are expected to increase, while quality losses (reduced protein, increased NDF and decreased digestibility) can be alleviated and possibly mitigated (Payant *et al*., 20121, Thivierge *et al*.,2016, Binggeli *et al*., unpublished). Overall, all future predictions show an increase in alfalfa proportion relative to timothy in mixtures. This modification in forage proportion of the mixture also contributes to maintaining a good nutritive value of the mixture, but can have an impact on diet formulation in some cases .On the other hand, in very intense climate change with increase in length and frequency of periods without rain, these yield gains are reduced, as water stress and even heat stress also appeares on alfalfa (Thivierge *et al*., 2016, Binggeli *et al*., unpublished).

Adaptations for Perennial Forages

Several strategies are being considered to help maintain forage survival, quality and yield in a changing climate. First and foremost, with the faster accumulation of growing degree days, it would be important to adjust the seeding and cutting dates accordingly (Thivierge *et al*., 2023). Seeding is projected to occur earlier, if soil moisture allows field work as spring is the season of snow melting and typically undergo frequent rainfall events. The interval between forage harvests is projected to be shorter, allowing a greater number of harvests per year. These adjustments will contribute to maintaining forage quality and increase forage yield (Thivierge *et al*., 2016, 2023). With increases in temperatures and drought period duration and frequency, summer regrowth of timothy could be limited in the future. To counter this, using other grass species in mixture with timothy is recommended (Thivierge *et al*., 2023). Grasses other than timothy were compared under historical and future climate in recent studies. Under historical climate, several alfalfa-based binary forage mixtures have been tested on three sites for three years (Pomerleau-Lacasse *et al*., 2019). Alfalfa-meadow fescue, alfalfa-tall fescue and alfalfa-meadow bromegrass mixtures represent possible alternatives to the alfalfa-timothy mixture since they had comparable seasonal yields, their persistence was good during the first three years of production. The estimated milk production per hectare associated with these mixtures was also similar to that of the alfalfa-timothy mixture. Alfalfa-based mixtures including the tested cultivars of festulolium and perennial ryegrass do not appear to be interesting alternatives to timothy in Quebec, mainly because of poor winter survival (Pomerleau-Lacasse *et al*., 2019). Under projected future conditions, the alfalfa-tall fescue mixture is expected to lead to the largest yield increase, when compared to other alfalfa-grass mixtures (Payant *et al*. 2021).

However, some dairy producers are reluctant to use tall fescue in their ration for fear of a reduced palatability. In addition to high yield, the main advantages of tall fescue over timothy are the higher optimal growth temperature, and deeper root system, helping for summer regrowth and drought survival (Thivierge *et al*., 2023). Tall fescue, however, can indirectly decrease the nutritive value of mixtures (higher NDF content, lower CP content, and lower energy content), compared with other alfalfa-grass mixtures (Pomerleau-Lacasse *et al*., 2019), since its competitiveness and resistance can lead to lower relative content of alfalfa. The potential decrease in nutritive value may necessitate significant adjustments to the diet. For example, lower energy and protein content of forages would require additional concentrate to fulfill cow requirements (Ouellet *et al*., 2021), leading to increased costs. An animal experiment was carried out to test use of timothy and tall fescue in a mixture with alfalfa or as pure species (Richard *et al*., 2019). This experiment showed that cows fed timothy or tall fescue had similar dry matter intake (23.5 kg of DM) and milk yield (MY; 27.5 kg). The use of mixture (MY, 28.3 kg) versus pure species (MY, 26.6 kg) rather affected results for these parameters. It was concluded that, with optimal harvesting practices and maintaining the same concentrate level in diet formulation, timothy and tall fescue could result in comparable cow performance (Richard *et al*., 2019; Ouellet *et al*., 2021)

We were also interested in the possibility of improving summer regrowth of timothy grass through genetic selection. It was interesting to see that there is some variability in regrowth performance between timothy populations around the world, so genetic selection could be considered in order to develop cultivars with high recovery (Claessens *et al*., 2019). Two management strategies were also identified to enhance winter survival of alfalfa, avoiding fall harvest and raising cutting height (Thivierge *et al*., 2023). These two strategies offer benefits by allowing the plant to accumulate more carbohydrates and nitrogen reserves, aiding its spring regrowth.

Thermal Stress on Animals

Climate change will also affect cows' welfare. Heat stress can have detrimental effects on dairy cows, including decreased milk production, impaired reproductive performance, and compromised health and welfare. The animal's level of thermal stress is often assessed by calculating a bioclimatic index combining the effect of temperature and humidity: the temperature-humidity index (THI). In the literature, several THI thresholds ranging from 58 to 72 are associated with the appearance of negative impacts linked to thermal stress (Ravagnolo *et al*., 2002; Lambertz *et al*., 2014; Campos *et al*., 2022). These thresholds vary according to the studied response and other factors related to thermotolerance of the animals, including their productivity and the climate in which they are raised.

Results of our recent projects have demonstrated that thermal stress is already a problem associated with the current climatic region of Canada with significant decreases in milk fat and protein yields observed under elevated THI (Ouellet *et al*., 2019). Using a THI threshold of 62, there were, on average, 106 days susceptible to causing heat stress in the agricultural regions of the province of Quebec for the years 2010-2019 (Campos *et al*., 2022). Furthermore, considering increases in temperatures and heatwave episodes associated with climate change, this problem could be exacerbated in the future with an increase in incidence and intensity of episodes of heat stress in Quebec and Canadian herds (Ouellet *et al*., 2020). With the projected climatic conditions mentioned above, we calculated an increase in average in-barn THI from 2 to 6 points. Our calculations also showed that the number of days with THI above 65 could increase by more than 30 days in the near future and by more than 50 days in the distant future in the four Canadian regions studied (VanderZaag *et al*. 2023, Binggeli *et al*., unpublished). These results vary depending on the greenhouse gas scenarios and climate simulations used. These THI increases in intensity and frequency could lead to a reduction in yearly fat and protein production between 0.3 and 5.2 kg per cow, depending on the intensity and region, compared to actual production levels, if no mitigation measures are implemented (Campos *et al*., 2022). Milk components are driving the on-farm milk price and, as such, these reductions will have a direct impact on farm net income. To alleviate these climatic effects on the animals, different cooling strategies were explored: water-based methods (misting and sprinkler) and recirculation methods (complementary fan). Water-based methods are shown to be ineffective in the eastern regions of Canada, where relative humidity is already high, but more effective in drier central regions, due to the increase in in-barn humidity (Fournel *et al*., 2017). However, these drier regions have less water reserves and are at risk of water shortages, which are increased with climate change. As such, recirculation method aiming at increasing airspeed at the animal level should be prioritized.

Economic and Environmental Impacts at the Whole-Farm level

As mentioned, with climate change, perennial forage yields tend to increase. If so, less land is needed to grow forage required for the herd (Payant *et al*., unpublished; Binggeli *et al*. unpublished). Climate change also allows an increase yield of corn silage (Thivierge *et al*., 2017; Payant *et al*., 2021), a crop increasingly used on dairy farms (Statistic Canada, 2023) for its benefits associated with management simplicity and consistent high yields and energy content.

With the aforementioned perennial forage yield increase and the production of corn silage, it is a possibility for dairy farms to allocate more land surface to cash crops, such as cereal, corn grain, soybeans and colza (Thivierge *et al*., 2017; Payant *et al*., unpubl.; Binggeli *et al*. unpubl.). Our whole-farm simulation showed that it would lead to an increase in net income (10 to 130%), associated with both a reduction of concentrate feed purchased and an increase in crop sold depending on the region and the total land area available on the farm. However, in more intense climate change scenarios, as yields of all crops are lower compared to the more moderate climate change scenarios, benefits would be lower. The amplitudes of the results are also lower in simulations with a more moderate climate change for the warmest and driest regions. At farm level, these changes would also lead to a 1 to 6% increase in total GHG emitted, although most of this increase is allocated to crops sold, as GHG allocated to milk is expected to be reduced by 1 to 9% (Payant *et al*., unpubl.; Binggeli *et al*., unpubl.).

Additionally, when using other grasses in replacement for timothy, only small differences are predicted (Ouellet *et al*., 2021, Payant *et al*., unpublished). Tall fescue, having the highest yields and better resistance to climate change, tends to have a slight advantage on net income in most regions and climatic projection. The differences in GHG emissions are also similar within a region of the same period, for both total and milk-allocated emissions. But overall, choice of species in binary combination with alfalfa should be established depending on region and quality of harvest. However, more complex mixtures should be explored as all species have some advantage over the others, and thus can compensate for higher climatic variation and stabilize composition and yield, also helping on diet formulation.

The inclusion of corn silage in diets in combination with alfalfa and grass silage usually lead to lower cost and higher net incomes (~100% increase at 40% of inclusion). In our simulation, the partial inclusion of corn silage in diet also reduced GHG emissions allocated to milk compared to grass-based diets (2 to 4% at optimum). Indeed, the need for purchased feeds is reduced, as well as the environmental impact coming with those feeds, when corn silage is included in the diet. Also, having a forage with more starch has a direct negative impact on methane emission. But, going over a 40% inclusion in diets, GHG emissions start to increase above grass-based diets (Charbonneau *et al*., 2022). Furthermore, the reduction of perennial forages grown reduces the C sequestration potential, decreasing GHG mitigation potential.

Conclusions

While climate change is expected to negatively affect various sectors of the economy and agriculture in southern regions, the projections for dairy farms in the northernmost regions offer a more nuanced perspective. Although initial projections, based on averages, suggest a potential increase in farm net income under Canadian climates due to factors like more frequent perennial forage cuts and the possible integration of crops requiring higher growing degree days, further simulation uncovers potential challenges. As simulations progress into the future with increasingly intense climate change scenarios, adverse effects on crop yields due to excessive temperature and increased water stress are anticipated, even in northern regions like Canada. Additionally, the likelihood of meteorological extremes, such as droughts and intense rainfall events, is expected to escalate with climate change. Moreover, dairy producers will continue to face intra-annual variations in weather conditions, presenting a significant challenge. Consequently, enhancing farm resilience to cope with these uncertainties becomes imperative.

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When optimization goals for forage harvest in a forage dominant feeding system are not achieved – consequences and preventive measures

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Introduction

This is a follow-up paper from Sveinbjörnsson (2022) that aims at defining what happens when forage harvest optimization goals are not achieved in a sheep production system with little or no concentrate supplementation. Consequences for animal performance will be evaluated.

Model description and background information

The same feed plan structure is used as in Sveinbjörnsson (2022) but only for adult ewes, and is extended through the period of lamb growth until weaning at roughly 4 months of age. The prediction of forage dry matter intake ($fDMI_{ewe}$, kg DM d^{-1}) is by the model:

$$fDMI_{ewe} = 0.0263 \times \frac{EV}{BW} \times DMI\text{ factor}$$

where EV = energy value of the forage, FE_m kg DM^{-1}; BW = ewe body weight, kg; and DMI factor adjusts fDMI proportionally in relation to the effects of stage in the production cycle on fDMI. Values for this DMI factor through the indoor feeding period are reported in Sveinbjörnsson (2022). Values for different parts of the lactation period are based on Foot and Russel (1979), who analyzed the specific effects of stage in production cycle on forage feed intake of ewes by feeding the same forage through the whole production cycle.

The most practical measure of animal performance for this study is the weaning weight of the lambs. That is to a great extent influenced by the quality (and quantity) of summer grazing, but also to some extent to body condition of the ewes before lambing, which is a result of winter feeding. Quantitative data for the influence of a) amount of fat in the ewes body and b) level of her energy intake on milk yield and rate of fat loss was presented by Robinson (1990; fig. 8); for twin-suckling ewes of 70 kg BW. This data is is very relevant for our study, as the mature weight of Icelandic ewes at average body condition (BCS=3) is estimated as ~70 kg (Sveinbjörnsson, 2024). From that data the following equation was derived:

$$MY = 0.14 \times (ME - 5) + (BF - 5) \times (0.48 \times EL^2 - 0.96 \times EL + 0.48),$$

where: MY=milk yield kg d^{-1} (ewe with twins); ME= daily intake of metabolisable energy MJ d^{-1} ewe^{-1}; BF= kg body fat kg ewe^{-1}; where 5 kg BF corresponds to body condition score 1, and each whole unit BCS above 1 corresponds to 6 kg BF; EL=the proportion of energy required to be supplied by the ration. From Robinson (1990) and other data, it can be assumed that lambs will grow about 200 g per kg milk in their first weeks of life. In the model presented here, it is also assumed that lamb growth per kg milk decreases proportionally to increases in maintenance requirements. The lamb requirements are based on CSIRO (1990).

In the present modelling study, lamb life to weaning is divided up into four periods: **Period 1:** first two weeks, confinement in- and outdoors, ewes fed best quality forage and introduced to grazing; **Period 2:** week 3-6, grazing highly nutritious cultivated grassland; lamb growth still entirely based on ewe milk yield; **Period 3:** week 7-12, rangeland grazing; increasing part of lamb nutrition comes from grazing and a decreasing part from ewes milk; **Period 4:** week 13-18, rangeland grazing, lambs are assumed to sustain mainly from grazing. For each of these four periods two important ratios are defined:

R_M: the **r**atio of available net energy to the ewe (minus maintenance) that goes into **m**ilk.

R_LF: the **r**atio of the **l**ambs **f**orage intake capacity that is utilized.

Except for the R_LF adjustment, the formula for calculating forage DMI intake of the lambs is based on the general relationship between sheep forage DMI and DM digestibility reported by Pulina et al. (2013) :

$$fDMI_{lamb} = (4.22 \times DMD\% - 184.33) \times R\_LF$$

Lamb growth response to milk and forage energy is predicted according to CSIRO (1990), which accounts for the increasing energy (and decreasing protein) requirement per kg growth as the animal approaches maturity. The mature weight of female sheep of the Icelandic sheep breed is estimated as 70 kg (Sveinbjörnsson, 2024) and with a ratio 1.3 found for British breeds (Friggens et al.1997), the corresponding value for males is calculated as 91 kg. The model results below are for ewes nursing twins, one female and one male lamb.

Results and Discussion

Table 1 reports key numbers for each period based on a feed plan with ”perfect fit” between animal energy requirements and forage energy values for each period of indoor feeding, as in Sveinbjörnsson (2022).

Table 1 Summary of indoor feed plan, outdoor grazing, amount of fat in the ewes body at the beginning of each period, the ewes milk yield and the LW of her twin lambs at the end of each period. The ratios R_M and R_LF are explained in the text above

	DMI factor	Forage EV, FEm kg DM^{-1}	Forage DMI kg d^{-1}	FEm forage	Body fat kg	R_M	R_LF	Milk kg ewe d^{-1}	Male lamb LW kg	Female lamb LW kg
Autumn & mating	1.10	0.81	1.58	1.28	13.2					
Untill 90 days pregnant	1.00	0.71	1.33	0.95	16.2					
Day 91-115 of pregn.	1.10	0.81	1.74	1.41	18.9					
Day 116-130 of pregn.	1.05	0.80	1.67	1.34	20.2					
Day 131-144 of pregn.	0.90	0.84	1.51	1.27	20.7					
Lactation										
Period 1 (2 weeks)	1.20	0.90	2.15	1.94	20.7	1.00	0.00	3.0	8.2	7.8
Period 2 (4 weeks)	1.56	**0.90**	2.77	2.33	20.3	1.00	0.00	3.2	16.6	15.8
Period 3 (6 weeks)	1.56	**0.80**	2.32	1.84	16.7	0.60	0.40	1.8	30.6	28.3
Period 4 (6 weeks)	1.20	**0.70**	1.47	1.08	13.7	0.20	0.60	1.5	44.9	39.7

The values for R_M were chosen arbitrarily, but with respect to production system. It is assumed that in the first 6 weeks of lactation (Periods 1 and 2), when ewes live in a relatively protected environment, all available net energy (minus normal maintenance) is used for milk production. After that, the sheep are moved to extensive grasslands, where there is a considerable energy expenditure for movement to aqquire good quality and enough quantity of pasture (CSIRO, 1990). Therefore, in Period 3 R_M is set at 0.6. It is further lowered to 0.2 in Period 4, as ewes then will have to start depositing rather than mobilising fat. Values for R_LF are also arbitrarily chosen, assuming that lambs are entirely dependent on their mothers milk in Periods 1-2, but will after that, rely increasingly on forage grazing as ewes milk yield decreases and lambs rumen develops towards it full function.

	Body fat	Lamb LW at weaning	
EV diff	lact. wk. 13	Male	Female
0	13.7	44.9	39.7
-0.05	10.2	42.6	37.6
-0.1	6.9	40.5	35.6

More work will be needed to modell R_M and R_LF or corresponding concepts. Keeping these ratios constant in the model, it is possible to get some idea of the effects of actual forage quality over the indoor feeding season on ewe and lamb performance. An example of such results is in Table 2. When the difference in EV is -0.05, the total effect on weaning weight is 4.4 kg, but 8.5 kg if the EV difference is -0.1. That can all be made up with lamb finishing at a cost. More difficult is the effect on ewe body fat, which at the -0.1 difference will go further down than can be recommended. Although, in most cases Icelandic sheep farmers do good in forage harvesting, there have been years when the quality is close to the -0.1 difference. The consequences have turned out to be worse for ewe health and longevity than lamb growth, as the present study suggests. Further work is required to model timing of mobilisation and its relationship with diet quality in more detail the.

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Effect of rapeseed products in diets and iodine supply to dairy cows on iodine concentration in milk – a field study in ten Swedish dairy herds

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Introduction

Dairy products, among other foods, are important iodine sources for humans (Trøan *et al*., 2015; NASEM, 2021). The iodine content in drinking milk has declined over the last two decades, and is 11.8 µg/100 g according to the Food database (Swedish Food Agency, 2024). The Food database is widely used by dietitians when formulating public meals (e.g. meals in schools, hospitals, nursing homes, etc.). The nutritional content of milk in the Food database is based on analysis on bulktank milk from Swedish dairies (Lindmark Månsson, 2012). In order to label the package, the iodine content must be at least 7.5% of daily reference human intake, i.e. 11.25 µg/100 g (EU, 2011). This means that iodine content in Swedish milk was very close to the minimum limit. Finland and Norway are more on the safe side, since drinking milk packages are labelled with iodine content of 16 µg/100 g (Valio, 2024; TINE, 2024).

Adequate intake of iodine for dairy cattle is 0.5 mg/kg dry matter (DM) when the diet is free from goitrogenic substances, and 1 mg/kg DM when feeds containing goitrogens are fed according to NASEM (2021). Products from double-low rapeseed contain low levels of goitrogens (3-9 mmol glucosinolates per kg; personal communication I. Lundmark AAK Sweden AB) and are widely used in commercial compound feeds to dairy cattle in Sweden (Gustafsson, 2017). A meta-analysis by Trøan *et al*. (2015) resulted in a prediction model for iodine content in milk based on dairy cow diet's, e.g. rapeseed products, and thereby content of iodine. The results from Trøan *et al*. (2015) agree well with the iodine recommendation, including goitrogens in the diet, of 1 mg/kg DM (NASEM, 2021). However, results from the experiment by Trøan *et al*. (2018), where dairy cows were fed up to two kilo of rapeseed expeller per day lowered milk iodine more than the prediction from 2015. The present study evaluated dietary iodine supply, rapeseed in diet and iodine in milk to dairy cows on ten Swedish dairy herds. We aimed to suggest a target of iodine in Swedish milk and how it could be achieved.

Materials and Methods

Tank milk was sampled in ten conventional dairy herds in the south of Sweden. In one of the herds, tank milk was collected on two occasions, at the first occasion, the mineral feed had normal iodine content of 180 mg/kg, and on the second time the mineral feed contained 680 mg/kg. The diet of an average yielding cow in each herd was registered. Content of rapeseed products, as well as total iodine and iodine additive of commercial compound feed used in the herds were obtained from the feed manufacturers. The iodine content of home-grown feeds are generally low in Sweden and was assumed to be the average for the area (Lätt, 2019).

The target value for iodine in milk was selected based on the limit value for labelling (11.25 µg/100 g; EU, 2011) with the addition of two standard deviations.

An equation for predicting milk iodine concentration, based on iodine concentration and content of rapeseed products in the diet, similar to Trøan *et al*. (2015), was performed in

Excel using the SOLVER function. In total, we had 15 observations, 11 from the current study and four from the experiment by Trøan *et al* (2018).

Results and Discussion

The herds averages of rapeseed products offered per cow and day ranged between 1.4 and 5.4 kilo. All herds provided the iodine recommendation of 1 mg/kg DM (NASEM, 2021) or more in the diets (Table 1). Increased dietary iodine concentration increased iodine content in milk (r = 0.77), but an increased intake of rapeseed products had negative effect (r = 0.51) (Table 1). However, only four of the observations were above the limit for labelling on milk packaging according to the EU (2011). The feed manufacturers reported that iodine additive was in the form of calcium iodate (CaI), which is approved in the EU (2015). Of total iodine dietary supply, 73 to 94% was derived from the additive CaI (Table 1).

Table 1. Iodine (I) content in tank milk from10 herds (A-J) was an average of two samples per herd. Total I and additive I (calcium iodate, CaI) and amount of rapeseed procucts in the diet to the dairy cows

	A	B	C	D	E	F	G	H	I	J^1	J^2
Tank milk											
I, µg/100 g	4.7	6.7	7.6	10.6	10.9	10.7	11.8	16.2	16.9	10.2	18.7
Diet											
I total, mg/kg DM	1.1	1.4	1.1	1.0	1.3	2.2	1.4	1.9	2.0	1.1	2.9
I (CaI), mg/kg DM	0.9	1.2	0.8	0.8	1.0	1.9	1.1	1.8	1.7	0.9	2.7
Rapeseed product, g/kg DM	170	122	151	79	178	129	117	61	113	94	94
Rapeseed products, kg/day	4.5	2.9	3.8	2.1	5.4	2.7	2.8	1.4	2.7	2.5	2.5

[1]Herd J gave 90 gram mineral feeds per cow and day, which consisted 180 mg I/kg, [2]Herd J gave 90 gram mineral feeds consisting 680 mg I/kg. Tank milk was sampled 14 days after switching mineral feed.

Fitting the data from this study and data from Trøan et al (2018) resulted in Equation 1 and Figure 1. The model is valid between 0 to 180 g rapseed product /kg DM.

$$I\_milk = 1/10 \times e^{(4.579+1.104\times I\_diet-0.01418\times R-0.2818\times(I\_diet^{\wedge}2)+0.00004260\times(R^{\wedge}2)+0.0317\times(I\_diet^{\wedge}3))} \quad \text{Eq. 1}$$

where: I_milk is iodine in milk (µg/100 g), I_diet is iodine in diet (mg/kg DM) and R is rapeseed products in diet (g/kg DM).

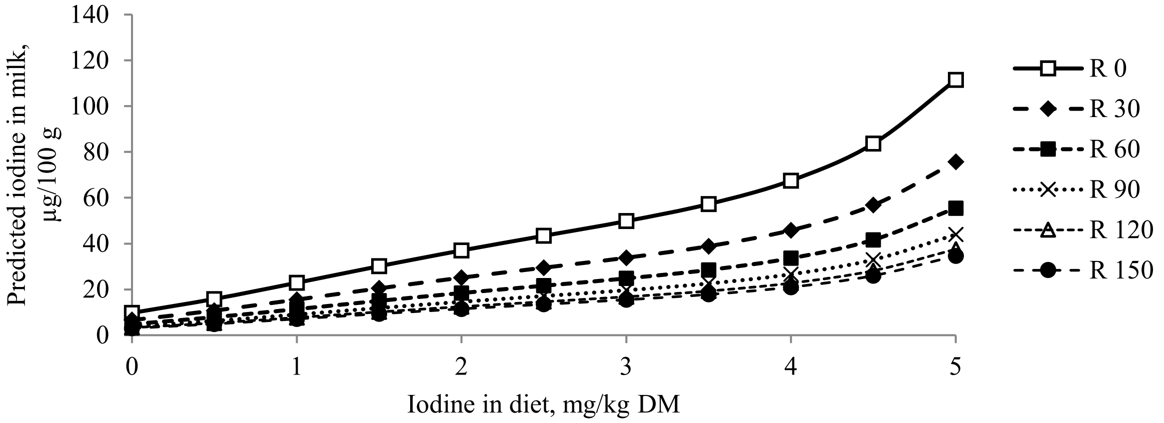

Figure 1. Predicted iodine concentration in milk (µg/100 g; according to Equation 1) at rapeseed cake or meal intake (R) of 0, 30, 60, 90, 120, and 150 g/kg DM and total dietary iodine intake of 0 to 5 mg I/kg DM.

In order not to fall below the limit of 11.25 µg/100g milk (EU, 2011), we suggest the target of iodine in milk to be two standard deviations above the limit. Standard devation of iodine concentration in milk was 2.3 µg/100g according to Lindmark Månsson (2012) based on bulktank milk from seven Swedish dairies sampled twice (January and July 2009). In other words, the target is proposed to be an iodine content of 16 µg/100g milk which is the same concentration labelled in both Norway and Finland (TINE, 2024; Valio, 2024).

Our prediction (Equation 1) indicates that dietary iodine for dairy cows should be 2 to 3 mg/kg DM when inclusion of rapeseed products are between 80 and 150 g/kg DM to achieve a concentration of 16 µg/100g milk. The feed industry in Finland and Norway aim at similar levels (personal communication M. Mughal, Luke; L.Karlsson Felleskjøpet AS; K.R. Vik, Fiskå Mølle). Since home grown forage in Sweden generally is low in iodine (Lätt, 2019), with a forage proportion of approximately 50% in a dairy cow diet (DM-basis), the level of concentrate ought to be almost double, *i.e.* 5 mg I/kg DM concentrate. Note that mineral feeds should also be considered since that also contains iodine additives. The results of this study have encouraged Swedish feed industry to increase iodine in compound feed during 2023.

Conclusions

To be on the safe side, the target of iodine in milk is proposed to be 16 µg per100g milk. To achieve 16 µg iodine per 100 g of milk, results of the current study show that 2 mg iodine per kg DM is needed when a diet contains 80 g rapeseed product per kg DM, and 3 mg iodine per kg DM when a diet contains 150 g rapeseed product or more per kg DM.

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Effect of molybdenum in farm-grown feed and copper supply to dairy cows on copper concentration in milk, urine, faeces, hair and liver – a field study in 10 dairy herds

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Introduction

Elevated levels of molybdenum together with sulphur in diets interfere with copper absorption in cattle (NASEM, 2021). This is the case for certain areas in Sweden, where molybdenum is high in soil and hence in feeds grown on the farm (Lätt, 2019). Copper supply should increase to animals where molybdenum is elevated in the diet in order to avoid shortage of copper. Liver, blood plasma, hair and milk can be analysed for assessment of copper status in dairy cows (Puls, 1993), whereas livers sampled in living animals is the best indicator (INRA, 2019).

In this study, we evaluated dairy cows' copper status with on-farm noninvasive samples and livers sampled at slaughter house were compared with dietary copper supply, where molybdenium varied in the diet.

Materials and Methods

In ten herds, all farm-grown feeds were sampled in advance. Feed consumption, diets to the lactating dairy cows and milk production were registered by the farmers. Milk, urine, faeces and hair were sampled in five random cows in each herd. The caudal lobe of the liver was sampled at a slaughter house on slaughtered dairy cows from the same herds within three months.

Farm-grown feeds were analysed by Eurofins (Wageningen, the Netherlands), milk, urine, faeces, hair and liver samples were analysed by ALS Global (Luleå, Sweden). Dietary copper surplus and shortage (copper intake minus copper requirement) and copper absorption based on dietary molybdenum and sulphur was calculated according to the American mineral requirements (NASEM, 2021) using a NorFor based tool (IndividRAM®, Växa, NorFor FRC version 2:10). Assessment of adequate and maximal tolerable levels of dietary molybdenum, sulphur and copper was done according to NRC (2005) and NASEM (2021). Assessment of deficient, marginal, adequate, high or toxic copper concentration in milk, hair and liver was done according to Puls (1993).

Correlations between dietary available copper and copper concentration in milk urine, faeces, hair and liver were calculated in Microsoft Excel 365. Detailed information on material and methods are described by Stensson (2023) and Hansen (2023).

Results and Discussion

Molybdenum, sulphur and copper values of diets and copper in samples of milk, urine, faeces, hair and liver from each herd are in Table 1.

Assessment of copper concentration in milk, hair and liver showed neither deficiency nor toxicity of copper at herd level. On three farms, both milk and hair samples were assessed to have marginally low copper levels, while the liver samples did not confirm this. However, the same cows were sampled for milk and hair, while liver samples were derived from other cows (Table 1).

Table 1 Molybdenum (Mo), copper (Cu) and sulphur (S) concentration on dry matter (DM) basis in diets to lactating dairy cows from 10 herds (A-J). Dieatry copper status (Cu status) was calculated as difference between average dietary copper supply and cows´ copper requirement. Average copper concentration in samples of milk, urine, faeces, hair from a number (n) of cows. Average copper concentration in liver samples from a number (N) of cows

	A	B	C	D	E	F	G	H	I	J
Diet levels										
Mo, mg/kg DM	0.4	0.5	0.7	0.8	0.8	2.7	5.9[1]	6.2[1]	6.9[1]	7.7[1]
Cu, mg/kg DM	12	11	13	10	16	16	16	19	10	11
S, g/kg DM	2.1	2.9	2.3	2.5	2.6	2.4	2.1	1.6[2]	1.4[2]	1.9[2]
Cu status, g/day	106	84	104	18	235	111	45	134	-85[3]	-129[3]
Copper concentration										
n	5	5	5	5	5	5	5	5	5	5
Milk, µg/L	95	51	30[4]	150	81	42[4]	62	80	42[4]	67
Urine, µg/L	25	8	18	17	24	18	22	27	12	10
Faeces, mg/kg DM	14	12	13	13	14	13	13	13	13	14
Hair, mg/kg	13.8	10.1	8.1[4]	12.0	8.7	7.9[4]	9.5	7.6[4]	7.8[4]	8.6
N	3	4	5	5	5	6	0	5	1	2
Liver, mg/kg	190	99	248[5]	112	156	137		133	132	78

[1]Above maximal tolerable level of dietary Mo of 5 mg/kg DM (NRC, 2005). [2]below adequate levels of dietary S of 2 g/kg DM (NASEM, 2021). [3]copper supply lower than requirement (NASEM, 2021). [4]marginal lower than adequate and higher than deficient in copper 4.3-8.3 mg/kg hair, 20-50 µg/L milk (Puls, 1993), [5]higher than adequate and lower than toxic copper in liver 200-250 mg/kg (Puls, 1993).

Dietary copper status, defined as supply minus requirement, was correlated with copper concentration in urine (Figure 1). When dietary supply minus requirement of copper was in balance (zero), the copper concentration in urine was 14 µg/L for herds with low molybdenum diets, and 17 µg/L for herds with elevated molybdenum diets.

Dietary copper status at herd level did not correlate with copper concentration in milk, hair and faeces, and had a low correlation with copper concentration in liver.

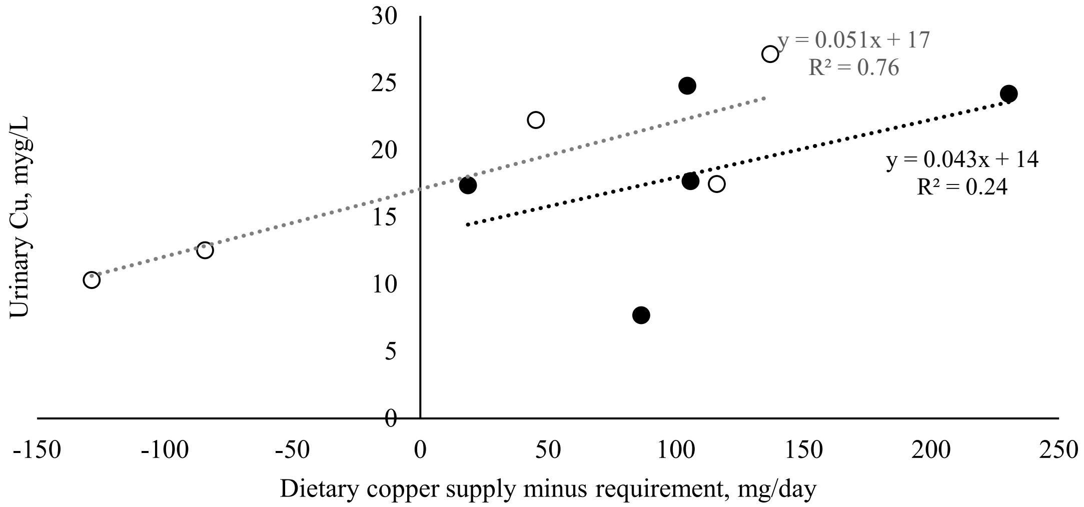

Figure 1 Urinary copper (Cu; average of five cows) is plotted against the dietary copper status for lactating cows at herd level (Cu intake minus Cu requirement). One dot is one herd with low dietary molybdenum (less than 1 mg/kg DM, black) and elevated dietary molybdenum (white). The intercepts of the trendlines indicate the marginal copper concentration in urine for adequate level of copper in diet.

Conclusions

Our results suggest that copper in urine could be an indicator of copper status in lactating dairy cows at herd level. Copper in milk, hair and faeces did not correlate and liver had very low correlation to dietary copper status.

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Effect of particle size reduction by extrusion on intake and digestion of reed silage in dairy heifers

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Introduction

Common reed (*Phragmites australis*) can reach a height of 1-4 m from its rhizomes under Swedish conditions and is naturally occurring in wetlands and coastal areas. Reed is an important species in aquatic ecosystems but due to euthrofication and reduced grazing it may expand into dense and homogeneous belts which reduces biodiversity and ecosystem quality (Pitkänen *et al*., 2013). Harvest of reed can be a way of restoring these ecosystems and remove nutrients from aqatic environments.

Historically, reed has ben used both for grazing and as a winter feed. It may serve as a an alternative in situations with forage shortage. Reed is a fibrous crop with low digestibility which constrains intake and utilisation and limits its use for demanding animals. Particle size reduction by extrusion, where screws shear fibrous plant material under pressure, aims to open up cell structures for improved microbial degredation. This may be a way to improve intake and digestibility in cattle. The aim of this research was to evaluate the effect of extrusion on intake and digestibility of common reed fed to dairy heifers.

Materials and Methods

The experiment was conducted at the Swedish Livestock Research Centre, SLU and was approved by Uppsala Ethics Committee for Animal Research (Dnr. 5.8.18–01637/2022). Reed was harvested from middle of July until the beginning of august in two areas in Ekoln (59°45' N, 17°36' E), the most northern bay of Lake Mälaren, close to Uppsala, and one area in Kyrkfjärden (59°26' N, 18°11' E), a small bay in the inner parts of Stockholm Archipelago. The crop was cut just above water surface with a flail chopper mounted on an airboat, a flat bottomed boat propelled by an aircraft propeller. In the chopper, the crop was sprayed with a silage additive containing lactic acid bacteria (Xtrasil bio ultra, Konsil Scandinavia AB, Tvååker, Sweden), which included Lactobacillus Plantarum, Lactobacillus Paracasei, Lactobacillus Brevis) and collected in builder bags of about 1000 l capcity. The bags were transported to land and there the reed was baled in a small square baler and wrapped in plastic film. At feed out, bales were opened three times per week and the reed silage was chopped in an impact shredder. Half of the chopped silage was processed in an extruder (Bio-Extruder MSZ-B15e, LEHMANN Maschinenbau GmbH). Prepared silage was stored in a frigarated container at a temperature close to 0°C until 12 h before feeding.

Six dairy heifers (555 ± 47 kg) was used in a change-over trial with three 2-week periods, the first week for adaptation and the second for sampling. The animals were housed in a section of a free stall barn equipped with feed bins for automatic intake recording (CRFI ,Biocontrol A/S, Rakkestad, Norway) and had free access to water, a mineral mix (Mixa Optimal, Lantmännen, Sweden) and blocks of sodium chloride. Each animal used only one and the same feed bin through out the experiment. Chopped or extruded reed silage was offered twice a day to ensure *ad libitum* intake with at least 10% orts. Intake registration and sampling of feed, orts and faeces were carried out during 5 days in the second week of each experimental period. All samples were frozen at -20°C directly after sampling and later pooled within experimental period.

Silages and orts were dried at 60ºC for 18 h in a forced-air oven for DM determination, milled to pass a 1-mm screen in a hammer mill and analysed for content of, ash, crude protein (CP), neutral detergent fibre (NDF) and acid insoluble ash (AIA, internal marker for digestibility estimations). Silages were further analysed for fermentation characteristics and mineral content. Feces samples were freeze dried, and analysed for ash, CP, NDF and AIA.

Statistical analysis was perfomed on period averages by Proc Mixed (SAS Inst. Inc., Cary, NC; version 9.4) with a model including period and treatment as fixed factors and animal as random factor.

Results and Discussion

Fermentation quality was satisfactory (Table 1) which support previous findings that ensiling of reed my result in acceptable silage (Rustas *et al.*, 2022).

Table 1 Composition of reed silages fed to dairy heifers

		Chopped		Extruded	
		average	SD	average	SD
Dry matter	%	36	6	38	7
Ash	g/kg DM	50	5	48	5
Crude protein (CP)	g/kg DM	99	27	95	31
NDF	g/kg DM	657	33	662	37
pH		3.9	0.1	3.9	0.1
Lactic acid	g/kg DM	41.9	5.7	39.0	6.3
Acetic acid	g/kg DM	8.1	1.8	8.2	2.2
Propionic acid	g/kg DM	0.3	0.2	0.3	0.2
Butyric acid	g/kg DM	0.2	0.0	0.2	0.0
Ethanol	g/kg DM	10.0	4.2	6.4	3.6
NH_4-N	% of CP	11.1	2.9	11.9	3.0
Ca	g/kg DM	4.2	1.9	3.9	2.0
K	g/kg DM	10.9	0.4	10.8	0.6
Mg	g/kg DM	1.1	0.2	1.1	0.2
Na	g/kg DM	1.8	0.4	1.7	0.4
P	g/kg DM	1.5	0.5	1.4	0.5
S	g/kg DM	2.6	0.6	2.6	0.6

Dry matter intake increased by a bit more than 20% after extrusion (Table 2). This is in line with earlier findings where extrusion of grass silage caused increased intake in dairy cows (Rustas *et al.*, 2021). Extrusion results in particle size reduction (Managos *et al.*, 2022) which is related to increased intake in growing cattle (Ingvartsen, 1994). Digestibility did not differ between treatments (P>0.36). High fiber content and low digestibility probably explained the relatively low dry matter intake (Allen, 1996) and although it increased after extrusion the calculated energy supply was not enough to cover the animals' maintenance requirements (Spörndly, 20023).

Table 2 Feed intake and digestibility of reed silage fed to dairy heifers

	Chopped	Extruded	SED	*P*-value
Intake				
Dry matter, kg/d	5.7	7.0	0.27	< 0.01
Dry matter, % of live weight	1.0	1.3	0.05	< 0.01
NDF, kg/d	3.8	4.7	0.18	<0.001
NDF, % of live weight	0.7	0.8	0.69	<0.001
Digestibility, %				
Dry matter	50	55	5.7	0.40
Organic matter	52	56	5.5	0.45
Crude protein	64	61	5.1	0.57
NDF	45	51	6.6	0.36

Conclusions

Extrusion of reed silage increased intake in dairy heifers but in this study, no effects on digestibility was found. Despite the positive effect of extrusion, animal feed intake was not enough to cover estimated energy requirement for maintenance.

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Effects of silage particle size reduction on feed total tract retention time and magnesium absorption in dairy cows

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Introduction

Magnesium is an important mineral element for dairy cows. Uptake of magnesium occurs mainly in the reticulorumen. There are no regulatory mechanisms adjusting magnesium uptake to the need of the cow, instead excess magnesium is excreted in the urine. It is well known that several factors can affect magnesium uptake in the rumen, *e.g.*, rumen pH, potassium concentration in the diet and solubility of added magnesium (Martens & Schweigel, 2000). There are indications that increased feed passage rate and hence decreased ruminal retention time may decrease magnesium uptake (Oberson *et al.*, 2019). One way to alter feed retention time in the rumen is to alter particle size, as smaller particles are more rapidly passed on to the omasum (Kaske & von Engelhardt, 1990).

In this study, we wanted to assess the effect of decreased silage particle size on uptake of magnesium in dairy cows, measured by urine excretion of magnesium.

Materials and Methods

This experiment was designed as a change over trial, with 8 cows and 4 treatments. The cows were all lactating, and 4 of them were equipped with a rumen cannula. They were housed in a tie stall barn, and provided with free access to water and salt licks. The treatments consisted of two grass silages cut from the same field on the 13th of June (early cut) and 23rd of June (late cut). The silages were chopped in a mixer wagon before feeding and half of it was extruded in a twin-screw co-rotating bio-extruder (Bio-Extruder MSZ-B15e, LEHMANN Maschinenbau GmbH). Particle size in the processed silages differed, as the extruded silage had markedly reduced particle size when analysed with a Penn state particle separator, with 0 and 23 % retained on the19-mm screen for extruded and chopped silage, respectively, and no difference on 8-mm screen (45 and 43%, respectively). Silages were fed individually and provided *ad libitum*. In addition to the silages, the cows were fed 6 kg of a pelleted compound feed and 2 kg of soybean meal. The experimental periods lasted three weeks, of which the last week was used for sampling. Cows were milked twice daily and volume of milk was recorded. Samples of the feeds used were taken, dried and analysed for magnesium concentration as well as dry matter. Urine samples were taken daily during 4 days, diluted with water 1:10 and analysed for magnesium and creatinine, used as a marker of urine production. To assess passage rate, the four cannulated cows were given a bolus dose of chromium mordanted NDF. Fecal samples were then taken at 24 occasions over 1 week.

Urine magnesium excretion was estimated assuming a creatinine excretion rate of 24.1 mg /kg BW (Chizzotti *et al.*, 2008), and milk magnesium concentration was estimated to be 0.092 g/kg of milk based on previously collected data (Kronqvist et al., unpublished). Chromium concentration in fecal samples was used together with time of sampling to fit curves that were used to estimate total tract mean retention time for the fiber fraction of the diet, as described by Eklund (2020).

Data were modelled using Proc Mixed in SAS (SAS 9.4). Fixed factors included in the model were silage cut, silage processing, period and cow, and the interaction between silage processing and cut. Significant differences were declared at $p<0.05$. Absolute values for magnesium excretion in urine, as well as proportions of magnesium intake excreted through urine and milk, were analysed. Proc Corr (SAS 9.4) was used to evaluate correlations between magnesium excretion in urine and total mean retention time.

Results and Discussion

Results regarding dry matter intake and dry matter digestibility were presented in Managos (2020). For data regarding magnesium output, see Table 1. When cows were fed the early cut silage, total magnesium intake was slightly higher compared to when they were fed the late cut silage. Magnesium intake for all cows was well above recommendations from NorFor (Volden, 2011). This was reflected by magnesium excretion in urine, which was calculated to be above the limit of 2.5 g/day that, according to Mayland (1988), indicates magnesium sufficiency. Neither processing nor cut affected magnesium excretion in urine. As excess magnesium is excreted in urine, it was expected that an altered magnesium absorption would be reflected in urinary excretion. As we hypothesized that extrusion of silage would decrease magnesium absorption, because of increased passage rate through the rumen, we expected decreased urine excretion of magnesium from cows fed the extruded silage. This was not supported by the data.

Table 1 Magnesium excretion in feces, urine and milk, as well as fiber total mean retention time (TMRT) from cows fed silage cut early or late, fed chopped or extruded

	Chopped		Extruded		P-values		
	Early cut	Late cut	Early cut	Late cut	Cut	Process	C*P
Mg intake, g/day	63.2	60.4	62.9	61.1	0.02	0.83	0.54
Mg urine, % of intake	7	7	8	8	0.29	0.17	0.57
Mg milk, % of intake	4	4	4	4	0.70	0.05	0.24
Mg in urine, g/day	4.7	4.7	5.0	5.2	0.72	0.23	0.60
TMRT^3	48.2	46	51.6	47.3	0.24	0.38	0.69

There was also no correlation between total tract mean retention time of fiber and magnesium excreted in urine, neither measured as total excretion in g/day (Figure 1, r=0.17) nor measured as a proportion of magnesium intake (data not shown). As most of the variation in total tract retention time is caused by variation in rumen retention time, lowering the TMRT indicates lower retention time in the rumen, possibly resulting in less magnesium being absorbed. However, this was not found in this experiment. We did also not find any effect of extrusion on total mean retention time of the diet, which was unexpected as the particle size composition was clearly altered by the extrusion, and also digestibility of dry matter was decreased with extrusion (Managos, 2020). The reason for this may be that there were too few observations.

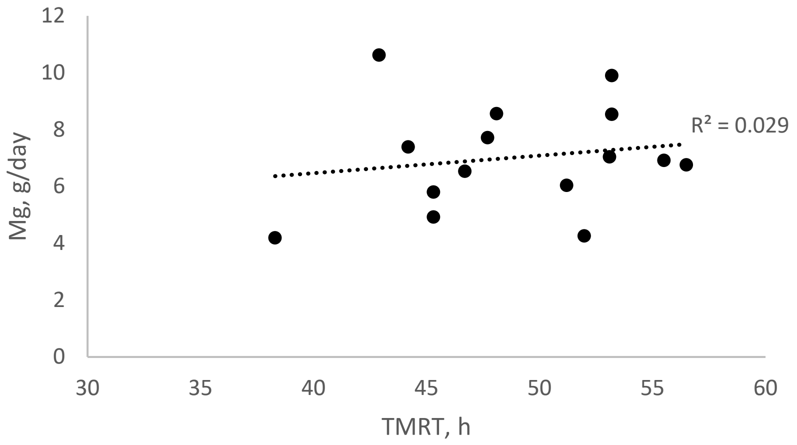

Figure 1 Urine magnesium excretion, g/day, as a function of total tract mean retention time of feed, TMRT, h.

Conclusions

Although magnesium intake was higher in cows fed the early cut silage compared to cows fed the late cut silage, there was no effect of extrusion of silage on magnesium absorption as measured by excretion in urine, or in magnesium excretion in milk. In addition, there were no effects on total tract mean retention time of fiber from extrusion or silage cutting time. Magnesium absorption, as measured by excretion of magnesium in the urine, did not correlate with total mean retention time of the feed.

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Effect of wilting and use of silage additive in grass silage on feed intake and milk production in Norwegian Red dairy cows

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Introduction

Restriction of fermentation of grass silages through use of silage additives (e.g. formic acid), or through modulating degree of wilting prior to baling, or a combination thereof, improve the metabolizable protein value of silages (Nadeau et al, 2019; Rupp et al., 2021). This improvement is expected through preservation of water soluble carbohydrates and a reduced proteolysis of plant protein during silage fermentation. The supply of metabolizable protein coupled with energy, in dairy cow diets is the driver of milk production. Here, we present effects of the degree of wilting of grass silage combined with the use of formic acid-based silage additive on feed intake, milk yield and milk composition with Norwegian Red (NRF) cows given *ad libitum* access to grass silage augmented with a fixed amount of concentrate.

Materials and Methods

A production experiment was conducted using 48 NRF cows fed four grass silage treatments prepared aiming at two wilting levels, i.e. 250 g DM /kg fresh matter (L) and 450 g DM/kg fresh matter (H). The silages were preserved either with no added preservative (-) or with the use of a formic acid-based silage additive (GrasAAT PLUS) at 6 L/ton fresh matter (+) resulting in: L-, L+, H-, H+ dietary treatments, respectively. The experiment was continuous over 8 weeks with the two first weeks used as a covariate period where all animals were fed a mixture of the four silage qualities followed by 6 weeks where the animals were split in to the 4 treatments based on days in milk, milk yield, silage intake and body weight (BW). In addition, animals were split into 3 blocks, one for first lactating, one for "low" yielding and one for "high" yielding cows, respectively.

Feed intake and milk production was monitored daily. Weekly samples of silage were pooled into one sample from the covariate period and two times for each treatment in the registration period, giving 9 samples in total. Concentrate samples were collected to give one composite sample in the covariate period, and two samples in the registration period. Cows were milked using AMS (DeLaval) and milk samples were taken once in the covariate period and twice in the registration period by sampling milk from a minimum of three consecutive milkings from each cow. Energy corrected milk (ECM) yield was calculated based on mean milk chemical composition and milk yield according to Sjaunja et al. (1991).

Data were later analysed using the Mixed procedure of SAS (SAS for Windows, *v.*9.4, SAS Institute Inc.). In addition to the covariate, block, silage DM, silage additive and the interaction between them was used as fixed effects, whereas cow within block was regarded as a random effect. The two samples within animal were considered repeated measurements.

Results and Discussion

Silage chemical compositions, feed intake and milk production data are presented in Table 1.

Table 1 Chemical composition of the grass silages, total DM intake, silage DM intake, milk yield and milk composition from Norwegian Red dairy cows fed grass silages prepared at two wilting levels (low DM = L; and high DM = H) preserved without (-) or with (+) formic acid-based silage additive

						P-value		
	L-	L+	H-	H+	SEM	Wilt	Additive	Wilt*Additive
Silage composition								
DM content (g/kg fresh)	209	219	320	324	-	-	-	-
Crude protein, g/kg DM	165	163	159	159	-	-	-	-
aNDFom, g/kg DM	530	513	530	514	-	-	-	-
WSC, g/kg DM	0.7	12.0	6.4	27.4	-	-	-	-
Lactic acid, g/kg DM	56	35	34	15	-	-	-	-
NH_3-N, g/kg N	188	140	143	122	-	-	-	-
pH	4.65	4.40	4.95	4.85	-	-	-	-
Feed intake, kg DM/d								
Total intake	20.2	21.0	22.0	21.7	0.25	0.001	0.528	0.157
Silage intake	13.6	14.3	15.2	15.0	0.25	0.002	0.429	0.235
Concentrate intake	6.74	6.68	6.75	6.72	-	-	-	-
Milk yield and milk component yields								
Milk yield, kg/d	28.8	29.0	27.9	29.5	0.32	0.750	0.055	0.117
Energy corrected yield, kg/d	29.7	31.4	30.3	31.6	0.41	0.523	0.016	0.744
Fat yield, g/d	1204	1290	1244	1301	21.0	0.401	0.022	0.634
Protein yield, g/d	961	1038	986	1067	16.5	0.265	0.002	0.929
Lactose yield, g/d	1380	1377	1343	1413	17.2	0.973	0.180	0.143
Milk composition, g/kg milk								
Fat	41.7	43.8	44.6	45.3	0.74	0.049	0.202	0.543
Protein	33.2	35.8	35.0	36.3	0.43	0.059	0.002	0.312
Lactose	47.9	47.9	47.4	47.9	0.22	0.338	0.415	0.443

aNDFom = neutral detergent fiber analyzed using amylase and correction for residual ash, NH_3-N = ammonia nitrogen in the grass silages, WSC = water soluble carbohydrates. SEM = Standard error of LSmeans (for interaction multiply by $\sqrt{2}$)

The achieved grass wilting levels and the difference in DM contents between the L and H silages were less than anticipated due to prevailing weather condition at the time of silage preparation. Furthermore, the observed levels of WSC in the silages, albeit differences between the wilting groups or in the presence or absence of silage additive, was lower than what is usually observed in grass silages (Randby *et al.*, 2020) or in grass hays made at different maturities (Stang *et al.*, 2023). Nevertheless, levels of lactic acid indicate that fermentation was restricted by wilting and by the formic acid additive, preserving WSC. For intake and performance parameters tested here, the interaction effects of wilting level and the use of additive did not differ ($P>0.05$). Regarding feed intake, both total DMI and silage intake improved by wilting ($P<0.01$) but were not affected by silage additive ($P>0.05$). This increased intake of silage or total DM had no effect on milk production (milk yield and ECM) with only marginal improvement in milk composition (i.e., milk fat and protein).

On the contrary, the use of the silage additive (i.e., GrasAAT PLUS) marginally improved milk yield and increased ECM, milk fat and milk protein yield ($P<0.05$) in the absence of any positive effects on feed intake ($P>0.05$). This can be contributed to increased digestibility of the consumed DM, or improved supply of metabolizable protein for milk production, or a combination of both at the intake levels achieved in the study. The relatively higher WSC and lower NH_3-N levels in the silages with formic acid-based additive suggest an improved metabolizable protein supply aligning well with the increased milk and ECM yields.

Conclusions

The higher DM content achieved by wilting showed only marginal effects on milk composition or performance parameters probably due to the difference in DM being too small to modulate protein values of the grass silages. The observed improvement in milk composition and ECM yield of cows consuming the formic acid-based silage additive could have been due to an increased DM digestibility and improved microbial crude protein synthesis leading to a higher metabolizable protein supply for milk production.

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Effect of restrictedly fermented grass silage on rumen metabolism and nitrogen utilisation

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Introduction

Grass silage fermentation can be restricted by wilting, use of acid-based silage additive or both (Charmley, 2001). More residual water-soluble carbohydrates (WSC) (Johansen *et al.*, 2017; Rupp *et al.*, 2021) as well as less proteolysis in the silage (Nadeau *et al.*, 2019; Sousa *et al.*, 2019) can increase the metabolizable protein value of restrictedly fermented silages compared to more extensively fermented silages. However, the results in milk production response to more restrictedly fermented silages have not been consistent (Jaakkola *et al.*, 2006; Heikkilä *et al.*, 2010). More research regarding influence of silage fermentation pattern on rumen metabolism and nitrogen (N) utilisation in dairy cows is therefore needed.

Materials and Methods

A metabolism trial was conducted with 8 lactating, rumen cannulated Norwegian Red dairy cows using a duplicated (two blocks: low vs. high yielding cows) four times four Latin square design (four silage treatments over four periods). Each period lasted 21 days with the first 10 days for adaption. The silage treatments were two levels of wilting (aiming for 250 (L) and 450 (H) g dry matter (DM)/kg), without (-) or with (+) use of silage additive (GrasAAT PLUS, 6 L/ton fresh matter); labelled L-, L+, H-, H+, respectively. Feed intake and milk production was monitored daily. Reticular sampling was used to measure rumen outflow and apparent rumen digestibility of DM, organic matter and ash corrected neutral detergent fibre (aNDFom) was estimated. Samples were taken from the rumen fluid every hour between 0600 and 1300 h (d 19 - 20) and analysed for VFAs and ammonium N fermentation pattern. Based on daily milk yield and total collection of urine and faeces (d 10-13), output of N in milk, urine and faeces was estimated. Microbial N yield was calculated from output of purine derivatives in urine.

Results were run in the statistical program IBM SPSS, using a linear mixed model. Fixed effects were silage DM, silage additive and the interaction between them, period and block. Cow within block was used as random effect.

Results and Discussion

The composition (g/kg DM unless otherwise stated) of the L-, L+, H- and H + silages were as follows: DM (g/fresh matter): 224, 230, 317, 324, crude protein: 159, 152, 156, 155, WSC: 2.4, 10.8, 3.80, 22.7, pH (no unit): 4.68, 4.40, 4.95, 4.83, lactic acid: 54.5, 32.8, 39.5, 15.3 and ammonia N (g/kg N): 175, 125, 141, 113.

Table 1 Dry matter (DM) and silage DM intake, production level and rumen metabolism for cows fed low (L) and high (H) DM silage, preserved without (-) or with (+) formic acid-based silage additive.

						P-value		
	L-	L+	H-	H+	SEM	DM	Additive	Interaction
DM intake, kg/d	22.7	23.4	22.7	23.5	0.588	0.863	0.042	0.839
Silage DM intake, kg DM/d	15.4	16.2	15.5	16.5	0.51	0.650	0.006	0.671
Milk yield, kg/d	27.1	28.4	26.6	28.4	1.295	0.561	<0.001	0.537
Apparent rumen digestibility, g/kg of intake								
DM	268	305	257	325	23.1	0.822	0.019	0.452
Organic matter	408	424	398	451	20.0	0.580	0.044	0.260
aNDFom	578	589	583	597	14.0	0.565	0.316	0.898
Rumen fluid fermentation product								
Ammonium nitrogen, mg/L	163	131	161	135	8.7	0.903	<0.001	0.651
Total acids, mmol/L	104	104	103	101	2.1	0.252	0.668	0.750
Acetic acid, molar %	63.7	64.2	64.1	63.7	0.41	0.923	0.870	0.202
Propionic acid, molar %	19.0	19.2	18.6	19.3	0.309	0.493	0.039	0.172
Isobutyric acid, molar %	1.02	0.90	1.08	0.97	0.023	0.005	<0.001	0.676
Butyric acid, molar %	13.4	13.3	13.2	13.2	0.30	0.579	0.875	0.726
Isovaleric acid, molar %	1.55	1.17	1.51	1.32	0.058	0.194	<0.001	0.033
Valeric acid, molar %	1.38	1.34	1.52	1.47	0.040	<0.001	0.135	0.918

aNDFom = ash corrected neutral detergent fibre treated with a heat stable amylase.

Due to harvesting conditions, the DM difference between L and H silages were only half the intended and may explain the general lack of effects of silage DM (Table 1). The higher intake of + silage, likely due to the higher rumen digestibility of DM and organic matter, resulted in increased milk yield (Table 1). Moreover, the rumen fluid of cows fed + silages contained less ammonium N and iso acids but higher amount of propionic acid, - the latter is a precursor to milk lactose (Aschenbach *et al.*, 2010). The results for use of silage additive

Table 2 Nitrogen (N) utilisation for cows fed low (L) and high (H) DM silage, preserved without (-) or with (+) formic acid-based silage additive.

						P values		
	L-	L+	H-	H+	SEM	DM	Additive	Interaction
N intake, g/d	587	586	580	598	14.5	0.751	0.331	0.305
N outflow rumen, g/d	579	599	570	567	24.1	0.296	0.675	0.568
Microbial N yield, g/d	260	285	253	223	15.7	0.634	0.516	0.767
N balance (% of intake)								
Milk	23.4	26.2	23.2	25.5	1.07	0.531	0.002	0.725
Urine	38.2	34.7	36.6	34.7	1.34	0.227	<0.001	0.263
Faeces	25.8	27.4	27.9	27.7	0.65	0.056	0.241	0.136
Total N excreted	87.5	88.3	87.7	87.8	0.89	0.895	0.573	0.660

are in line with the study of Jatkauskas and Vrotniakienė (2006). There were no differences for microbial N yield or N flow out of rumen (Table 2), but use of silage additive increased the milk N efficiency and reduced proportion of N excreted in urine.

Conclusions

The use of the formic acid-based silage additive increased rumen apparent digestibility of DM and organic matter, as well as proportion of propionic acid in the rumen fluid. The change in rumen digestibility and ruminal fermentation profile likely caused the higher silage DM intake and milk production, as well as a higher milk N use efficiency. No major differences were observed when changing silage DM, but the magnitude of difference in silage DM was only half the intended, due to challenging weather during harvest.

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Beef-on-dairy heifers grazing semi-natural grasslands can produce tender beef

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Introduction

Consumer demand for Swedish beef is still high and the Swedish produced proportion of consumed meat is still increasing (Lannhard Öberg, 2024). Therefore, this study was an attempt to contribute to this demand by developing innovative ways to satisfy the Swedish market with Swedish beef of high and uniform eating quality. It is well known that eating quality of beef is highly related to the degree of marbling, *i. e.* intramuscular fat; it is a strong predictor of eating quality and correlates positively with juiciness, tenderness, flavour, and overall acceptability (Savell *et al*., 1987).

A beef cattle body becomes fatter with increasing weight and age. As the growing animal deposits fat in an predetermined order, starting with filling up the abdominal and subcutaneous fat stores, *i. e*. trim fat, before depositing intramuscular fat, it is almost impossible to obtain a high amount of intramuscular fat in the meat without the carcass getting too fat. These excessive fat deposits is a waste for the processors, a cost for the farmer, and an unnecessary environmental impact from a too long animal rearing period. The aim of this study was therefore to investigate the effects of dam breed, sire breed, and intensity of production system on meat quality characteristics from dairy × beef heifer production based on forage and a well-managed seminatural grassland.

Materials and Methods

The experiment was conducted at the SLU Götala Beef and Lamb Research Centre, Skara, south-western Sweden (58°42′N, 13°21′E; elevation 150 m asl.) and the experimental protocol and execution were approved by the Ethics Committee on Animal Experiments in Gothenburg (ID number 002530). During indoor periods, the animals, consisting of 72 dairy × beef heifers acquired from commercial farms, were kept in groups of six in pens with deep straw bedding, while during grazing periods all animals grazed semi-natural grassland. The animals were followed from weaning to slaughter in an experiment with a 2 × 2 × 2 factorial design, comparing two sire breeds (Angus (ANG) and Charolais (CHA)), two dam breeds (Swedish Red (SRB) and Swedish Holstein (HOL)) at two production systems (moderately high (H) and Low (L) indoor feed intensity). Apart from indoor feed intensity, the two production systems also differed in terms of slaughter age and number of summers on grass. But, the two systems were chosen to reflect possible rearing strategies combining grazing for nature conservation and production of market-oriented carcasses. The heifer calves entered the experimental stations at 11 to 14 weeks of age, and animals on production system L were slaughtered at an average age of 824 ± 5 days, or 27 months, and those on H were slaughtered at an average age of 613 ± 7 days, or 20 months. For details regarding diet composition and production response, see Hessle *et al.* (2024), where no difference in feed intake was found between the systems.

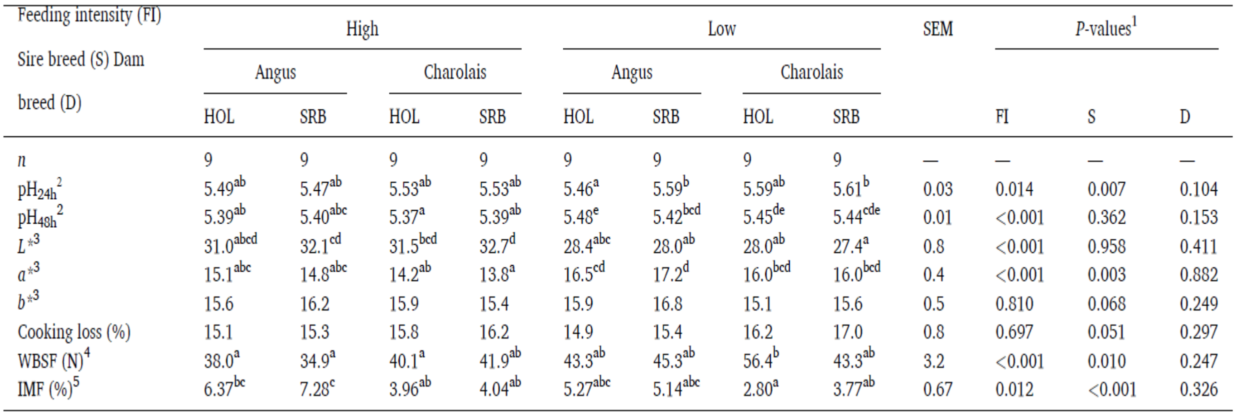
The heifers were transported to a local commercial abattoir for slaughter. After slaughter, carcasses were divided along the vertebral column and weighed. Carcass conformation and fat cover were graded according to the European Union Carcass Classification Scheme EUROP. Marbling was determined visually between the 10th and 11th ribs on right hindquarters in M. longissimus dorsi on the Swedish 5-point scale with steps from 1 (no marbling) to 5 (slightly abundant). Trim fat was weighed and pH 24 h post-mortem was measured at the same place af the carcass (pH_{24h}).

At 48 h post-mortem, samples from the loin (*M. longissimus lumborum*) were removed from the carcasses for meansurements of ultimate pH (pH_{48h}), in duplicate and for colour. Colour was measured after 60 min of blooming (Caldwell *et al.*, 2017) at five sites per slice, using a CM-600d spectrophotometer (Konica Minolta, Osaka, Japan). Instrumental tenderness was measured as shear-force using a Warner-Bratzler knife (WBSF) using a texture analyser (TMS-Pro-Texture Analyser; Food Technology Corporation, Sterling, Virginia, USA). Samples, after 5 days of aging (7 days post mortem) at 4°C, were first cut to size (5 × 4 × 8 cm, in a total 8 samples per animal), weighed, vacuum packed, and heat treated in circulating water (10 min at 4°C, 60 min at 62°C, and 30 min at 4°C). Thereafter rinsed with water, blotted dry with paper towels, and reweighed to determine cooking loss. The mean maximum force of replicates was recorded. For analysing intramuscular fat (IMF), meat slices (40–50 g) were cut into smaller pieces and homogenized. IMF was extracted using a combination of the Weibull-Berntrop gravimetric method and SOXTHERM® (rapid soxhlet extraction). The sample IMF% was determined from the amount of fat extracted relative to the sample weight. For details, see Drachmann *et al.* (2024).

Results and Discussion

The technological meat quality in the loin differed between production system H and L (Table 1). Generally, beef from production system L was darker, more red and tougher (higher WBSF and lower IMF%) compared to H. Beef from ANG crossbreeds was more red and tender (lower WBSF and higher IMF%) compared with beef from CHA crossbreeds. Regarding the production system used here, Hessle *et al* (2024) concluded that beef production with dairy × beef heifers based on forage and semi-natural grasslands is a multifunctional system where nature conservation effects of grazing and market-oriented carcasses can be achieved simultaneously.

Table 1 Technological meat quality of *M. longissimus lumborum* from heifers with the effects of feeding intensity (moderately high and low), sire breed (Angus and Charolais) and dam breed (HOL—Swedish Holstein; SRB—Swedish Red-and-White). Results are presented as least-squares means, standard error of the mean (SEM) with P-values



Feeding intensity (FI)	High				Low				SEM	*P*-values[1]		
Sire breed (S) Dam	Angus		Charolais		Angus		Charolais					
breed (D)	HOL	SRB	HOL	SRB	HOL	SRB	HOL	SRB		FI	S	D
n	9	9	9	9	9	9	9	9	—	—	—	—
pH_{24h}[2]	5.49^{ab}	5.47^{ab}	5.53^{ab}	5.53^{ab}	5.46^{a}	5.59^{b}	5.59^{ab}	5.61^{b}	0.03	0.014	0.007	0.104
pH_{48h}[2]	5.39^{ab}	5.40^{abc}	5.37^{a}	5.39^{ab}	5.48^{e}	5.42^{bcd}	5.45^{de}	5.44^{cde}	0.01	<0.001	0.362	0.153
*L**[3]	31.0^{abcd}	32.1^{cd}	31.5^{bcd}	32.7^{d}	28.4^{abc}	28.0^{ab}	28.0^{ab}	27.4^{a}	0.8	<0.001	0.958	0.411
*a**[3]	15.1^{abc}	14.8^{abc}	14.2^{ab}	13.8^{a}	16.5^{cd}	17.2^{d}	16.0^{bcd}	16.0^{bcd}	0.4	<0.001	0.003	0.882
*b**[3]	15.6	16.2	15.9	15.4	15.9	16.8	15.1	15.6	0.5	0.810	0.068	0.249
Cooking loss (%)	15.1	15.3	15.8	16.2	14.9	15.4	16.2	17.0	0.8	0.697	0.051	0.297
WBSF (N)[4]	38.0^{a}	34.9^{a}	40.1^{a}	41.9^{ab}	43.3^{ab}	45.3^{ab}	56.4^{b}	43.3^{ab}	3.2	<0.001	0.010	0.247
IMF (%)[5]	6.37^{bc}	7.28^{c}	3.96^{ab}	4.04^{ab}	5.27^{abc}	5.14^{abc}	2.80^{a}	3.77^{ab}	0.67	0.012	<0.001	0.326

[1] Interactions not shown. Significant interaction between FI × S × D for b* (P = 0.013). [2] pH was measured in the centre of the muscle 24 and 48 h post-mortem. [3] L* (lightness), a* (redness), b* (yellowness) measured on CIE 1976 L*a*b* scale. [4] Warner-Bratzler shear force, peak force. [5] Intramuscular fat concentration assessed by chemical analysis. a-c: values within a row with different superscripts differ significantly at $p < 0.05$.

Conclusions

Beef-on-dairy heifers reared on forage and semi-natural grasslands with one or two grazing seasons can deliver high quality beef. Beef from heifers reared under moderately high feeding intensity was less tough and had higher IMF% but was lighter and less red compared with heifers reared under low feed intensity. Beef from Angus crossbreeds was redder (b*) with lower shear force (WBSF), *i.e.* more tender and had a higher intramuscular fat content (IMF%) than Charolais. These characteristics are generally associated with superior meat quality. Notably, beef from Charolais crossbreeds in low feed intensity production system (L) was of considerably lower quality, compared to the better-performing Angus crossbreeds and Charolais crossbreeds in the moderately high production system (H). While sire breeds differed on some important meat quality traits, meat quality of Swedish Holstein (HOL) and Swedish Red (SRB) crossbreeds was comparable.

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Ruminal pH data analysis in lactating dairy cows fed with a SARA-inducing diet

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Introduction

Feeding high amounts of easily fermentable carbohydrates, as starch, may lead to a decrease in ruminal pH resulting in conditions such as ruminal acidosis (RA) and subacute ruminal acidosis (SARA) (McAuliffe *et al*. 2022). Unlike clinical RA, SARA results from a repeated moderate drop in rumen pH over a prolonged period of time (Enemark 2008). Signs of SARA include, among others, a decrease in dry matter intake, lamenesses, milk fat depression, loose faeces, abscesses in the liver and other organs and also a high culling rate in the herd. SARA usually goes undetected due to it´s subtle signs. The lack of diagnosis cause negative welfare impact and economic losses (Plaizier *et al*. 2008). With the objective to identify suitable biological markers to diagnose SARA, we conducted a study with lactating dairy cows fed either a control diet or a SARA inducing diet. Along the study, rumen pH for each cow was monitored by using a rumen bolus capable of register pH continuously at 10 min intervals. In the present paper, we present data of rumen pH and discuss how to interpretate the data provided by it.

Materials and Methods

A total of 22 primiparous cows (7 Swedish Holstein (SH) and 15 Swedish Red (SR) breeds) averaging (± SD) 142 ± 40.2 days in milk (DIM) and milk yield of 29.8 ± 5.45 kg/day were used in the study. The cows were housed in a free stall housing system at the Swedish Livestock Research Center at Lövsta, Uppsala. The study was performed in three periods lasting a total of 52 days. At the beginning of the study, cows were fed the same diet as they normally had, 48:52 concentrate to silage ratio for 9 days (CONT1). From day 10 to day 32 (23 days), cows were fed a diet with a 64:36 concentrate to silage ratio (SAR2). Finally, from day 33 to day 52, recovery period, cows were again fed with control diet (CONT3).

All cows had free access to the mix. Feeds were delivered at 7:30 and 15:00 h each day. The concentrates were pelleted and provided both in a trough as TMR and individually in the milking unit (0.5 kg/cow/day). Individual daily forage intake was recorded automatically by forage troughs on weight scales (CRFI, BioControl Norway A/S, Rakkestad, Norway). Milking was done voluntarily in a single-station automatic milking system (VMS, DeLaval International AB, Tumba, Sweden). On Day 1 of the study, one pH bolus (Moonsyst cattle monitoring, Ireland) was applied per cow. Before application, the pre-calibrated boluses were activated, cross-referenced with the cow's individual ear tag number, and connection to the base station was ensured. Cows were locked in a self-locking grid, heads were restrained manually by the person and bolus was applied with the help of a balling gun. The mooncyst rumen bolus most likely remained in the reticulo-rumen. Boluses monitored the rumen pH continuously at intervals of 10 minutes. Data handling was done by Microsoft Excel 2311 and R studio Version 4.3.2. . To analyze the normality of the data a Shapiro wilktest was performed. Data on mean ruminal pH, max, min, and time (h) under pH 6 were statiscally analysed by ANOVA, according to a repeated measurements model. Differences were considered different at $p<0.05$.

Results and Discussion

Cows used in the present experiment were primiparous because primiparous cows are more susceptible than multiparous cows to changes in rumen pH in response to changes in diet.

Data on rumen pH (mean, maximum and minimum values, and time below pH 6) are presented in Table 1. The mean values of ruminal pH was the lowest in SAR2 and highest in CONT3 (Table 1). While no differences were observed among mean max pH values among treatments, mean min pH values was lower in SAR2 than in both of the CONT treatments.

Table 1: Effect on ruminal pH levels in dairy cows fed diet with different concentrate to forage rations (48:52 for CONT1 and CONT3; and 64:36 for SAR2)

Parameters	CONT1	SAR2	CONT3	SEM[1]	P value
Mean ruminal pH	6.40^{b}	6.31^{c}	6.46^{a}	0.015	<0.05
Mean Max rumen pH	7.10	7.13	7.12	0.022	>0.05
Mean Min rumen pH	5.87^{a}	5.66^{b}	5.86^{a}	0.020	<0.05
Time below pH 6 (h/d)	0.53^{b}	2.86^{a}	0.33^{c}	0.217	<0.05

[1] SEM: standard error of the mean;

[a,b,c] Values within a row with different superscripts differ significantly at $P < 0.05$.

Mean minimum pH values, even for SAR2, were above pH 5.4, considered as a threshold under which rumen metabolism (organic matter digestibility and microbial protein synthesis; (Dijkstra *et al.* 2020) may be impaired. Among the different variables analysed in the present study, the biggest difference was observed in time (h/d) that ruminal pH remained under 6. Thus, when cows were fed the SARA inducing diet, ruminal pH remained under 6 almost 7 times longer than when fed the control (low concentrate) diets. However, it is worth noticing, that as reported by others (Dijkstra *et al.*, 2020), there was a big variation among cows with regard of time when ruminal pH was lower than 6 in the SAR2 treatment. In the present study, time ranged from 0 to almost 17 h/d (Figure 1). The difference among individual cows, may be due to different factors, such as feeding behavior, buffering capacity, volatile fatty acids absorption rate, digesta passage rate, etc. *(Dijkstra et al.*, 2020).

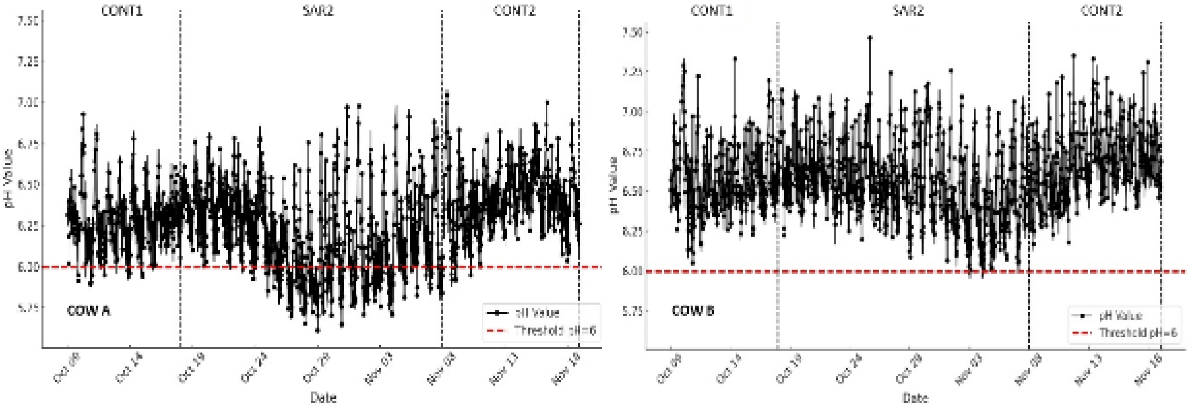

Fig 1 An example of the variation registered in rumen pH in two cows along the whole experiment.

In general, maximum and minimum ruminal pH recorded in the present study were higher compared to other studies (Baek *et al*. 2022). A reason for that could have been the location of the sensor in the reticulo-rumen as it can affect values of pH recorded. Studies have shown that reticular pH is higher than ruminal pH (Klevenhusen *et al*. 2014; Dijkstra *et al*. 2020**).** According to the manufacturer, the sensor used in the present study stays in the reticulum.

Conclusions

In the conditions of this study, the rumen pH sensor used was capable to detect differences in mean daily ruminal pH, minimum pH, and time (h/d) when pH was lower than 6, when lactating dairy cows were fed a SARA inducing diet as compared to a control diet. As the magnitude of the differences among diets was the biggest for time under pH 6, it appears that this variable is more reliable than mean pH values.

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Effect of different additives and harvesting date on fermentation characteristics of maize silage

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Introduction

Maize is widely grown in many parts of the world and a major component in silage-based diets for dairy cows due to its stable dry matter (DM) yield, high nutritional value, and good ensiling characteristics. However, maize silage often suffers from aerobic deterioration during feed-out, which leads to considerable losses in DM and feed quality (Bernardes *et al.* 2021). Therefore, the use of inoculants, such as *Lactobacillus buchneri*, or chemical additives are common methods to improve the aerobic stability of silages (da Silve *et al.* 2017) and thus maintain or even improve feeding value as well as animal productivity (Bernardes *et al.* 2021). The objective of this study was to examine the effects of different additives on DM losses and fermentation characteristics, such as acetic acid (AA) and 1,2-propanediol (1,2PD), during fermentation under varying ensiling conditions.

Material and Methods

Chopped maize (*Zea maize* cv. Benidiktio) was ensiled with two different DM levels (DML) of 350 (D1) and 385 g/kg (D2), respectively configured by different harvest dates (16./23.09.2020) and treated with or without additives prior sealing. Before packing, fresh plant material of each DML was divided into three identical groups for the following additive treatment, including a control (CON) without additive, a chemical additive (SALT) containing sodium benzoate (889g/kg) and (111g/kg) potassium sorbate and an inoculant containing *Lactobacillus buchneri* $1.0*10^{11}$ CFU/g; *Enterococcus faecium* $2.0*10^{10}$ CFU/g (LBEF, Kofasil S 1.2). The additive was applied at 0.5 kg/t of fresh matter (FM) (diluted in 2 l of water) and the inoculant at a rate of 1 g/t FM (dissolved in 1 l of water). Both additives were sprayed manually on the forage. Subsequently, treated plant material was compacted in 1.5 l glass jars and stored for 90 days at controlled ambient temperature (25 °C). All treatments were ensiled in triplicate. Samples were taken to analyse chemical composition as well as fermentation characteristics (lactic acid (LA), AA, ethanol, ammonia-nitrogen, 1,2PD and pH) using standard laboratory methods. Moreover, occurring DM losses during storage period were determined (Weissbach 2005). The statistical analysis was performed with the GLM procedure of SAS version 9.4, considering the effects of additive treatment, harvesting date and their interaction. When calculated differences were significant ($P<0.05$), REGWQ test was used for pairwise comparisons between means.

Results and Discussion

The set DM levels resulted in a significant increase of DM while protein solubility and net energy of lactation decreased with progressing maturity level ($p<0.01$) The energy density is not in line with the typical ripening process and the measured starch content. But the silage additives had a positive effect onto the amount of starch in the silage. After 90 days of ensiling, DM fermentation losses were affected by both DML and additive treatment ($p<0.01$), whereby higher figures were found for LBEF (7.3/5.7% of DM) compared to the controls and SALT treatments (tab. 1). These higher FL of *Lactobacillus buchneri* inoculated silages during fermentation related to the hetero fermentation pathway and the associated CO_2 formation (Driehuis 1996). Concerning the silage pH, an effect of additive could be observed as well (fig. 1). The LBEF treatment led to a higher final pH in both DML ($p<0.01$), which is most likely attributed to a lower concentration of LA (Driehuis 1996) compared to the control and the SALT treatments. The acetic acid concentration differed considerably between the treatments and ranged between 13 g/kg DM in D2CON to 49 g/kg DM in D1LBEF and was significantly increased by the inoculation. From the higher AA values, it may conclude a higher aerobic stability of the silage during feed-out (Nishino *et al*. 2003), but it was not measured in the trial. Likewise, a substantial formation of 1,2PD was determined in LBEF silages (>29 g/kg DM), whereas in D1CON, D2CON as well as D1SALT concentrations were below 10 g/kg DM. These findings are related to the degradation of lactic acid by *Lactobacillus buchneri* into equimolar amounts of 1,2PD and

Table 1 ADFom, starch content (XS), protein solubility (PL) energy density (ED), and DM fermentation losses (FL) of a different treated maize silage

	DML (g/kg DM)	ADFom (g/kg DM)	XS (g/kg DM)	PL (% of CP)	ED MJ NEL/ kg DM	FL (%)
Control	352,2^{a}	235,1^{b}	295,6^{a}	65,0^{a}	6,7^{a}	4,7^{a}
LBEF	343,3^{a}	228,6^{a}	325,9^{b}	64,3^{a}	6,9^{b}	7,3^{b}
SALT	356,1^{a}	229,0^{a}	297,9^{a}	62,7^{a}	6,8^{b}	4,5^{a}
Control	386,7^{b}	229,1^{a}	303,4^{a}	59,9^{b}	6,6^{a}	4,4^{a}
LBEF	380,4^{b}	229,1^{a}	317,6^{b}	59,0^{b}	6,6^{a}	5,7^{b}
SALT	389,0^{b}	225,4^{a}	319,2^{b}	58,7^{b}	6,6^{a}	4,8^{b}

different letters indicate significant differences between treatments for each parameter, $p<0.05$
SALT= sodium benzoate and potassium sorbate, LBEF= *Lactobacillus buchneri* $1.0*10^{11}$ CFU/g; *Enterococcus faecium* $2.0*10^{10}$ CFU/g, CP=crude protein.

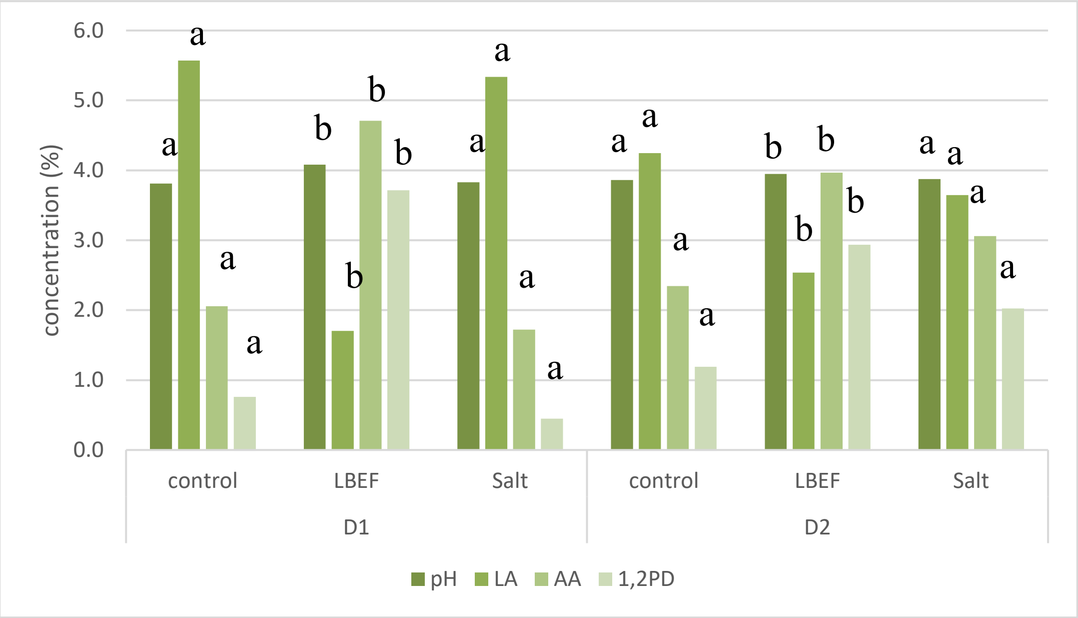

Figure 1 Effect of different DM levels of a maize silage and different additives onto fermentation acids and 1,2-propanediol. Different letters indicate significant differences between treatments for each parameter, $p<0.05$.

acetic acid and trace amounts of ethanol (Oude Elfering 2001). Ethanol was significantly higher in the LBEF silages also (data not shown). This substantial formation of 12PD may have a positive effect on a risk reduction of ketosis *post-partum* (Lau *et al*. 2018).

Conclusion

The application of LBEF resulted in a conversion of LA to AA and 12PD in comparison to SALT and the control. Thus, it can be concluded that LBEF treated silage showed both a potential to improve silage aerobic stability due to increased AA concentration and an enhanced nutritive value because of the elevated 12PD content in the silage. As a result of the heterofermentative pathway higher DM losses during the fermentation are given. But these losses will be figured out by prevented aerobic losses during feed-out. This finding we had at both different harvest dates, DML. In tendency the additive treatments resulted in a higher starch content.

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Field survey on the silage quality of Finnish farms

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Introduction

The use of good silage practices improves the preservation and quality of the silages. However, several factors, such as use of additives, cut, wilting period and species, can affect the final result of the silage. The nutritional and hygienic quality of silages from Finnish farms can vary considerably depending on the production methods used. This survey is part of the Sustainable Silage project funded by the Interreg Central Baltic programme (2023 – 2025), where Estonian, Finnish and Latvian partners strive together to develop the silage chain for reduced environmental impact covering simultaneously silage quality and economic aspects. An important part of the project is the pilot farms in each country (total of 25). This study is based on silage samples collected from the Finnish pilot farms. The aim was to conduct a farm survey on the overview of key silage production practices and establish links between silage parameters to provide opportunities to improve the nutritional and hygienic quality of silages at farm scale.

Materials and Methods

This experiment was carried out at the Natural Resources Institute Finland (Luke), Jokioinen, Finland in cooperation with ProAgria Western Finland and pilot farmers of the Sustainable Silage project. Silage samples were collected from 7 farms totalling 18 samples. Samples were collected on two occasions, the first in summer 2023 for silages produced in summer 2022 and in autumn 2023 for silages produced in summer 2023. Approximately 2 kg of silage samples were delivered to Luke. The samples were analysed for dry matter, pH, ammonia-N, ethanol, water soluble carbohydrates, crude protein, ash, lactic acid, volatile fatty acids, aerobic stability, mould score after aerobic exposure, yeasts and moulds in the laboratory of Luke as described by Franco *et al.* (2022). Additionally, information such as plant species, cut and type of additive used were collected. In order to establish links between silage characteristics, a correlation was performed among all parameters using the CORR procedure of SAS 9.4 for each individual silage sample.

Results and Discussion

Most of the silages (n = 15) were produced with a variety of grasses and legumes, including timothy, meadow fescue, ryegrass, tall fescue, white clover and red clover. Only two silages were produced with maize and one silage of whole crop vetch and spring rye. The majority of silages (n = 11) were produced from the first cut, followed by the second cut (n = 4) and then a minority from the third cut (n = 3). As commonly found on farms in Finland, most producers preserved their silage using formic acid based additives (61%), while some producers used lactic acid bacteria inoculants (31%) and rarely those that do not use any additives (8%).

Chemical composition and fermentation quality of the silages are shown in Table 1. The whole crop vetch and spring rye had the highest dry matter among the silages, followed by the maize silages and then grass silages. Most silages were considered well preserved taking into account the low pH, low concentrations of ammonia-N and ethanol, as well as sufficient production of lactic acid and moderate proportion of acetic acid, which contributed to silages with long aerobic stability.

Table 1 Chemical composition, fermentation quality, aerobic stability and microbial quality of the farm silages

	Grass and legume silages (15)		Maize silage (2)		Whole crop silage (1)
	Mean + SD	Min, Max	Mean + SD	Min, Max	
Dry matter (DM), g/kg	327 ± 112.1	208, 635	320 ± 34.2	296, 345	350
pH	4.24 ± 0.411	3.81, 5.12	3.89 ± 0.148	3.78, 3.99	3.75
Ammonia-N, g/kg N	50 ± 22.8	23, 111	62 ± 0.8	61, 62	59
In DM, g/kg					
Ethanol	7.7 ± 7.07	1.7, 23.5	2.8 ± 0.40	2.5, 3.1	5.7
Water soluble carbohydrates	60 ± 69.9	6, 237	15 ± 13.1	6, 25	17
Crude protein	140 ± 21.5	98, 166	87 ± 1.0	86, 87	140
Ash	82 ± 15.8	65, 123	42 ± 0.3	41, 42	61
Lactic acid	51 ± 29.3	6, 101	45 ± 9.6	38, 52	67
Acetic acid	21.4 ± 16.37	4.6, 67.7	24.7 ± 12.06	16.2, 33.3	15.3
Propionic acid	0.41 ± 0.424	0.09, 1.68	0.28 ± 0.149	0.17, 0.38	1.23
Butyric acid	0.33 ± 0.446	0.05, 1.63	0.01 ± 0.007	0.09, 0.10	0.11
Aerobic stability 2 °C, hours	257 ± 104.9	81, 385	216 ± 238.9	47, 385	385
Mould score, 1 to 5	3 ± 1.7	1, 5	2 ± 1.4	1, 3	1
Yeast, cfu/g	140 ± 105.6	100, 400	795 ± 982.9	100, 1490	100
Mould, cfu/g	140 ± 105.6	100, 400	100 ± 0	100, 100	100

SD, standard deviation.

The correlations between the different parameters of the silages are shown in Figure 1. Most of the parameters studied were not significantly correlated with each other and especially with aerobic stability. The dry matter content of the silages was negatively correlated with the amount of lactic acid, thus inferring that the higher the dry matter of the silages, the lower the lactic acid production, probably due to the limitation of low water activity on fermentation. Consequently, the dry matter showed a positive correlation with the water soluble carbohydrate content and pH of the final silages, as with the limitation of fermentation due to high dry matter, the water soluble carbohydrates are preserved and not degraded, resulting in high pH silages. The positive correlation between ethanol and acetic acid indicated that the greater the ethanol production, the greater the acetic acid production.

The lactic acid concentration of the silages showed a negative correlation with several parameters, such as water soluble carbohydrates, pH and the presence of mould in the silages. The correlation results indicated that the greater the production of acetic acid, the longer the aerobic stability when the silages are exposed to air. Propionic acid was strongly and positively correlated with the production of butyric acid. The presence of moulds and yeasts had a negative correlation with aerobic stability, indicating that they are precursors to silage deterioration.

	Dry matter	pH	Ammonia	Ethanol	Water soluble carbohydrates	Crude protein	Ash	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Aerobic stability	Mould score	Yeast
pH	**0.81**													
	<0.01													
Ammonia	-0.12	0.12												
	0.63	0.64												
Ethanol	-0.33	-0.16	0.35											
	0.19	0.53	0.15											
Water soluble carbohydrates	**0.62**	**0.53**	-0.38	-0.20										
	0.01	**0.03**	0.12	0.44										
Crude protein	**-0.48**	-0.25	-0.11	0.38	-0.31									
	0.04	0.31	0.67	0.12	0.22									
Ash	-0.22	0.18	0.01	0.11	-0.09	**0.61**								
	0.39	0.48	0.96	0.66	0.73	**<0.01**								
Lactic acid	**-0.84**	**-0.77**	0.02	0.37	**-0.64**	**0.52**	0.22							
	<0.01	**<0.01**	0.93	0.13	**<0.01**	**0.03**	0.39							
Acetic acid	-0.29	-0.16	0.45	**0.67**	-0.43	0.25	-0.03	0.22						
	0.24	0.53	0.06	**<0.01**	0.08	0.31	0.90	0.39						
Propionic acid	-0.09	-0.02	0.28	0.36	0.05	-0.04	-0.16	0.07	0.14					
	0.73	0.94	0.27	0.15	0.86	0.86	0.53	0.78	0.57					
Butyric acid	-0.05	0.20	0.23	0.30	0.15	-0.07	-0.03	-0.12	0.23	**0.77**				
	0.84	0.42	0.36	0.22	0.54	0.79	0.89	0.63	0.35	**<0.01**				
Aerobic stability	-0.25	-0.36	0.07	0.31	-0.28	0.27	-0.26	0.22	**0.52**	0.12	0.12			
	0.32	0.15	0.78	0.21	0.26	0.28	0.29	0.38	**0.03**	0.64	0.65			
Mould score	0.19	0.37	-0.19	-0.16	0.36	-0.14	0.20	-0.05	**-0.47**	0.07	0.09	**-0.71**		
	0.44	0.13	0.44	0.54	0.14	0.57	0.42	0.83	**0.04**	0.77	0.71	**<0.01**		
Yeast	0.22	0.09	0.11	-0.23	-0.14	-0.46	-0.33	-0.22	-0.17	-0.11	-0.17	**-0.57**	0.14	
	0.39	0.72	0.66	0.36	0.58	0.06	0.18	0.39	0.49	0.67	0.50	**0.01**	0.58	
Mould	**0.63**	**0.68**	-0.07	-0.17	0.44	**-0.50**	-0.19	**-0.52**	-0.34	0.37	**0.48**	-0.22	**0.48**	0.04
	0.01	<0.01	0.77	0.51	0.07	**0.04**	0.46	**0.03**	0.17	0.13	**0.04**	0.37	**0.04**	0.87

Figure 1 Pearson correlations between the chemical composition, fermentation quality, aerobic stability and microbial quality parameters of the silage samples. Significant correlations are highlighted in bold. Correlation coefficient is followed by its P-value.

Conclusions

This study identified through correlations that well-preserved silages at farm level, with less contamination by mould and yeast, have longer aerobic stability. Additionally, the aerobic stability of the silages was increased by a greater production of acetic acid.

Acknowledgements

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Effects of cultivars and additives on preservation quality and antinutritional factors of crimped ensiled faba bean seeds

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Introduction

One of the goals of the European Union is to achieve protein self-sufficiency. Faba bean is an annual legume whose profile of amino acids is similar to that of soybean, and that could, therefore, substitute soybean in livestock feeding. Due to its rather long growing time and humid weather conditions during autumn, there may be difficulties in harvesting fully ripened seeds under Northern conditions. Thus, crimping and ensiling could provide a more competitive option for feed preservation than drying. Further, utilization of faba beans is limited especially in pig and poultry feeding due to antinutritional factors, such as vicine and convicine, tannins and oligosaccharides. Crimping and ensiling could be a cost-effective option for preserving faba bean seeds and simultaneously degrading their antinutritional factors (Rinne *et al*., 2020).

Materials and Methods

The faba bean seeds used in this experiment were obtained from Boreal Plant Breeding Ltd. (Jokioinen, Finland). The cultivars selected were Kontu, which is characterized by high vicine and convicine (V+C) concentrations and Vire, which was developed through plant breeding to contain lower levels of V+C. Dried beans were moistened for 1 day with tap water to reach a moisture content of ca. 350 g/kg and crimped using a pilot scale crimper (Nipere Ltd., Teuva, Finland). The moist beans were ensiled using 3 additive treatments that included Control (without additive), a combination of hetero- & homofermentative lactic acid bacteria inoculant (LAB; Kofasil Duo, Addcon, Bitterfeld-Wolfen, Germany) at 1 g/t [2×10^5 cfu / g fresh matter] and a formic and propionic acid based additive (FAPA; AIV Ässä Na, Eastman, Oulu, Finland at 5 l/t). Five replicates per treatment were used so that 4 replicates were plastic vacuum bags and 1 replicate was a 5 L capacity cylindrical silo. After 80 days of storage, the samples were analysed for fermentation quality, aerobic stability and antinutritional factors as described by Rinne *et al*. (2020). Statistical analyses were performed using a PROC MIXED statement of SAS 9.4 with variety and additive as fixed effects and replicate as a random effect. Tukey test was used to establish pairwise comparisons of treatment means and treatment effects were further analysed using contrasts.

Table 1 Chemical composition of the two faba bean cultivars before ensiling

Item	Kontu	Vire
Dry matter (DM), g/kg	612	655
Buffering capacity, g lactic acid/100 g	5.3	5.5
In DM, g/kg		
Ash	37	38
Crude protein	326	277
Starch	379	404
Water soluble carbohydrates	44	47
Neutral detergent fibre	123	126
Vicine	5.53	0.63
Convicine	3.23	0.03
Condensed tannins (proanthocyanidins), mg/100 g	366 (7.85; 36/64)[1]	1620 (10.6; 30/70)

[1] Presented as follows: Content (degree of polymerization; procyanidins / prodelphinidins).

Table 2 Preservation quality of two varieties of crimped faba bean (Kontu and Vire) ensiled without additive (C) or using a lactic acid bacteria inoculant (LAB) or formic and propionic acid-based additive (FAPA)

Additive	C		LAB		FAPA		SEM[1]	Statistical significance			
Variety	Kontu	Vire	Kontu	Vire	Kontu	Vire		Variety	C vs LAB	C vs FAPA	LAB vs FAPA
Dry matter (DM), g/kg	630^{b}	656^{a}	632^{b}	661^{a}	636^{b}	662^{a}	3.4	<0.001	0.282	0.068	0.421
Moisture content, g/kg	370^{a}	344^{b}	368^{a}	339^{b}	364^{a}	338^{b}	3.4	<0.001	0.282	0.068	0.421
pH	4.90^{b}	5.11^{a}	4.61^{c}	4.64^{c}	4.56^{c}	4.65^{c}	0.046	0.008	<0.001	<0.001	0.718
Ammonia N, g/kg N	35.8^{a}	29.0^{bc}	34.5^{ab}	25.3^{c}	12.0^{d}	11.3^{d}	1.36	<0.001	0.080	<0.001	<0.001
In DM, g/kg											
Ethanol	9.1^{b}	14.5^{a}	5.4^{d}	7.6^{c}	2.2^{e}	3.0^{e}	0.30	<0.001	<0.001	<0.001	<0.001
Water soluble carbohydrates	1.0^{b}	1.5^{b}	1.1^{b}	0.5^{b}	23.4^{a}	22.9^{a}	1.57	0.860	0.772	<0.001	<0.001
Lactic acid	31.5^{a}	28.1^{ab}	35.9^{a}	35.7^{a}	18.7^{b}	17.4^{b}	2.40	0.412	0.020	<0.001	<0.001
Acetic acid	6.7^{abc}	7.3^{ab}	7.6^{ab}	8.8^{a}	3.1^{c}	4.1^{bc}	0.80	0.160	0.138	<0.001	<0.001
Propionic acid	0.11^{a}	0.04^{a}	0.04^{a}	0.04^{a}	0^{b}	0^{b}	0.020	0.030	0.094	<0.001	<0.001
Butyric acid	0.04^{a}	0.02^{b}	0.01^{b}	0.01^{b}	0.01^{b}	0.01^{b}	0.003	0.002	<0.001	<0.001	0.702
Vicine	0.03^{c}	0.06^{c}	0.07^{c}	0^{c}	2.81^{a}	0.54^{b}	0.035	<0.001	0.826	<0.001	<0.001
Convicine	0.25^{c}	0^{c}	1.16^{b}	0^{c}	2.64^{a}	0.03^{c}	0.075	<0.001	<0.001	<0.001	<0.001
Condensed tannins, mg/100g[2]	73	736	123	951	139	942	--				
Aerobic stability 2°C, hours[3]	172	186	196	223	218	227	21.0	0.261	0.089	0.021	0.474

[a,b,c,d,e] Values within a row with different superscripts differ significantly at $P < 0.05$

[1] SEM, standard error of the mean

[2] Condensed tannins (proanthocyanidins) were analysed only from a single sample per treatment

[3] The length of the observation period was 238 h

Results and Discussion

The chemical composition of crimped faba beans is in Table 1. Expectedly, Vire had lower V+C concentration than Kontu, but also lower CP and higher starch and tannin concentrations. After ensiling, Kontu had slightly higher moisture content than Vire (Table 2). Vire had lower ammonia-N concentration in both Control and LAB-treated seeds compared to Kontu but did not differ when treated with FAPA. In contrast, ethanol concentration was higher in Vire than Kontu only with Control and LAB. Both LAB and FAPA additive treatments decreased pH and ethanol concentration compared to Control, although Control could also be regarded as well preserved. The lower concentrations of ammonia, ethanol, lactic acid and acetic acid in FAPA-treated seeds compared to Control and LAB, irrespective of the cultivar studied, suggest a restriction in fermentation. Ensiling was effective in reducing the antinutritional factors, but the restriction of fermentation by FAPA seemed to also protect V+C from degradation which was more pronounced in Kontu compared to Vire. Tannin degradation was not influenced by the additives used (Figure 1).

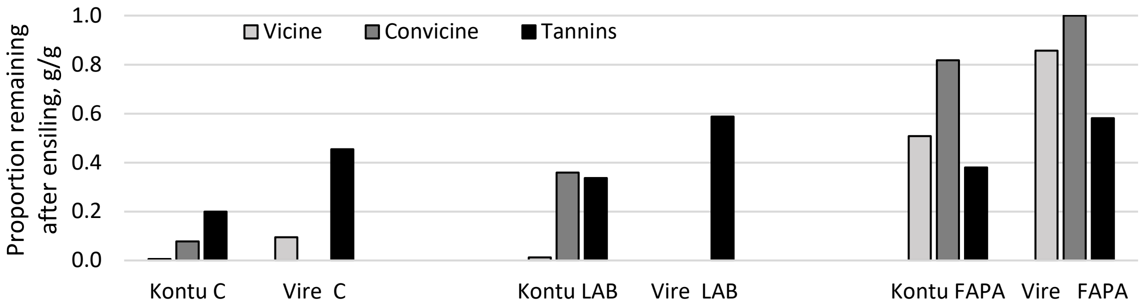

Figure 1 Proportion of antinutritional factors remaining after ensiling two varieties of crimped faba bean (Kontu and Vire) ensiled without additive (C) or using lactic acid bacteria inoculant (LAB) or formic and propionic acid-based additive (FAPA). Vicine: Variety <0.001; C vs LAB = 0.364; C vs FAPA <0.001; LAB vs FAPA <0.001; SEM = 0.043; Convicine: Variety <0.001; C vs LAB <0.001; C vs FAPA <0.001; LAB vs FAPA <0.001; SEM = 0.026.

Conclusions

Crimping and ensiling moist faba beans offers a practical alternative to drying seeds and preservation quality was further improved by using LAB and FAPA additives. The Vire cultivar contained markedly lower V+C concentrations than Kontu, and they were effectively degraded in both cultivars during fermentation except when FAPA was used as an additive, probably due to restriction of fermentation. Tannin concentration in Vire was higher and seemed to be more resistant to degradation than in Kontu, but degradation was unaffected by additives used.

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Grass for biorefinery: effect of additive treatment on fermentation quality of ensiled intact grass and pulp

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Introduction

Novel business models are being developed to valorize green biomasses in multiple ways. The concept of green biorefinery involves mechanically separating grass into liquid and solid fractions. After the removal of soluble components by mechanical pressing, the pulp can be stabilized by ensiling which can be used *e.g.*, as ruminant feed (Savonen *et al*., 2020). Furthermore, green biorefinery presents an alternative to utilizing grass with low dry matter (DM) content which can minimize the risk of effluent production and poor fermentation through increased DM concentration. The aim of the current experiment was to evaluate the preservation quality of ensiled pulp compared to intact grass, and how additives with different modes of action affect it.

Materials and Methods

The grass material was harvested on 15 June 2023 from a primary growth of pure timothy (*Phleum pratense*) stand grown in Jokioinen, Finland (60°48′N 023°29′E). The grass was mowed and precision chopped immediately after cutting. Growing conditions were cool and very dry during early summer. The fresh grass material was processed and separated into liquid and solid fractions (pulp) using a pilot scale single screw press (Smicon MAS SP300 filter press, Haderslev, Denmark). Water was added to the fresh grass material during the pressing process to “wash” out soluble components into the liquid due to its high DM content. The ensiling experiment was performed using both intact grass material (FR) and pulp obtained from the green biorefinery process which were ensiled using 3 additive treatments. Additive treatments consisted of: Control (without additive), a combination of homo- and heterofermentative lactic acid bacteria (LAB; Kofasil Duo, Addcon, Bitterfeld-Wolfen, Germany at 1 g/t [2×10^5 cfu / g fresh matter]) and a formic and propionic acid-based additive (FAPA; AIV Ässä Na, Eastman, Oulu, Finland at 5 l/t). Four replicates were used for each treatment, stored in plastic vacuum bags for 88 days. After opening, samples were analysed for fermentation quality, aerobic stability and chemical composition. The experimental data was analysed statistically using SAS MIXED procedure (SAS 9.4) with material type, additive and their interaction used as fixed effects and replicate as random effect. Tukey test was also done and statistical significance was considered at $P < 0.05$.

Table 1 Chemical composition of the intact grass and pulp materials before ensiling

Item	Fresh	Pulp
Dry matter (DM), g/kg	276	249
Buffering capacity, g lactic acid/100 g DM	4.89	3.90
Fermentation coefficient	55	58
In g/kg DM		
Ash	70	66
Crude protein	146	145
Water soluble carbohydrates	169	160
Nitrate-N	0.011	0.006
Neutral detergent fibre	492	549
Organic matter digestibility, g/g OM	0.837	0.834
D-value, g/kg DM	778	779

Table 2 Chemical composition and fermentation quality of ensiled intact grass and pulp treated without additive (Control), lactic acid bacteria (LAB) or formic and propionic acid based additive (FAPA)

Additive (Add)	Control		LAB		FAPA		SEM[1]	Statistical significance		
Material type (MT)	Fresh	Pulp	Fresh	Pulp	Fresh	Pulp		MT	Add	MT×Add
Dry matter (DM), g/kg	268^{b}	244^{c}	277^{ab}	242^{c}	279^{a}	249^{c}	2.2	<0.001	0.006	0.073
pH	4.05^{a}	3.74^{cd}	3.76^{cd}	3.92^{b}	3.81^{c}	3.71^{d}	0.018	<0.001	<0.001	<0.001
Ammonia N, g/kg total N	47.9^{a}	32.3^{bc}	33.6^{b}	33.4^{b}	28.3^{cd}	24.7^{d}	0.92	<0.001	<0.001	<0.001
In g/kg DM										
Ethanol	31.8^{b}	43.5^{a}	14.7^{c}	13.3^{cd}	9.6^{d}	8.9^{d}	1.12	0.002	<0.001	<0.001
Water soluble carbohydrate	50.4^{ab}	23.6^{c}	70.8^{a}	44.4^{bc}	43.8^{bc}	38.1^{bc}	5.75	<0.001	0.003	0.109
Lactic acid (LA)	66.5^{b}	97.3^{a}	105.5^{a}	65.3^{b}	63.1^{b}	59.9^{b}	2.65	0.069	<0.001	<0.001
Acetic acid (AA)	20.6^{bc}	15.8^{c}	25.3^{b}	47.1^{a}	26.8^{b}	27.0^{b}	1.53	<0.001	<0.001	<0.001
Propionic acid	0.06^{a}	0.04^{b}	0.10^{b}	0.04^{b}	0^{c}	0^{c}	0.008	0.002	<0.001	0.005
Butyric acid	2.46^{a}	0.05^{b}	0.04^{b}	0.04^{b}	0.06^{b}	0.05^{b}	0.053	<0.001	<0.001	<0.001
Total volatile fatty acids	23.2^{b}	15.9^{c}	25.4^{b}	47.2^{a}	26.8^{b}	27.0^{b}	1.52	0.001	<0.001	<0.001
Total fermentation acids	89.7^{c}	113.2^{b}	130.9^{a}	112.5^{b}	89.9^{c}	86.9^{c}	3.53	0.804	<0.001	<0.001
Total fermentation products	121.5^{b}	156.8^{a}	145.5^{a}	125.7^{b}	99.5^{c}	95.9^{c}	2.91	0.113	<0.001	<0.001
LA to AA ratio	3.42^{b}	6.16^{a}	4.20^{b}	1.40^{d}	2.36^{c}	2.23^{cd}	0.211	0.709	<0.001	<0.001
Aerobic stability, 2°C, h[2]	87.6^{b}	41.6^{b}	171.7^{a}	155.8^{a}	171.7^{a}	171.7^{a}	10.80	0.025	<0.001	0.112
Aerobic stability, 3°C, h[2]	101.0^{b}	92.0^{b}	171.7^{a}	171.7^{a}	171.7^{a}	171.7^{a}	15.47	0.815	<0.001	0.945

^{a,b,c,d,}Values within a row with different superscripts differ significantly at $P < 0.05$

[1] SEM, standard error of the mean

[2] The length of the observation period was 171.7 h.

Results and Discussion

The chemical composition of the fresh grass and pulp are in Table 1. Due to dry growing conditions, DM content of FR was relatively high while addition of water during the liquid-solid separation reduced DM in the pulp by 10% but with a similar crude protein (CP) concentration. We expected the pulp to be drier with lower CP than FR, but the press used was of low efficiency. Both materials were regarded as easy to ensile due to a high value of the fermentation coefficient which was above 45 (threshold for easy to ensile raw material). The high NDF concentration in the pulp was expected due to removal of solubles during mechanical processing (Franco *et al*., 2019) while the D-value was unaffected. The proportion of ammonia N in total N of all silages was below 50 g/kg N which indicates that they were all fermented. FR Control showed elevated butyric acid concentration, which was eliminated by additive treatments and pulping. Ethanol concentration was elevated in Control compared to other additive treatments, and even higher in pulp than FR. LAB decreased lactic acid production on pulp compared to Control while an opposite effect was observed in FR.

Conclusions

Ensiling of pulp was not markedly impaired compared to fresh grass indicating that ensiling is a viable approach to stabilize the solid fraction of green biomass after removal of solubles. In case of no additive use, pulp even improved fermentation quality as indicated by elimination of butyric acid formation. When treated with LAB, the pulp had a high acetic acid concentration. FAPA produced consistently good fermentation quality in both raw materials. Use of both additives improved the aerobic stability of the silages.

Acknowledgements

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Examining the effect of silage inoculants containing only homo-fermentative or a combination of homo- and hetero-fermentative strains on fermentation of grass ensiled immediately after cutting or wilted for one day and rewetted by rain or one day wilted, rewetted by rain and wilted another day

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Introduction

Wilting grass is a common practice to improve fermentation and minimise the risk of Clostridia growth in silage. However, controlled wilting to an optimal dry matter content can be difficult due to the weather. Grass can either become too wet or dry, and a rain shower can ruin the effects of careful preparation. This study aimed to determine whether specific biological silage inoculants enable a butyric acid-free and aerobically stable silage, regardless of whether when ensiled immediately after cutting, after wilting and a rain shower, or after a rain shower and an additional day of wilting.

Materials and Methods

The herbage originated from one field located in the northwest of Germany. The dominant crops of the meadow were perennial ryegrass (*Lolium perenne*), timothy grass (*Poa pratensis* L.), meadow foxtail (*Alopecurus pratensis*) and tall oat grass (*Arrhenatherum elatius*) at maturity stage between middle and end of heading. The grass was cut in the afternoon. One part of the grass was collected and ensiled immediately after the cut (UNW). Another part of the grass was ensiled after a natural short rain of 2 mm after one day of wilting (RAIN). The third part of the grass was wilted again by leaving it on the field for another day and then ensiled (DRY). All three harvest conditions were ensiled untreated (CON) or treated with three bacterial inoculants. MC treatment was inoculated with SiloSolve® MC, containing *Enterococcus lactis* (DSM22502), *Lactiplantibacillus plantarum* (DSM26571) and *Lactococcus lactis* (NCIMB30117). AS treatment was inoculated with SiloSolve® AS, containing *Lentilactobacillus buchneri* (DSM22501), *Lactiplantibacillus plantarum* (DSM26571) and *Enterococcus lactis* (DSM22502). FC treatment was inoculated with SiloSolve® FC, containing *Lentilactobacillus buchneri* (DSM22501) and *Lactococcus lactis* (DSM11037). The inoculation rate was 150,000 total CFU/g forage. Ten mini-silos (1.5-l volume) for each treatment and harvest condition (3 harvest conditions x 4 treatments x 10 mini-silos) were filled with crop (target density 150 kg DM/m^3 for UNW and RAIN and 240 kg DM/m^3 for DRY). After 70 days of fermentation at room temperature, five mini-silos per treatment were opened and sampled for proximate analysis, forage hygiene, and the fermentation profile. The content from the other five mini-silos was subjected to an aerobic stability test. Aerobic stability was defined as the time (hours) for the silage temperature to exceed 3.0°C above the ambient temperature. Data were analysed as a completely randomised design using PROC GLM procedure (SAS 9.4) with treatment as a fixed effect separately for each dry matter level. The effects were considered statistically significant when $P < 0.05$.

Results and Discussion

The crop before ensiling had a sugar content >20 % dry matter (DM) for all three DM levels. The fermentability coefficient (Schmidt et al., 1971) was 60.6 for UNW, 51.4 for RAIN, and 86.9 for DRY grass. The grass could be considered "easy to ensile", irrespective of DM level. Results of the fermentation of UNW grass are in Table 1.

Table 1 Characteristics of grass silages ensiled immediately after the cut untreated (CON) or treated with SiloSolve® MC (MC), SiloSolve® AS (AS), and SiloSolve® FC (FC)

Item	Unit	CON	MC	AS	FC	SEM
DMcorr[1]	%	21.6^{a}	20.0^{bc}	20.5^{b}	19.6^{c}	0.28
DM loss	%	8.15^{b}	9.03^{b}	5.48^{c}	11.09^{a}	0.55
pH		4.12^{b}	3.85^{c}	3.86^{c}	4.35^{a}	0.01
Lactic acid	% DM	8.04^{c}	13.84^{a}	12.92^{b}	3.33^{d}	0.19
Acetic acid	% DM	3.84^{b}	0.64^{d}	2.08^{b}	9.36^{a}	0.08
n-Butyric acid	% DM	0.29^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.05
Ammonia-N	% total N	7.80^{a}	3.69^{b}	5.75^{b}	7.46^{ab}	0.25
Ethanol	% DM	1.03^{b}	5.39^{a}	0.93^{b}	1.46^{b}	0.05
1,2-Propanediol	% DM	0.72^{b}	0.00^{c}	0.00^{c}	6.60^{a}	0.08
Yeasts	log$_{10}$ CFU/g	Nd[2]	5.49^{a}	3.06^{b}	Nd	0.22
Aerobic stability	hours	220^{b}	29^{d}	136^{c}	602^{a}	28.8

[1] DMcorr = dry matter content corrected for volatiles; [2] Nd = value below the limit of detection (< 2 log$_{10}$ CFU/g) Means with different superscripts within the row differed at $P < 0.05$.

All inoculants prevented formation of butyric acid in the UNW grass silage. MC silage, fermented with only homofermentative strains and had the shortest aerobic stability.

Table 2 Characteristics of grass silages ensiled after 24 hours of wilting and immediately after rain shower, untreated or treated with SiloSolve® MC (MC), SiloSolve® AS (AS), and SiloSolve® FC (FC)

Item	Unit	CON	MC	AS	FC	SEM
DMcorr	%	21.1^{b}	22.4^{a}	22.6^{a}	22.2^{a}	0.19
DM loss	%	11.16^{a}	4.51^{b}	6.17^{b}	10.93^{a}	0.11
pH		4.22^{b}	3.95^{c}	3.94^{c}	4.51^{a}	0.01
Lactic acid	% DM	9.61^{b}	13.31^{a}	13.01^{a}	4.07^{c}	0.30
Acetic acid	% DM	4.65^{b}	0.68^{d}	2.11^{c}	7.65^{a}	0.12
n-Butyric acid	% DM	0.60^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.03
Ammonia-N	% total N	10.29^{a}	4.31^{c}	7.25^{b}	11.03^{a}	0.44
Ethanol	% DM	1.65^{a}	0.73^{c}	0.96^{b}	1.64^{a}	0.04
1,2-Propanediol	% DM	0.82^{b}	0.00^{d}	0.39^{c}	5.52^{a}	0.03
Yeasts	log$_{10}$ CFU/g	Nd[2]	5.16	Nd	Nd	0.12
Aerobic stability	hours	680^{a}	119^{c}	144^{c}	382^{b}	20.27

[1] DMcorr = dry matter content corrected for volatiles; [2] Nd = value below the limit of detection (< 2 log$_{10}$ CFU/g) Means with different superscripts within the row differed significantly at $P < 0.05$.

All inoculants prevented formation of butyric acid in the grass silage ensiled after the rain shower (Table 2). Untreated silage had an increased level of butyric acid ($p<0.05$)and the most prolonged aerobic stability compared to inoculated silages. The effects of the inoculants on other fermentation parameters were similar to those of the silages ensiled directly (UNW).

Table 3 Characteristics of grass silages ensiled after an additional day of wilting untreated or treated with SiloSolve® MC (MC), SiloSolve® AS (AS), and SiloSolve® FC (FC)

Item	Unit	CON	MC	AS	FC	SEM
DMcorr	%	55.4^{a}	53.8^{b}	55.0^{a}	55.6^{a}	0.27
DM loss	%	6.27^{a}	4.37^{b}	4.34^{b}	4.99^{b}	0.25
pH		5.53^{a}	4.55^{d}	4.62^{c}	4.67^{b}	0.01
Lactic acid	% DM	1.75^{d}	6.20^{a}	5.84^{b}	3.53^{c}	0.07
Acetic acid	% DM	0.60^{b}	0.41^{c}	0.45^{c}	1.71^{a}	0.02
Ammonia-N	% total N	4.69^{b}	4.12^{c}	4.21^{c}	5.18^{a}	0.05
Ethanol	% DM	2.31^{a}	0.69^{b}	0.65^{b}	0.69^{b}	0.18
Yeasts	$\log_{10}$ CFU/g	3.63^{a}	2.33^{b}	2.41^{ab}	Nd[2]	0.42
Aerobic stability	hours	151^{c}	231^{b}	235^{b}	$>710^{a}$	12.14

[1]DMcorr = dry matter content corrected for volatiles; [2]Nd = value below the limit of detection (< 2 $\log_{10}$ CFU/g) Means with different superscripts within the row differed significantly at $P < 0.05$.

An additional day of wilting resulted in butyric acid-free silages across all treatments (data not shown). However, CON silage had the shortest aerobic stability (Table 3). FC silage had a moderately increased acetic acid level and the lowest contamination of yeasts and remained stable until the end of the aerobic stability test, which lasted 30 days.

L. buchneri converts lactic acid to acetic acid, 1,2-propanediol and ethanol (Oude Elferink et al., 2001). Our study showed that the extent of this process in the silage depends on DM content and other LAB strains added to the silage inoculant. *Lactococcus lactis* as a second strain and low DM led to high levels of acetic acid and 1,2-propanediol. In contrast, *L. plantarum* and *E. lactis* seemed to impact *L. buchneri,* resulting in lower levels of acetic acid in the silage and shorter aerobic stability.

Conclusions

Silage inoculants may prevent butyric acid formation by Clostridia if high sugar containing grass should be ensiled under non-optimal conditions. Inoculation with strains producing mainly lactic acid may negatively impact aerobic stability. If the weather allows for a quick wilting of the grass after a rain shower, inoculation with SiloSolve® FC can be recommended as the best technique, allowing prolonged aerobic stability in addition to good fermentation.

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Vacuum storage of moist grain

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Introduction

In Sweden, grain is generally harvested with a high water content preventing stable storage. To avoid negative consequences of microbial growth, grain needs to be dried directly after harvest. However, by preserving grain moist, energy-consuming drying can be avoided. Wet storage methods are mostly based on adding preservatives often combined with restricted oxygen supply. Propionic acid and their mixtures are the most widely used preservatives for feed grains (Jonsson & Pettersson, 1999). Airtight storage systems allow use of other treatmemts such as bacterial innoculants. In normal airtight storage, the respiratory activity of grain is used to create an oxygen-free environment that maintains good grain hygiene. However, this process prolongs survival of unwanted microflora (mould, enterobacteria) and thereby reduces the potential for added microflora to dominate the storage process. To speed up airtightness, the air in the storage space can be removed in order to establish vacuum. The aim ot this study was to evaluate a potential of vacuum in storage of moist grain treated with a variety of mictobial addditives.

Materials and Methods

Dried winter wheat grain was used in the experiments. The grain was moisturized to a water content of 26% by adding water to the grain placed in 125 L plastic bags and mixed properly. Two doses of the yeast *Wickerhamomyces anomalus* (previously named *Pichia anomala* or *Hansenula anomala*) J121 and two doses of a lactic acid bacteria (LAB) based additive were tested and compared to traditional treatment with propionic acid and to an untreated control. *W. anomalus* J121 was obtained from the Swedish University of Agricultural Sciences (SLU), Department of Molecular Sciences. The LAB treatment consisted of a mixture of *Lactobacillus lactis* and *Lactobacillus buchneri* (both in concentration 6.2x10^{10} cfu/g, Silosolve FC by Chr. Hansen, Czech Republic). Untreated grain was used as a negative control while propionic acid-treated grain was used as a positive control (Table 1). Treated grain was stored in vacuum bags (6 liters) where air was sucked out through a valve using a vacuum cleaner. To evaluate effectiveness of vacuum storage, all treatments were also stored in lab silos (glass jars with a volume of 1.7 liters) equipped with fermentation lock on the lid to prevent air entry. Both bags and silos were stored for 180 days at temperatures ranging from 3 to 21 °C. Microbial and chemical analyses were performed on grain prior and efter storage. Dry matter content was analyses by drying of samples in 130°C for 19 hours, water activity was measted using an AquaLab (Decagon Devices Inc. Pullman, Washington, USA). Analysis of yeast and mould colonies was performed by cultivation of homogensied samples on malt extract agar (MEA) in 25 °C for 5 days. Plates for yeast identification was supplemented with chloramphenicol and for molds cycloheximide was also added to inhibit yeast overgrowth. Silos were weighed at the time of filling and at the end of storage to determine weight losses. Statistical analyzes were performed using the GLM procedure in the SAS computer package (SAS Institute Inc., 1990). Analysis of variance in a completely randomized design was used to evaluate the effect of application addition on silage quality. Differences between effects were calculated by t-test and expressed as least significant difference (LSD, P <0.05).

Tabel 1 List of treatments used in the test

Treatment	Dosage (l/t)
Control	-
Propionic acid (90%)	5
LAB 1 (*L. lactis, L. buchneri*; 10^5 cfu/g)	2
LAB 2 (*L. lactis, L. buchneri*; 10^6 cfu/g)	2
Yeast 1 (*W. anomalus*; 10^3 cfu/g)	2
Yeast 2 (*W. anomalus*; 10^4 cfu/g)	2

Results and Discussion

The chemical and microbial composition of harvested winter wheat before and after soaking is presented in Table 2. Winter wheat contained 11.5% water content, which corresponded to water activity of 0.494. Microbial composition showed fairly low numbers of molds with a high number of yeasts, which was not affected by water addition.

Table 2 Water content, water activity (a_w) and microbial composition of wheat grain before test

Wheat status	Water content	a_w	Yeasts	Moulds
	%		log cfu/g	
Before water addition (n=2)	11.5	0.494	5.45	3.71
After water addition (n=3)	26.2	0.939	5.34	2.67

Microbial analyses showed that the vacuum bags were generally tight as no significant mold presence was observed in the majority of samples (Tables 3, 4). Differences between silos and vacuum bags were negligible. Theoretically, a better result was expected from vacuum bags. The low mould count in the grain was probably responsible for the lack of effect of vacuum. On the other hand, a high prevalence of yeasts was observed in all treatments, except in the propionic acid treatment. An increased presence of yeasts is not suprising in treatments with *W. anomalus*. *W. anomalus* acts by out-competing other microflora in presence of oxygen. This phenomenon was observed in a previous study (Melin et al. 2006) where the amount of added mould spores was actually reduced after storage with *W. anomalus* compared to the control.

Addition LAB in this study had no effect on growth of yeasts. This is in contrast to da Silva et al (2018) who used identical dosages. However, da Silva et al (2018) used maize grain at a higher water content (36%), which probably promoted growth of LAB. Another difference may have been the nutritional composition of the cereals. More mature grain, as in our study, contains a smaller proportion of readily available substrate (carbohydrates) for LAB, eliminating their preservation ability. In addition, abundance of yeasts in the wheat grain gave a competitive advantage to them over LAB.

Table 3 Chemical and microbial composition of wheat garin after 180 days of storage in vacuum bags (n=3)

Treatment	Water content	a_w	Mould	Yeast	Losses
	%		log cfu/g		% DM
Control	26.9	0.957	1.3	6.4	0.021
Propionic acid	26.3	0.935	<1.0	<1.0	0.005
LAB 1	27.1	0.968	<1.0	6.2	0.021
LAB 2	26.9	0.962	<1.0	6.1	0.022
Yeast 1	26.9	0.960	<1.0	7.2	0.020
Yeast 2	26.8	0.960	<1.0	7.5	0.021
LSD	**0.28**	**0.0156**	**0.76**	**0.32**	**0.003**

Table 4 Chemical and microbial composition of wheat grain after 180 days of storage in silos (n=3)

Treatment	Water content	a_w	Mould	Yeast	Losses
	%		log cfu/g		% DM
Control	27.1	0.957	1.0	7.0	0.022
Propionic acid	26.4	0.935	<1.0	<1.0	0.008
LAB 1	27.0	0.951	<1.0	7.1	0.021
LAB 2	27.0	0.950	<1.0	6.7	0.021
Yeast 1	26.9	0.951	<1.0	7.0	0.020
Yeast 2	26.7	0.952	<1.0	7.0	0.023
LSD	**0.68**	**0.0049**	**0.44**	**0.32**	**0.005**

Conclusions

This study showed that vacuum was not better than other airtight storage. It is possible that this result was influenced by initial low contamination of mould in grain and that the limited amount of molds was out-competed by the yeast. Netiher vacuum nor lactic acid bacteria addition had any effect on yeast growth.

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Rate of NDF degradation from static measures

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Introduction

Feed evaluation systems like NorFor (Volden, 2011a) model nutrient degradation in the rumen based on degradation parameters (potential degradation and fractional rate of degradation (kd)). The basis for model building and parameterization in NorFor (Volden, 2011a) was *in situ* measures for protein, starch and NDF degradation in the rumen. Protein and concentrate NDF kd are still based on *in situ* (table values). For starch, there has been a change to table values, where degradation rates are back calculated from *in vivo* measures based on Moharrery *et al*. (2014). Forage NDF kd was already included at the introduction of NorFor (Volden, 2011b) based on back calculations (Huhtanen *et al*., 2006; Weisbjerg, 2006) using assumptions on neutral detergent solubles (NDS) digestibility (Weisbjerg, 2004) combined with measures or estimates of concentration of ash, NDF, indigestible NDF and digestibility of organic matter.

The aim of the present study was to test this method against *in situ* measures on a sample set of grasses highly varying in development stage.

Materials and Methods

In 2021, 24 plots of monocultures of seven grasses (seven species with 2 to 8 varieties within each species) were harvested at Research Centre Foulum (Denmark) in primary growth at three developmental stages (early, normal and late; 16 days between harvests). The seven grass species were perennial ryegrass (*Lolium perenne* L.), hybrid ryegrass (*Lolium hybridum*), tall fescue (*Festuca arundinacea* Schreb.), festulolium (*Festulolium pabulare*, festuca and lolium types), meadow fescue (*Festuca pratensis* Huds.), timothy (*Phleum pratense* L.), and orchard grass (*Dactylis glomerata* L.). Samples were dried immediately after harvest and then milled (1.5 mm in cutter mill). Milled samples were incubated (Dacron bags with 38 µm pore size) in the rumen in triplicate (one bag in each of three cows) at 0, 2, 4, 8, 16, 24, 48, 96, and 168 h, although some samples at early harvest with limited sample size were incubated using fewer incubation times. Indigestible NDF (INDF) was measured after 288 h incubation in Dacron bags with 12 µm pore size. Sample residues were transferred quantitatively and analysed for ash-free NDF (including heat stable α-amylase). Chemical analyses and *in vitro* (rumen fluid) digestibilities were performed. NDF degradation parameters were estimated, and *in vitro* digestibility transformed to *in vivo*, according to Åkerlind *et al*. (2011).

Backwards estimation of kd was performed using below set of equations:

Neutral detergent solubles (NDS) digestibility is estimated as (Weisbjerg *et al*., 2004):
Apparent NDS digestibility (NDSD, %) = 101.3 – [902/NDS (% DM)].
With known organic matter (OM) digestibility (OMD), undigested NDF is estimated as the difference between undigested OM and undigested NDS:
Undigested NDF (% DM) =
[OM (% DM)*[100-[OMD (%)]]/100] - [NDS (% DM)*[100-[NDSD (%)]]/100].
Digested NDF and NDF digestibility (NDFD) is then calculated as:

Digested NDF (% DM) = NDF (% DM) – undigested NDF (% DM).
NDFD (%) = [100*[NDF (% DM)] - [undigested NDF (% DM]]/[NDF (% DM)].
Potentially digestible NDF (DNDF) digestibility (D) = (kd /(kd +kr) [1 + kr /(kd + kp)]
This equation is solved according to kd (Huhtanen *et al.*, 2006):
$kd = [-(kp + kr)+[(kp + kr)^2+4Dkrkp/(1 - D)]^{0.5}]/2$
where, kr and kp are passage rates from the non-escapable and escapable rumen pools, based on a two-pool model. In the NorFor system (Volden, 2011b), total rumen retention time is assumed to be composed of 40% in the non-escapable pool and 60% in the escapable pool, resulting in passage rates of 4.20 and 2.78 %/h from the two pools, respectively, with a total mean retention time (MRT) of 60 hours.

Results and Discussion

Overall, the correlation between measured *in situ* kd and kd estimated by back calculation was positive but poor, and the grasses clustered according to development stage. Mean statistics per development stage are in Table 1 and show both measured *in situ* kd and kd calculated with an assumed MRT ranging from 20 to 60 hours for comparison.

Table 1 Summary statistics for early, normal, and late harvest of grasses in spring growth

	Early (n=20)	SD	Normal (n=24)	SD	Late (n=23)	SD
Ash (g/kg DM)	81	7	85	9	65	7
NDF (g/kg DM)	338	46	469	60	566	83
INDF (g/kg NDF)	55	18	67	14	132	37
D (g/kg)	860	31	800	33	770	40
OMD (g/kg OM)	841	21	776	34	704	60
kd *in situ* (%/h)	14.21	3.66	8.85	1.95	5.45	0.87
kd20 (%/h)	17.35	2.81	12.70	1.88	11.15	1.98
kd30 (%/h)	11.55	1.87	8.46	1.25	7.42	1.32
kd40 (%/h)	8.71	1.41	6.38	0.95	5.60	1.00
kd50 (%/h)	6.94	1.13	5.08	0.75	4.46	0.79
kd60 (%/h)	5.81	0.94	4.25	0.63	3.73	0.66

D = potentially digestible NDF (DNDF) digestibility; OMD = *in vivo* sheep organic matter digestibility; kd = fractional rate of DNDF degradation.

For early harvests, average *in situ* kd was between average kd estimated at MRT at 20 and 30 hours; for normal harvest it was between 20 and 30 hours and for late harvest between 40 and 50 hours. A distribution with 40% in non-escapable pool and 60% in escapable pool were used in the present calculations as in the NorFor system for all MRT. Earlier, the *in vitro* method (gravimetric) has been found to give higher kd values compared to the *in situ* method (Bossen *et al.*, 2008). And, *in vitro* (gas production) has been found to give kd values corresponding well to obtained *in vivo* digestibilities (Huhtanen *et al.*, 2008). Thereby, results obtained in the present study, where the *in situ* method seemed to overestimate kd for early harvested grass, were surprising, and very short (and probably unrealistic) MRT were required to obtain calculated kd comparable to *in situ* kd. Alternatively, other measures and assumptions used for the kd calculation were biased with development stage, e.g secondary loss of particles from the bags for early harvested grass. It however also indicates that the 60 h MRT used in the NorFor system might be an overestimation of MRT in sheep at maintenance and, sheep fed at maintenance, is the basis for the *in vitro*/*in vivo* digestibility measures used for the back calculations.

Conclusions

The present study indicates that estimation of kd for NDF in forages, based on back calculations, should be revisited regarding assumptions used in the calculations.

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Residual weight loss of grass and grass-clover silages dried at 103°C after pre-drying at 60°C

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Introduction

The NorFor feed evaluation system defines dry matter of forages as constant weight at 60°C corrected for loss of volatiles (Volden, 2011). In grass-based forages there are a large variation in residual weight loss at 103°C in milled samples pre-dried at 60°C. The weight loss at 103°C might represent water taken up by the samples when equilibrating with air or represent residual water and other components retained in the sample during drying at 60°C. The aim of the present study was to describe residual weight loss in grass and clover-grass silages pre-dried at 60°C. It was investigated if residual weight loss represents material retained in the sample during pre-drying or if it represents water gained after drying. It was also investigated if residual weight loss of grass silages are correlated to any routinely predicted chemical fraction or property of the samples and how correction for residual weight loss impact calculation of OMD based on an in vitro assay.

Materials and Methods

Residual dry matter loss in grass was investigated using 267 samples of grass and clover-grass silage submitted for routine analysis at Kvægbrugets Forsøgslaboratorium, SEGES Innovation P/S, Skejby, Denmark. The samples were reduced to 300 to 400 g and weighed into aluminum trays measuring 320 x 260 x 40 mm. For drying at 60°C, samples were incubated in a forced air oven for at least 40 h (Termaks, A/S Ninolab, Køge, Denmark; Model TS9430, fan speed 80%, air valve 100% open, 6 wire shelves per oven, sample trays covering approx. 71% of shelf surface, placed in alternating order on shelves). Samples were weighed out warm and milled on a 0.5-mm screen using a cyclone mill (Peppink 200CM, Peppink Mills b.v., Olst, Holland).

After milling, samples were thoroughly mixed inside PE bags, subjected to NIR scanning (near infrared spectroscopy; 97 mm cups on sample rotator using a Bruker MPA, Bruker, Ettlingen, Germany), and subsampled for residual dry matter loss within 3 to 4 h after milling. Approximately 50 g milled sample was weighed into aluminum trays measuring 214 x 110 x 54 mm. Trays were placed in a forced air oven at 103°C for 14 h with automatic timer shut down (Termaks, A/S Ninolab, Køge, Denmark; Model TS8136, air valve fully open, fan speed 4). If the oven temperature was below 90°C at the beginning of the next working day the oven was restarted at 103°C for 1 h before weighing. Samples were weighed out warm.

A subset of 24 samples was selected for investigations on properties of residual weight loss at 103°C. The main inclusion criterium was the amount of sample received, 1 kg of wet matter was required for inclusion of the sample. Two of the samples were NorFor grass silage references stored at -20°C. The selected samples originated from Denmark and Germany. Samples were reduced and subsampled by coning and quartering. Three subsamples each of 300 to 400 g wet weight were obtained from each original sample and weighed into aluminum trays as described above. Two trays were dried at 60°C (A & B) and one tray (C) directly dried at 103°C. Tray A was analyzed as a routine NIR sample as described above. Tray B was weighed in for drying at 103°C immediately after weighing out from 60°C and the sample was not removed from the tray between drying at 60°C and 103°C. Tray A was milled and scanned using NIR after 60°C drying. Tray B and C were milled and NIR scanned after drying at 103°C. After NIR scanning, all samples were subjected to determination of residual weight loss at 103°C as described above. Finally, all samples were combusted at 550°C for 6.5 h for determination of crude ash.

Dry matter of silages was calculated according to the simple NorFor correction = (dry mass at 60°C (g/kg) x 0.99) + 10. Dry matter determined at 103°C was not corrected. All silages were assigned a NorFor feed code at registration. The silages were either denoted first to fifth clover-grass cut if the information was provided with the sample. Samples of unknown cut were defined as either high or low digestible clover-grass silage. The summary statistics and skewness of the distribution of residual dry matter was calculated using the Proc Summary procedure of SAS. Pearson correlations between variables were calculated using the Proc Corr procedure of SAS. The effect of feed code on residual dry matter was tested by ANOVA using Proc Mixed in SAS using the pdiff option to separate means.

Results and Discussion

Residual dry matter was observed in the range from 873 to 975 g/kg with an average of 926 ± 20 g/kg and a negative skewness (-0.37). The residual dry matter at 103°C differed ($P < 0.01$) among silage types, with the lowest value observed for 1st cut grass-clover (909 ± 3 g/kg) and the highest value observed for low OMD grass-clover silage (950 ± 3 g/kg). Silages designated second to fifth cut as well as high OMD silages (often mixtures of different cuts) had similar average residual dry matter (927 ± 1 g/kg).

The correlation coefficients between residual dry matter at 103°C and the chemical composition of silages predicted based on NIR scanning are in Table 1. Moderate correlations ($r > |0.5|$) between residual dry matter and OMD, soluble crude protein, and sugar were observed. These correlations indicate that the effect of harvest on residual dry matter is not an effect of the regrowth of the grass, however, an effect of the chemical composition of the silage. The strongest correlation to residual dry matter was observed for predicted OMD in the samples ($r = -0.69$, $P < 0.01$). The relatively strong correlation between OMD and residual dry matter is of concern because residual water in the sample might be released during in vitro digestion of the samples and might therefore lead to relative overestimation of OMD in high digestible samples compared to samples with low digestibility. The composition of observed residual dry matter loss at 103°C is not known. However, if it is assumed that dry matter lost at 103°C after pre-drying at 60°C is water and it is assumed that residual water in the samples is completely soluble and/or lost in drying the residue after in vitro digestion of silage samples, the potential error induced from not correcting for residual dry matter loss of silage samples in determining OMD are 2 to 3 OMD units.

To evaluate if residual dry matter loss at 103°C represents water taken up by the sample during equilibration with air or represents an intrinsic property of the sample, residual dry matter of samples with minimum handling were compared with residual dry matter of samples that were milled, thoroughly mixed in PE bags, and transferred to and from NIR scanning cups before being weighed into trays for drying at 103°C. The residual dry matter of milled and NIR scanned samples (A procedure) were highly correlated ($p = 0.92$; $P < 0,01$) with residual dry matter of samples dried at 103°C immediately after drying at 60°C (B procedure) suggesting that the dry matter loss at 103°C is not representing weight gained from equilibration of the samples with moisture in air. No correlation ($p = 0.65$, $r = -0.1$) was observed between residual dry matter after milling using procedure A and C.

Table 1 Correlations between residual dry matter at 103°C (g/kg) and predicted composition of grass-clover silages after pre-drying of samples at 60°C, milling through a 0.5 mm screen, and transferred to NIR scanning cups

Variable	Correlation to residual dry matter	P value	Number of observations
Dry matter at 60°C	-0.13	0.03	267
Ash	0.03	0.59	267
OMD	-0.69	< 0.01	267
Crude protein	0.10	0.09	267
Soluble crude protein	-0.60	< 0.01	267
NDF	0.46	< 0.01	267
Sugar	-0.57	< 0.01	267
Lactic acid	-0.29	< 0.01	267
pH	0.23	< 0.01	267
Clover content	0.22	< 0.01	178

Visual inspection of the 2nd derivative of the collected spectra indicated great variation between samples at 5.250 cm^{-1} (first overtone for water) and 7.050 cm^{-1} (second overtone for water). Calibration models developed using narrow ranges of the spectra (5.000 to 6.000 cm^{-1} and 6.500 to 7.500 cm^{-1}) predicted residual water in samples dried at 60°C with a root mean square error of cross validation (RMSECV) of 14 and 12 g/kg, respectively.

Conclusions

Clover-grass silages dried at 60°C had residual dry matter of 873 to 975 g/kg when dried at 103°C. The weight loss observed at 103°C was not caused by weight gained from equilibrating with air. The residual dry matter was less for 1st cuts compared with later cuts and is correlated with predicted OMD as well as soluble crude protein, NDF, sugar, lactic acid, pH, and clover content. Evaluation of NIR spectra indicated that a large fraction of the residual weight loss might be water. Correction for residual dry matter might be of importance to improve precision of OMD and chemical methods used in the NorFor feed evaluation system.

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The use of feed in the Swedish livestock system

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Introduction

This article is based on research and a report commissioned by WWF Sweden (Rundgren, 2023). The objective of that report was to be a basis for future quantification of the impact of Swedish livestock production on climate, biodiversity and other environmental factors.

The flow of feed to livestock is the biggest flow within the Swedish agriculture system, with approximately 55% of the total harvest from croplands being directly used as feed. Permanent grasslands represent an additional flow and there are considerable streams of by-products from food and bio-energy industries used as feed (Rundgren 2021). To understand these flows of feed is therefore a key for understanding the Swedish food system and the role of livestock in this.

Use of feed, and how it is produced, is the major factor for the environmental impact of livestock. Lifecycle assessments (LCA) for livestock production are mostly based on models or sample farms and not on real feed use for a whole livestock chain. In addition, feed used is often expressed per kg of carcass weight or sometimes boneless meat, but the quantity of edible meat can differ substantially from that. This means that LCAs for meat may not represent the real situation. In addition, feed use is also a major determinant of the economic result of livestock keeping.

The study is the first attempt to quantify the total actual use of feed for all important livestock production systems in Sweden and to put that in relation to the food that is produced, including the protein conversion ratio.

Materials and Methods

First, total feed use was determined, differentiated for the species. In order to assure that data was robust and reliable, data was collected both bottom-up and top-down. The total quantity of feed from the calculation bottom-up and top-down should match. If not, there was some error in the data, which merited further investigation and final judgement.

Bottom-up data was based on rations (from LCAs, economic production models (SWE:bidragskalkyler and advisory materials) if possible, from several sources, which were compared and combined. Feeds used for parent animals, replacement animals, eggs, etc. were included. For pigs, there was a recent comprehensive LCA (Landquist *et al,* 2020 and Landquist, 2023) made with a similar approach for the feed use, i.e. determining the actual total feed use in the pig sector. That data was used with minor adjustments.

The rations were multiplied by the number of animals that, on average, had consumed the rations, including estimates of animals that die before slaughter either on farm, during transport or because of disease.

For cattle, the bovine registry (CDB) made it possible to trace all animals and their fate, including movements and age of slaughter or death. For other animals, industry sources and slaughter statistics have been used. Number of animals culled as part of government interventions to control disease were provided for a 5-year period by the Swedish Board of Agriculture (SBA).

For feed used, an additional 0.5% of waste/loss in storage and handling was applied for non-roughages. A tool from Hushållningssällskapet (2021) set in-farm storage waste of feed grain at 2%. A lot of grain is supplied also from farms to the feed industry where losses are lower. On the other hand, commercial farmers may store grain for longer periods and sell it later on. The waste factor is only applied to grains and pulses as there would be many complications to use it also for by-products. A 9% loss was calculated for storage of silage based on Petterson et al (2009) and Spörndly and Nylund (2017). Waste at feeding was added for ruminants' consumption of silage according to industry estimates, while no other waste at feeding was considered for other animals. Use of seed for the production of feed was not been included.

For the top-down calculation, the total quantity of feed available was calculated from SBA harvest statistics, feed industry statistics 2018-2021, trade statistics 2019-2021, SBA grain balance 2017/2018 and investigations of all major food industries which have by-products used for feed by Rundgren (2021). The feed industry statistics had indications of which feedstuff that is used for which kind of animal (Foder och Spannmål, 2023). For the total harvest, various sources have been used to allocate the harvest to different uses: export, seed, left on field, grazed, fed on farms (including sold to other farmers), to feed industry, bedding, used by non-food industries (starch, biofuels etc.), food industry and shops, direct consumption (sold to consumers from farms), biogas, combustion and on farm losses. In order to make it possible to compare data with total quantities of feed available, rations for all other animals, horses, goats, reindeer, fish and pets have also been calculated from various industry sources, according to Rundgren (2021).

The national statistics of slaughter, milk deliveries and egg packing were used to make calculation of total volume produced in average 2018-2022. Estimates of home consumption and losses underway were added.

Carcass weight is the basis for payment in Sweden, and the official slaughter statistics record carcass weights. *Edible meat* was calculated as boneless meat share of carcass weight plus details not part of the carcass, which are mainly used as food either in Sweden or exported as food, according to industry sources. Other products (e.g. tripe) are edible in principle and eaten in some cultures but only to a very limited extent in Sweden and are not exported as food. Those are classified as *potentially edible.* Notably, there are technologies to convert bones into edible food products, both traditional ones as production of stock or marrow from bones or more modern methods such as production of hydrolysates (Pap *et al*., 2022, Sandström *et al*., 2022). This has, however, not been considered in this study. To allow a comparison with milk and eggs, the edibel share of milk was set at 100% (losses and milk given to calves etc. is already included in the feed use) and for egg, the weight of the shell, approximately 10.5%, was deducted from the total.

For the partitioning of feed between edible and various by-products as well as the partitioning between meat and milk, biophysical allocation was used as far as possible. Economic allocation was, however, used for some by-products, based on data from industry sources. There was no allocation for manure. A separate partitioning (both biophysical and economic) of feed use for cattle between meat and ecosystem services such as maintaining the landscape, bio-diversity, carbon sequestration and soil improvements was also made.

The preliminary results were communicated to industry representatives who could clarify or verify some figures.

The share of edible protein in the various feed stuffs were based on the actual situation in Sweden. For example, most wheat is consumed as white flour or as spirits, in both cases a substantial part of the crop is "not edible", *i.e.* the wheat bran and distillers' grain. Those by-products were considered not edible even if they could be theoretically edible. In the analysis, only spirits for human consumption corresponding to the actual total Swedish consumption was included as human edible and not the substantial quantities that are exported. When whole wheat is used as feed, only the share of wheat for human consumption in what is actually used for human food and drink was thus calculated as human edible. In addition, the proportion of wheat that, in average, hasn't sufficient quality for the food market, was considered not edible.

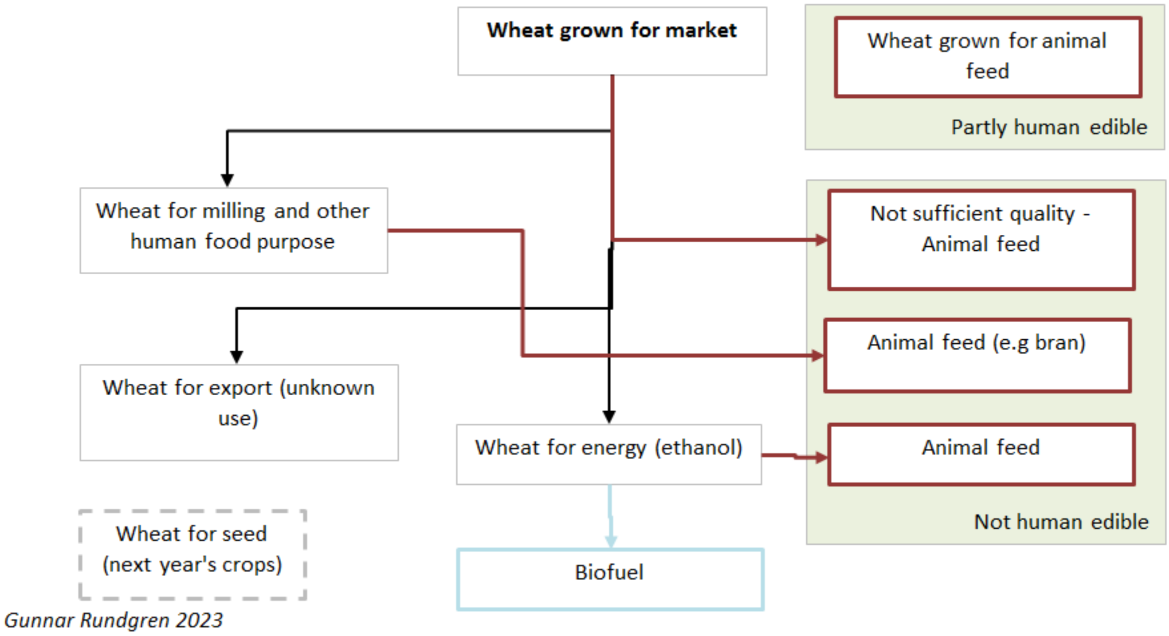

Figure 1 Use of wheat harvest.

Results and Discussion

Compared to other animals, a very high share of the pig (both expressed in relation to live weight and carcass weight) is edible (Table 1). The difference between human edible and potentially edible indicates a potential for increasing the valorisation of a bigger part of the animal as human food. This potential is biggest for beef followed by sheep and chicken. It is clearly a low-hanging fruit when it comes to lowering the ecological footprint of livestock production if a bigger part of the animal is actually consumed.

Table 1 Relationship between live weight, carcass weight, edible meat and potentially edible meat

	Live weight	Carcass weight	Edible	Potentially edible
Beef	189	100	82	103
Lamb	238	100	88	101
Chicken	147	100	75	88
Pig	133	100	103	108

Actual feed use was mostly considerably higher than theoretical feed rations, which were based on optimal conditions. It should be noted that representatives from both the egg and chicken industries objected to the results which showed an 8% higher feed use than the figures presented by the industry itself. They were, however, not able or willing to present alternative data that could change the calculations.

Milk had the lowest feed use of the studied products, 0.77 kg of feed (DM) is used to produce 1 kg of milk (Table 2). Even calculated on a dry matter basis, it still used less feed than egg production, which otherwise has a high feed conversion ratio. For the meats, feed use per kg edible meat was lowest, and almost the same, for chicken and pork. This is perhaps surprising as feed conversion of chicken is mostly said to be superior of other animals.

Meat from cattle and sheep has very high feed use compared to meat from monogastic animals. This is a result of the digestive system of ruminants. While consuming a lot, they also have the ability to digest feed which is too coarse for the monogastric animals, pigs and hens. This also results in in a lower use of human edible protein to produce human edible protein in the form of animal products. Monogastric animals use feed protein that is more easily digested. Milk has the highest ratio of 2.4 kg of human edible protein for each kg of edible protein used as feed. Notably, the calculation of protein conversion has not taken into account the higher nutritional value of animal proteins compared to the protein in their feed. When taking protein quality (measured by the Digestible Indispensable Amino Acid Score) into account, the edible protein converstion ratio (EPCR) of pork in Australia went from 0.70 to 3.26 according to van Barneveld *et al.* (2023).

Table 2 Feed use (DM) and edible protein conversion ratio

	kg per CW	kg per kg HE	kg per kg PE	EPCR
Egg	NA	2.43	2.43	0.67
Chicken	2.41	3.26	2.80	0.69
Milk	NA	0.77	NA	2.39
Beef	19.65	24.59	19.53	0.90
Pork	3.25	3.21	3.13	0.83

CW=carcass weight;

HE=human edible;

PE=potentially edible;
EPCR=edible protein conversion ratio.

It should be noted that for pure grass-fed ruminants, the edible protein conversion ratio would be limitless as they consume no human edible protein. For sheep, data was of rather low quality and the sector is very varied with many different farms, breeds and production models. Many also keep sheep mostly for landscape management or as pets and don’t supply any meat at all. Therefore, sheep data is not presented here. For beef, there is also considerable uncertainties relating to the many actors and big variations in the sector. The production of pork and milk, and even more so chicken and eggs, is more standardized and more large-scale which makes the data more certain.

With a biophysical allocation of feed for maintaining semi-natural grasslands, feed use for meat from suckler cows and their offspring would be reduced with more than 50%. With an economical allocation based on environmental payments and payments for carcass, feed consumption for meat from the whole ruminant sector would be reduced with 21%.

61% of the total feed use in Sweden is roughage and grazing of which none is human edible (Table 3), 21% of the feed used is grain, 12% by-products and oilseed cakes, 1% pulses and 4% mineral feeds, vitamins, fish meal and others. Wheat is the main grain used by Swedish livestock followed by barley. Maize is in little use. The share of the wheat used as feed was calculated to be 49% human edible.

Table 3 Share of used protein that is human edible protein

Wheat	49%
Wheat by-products	0%
Oats	32%
Oats by products	0%
Barley	45%
Barley by-products	0%
Rye	67%
Maize/corn	60%
Maize by-products	0%
Peas	75%
Fava Beans	50%
Soy beans	70%
Rape seed	0%
Oil seed cakes (not soy)	0%
Soy meal	65%
Roughage	0%
Fish meal	0%

Our results from Sweden are quite similar as those of Laisse *et al.* (2018) from France, even though they are based on models rather than actual use.

The results demonstrate that “efficiency” is context specific and is based on undeclared value judgments and assumptions. Feed conversion and protein conversion ratios are often opposite both in comparisons among different animals as well as among different systems for the same animal. Ruminants fed a high share of concentrates (feed lot style) will have a much lower feed use but also a lower conversion of edible protein, compared to ruminants on a roughage diet (suckler cows and their offspring). Monogastric animals have low feed use but also a low edible protein conversion ratio, while ruminants consume much more feed but less human edible feed. Much of the increased feed conversion efficiency of livestock production has been a result of less use of roughage, straw, food waste, scraps and other low quality feeds and increased use of high quality feeds. Mottet *et al.* (2017), based on global averages, demonstrated that t use of human edible feed per kg of protein is much higher for monogastics than ruminants and much higher for industrial systems than in backyard production. What is a weakness from one perspective such as feed use per kg edible product can be a strength from another perspective as in the case of grazing cattle and sheep which maintain biodiversity. Also, recycling of nutrients and organic matter to croplands is an important factor for long term soil fertility and it is well established that ruminants in Sweden contribute more to organic matter in soil, both by their manure and associated forage production.See for example Henryson *et al.* (2022) and Poeplau *et a.l* (2017).

While feed use is important, one should not draw too many conclusions about respective products as there are many linkages in production systems, *e.g.* impact on crop rotation, recycling of nutrients in manure and in by-products and the close ties between milk and beef, where one can't produce milk without also producing meat. The study indicates that one-dimensional indicators, such as feed use, don't capture the complexities of the agriculture and food system, and this also makes it questionable to use data for comparisons among products and countries. This is further emphasized when taking impact of allocation choices into account.

Determination of what is human-edible in this study is based on current market factors both for meat and for feed, and doesn't express what is theoretically edible. On the one hand, it can be argued that the edible protein share in feed is underestimated. On the other hand, the calculations assumes that humans could consume all the edible food that currently is consumed by animals. But, obviously, that is not the case as the production of grains is much higher than human needs. Assuming that there would be land over if all the human edible raw materials presently used for feed would be consumed by people, landowners are likely to still want the land to be productive in one way or the other. The primary use of that would probably be for bio-energy, derived from wheat, rape seed, sugar cane, maize, soybeans and oil palm. If there were no animals, they could not consume the leftovers and, *on an aggregate level*, the edible protein conversion ratio of the whole agriculture system might become very low in such a scenario. This implies that one should be careful not to jump to conclusions about the efficiency or lack of efficiency of livestock in general.

Conclusions

Milk is the animal product with the lowest use of feed (DM) per unit of edible products. This is followed by eggs, pork and chicken. The feed use of beef is an order of magnitude higher. For det conversion of edible protein milk is again most efficient and beef has a higher conversion ratio than eggs, chicken and pork. In order to get a better picture of the role of various production systems in the agriculture and food system one need to consider several other indicators.

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Fish meal replacement with torula yeast (*Cyberlindnera jadinii*) and enzymatic treatment of the aquatic feed model can affect the flow resistance in the pelleting die and alter physical properties of the pellets

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Introduction

Expansion of aquaculture requires increased quantities of alternative and sustainable protein sources (Aslaksen *et al.*, 2007). Fishmeal remains an important part of aquatic feed diets and the feed manufacturing industry is a substantial consumer of fishmeal. Therefore, this growing industry, together with research institutions, tries to target the best possible alternative candidates to replace fishmeal. Out of many novel feed ingredients, yeast has positioned itself among the important ones (Huyben *et al.* 2017; Vidakovic *et al.*, 2019; Agboola *et al.* 2020). Yeasts have an optimal composition of amino acids for farmed shrimp (Gamboa-Delgado *et al.* 2015). Yeasts can be successfully used as alternatives to fishmeal or soybean meal up to 24% without impacting growth (Guo *et al.*, 2019) or health condition (Jin *et al.*, 2018) of aquatic animals. However, replacing conventional aquatic feed ingredients in diets may influence the physical quality of aquatic feed pellets and thus loss of feed nutrients in the water. This may contribute to water pollution and consequently increase stress on the farmed animals and reduce production (Ferreira *et al.*, 2011). The goal of the study was to assess the impact of substituting fishmeal with Cyberlindnera jadinii, supplemented with enzymes and minimal water content, on the resistance of the feed material during pelleting. The experiments were designed to elucidate how replacing fishmeal with yeast and treating it with enzymes could influence the physical characteristics of aquatic feed pellets.

Materials and Methods

Raw ingredients typically used for shrimp feed manufacturing were obtained from the Center for Feed Technology, Norwegian University of Life Sciences, Ås, Norway. *Cyberlindnera jadinii* had a crude protein of 470 g/kg and gross energy of 19.9 MJ/kg. Fishmeal had a crude protein of 684 g/kg and gross energy of 19.4 MJ/kg. Raw ingredients had a particle size under 1 mm before the manufacturing. Mixing of the raw materials was done using a mixer Diosna (P1/6, Germany). During the mixing of the formulated diets, a 10th part of distilled water per unit was added by spray onto the mash with a nozzle (Model 970, Düsen-Schlick). Water was used to integrate enough moisture for further processes and to help spray the enzymatic cocktail comprised of protease and endo-exo 1.3-beta-glucanase, adequately mixed before addition to water. All diets with added 0% yeast as a negative control; 2.5; 5; 10 and 20% yeast instead of fishmeal, except the positive control diet, were mixed with the enzymatic cocktail for 800 seconds. From each mixed diet, three samples were randomly taken to obtain a representative homogeneous sample. Samples were analyzed for moisture content by the EU, No. 152/2009 method. The average moisture for all the trials was 13%. Thereafter the mixed mash was vacuum-packed to avoid moisture loss before further work.

The manufacturing of pellets was done by weighing out 30 samples per diet. The feed mash for each pellet was weighed out as 0.13 g in the Eppendorf tubes, which were further placed into the boiling water and conditioned for 3 minutes. This was to allow the water in the feed mash to become steam. Subsequently, the Eppendorf tubes were placed in the fridge at 4°C to cool down for 20 minutes before pelleting so that water could condense swiftly within the entire feed mash in the Eppendorf tube after conditioning. The single-die pelleting method was used for pellet manufacturing. Conditioned feed material from each Eppendorf tube was used to produce one pellet by pouring it into an 81ºC preheated blank single-die channel. Poured feed mash was heated for 3 minutes before compaction to ensure an equal temperature of the mash particles. Pelleting was done into the die hole with a diameter of 5.5 mm with a compression rod of a 5.45 mm diameter. During pelleting, the maximal force load for each pellet was 285 N. Maximum compaction pressure (Pa) at maximum force (N) during densification, compaction, and pellet ejection from the die were observed and recorded with NEXIGEN Plus software. The p-max was identified as the pressure needed to initiate pellet ejection from the pelleting die. Results of the tensile strength analyses were done by applying maximum force (F) on the compacted feed pellet under diametral compression for three randomly chosen pellets for each treatment. Underwater pellet swelling analyses were done to indicate pellet cohesivity (particle detachment), linked to the swelling rate of the pellet. Surface roughness analyses were done to understand if the replacement of the fish meal with yeast or the addition of enzymes may influence any changes to particle packing and thus surface-change of the pellets.

Results and Discussion

Flow resistance (p-max) in the pelleting die showed a significant increase when feed with 20% yeast was treated with enzymes (Table 1). This indicates significantly higher usage of the electricity during compaction ($p<0.01$). The tensile strength of pellets with 20% yeast, either treated or not treated with enzymes, significantly increased (Table 1). Tensile strength was strongly correlated to p-max (R^2 0,99; $p<0.001$) (Figure 2)

Table 1 Mean values for p-max, tensile strength, and underwater pellet swelling (UPS) for both pellets without and with added enzymes

Added yeast	p-max (MPa/mm²) no enzymes	p-max (MPa/mm²) with enzymes	Tensile strength (N/mm²) no enzymes	Tensile strength (N/mm²) with enzymes	UPS (mm²) no enzymes		UPS (mm²) with enzymes	
					1 min.	20 min.	1 min.	20 min.
0% no enz.	18.2^{b}	18.9^{bc}	19.1^{c}	16.9^{bc}	30.3^{ab}	40.2^{ab}	38.3^{b}	80.5^{abc}
0% with enz.	-	13.65^{c}	-	7.3^{c}	-	-	62.6^{a}	109.6^{a}
2.5%	16.6^{b}	14,56^{bc}	35.1^{c}	9.3^{bc}	36.1^{a}	40.8^{ab}	52.5^{ab}	100.3^{ab}
5 %	17.4^{b}	15.92^{bc}	31.7^{c}	8.9^{c}	28.4^{ab}	49.1^{ab}	43.5^{ab}	74.0^{abc}
10 %	19.7^{b}	22.75^{b}	45.2^{bc}	13.4^{b}	33.0^{ab}	47.2^{ab}	27.3^{b}	73.9^{bc}
20 %	23.9^{b}	36.4^{a}	71.3^{b}	20.2^{a}	28.8^{ab}	51.0^{a}	26.7^{b}	66.4^{c}

Data representing means. Different letters from ANOVA-Tukey statistical method indicate significant differences at 5% level. Presented p-values for p-max ($p<0.01$); Tensile strength ($p<0.001$); and UPS ($p<0.01$)

Enzymatic treatment showed a tendency to decrease underwater pellet swelling of the pellets with increased addition of yeast (Table 1). This indicates that pellets may stay longer underwater before being consumed. The longitudinal surface roughness decreased when pellets containing yeast and pellets with the materials treated with enzymes (Figure 1). This ensures that the raw materials will be packed better.

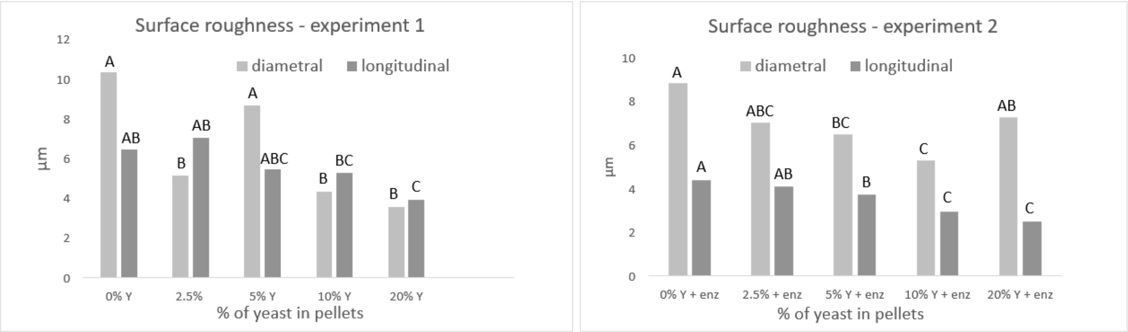

Figure 1 Diametral and longitudinal surface roughness of the pellets with replaced fish meal (experiment 1) and pellets with replaced fish meal and treated with enzymes (experiment 2). Results are presented as a mean value of 25 pellets for each treatment. Different sequential letters from the ANOVA-Tukey statistical method signify differences at a 5% level separately for diametral and for longitudinal measurements where p-values were lower than 0.001.

Conclusions

The impact of substituting fish meal with yeast at levels of up to 20% or treating feed with enzyme up to 10% of added yeast, exhibits no discernible effect on p-max. Introducing yeast at a concentration of 20% instead of fish meal, regardless of enzymatic treatment, contributes to the enhancement of tensile strength in feed pellets. However, enzymatic hydrolysis should be carefully used in formulating pelleted aquatic feed to mitigate underwater disintegration. The addition of an enzymatic mixture results in feed pellets containing 20% yeast displaying a reduced surface roughness, potentially mitigating pellet agglomeration during storage and improving pellet durability during air transportation to aquatic farms.

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Body condition score of Icelandic dairy cows

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Introduction

The ability of dairy cows to deposit and mobilize energy is important as it has effect on health, fertility, calving performance, milk yield and milk composition (Roche *et al*., 2009). Body condition of dairy cows is commonly assessed with a scoring system from 1 – 5. For Icelandic dairy cows this has not been a research subject and advise to farmers regarding body condition of the breed through the production cycle is based on studies on foreign dairy breeds.

Materials and Methods

To get an overview of body condition score (BCS) of Icelandic dairy cows through the production cycle and its effect on health, fertility and yield 7 dairy farms in Iceland were visited monthly over a 12-month period. All dairy cows on these farms were condition scored based on the scale of Ferguson *et al.* (1994). Data regarding yield, health and fertility were collected from the farms herd management systems and the national cattle recording system. Health information was based on certified veterinarian visits. On 3 farms live weight data was collected from a weighing system in the dairy robots (Lely A3, Lely Industries N.V., Maassluis, Netherlands) and on 1 farm the cows were weighed monthly with a platform weighing scale.

For statistical analysis two different datasets were created. Dataset 1 included all cows in the study, whereas Dataset 2 is a subset that only included the cows that calved in the time of the study. Dataset 1 included 7.762 condition scores of 906 individual cows and also information on live weight (LW), milk yield, days from calving and number of lactations. Dataset 2 included last BCS before parturation (Dry stage BCS), lowest BCS postpartum (Nadir), BCS loss (Dry stage BCS- Nadir), milk yield, fertility and health (number of documented veterinarian visits postpartum). The data were summarized for each of 662 individual cows. Statistical analysis were performed in Rstudio version 4.1.2, 64- bit (Rstudio Team, 2021). Dataset 2 was analysed using the following model: $Y_{ijklm} = \mu + A_i + B_j + \alpha_k + \beta_l + \varepsilon_{ijklm}$ where Y_{ijklm} is the response variable (milk yield, days from calving to first insemination, number of inseminations & veterinarian treatments); μ the overall mean; A_i the regression coefficient for BCS Prepartum; B_j the regression coefficient for BCS loss Postpartum; α_k the fixed effect of farm (1, 2, 3, 4, 5, 6 & 7) ; β_l is fixed effect of lactation (1, 2 & ≥3); and ε_{ijklm} is random error.

The following model was used to estimate the effect of BCS and days in milk (DIM) with data from Dataset 1 on live weight: $Y_{ijklmn} = \mu + A_i + B_j + \alpha_k + \beta_l + w_m + \varepsilon_{ijklmn}$ were Y_{ijklmn} is LW; μ the LW mean; A_i the regression coefficient for BCS; B_j the regression of DIM; α_k the fixed effect of farm (1, 2, 3 & 4) ; β_l is random effect of lactation (1, 2 & ≥3); w_m is random effect of individual cows; and ε_{ijklm} is random error.

Results and Discussion

Average body condition score (BCS) of Icelandic dairy cows in the study was 3.37. Cows on 2nd lactation had lower BCS (3.33) compared to cows on 1st lactation (3.38) and ≥3rd lactation (3.39). The BCS was highest during the dry period (3.52, 3.52 & 3.54 for 1st, 2nd and ≥3rd lactation) but decreased rapidly after parturition. Cows on 1st, 2nd and ≥3rd lactation lost on average 0.38, 0.45 and 0.61 BCS and reached the lowest BCS in 3rd, 4th and 6th week after parturation, respectively. After 300 days in milk (average of 300-330 DIM) cows on 1st & ≥3rd lactation had gained similar BCS as they had Prepartum (3.51 & 3.53) while cows on 2nd lactation gained significantly ($p<0.05$) less BCS (3.40). On all the farms in the study, cows on 2nd lactation were allocated the same concentrate feed allowances based on production as older cows (≥3rd lactation).

Live weight of cows for 1st, 2nd and ≥3rd lactation in the study differed ($p<0.0001$) with mean weights of 465, 491 and 524 kg. Weight differences of 2nd and ≥3rd lactation indicate that 2nd lactation cows were still growing during 2nd lactation and did generally not reach maturity until 3rd lactation. Growth and lower body weight affect nutrient requirements and intake capacity (Volden *et al.*, 2011), therefore, it could be beneficial for Icelandic farmers to formulate special feed ration for 2nd lactation cows.

Body condition loss postpartum affected milk yield across all stages of lactation (day 10 – 300 after parturition). According to regression analysis, a loss of 1 BCS postpartum increased milk yield by 1.4 – 5.0 kg per day depending on lactation stages. Dry period body condition only affected milk yield in the period 51 – 100 days after parturition with a 0.9 kg increase in milk yield per unit BCS (Table 1).

Table 1 Least square means of Nadir, BCS loss to Nadir and DIM to nadir, cows were grouped according to BCS postpartum to low (S ≤ 3.00), moderate (3.25-3.50) and excessive (≥3.75)

BCS Prepartum	**≤3.00**	**3.25 – 3.5**	**≥3.75**
BCS loss postpartum[1]			
Nadir	2.71^a	2.88^b	3.18^c
BCS loss to Nadir	0.21^a	0.54^b	0.67^c
DIM to Nadir	41.3^a	42.1^a	42.5^a

[1] Different letter represents significant difference among groups (P<0,05).

Body condition prepartum affected body condition loss postpartum significantly ($p< 0.05$) (Table 1). This indicates the need for moderate body condition prepartum (3.25-3.5) to support condition loss postpartum. Excessive BCS loss postpartum (≥1.25) increased significantly ($p< 0.05$) number of days to first insemination compared to cows with moderate condition loss (0.50-0.75). First insemination of moderate loss cows was on average 87 DIM but 107 DIM in the excessive loss group. BCS prepartum or BSC loss postpartum did not have any effect on number of inseminations in the study.

Cows with BCS of 3.75 or more during the dry period showed ($p<0.05$) increase in veterinarian treatments (17%) compared to cows with BCS 3.25 – 3.50 (9%). Mastitis was the most common cause of treatment in the study, 56% of the visits.

A relationship between body weight and condition score was found in the study. Regression slope indicated that a loss of 1 BCS accounted for 34.6 kg loss of live weight when all lactations were included. When only ≥3rd lactation cows were analysed, the regression slope indicated 42 kg live weight loss for a loss of 1 BCS. The default value used in the NorFor system is 45 kg/BCS (Norfor, 2022) which seems to fit well with mature cows but likely overestimates energy reserves of 1st & 2nd lactation cows.

Conclusions

Based on the results of this research, optimal dry period body condition of Icelandic dairy cow is 3.25 to 3.50 BCS. Optimal body condition loss in the first weeks of lactation was around 0.50 to 0.75 units. Greater loss could have negative effect on fertility and lower loss was connected to lower milk yield.

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