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Sveriges lantbruksuniversitet
Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

Rapport 306
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Foreword

We celebrated the 10-year anniversary of our Nordic Feed Science Conference three years ago. But, then came Corona and we had to make a halt. Plans for this year was to have it just before Midsummer but the Finnish national Agricultural Science days coincided with that period, so we decided to move to August just before start of the fall term. We hope that the weather will be as pleasant as June normally is in Uppsala and that you will enjoy your stay and make the most of the time in Uppsala.

We have received a total of 35 written contributions this year with the following distribution: 14 on Ruminant Nutrition, 10 on Conservation, 5 on Methods and Miscellaneous, 4 on Modelling and 3 commercial contributions from our sponsors.

In 2018, all Nordic countries, except Iceland, experienced a severe drought. This has resulted in research projects in several Nordic countries, and we will find contributions in the present conference on that topic.

The Russian invasion of Ukraine has made commodity prices to increase dramatically also for farmers. For that reason, Farm advisor Susanne Bååth Jacobsson will speak on the topic of profitability during turbulent times.

Two well-known speakers will address the topic of silage conservation – Professor Giorgio Borreani from Torino University and Professor Adgebola Adesogan from the University of Florida in Gainesville. Professor Adesogan is also the main speaker on the topic of improving fiber utilization. In addition, we look forward to a number of presentations on ruminant nutrition, models, methodologies and forage conservation.

Two industrial sponsors will be presenting what they can offer you at booths near the auditorium. Take your time and talk to them.

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/animal-nutrition-management/news/nordic-feed-science-conference-2022/proceedings/

Uppsala 2022-08-04

Peter Udén

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Improving forage harvesting and conservation affects dairy farm efficiency and sustainability

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Introduction

Agricultural production is responsible for 14% of human-induced emissions of greenhouse gases (GHG), with a relevant role coming from the livestock production sector (IPCC, 2007; 2019). The conversion of natural land and land management are significant net contributors to GHG emissions and climate change, but land ecosystems also work as GHG sinks (Tubiello et al., 2015; Le Quéré et al., 2018). Food from animal sources contributes 18% of the global calorie (kcal) consumption and 25% of the global protein consumption (FAOSTAT, 2016) and makes an important contribution to food security through the provision of high-quality protein and a variety of micro-nutrients that can be locally difficult to obtain from plant-source foods (Mottet et al., 2017). As a result of the expected increase in demand for animal products (Gerber et al., 2013), it will be a challenge to increase food production through a sustainable intensification approach in order to avoid further pressure on the natural environment (Tabacco et al., 2018). Land-based mitigation measures are some of the most important options currently available to reduce GHG emissions. Direct GHG from livestock are mainly due to the production phase (80 to 94%) and can be attributable to CH_4 (51%), N_2O (29%), and CO_2 (14%) (Gerber et al., 2013). Almost all methane is physiological (enteric fermentations) and thus difficult to mitigate. However, feed strategies based on the management of the forage system are of great importance because a land use change (LUC) may result in an increase or decrease in GHG emissions (Tabacco et al., 2018; Gislon et al., 2020). Thus, the analysis of the environmental impacts of a dairy farm may be reduced to the cradle-to-animal level to assess the impact of the forage system adopted to produce feeds (Peyraud et al., 2014; Tabacco et al., 2018). In order to achieve a sustainable food supply, agricultural producers need to identify cropping/forage systems, as well as feed growing/harvesting and livestock management practices that make the best use of the available resources and minimize the potential environmental impacts (Liebman et al., 2008; Gislon et al., 2020). The intensification of dairy farming systems has been accompanied by the development of corn silage and intensively fertilized grasses throughout Europe (Borreani et al., 2013), while the protein supplementation of dairy rations has mainly been left to purchased soybean meal, which is predominantly produced overseas (Peyraud et al., 2009; Lehuger et al., 2009). In the same way, most European countries have been affected by an intensification process, as a result of an increase in the number of dairy cows per hectare of land, the acquisition of genetically improved dairy cattle, and an increase in concentrates in the diet (Alvarez et al., 2008), and all this has resulted in significant effects on the efficiency, and thus on the economic results of farms.

In order to increase the production, technical, agronomic, and economic efficiencies of intensive dairy farming systems, it is necessary to increase the farm production of the net energy and protein per unit of cultivated area, while avoiding any increase in external inputs that could worsen the environmental impacts.

Improving forage quality by scheduling the cutting time

In order to maintain farm competitiveness, decrease feeding costs and increase farm protein and net energy self-sufficiency (Peyraud et al., 2014; Wolf, 2012), producers need to develop more sustainable cropping systems by considering crop sequencing and by capitalizing on external resources, such as the weather, neighboring farm interrelationships, markets, government programs and new technologies (Liebman et al., 2008; Tanaka et al., 2007). These objectives can be reached by introducing or valorizing forage legumes, double-crops, and meadows over a large part of the utilized agricultural area of a farm, and by paying particular attention to adopting optimized management practices (Tabacco et al., 2018). The key factor is to maximize the net energy for lactation and the protein produced on the farm surfaces, which should be contained in forages of high nutritional quality and with a low NDF content (Randby et al., 2012). This could be obtained by means of an early cutting and an improved cutting frequency, coupled with an efficient conservation system of forages, such as silage or haylage (Tabacco et al., 2018). The timing of forage harvesting influences the quality of legumes and grass forages, and it is necessary to optimize its potential for livestock production. The variation in nutritive value of the herbage is related to the environmental and physiological history of the crop and is in particular influenced by the maturity of the forage at the time of harvesting, which is the primary factor that influences the lignification of fiber and the nutritive value within a species (Kalu and Fick, 1983; Valente et al., 2000). The effect of stage of growth and DM content at harvest on forage protein content of permanent meadow in northern Italy is reported in Figure 1 (blue line). Furthermore, the quality of conserved forage in bunker silo or wrapped bales in relation to very early, early and late stages of maturity at harvesting and DM level reached with different wilting period is also reported in Figure 1. It is evident the great effect on protein content of forage due to early or late harvesting, and the reduction of protein content with increasing DM content at baling due to the increase of mechanical losses during field curing and harvesting.

In order to reduce the purchasing of feeds at a farm level through a valorization of the feeds produced on farmlands, without reducing the milk performances of animals, it is not sufficient to produce a large amount of DM and protein on the farm, it is also necessary to produce forages with high NDF degradability and low NDF content to reduce the rumen fill, and to improve the DM intake (Kammes and Allen, 2012) and rumen VFA production (Rinne et al., 2002; Zebeli et al., 2010). A correct feeding of high-quality forages leads to a reduction in the use of concentrates in the ration of dairy cows and improves the overall performance of the animals (Randby et al., 2012; Martinez et al., 2009; Kammes et al., 2012).

This means growing more crops (both annual and perennial) in the forage system and re-designing crop rotations and intercropping in such a way as to develop a more self-sufficient, integrated and closed-loop livestock and crop production system, using an agro-ecological approach with the final objective of achieving an eco-functional intensification of sustainable livestock production (Tilman et al., 2002; Guyader et al., 2016; Tabacco et al., 2018). Forage production systems that serve dairy farms should be modified to attain an increased protein self-sufficiency in order to partially or totally replace imported soybean and other protein concentrates, and to increase the use of crops that are not suitable for use as food and fiber for humans, but which are instead utilizable by livestock.

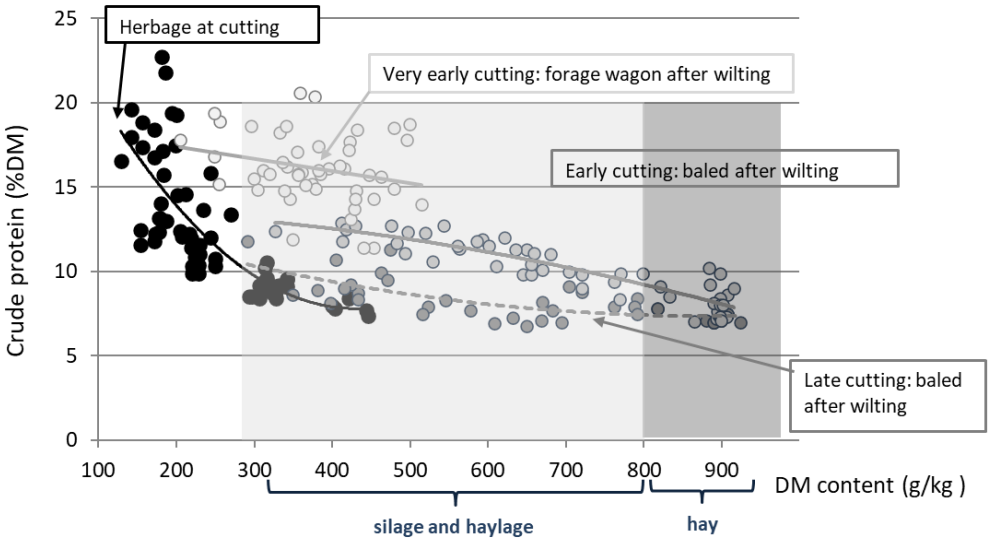


Figure 1 Protein content evolution of fresh herbage during growth (black line) and harvested at very early stage of development with forage wagon and ensiled in bunker (light grey line) or in wrapped bales harvested at early (mid grey) or late (dark grey dotted line) stage of growth in relation to different wilting period, thereby resulting in different DM content at harvesting (unpublished data from Forage Team – University of Turin, Italy).

Reducing on-farm silage conservation losses

To improve farm efficiency, the harvesting and conservation of silage is crucial to maintain the quality of forages produced in the field, and all the necessary efforts to reduce qualitative and DM losses, from harvesting to animal feeding, should be put in place (Borreani et al., 2018). After the dominance of lactic acid fermentation over butyric fermentation, ensuring anaerobiosis during conservation is the key point of the process, because, if air penetrates the silage mass, aerobic microorganisms can multiply, thereby resulting in aerobic deterioration and, consequently, in DM and quality losses. Thus, the main goal of improving conservation efficiency is to exclude air as soon as possible and to maintain the anaerobic environment throughout the conservation period. Bunker silos and round bales are the two most commonly used systems for silage conservation on farms throughout Europe. The use of bale silage has proved to be a good alternative to silage on small-to-medium farms to produce high quality forage (Hancock and Collins, 2006; Borreani et al., 2019), whereas many farms have adopted horizontal silos to store cereals and forages that have peripheral areas prone to aerobic spoilage (Borreani and Tabacco, 2010). One of the first factors that can affect the final quality of silage is the time in which a silo is sealed. Round bales are generally compacted and sealed in just a few minutes after the crop is harvested, whereas forage stored in bunker silos undergo longer periods before sealing, and the mass thus respires water soluble carbohydrates and other solubles (Randby and Bakken, 2021). Although some losses are unavoidable during conservation, good management practices can reduce or compensate for these losses by providing the quality forage needed for each animal group (Borreani et al., 2018). Several management factors should be adopted on a farm to maintain the quality of harvested forages in silages. In farm bunker silos, other than shortening the time in which the silo is sealed as much as possible, compaction, sizing silage within the walls, and choosing an adequate cover and sealing should be adopted to reduce the DM and quality losses during conservation (Muck and Holmes, 2000; Borreani and Tabacco, 2010). The practice of lining the walls of bunker silos with plastic, lapping the plastic over the forage, and then applying a top layer of plastic is highly recommended (Borreani et al., 2018). Reducing the air trapped in

silage can be achieved by choosing the right chopping length and compacting the forage material on top, usually through the use of heavy machinery. The silo face should be sized to allow at least the minimum required removal rate in relation to the climatic conditions and the type of silage crop. Silage quality can be improved by using a plastic film with high impermeability to oxygen, which, compared to traditional polyethylene films, allows the spoiling process to be minimized, especially in the peripheral areas more prone to aerobic deterioration (Borreani and Tabacco, 2014). Other than the quality of the plastic film, in order to exclude air penetration as much as possible, it is important to weigh down the silage using uniformly distributed heavy material in order to maintain contact between the plastic film and the silage (Borreani et al., 2007; Bernardes et al., 2012), to line the bunker walls (Lima et al., 2017) and to reduce the risk of mechanical damage of the cover by protecting it with a net or tarpaulin (Wilkinson and Davies, 2013). All these practices contribute to the goal of reducing losses to a minimum and maintaining the highest possible quality during the conservation period and the feed-out phase. The density of the silage is usually greater in bunker silos than in round baled silage.

The new kinds of agricultural compactors that are available offer new opportunities to ensile forages and other feeds (i.e. high moisture ear corn, corn silage etc.) in round bales, because they can quickly seal chopped material, thus obtaining equal or even higher densities than in a bunker (Borreani et al., 2019). An additional factor that can help reduce DM and quality losses is the use of chemical additives or inocula (Muck et al., 2018).

Several farms that have adopted the *vade mecum* reported in Table 1, have greatly reduced the DM and quality losses of silages conserved in bunkers or bales.

Table 1 *Vade mecum* of the correct practices to reduce DM and quality losses in horizontal silos and wrapped bales

Correct ensiling in horizontal silos	Correct ensiling in bales
Correct sizing of the silo face to ensure an adequate feed out rate	Choosing the correct DM content at ensiling (better above 40%)
Choosing a correct DM content at ensiling	Quickly wrapping the bales after baling
Assessing the need for adequate additives or inocula on a case-by-case basis	Assessing the need for adequate additives or inoculations on a case-by-case basis
Compacting forage for the earliest ensiling stages	Choosing an adequate number of plastic layers
Never filling silage over the silo walls	Using stretch film with adequate mechanical properties and stickiness
Using high-quality oxygen barrier plastic films	Using 3D wrapping and/or film nets
Weighting the silage cover and sealing the corners	Storing the bales on their ends
Driving over the entire top and side surface of piles (avoid steep ramps)	
Protecting the cover with a net or tarpaulin (reusable for 5 years)	Protecting the bales with a net or tarpaulin against birds and rodents
Avoiding opening too early (at least for 60 days)	

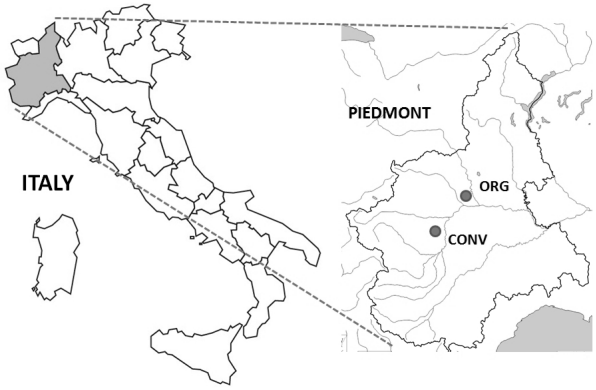
A dairy farm case study: reducing impacts by linking the forage system to the dairy herd requirements

In order to show and discuss how the increase in forage quality from farm cultivated land can impact the final results, in terms of milk production and quality, the efficient use of the utilized resources, and the environmental impacts of the whole production chain (in terms of

Carbon Footprint of 1 kg of fat, protein corrected milk, FPCM), data from two different commercial dairy farms (one conventional and one organic) have here been utilized as case studies. Over the last ten years, the Forage Team of the University of Turin, in collaboration with the Extension Service of the Regional Breeder Association of Piedmont (ARAP), has worked with several farmers on developing case studies to provide opportunities from which other livestock managers can learn.

The studied farm environments

The soils are deep and well drained on both farms, with prevailing loam and sandy loam textures in the 0-30 cm horizon on ORG Farm and CONV Farm, respectively. The elevation on ORG Farm and CONV Farm ranges from 224 to 237 and from 249 to 252 m above sea level, respectively. The local climate has been classified as temperate sub-continental, with a long potential growing season and a mean annual rainfall ranging from 700 to 1000 mm, mainly concentrated in two rainy periods that occur during spring (April and May) and autumn (September–November). The mean annual temperature is around 12.2°C. All the summer crops are fully irrigated on both farms, on average 3 to 4 times for corn, 2 to 3 times for alfalfa and 3 to 5 times for rotational and permanent swards.



Location of the two studied farms: ORG and CONV.

The aims of this study have been to achieve more sustainable production systems, by increasing the self-sufficiency and efficiency of farms through the improvement of the yield and quality of on-farm produced forages to reduce the farmers' dependency on market commodities, which, under extreme volatility, can result in large income fluctuations and process inefficiencies. In these two case-studies, the University-farm collaboration has lasted for at least 8 years, with the aim of understanding the overall performance of the farm systems through a pre- and post-evaluation of the effects of management improvements adopted at a farm scale. The specific information on agricultural practices applied on farms were collected from farmer interview. Farm inputs (purchased feeds, bedding materials, fertilizers, agrochemicals, seeds, plastics, electricity, fuel, oil, and lubricants) and farm outputs (kg of milk and meat sold, forages and cereal grains sold) were obtained from the analysis of all the farm invoices. The fresh matter yield was measured using the sum of the weights of all the loads from each field measured using platform truck-scales, that were located on the farms. All the on-farm produced feeds were sampled to determine dry matter (DM) content and nutritional quality in terms of crude protein (CP) and metabolizable energy (ME), following methods reported in Tabacco et al. (2018). The GWP was calculated using the impact assessment method proposed by the intergovernmental Panel on Climate Change

(IPCC, 2013) (100y, v1.03), contained in the SimaPro software (9.0 PhD PR´e Consultants), for a time horizon of 100 years, and considering the CO2 equivalent factors for GHG gases reported by IPCC (2013).

Over the years, both farms have changed their forage system management from a PRE to a POST situation, which have been compared in the present paper. The conventional farm (CONV-PRE) was previously mainly based on a whole plant corn silage forage system plus hays from forage crops harvested at a late stage of development (Italian ryegrass, alfalfa, and rotational or permanent meadows), with any exceeding corn being sold as a grain cash crop. This system is representative of the current cropping management of about 80% of the intensive dairy farms located in the Po Plain in Italy (Borreani et al., 2013; Tabacco et al., 2018; Gislon et al., 2020). In the new management situation (CONV-POST), corn harvested as whole crop silage has been substituted by corn harvested as whole ear silage; alfalfa is grown on about 30% of the utilized agricultural area (UAA), and it is harvested as wilted silage (40-50% DM) at an early stage of growth (late vegetative or early-bud), 6 to 7 times a year. Italian ryegrass is harvested once/twice a year as wilted silage (40-50% DM) at an early stage of growth (late elongation to boot stage, mid-April, and mid-May in the case of a second cut).

The forage system of the studied organic farm was previously completely based on permanent meadows, as is the case of many organic dairy farms in Italy. In the pre-change situation (ORG-PRE), rotational meadows, sown every 4 to 6 years, were mainly composed of grasses, and were harvested as hay at late stages of growth (from full flowering onwards), with 4 cuts a year. The new management practices (ORG-POST), concern the successful establishment of white clover in the swards as a result of over-sowing, increasing the sward age, harvesting at an early stage of growth (6 to 8 cuts a year every 25-30 d, from the beginning of April), and conservation of forages through ensiling. A better calibration of the nutrient input from livestock slurry and manure was implemented with the aim of favoring a better survival and development of forage legumes in the permanent swards.

The main variations in the implementation of the forage system and in the management of the forage harvesting and conservation from the PRE to POST situations are summarized in Table 2.

The structural characteristics of the two studied farms are reported in Table 3. The ORG farm cultivates 180 ha (all permanent meadows), and on average rears 195 Simmental milking cows, with a stocking rate of 2.0 livestock units (LU) per hectare, whereas the CONV farm has around 63 ha of UAA, and on average rears 98 Holstein milking cows, with a stocking rate of 2.9 LU per hectare.

Table 2 Management of the forage system, harvesting and conservation practices, from the PRE to POST situations, on the two case-study farms in northern Italy

Item	ORG		CONV	
	pre	**post**	**pre**	**post**
Corn double cropped with Italian ryegrass	-	-	baseline	increase
Corn for whole-crop silage	-	-	baseline	decrease
Corn harvested as high-moisture ear silage	-	-	baseline	increase
Alfalfa and Italian ryegrass hay (late maturity cut)	-	-	yes	no
Alfalfa and Italian ryegrass silage (early maturity cut)	-	-	no	yes
Rotational or permanent meadow hay (late maturity)	yes	no	yes	no
Rotational or permanent meadow silage (early maturity)	no	yes	no	yes
Over-sowing white clover in existing permanent swards	no	yes	-	-
Balancing nutrient (N, P, K) input	no	yes	no	yes

Table 3 Farm and forage characteristics of the PRE and POST period applications of the new management practices, on the two considered farms in northern Italy

	ORG		CONV					
	pre	**post**	**pre**	**post**	**SE**	**P**	**F**	**PxF**
Utilized agricultural area (UAA)	180	180	62	64	19.1	NS	***	NS
Rotational/permanent meadows (proportion)	1.00	1.00	0.33	0.39	0.10	**	***	*
UAA yearly ploughed (proportion)	0.00	0.00	0.78	0.72	0.14	NS	***	NS
UAA not treated with agrochemicals (proportion)	1.00	1.00	0.33	0.42	0.10	***	***	***
Lactating cows (n)	183	207	99	97	16.1	**	***	*
Stocking rate (Livestock unit/ha)	2.0	2.1	2.9	2.9	30.2	NS	***	NS

P, period effect; F, farm effect; PxF, interaction; NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

The change in forage quality over time

The adoption of harvesting at early cutting, the increase in cutting frequency, coupled with the efficiency of silage conservation, have allowed the farms to improve the forage quality, in terms of crude protein and NDF content. The evolution of silage quality (that is, of the CP

and NDF contents) for different cuttings in different harvest years for permanent meadows (ORG farm) is reported in Figures 2 and 3 and for alfalfa (CONV farm) in Figures 4 and 5 as examples. It can be observed that the intended goal of having silages a higher CP than 15% DM and lower NDF than 50% from meadows was almost reached for all the harvested silages. The ORG farm changed its cutting schedule (more frequent cutting) and conservation management (from hay to bunker silage). Similar results were obtained for the CONV farm, in which the intended goal of having alfalfa silage with a higher CP content than 20% DM and a lower NDF content than 45% DM was reached for almost all the cuttings in the 2019-2021 period. The CONV farm changed its harvesting and conservation practices, by paying more attention to scheduling the cuts to when the development stage of alfalfa was late vegetative or at least early bud development. The increased cutting frequencies allowed both farms to compensate for the lower DM yield from each cut, with no changes in the total amounts of DM produced yearly, which ranged from 7 to 10 t DM/ha and from 13 to 15 DM/ha, for permanent meadows (ORG farm) and alfalfa (CONV farm), respectively.

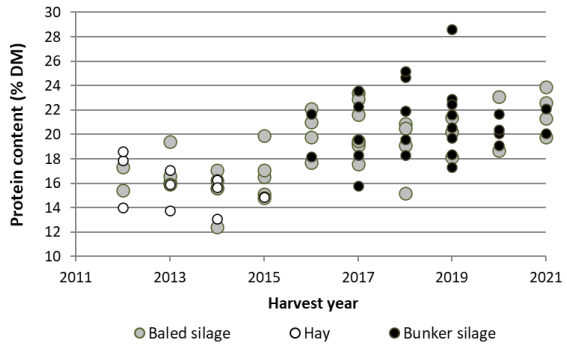


Figure 2. Evolution of the crude protein content in silages from permanent meadows, in relation to the conservation method (hay or bunker haylage) on the ORG Farm, for the 2016 to 2021 period.

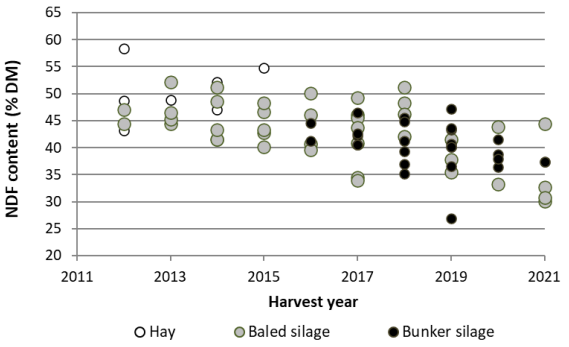


Figure 3. Evolution of the NDF content in silages from permanent meadows, in relation to the conservation method (hay or bunker haylage) on the ORG Farm, for the 2016 to 2021 period.

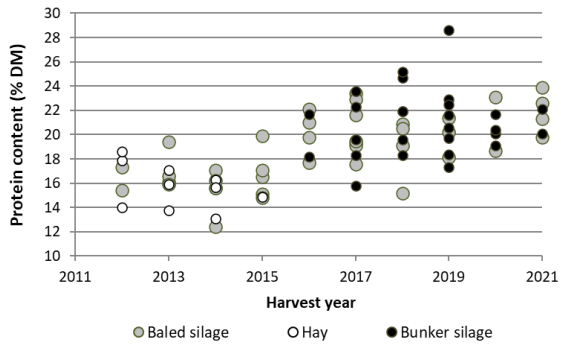


Figure 4. Evolution of the crude protein contents in alfalfa silages, in relation to the conservation method (hay, baled haylage or bunker silage) on the CONV Farm, for the 2012 to 2021 period.

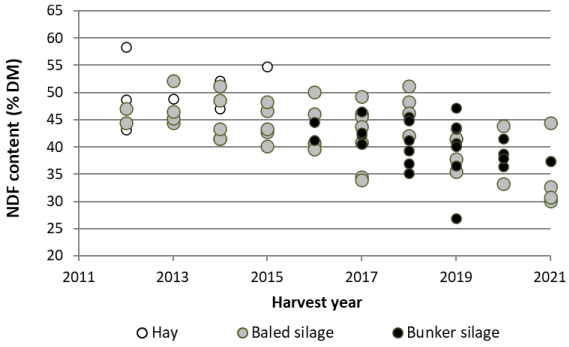


Figure 5. Evolution of the NDF content in permanent meadow silages, in relation to the conservation method (hay, baled haylage or bunker silage) on the CONV Farm, for the 2012 to 2021 period.

On the other hand, the amount of CP produced per hectare increased from 1105 kg/ha for the PRE- situation to 1296 kg/ha for the POST- situation and from 2240 kg/ha to 3130 kg/ha for the ORG farm meadows and CONV farm alfalfa, respectively.

Table 4 Milk production intensity, milk quality, dairy efficiency, herd requirements and CP and ME produced on farm in the PRE and POST application periods of the new management practices, on the two considered farms in northern Italy

	ORG		CONV					
	pre	post	pre	post	SE	P	F	PxF
Milk production intensity (t FPCM/ha)	6.6	8.1	18.2	22.1	2.16	***	***	*
Milk FPCM per cow (kg/d)	17.9	19.3	31.9	39.0	2.92	**	***	NS
Milk CP (%)	3.44	3.46	3.28	3.48	0.031	*	NS	NS
Milk fat (%)	4.06	4.18	3.83	3.81	0.056	NS	**	NS
DMI (kg/d)	17.5	15.8	24.1	25.2	1.33	NS	***	**
Dairy efficiency (kg FPCM/kg DMI)	1.02	1.22	1.32	1.55	0.064	**	***	NS
Total EM (GJ) requirements of the herd	18277	19705	10761	12983	1207	*	***	NS
Total CP (t) requirements of the herd	288	305	165	235	18.2	*	***	NS
EM (GJ) in purchased feeds	6786	5861	2774	3700	536	NS	***	**
CP (t) in purchased feeds	89	72	84	99	3.70	NS	*	*
EM (GJ/t FPCM) in purchased feeds	5.7	4.0	2.5	2.6	0.420	**	***	**
CP (kg/t FPCM) in purchased feeds	75	49	75	71	3.43	**	**	*
EM (GJ) supplied by on-farm feeds[1]	11491	13844	7987	9283	740	*	***	NS
CP (t) supplied by on-farm feeds[1]	199	233	80	135	19.9	*	***	NS
EM (GJ/ha) supplied by on-farm feeds	64	77	129	146	9.86	**	***	NS
CP (kg/ha) supplied by on-farm	1105	1297	1300	2126	85.1	*	**	NS

[1]calculated as the difference between the herd requirements and the amount of CP (t) or ME (GJ) from the purchased feedstuffs. CP, crude protein, DMI, dry matter intake, EM, metabolizable energy; FPCM, fat, protein corrected milk; P, period effect; F, farm effect; PxF, interaction; NS, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Cutting at an earlier stage of growth also increased the metabolizable energy (ME) content of the conserved forages fed to dairy cows with values which, on average, increased from 6.45 MJ/kg of DM for the PRE- situation to 8.82 MJ/kg of DM for the POST- situation in conserved forages from permanent meadows on the ORG farm, and from 7.77 MJ/kg of DM for the PRE- situation to 8.31 MJ/kg of DM for the POST- situation in conserved alfalfa forages on the CONV farm.

Milk production intensity and efficiency, and environmental impacts

The herd performances, CP, and ME requirements are reported in Table 4. The milk production intensity per hectare of UAA on average increased by more than 22% and 21%, on the ORG and CONV farms, respectively. The milk production per cow and the dairy efficiency also increased on both farms from the PRE to POST situation, whereas milk fat increased on the ORG farm and milk protein on the CONV farm. The new harvesting and conservation management provided more CP and ME from the UAA to satisfy the herd nutrient requirements. Less CP and ME were purchased off-farm per kg of FPCM produced

on the ORG farm, whereas the same amounts of CP and ME were purchased off-farm per kg of FPCM produced on the CONV farm. This was reflected by an overall lower carbon footprint per kg of FPCM, which showed an average reduction of about 10% on both farms (Table 5).

Table 5 Global warming potential (GWP) per kg of FPCM (kg CO_2-eq/kg FPCM) in the PRE and POST application periods of the new management practices, on the two considered farms in northern Italy

Item	ORG		CONV					
	pre	post	pre	post	SE	P	F	PxF
Total GWP	1.44	1.30	1.43	1.29	0.031	*	NS	NS
Purchased Feeds	0.40	0.34	0.53	0.63	0.038	NS	***	*
Methane (Enteric and from slurry)	0.89	0.80	0.58	0.45	0.058	*	***	NS
Nitrous oxide (direct and indirect)	0.10	0.10	0.15	0.11	0.019	NS	**	*
Other inputs	0.06	0.06	0.16	0.10	0.014	**	***	**

FPCM, fat, protein corrected milk; P, period effect; F, farm effect; PxF, interaction;
NS, not significant; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Plastic use on farm and perspectives for reducing waste

The ensiling technique is based on the use of plastic films, and approximately 45% of the plastic utilized in agriculture in Europe is destined for silage packaging (Borreani and Tabacco, 2017). From a survey conducted in Italy, it resulted that around 6 kg/ha of plastic is consumed yearly on dairy farms (Borreani and Tabacco, 2017) (Figure 6). These data agree with those reported in a study conducted in Ireland (Hamilton et al., 2005). Around 80% of the plastic consumed on dairy farms is represented by flexible plastic film that is used to wrap bales or to cover horizontal silos (Borreani and Tabacco, 2014).

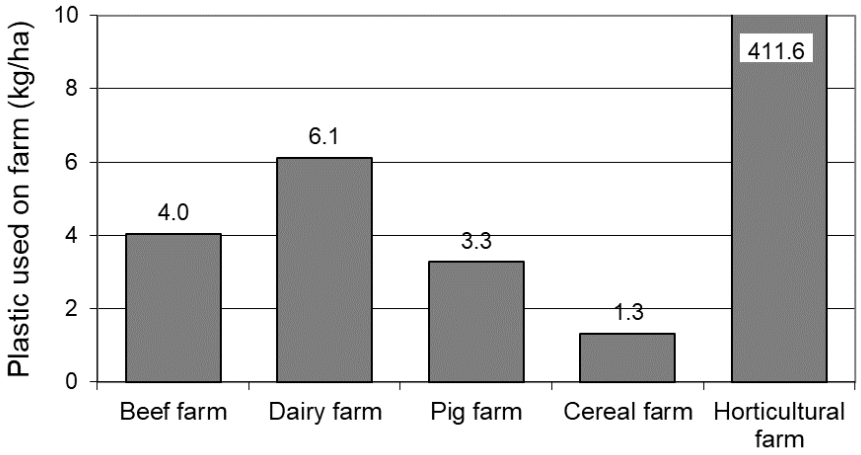


Figure 6. Plastic used on farms per hectare and year in northern Italy (Borreani and Tabacco, 2017).

The agricultural plastic films used for silage may be intrinsically difficult to recycle, due to their contamination by soil, sand, and/or silage (Holmes and Springman, 2009). The disposal of PE sheeting could represent a potential environmental concern because it is difficult to recycle and can basically only be used once (Kyrikou and Briassoulis, 2007). For these reasons, an efficient use of plastic for ensiling, its recycling, and the development of new biodegradable polymers that have suitable characteristics to produce films for ensiling (both

stretch films and plastic sheets to cover horizontal silos) and which could be composted on farm at the end of life, should be studied and developed (Borreani and Tabacco, 2017; Guerrini et al., 2017; Tabacco et al., 2020). In recent years, a great deal of attention has been paid to the search for cost-effective alternatives to replace non-biodegradable plastic films to cover silages, such as biodegradable materials with competitive mechanical properties (Borreani and Tabacco, 2015). Therefore, an alternative way of disposing of agricultural plastic wastes is through the biodegradation of fully biodegradable polymers that can be completely converted, by means of microorganisms, into carbon dioxide, water, minerals, and/or microbial biomass, without releasing any potentially harmful substances in the environment (Kyrikou and Briassoulis, 2007). Biodegradable polymers can be a solution to reduce the overall environmental impact of some applications and increase the quantity and quality of organic material recuperated via composting (green waste) (Guerrini et al., 2017). Earlier research identified and produced biobased biodegradable plastics to produce plastic films (Bastioli, 1998; Keller, 2000), and these plastics have recently been utilized to produce films of different thicknesses that might be suitable for covering silage (Borreani et al., 2010; Tabacco et al., 2020). Biodegradable coatings, such as straw, apple pulp or food industry waste, are other alternatives that can be used to cover silage, but until now these materials have been unsuccessful in forming a stable barrier against air for longer periods than 1 month under farm conditions (Brusewitz et al., 1991; Denoncourt et al., 2007).

For a plastic film to be suitable for silage conservation, it should have high mechanical properties (puncture resistance, tear resistance) to resist wind, hail, frost, and handling; a thickness ranging from 45 to 200 μm; high impermeability to oxygen (full anaerobiosis is necessary); physical strength properties that can be maintained over a long period of time (longer than 1 year) in a natural rain- and sun-exposed environment; UV protection (different degrees of protection in relation to the latitude); and costs related to the necessary quality requirements. Preliminary studies (Borreani and Tabacco, 2015; Tabacco et al., 2020) showed the possibility of covering horizontal silos with films made of innovative biodegradable polymers for about 5 months, with a good conservation of silage. Improvements in the future will involve improving oxygen impermeability and the stability of the new biodegradable films to obtain silages with a longer shelf life after air gains access to the silo during consumption, by delaying the growth of molds and reducing their detrimental effect on the safety and quality of silage (Guerrini et al., 2017). These studies have encouraged the development of biodegradable plastic films to cover silages under outdoor conditions through the improvement of biodegradable blends to enhance microbiological film stability over time, and also to evaluate a biodegradable UV stabilizer to maintain the performances of such films under outdoor conditions in both winter and summer.

Conclusions

Through the adoption of an early cutting harvesting, which involved increasing the cutting frequency of alfalfa and permanent meadows, coupled with the high efficiency of conserving forage by ensiling, the farm that utilizes its agricultural area to satisfy the nutrient requirements of its herd has achieved a considerable increase in crude protein (+17 and 25%) and metabolizable energy (+20 and 12%). This greater availability of feed nutrients has been accompanied by a reduction in the farm's dependency on market commodities and has had a beneficial effect on the global environmental impact of the milk production, in terms of GWP reduction (-10%). Considering similar commodity price trends to those of the last decade, this

analysis indicates that this forage system management approach will become an increasingly more profitable alternative to the conventional forage systems that are currently adopted by the majority of farms in northern Italy.

Acknowledgement

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Silage technologies and management of the future

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Introduction

Decades of research have focused on developing strategies to improve silage quality and minimize nutrient losses during ensiling. In Western countries particularly in North America and Europe, several parts of Central America and in certain Asian countries, silage is an important part of the diet of dairy cows, beef cattle and small ruminants. For instance, in the United States, silage accounts for up to 60% of the diet of dairy cows and 131 million tons of corn silage were produced in 2021. This is because silage is an excellent forage conservation strategy that allows winter feeding or housing of cows under confinement and provides the bulk of the energy, protein, and digestible fiber content of ruminant diets in many countries. While the concept of ensiling dates to back to 1000 to 1500 BC (Ecosyl, 2022), refinement in silage making practices and equipment in the last two decades have resulted in better preservation and greater nutritional quality and aerobic stability of ensiled forages. In this paper, we recommend future improvements in silage management and technology that will improve silage harvesting and packing, fermentation and preservation, nutritional value, and reduce its adverse impacts on the environment. The intention is not to provide an exhaustive discourse on future silage technologies and management strategies but to mention some innovative aspects that are imminent or should be developed for routine use by 2050. The specific innovations are described under each of the following silage production phases.

Forage monitoring

Knowledge of the potential yield, moisture concentration and nutritional quality aspects of forages during the growing season are important for making decisions about fertilizer application and timing of harvests for silage production. For example, various types of forage grasses for silage production are harvested every three to six weeks depending on whether forage quality or yield is prioritized, and fertilizer is applied after each harvest. Conventional methods of determining the timing of harvest range from subjective estimates of moisture content e.g., by the squeeze test, to using various devices to precisely measure forage moisture concentration. More insightful estimates of nutritional value require *in vivo* and *in vitro* methods that are labor intensive and time-consuming. Recently developed mobile/hand-held and online near-infrared reflectance spectroscopy (NIRS) devices allow rapid and reliable online monitoring of concentrate nutritional quality, providing almost immediate and much cheaper analytical results than conventional wet chemistry alternatives (Oliveira et al., 2020). Though mobile NIRS devices provide precise moisture content predictions for forages, their use for forage chemical analysis is limited by high moisture content, which results in less precise calibration and validation relationships. Hence, near term research is needed that will allow such mobile devices to be precisely used in the field to monitor forage nutritional quality, particularly from standing forage or fresh /undried forage samples.

The use of NIRS has paved the way for more advanced portable and field hyperspectral sensors for the estimation of forage quality and quantity (Pullanagari et al., 2012). For instance, fine-scale digital elevation models (DEMs) with satellite imagery are used for monitoring spatial variations in crop moisture and nutrient levels. Such techniques need to be

applied to forages because they overcome the main limitation of using NIR, i.e., it is not practical for spatial analysis of the yield or nutritional quality of an entire field of forage.

Recently, several studies have shown the potential of remote sensing technology using drones for estimation of forage parameters like biomass yield and attributes of entire fields or individual plants. Drones are rapid, low-cost alternatives for collecting remote-sensing data in agriculture (Näsi et al., 2018) and when equipped with appropriate sensors, they can be used to scan above the canopy to monitor crop yields, disease infestation, lodging, etc. For instance, drones can be used to assess spatial and temporal trends in forage yield, allowing avoidance of the need for weigh wagons or weighing scales for monitoring silage loads. This can allow more complex experimental designs on-farm with experimental units shorter than the length of the whole field (Matcham E., pers. comm.).

Currently drones are mostly used to scout or make variable rate fertilizer or pesticide recommendations based on greenness and disease and pest infestation levels and to monitor silage inventory. In addition, drones with moisture sensors can be used to sense the best time to harvest forages, while future enhancements that integrate remote capture of digital images may enable monitoring of the epiphytic microbial population and composition, which could inform the timing, type, and application rate of silage additives.

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Forage harvesting

Harvesting equipment should be equipped with sensors that automatically detect and avoid poisonous plants, dead animals that can cause botulism, and forages with high concentrations of toxins like nitrates, mycotoxins, and prussic acid. In addition, future harvesting equipment should ensure forage consistency and uniformity by automatically adjusting chop length to maintain a desired theoretical length of cut and ensure consistently high starch and fiber digestibility as the chopper moves through the field. Machine learning should also be integrated to monitor rates of additive application and automatically adjust them to ensure uniform application throughout the forage mass, regardless of the silo size.

Silo filling and compaction

High powered vacuums should be developed to ensure rapid and near complete oxygen exclusion from silos, thus providing conditions that inhibit the growth of microbes that predispose silage to aerobic spoilage and secondary fermentation.

Packing tractors equipped with density sensing devises should be integrated with strategically placed laser flow meters in silos to enable dynamic density estimation and achievement of silage density targets. Such equipment can highlight areas where additional compaction is critical to exclude oxygen thus inhibiting the growth of spoilage-causing yeast and mold. Automation of silage packing is highly desirable to increase objectivity of achieving the desired packing density as well as increasing the efficiency thus minimizing waste and losses of DM, energy, and nutrients.

Anaerobic fermentation and storage

Continuous monitoring of silage in silos could potentially assure greater quality of silage and benefit farmers economically. Despite extensive research in this area, automated precise systems for monitoring silage quality are not routinely available. Initial attempts to use sensor-based monitoring systems did not work due to their invasiveness resulting in breakage of the silage plastic films (Williams et al., 1997). Due to cost-effectiveness, reduced power consumption, and ease of implementation, wireless sensor networks are now capable of sending data from inaccessible or hostile environments (Khalifeh et al., 2021). For instance, Green et al. (2009) used wireless sensor networks for collecting temperature and humidity data within the silage. This system was further developed by adding oxygen sensors (Bochtis et al., 2011) and temperature and pH sensors (Thunen et al., 2019). Nevertheless, these have not been successfully used in farm management information systems despite the promise. Bauerdick et al. (2022) developed a novel silage monitoring system by collecting additional information along with temperature and oxygen for detecting deviations in the silage

environment caused by air breaches. Future research should be focused on implementing this technology for monitoring of silage quality under farm conditions. In addition, the extensive dataset collected over different silage types and qualities along with lab-based measurement of silage quality may be helpful in creating models to estimate actual DM losses based on measured changes in the silage environment and attributes such that more effective strategies to prevent silage losses and improve quality can be developed.

Future integration of metabolomic, metagenomic and meta transcriptomic techniques, will allow unprecedented understanding of the mode of silage fermentation and the mode of action of additives, which will lead to more targeted, consistently effective, and efficient additives. For instance, the potential of fibrolytic and proteolytic enzymes to enhance fiber and protein utilization in ruminant diets has not been realized. This is partly due to lack of understanding of basic factors relating to how to optimize enzyme penetration and deconstruct the cell wall, how to optimize enzyme stimulation of beneficial microbes by enhancing their attachment and efficacy as well as preventing crude problems like declaring that an enzyme with a cocktail of activities only contains the single measured activity. Future inoculant research should develop strategies that assure uniform application to forage masses, improve consistency of the targeted response such as improving fermentation, reducing DM, energy, and nutrient losses, increasing aerobic stability and increasing the feed safety of silage. Use of omic and newer analytical techniques to elucidate the detailed modes of action of these additives will allow better targeting, increased consistency, thereby reducing production costs and increasing affordability for routine use.

Better understanding of the microbiome will also enhance use of various novel additives including viruses, novel fungi, bacteria, protozoa and archaea to modulate and improve silage fermentation. Various underexploited chemical attributes of functional forages like their tannins and saponins, should be used to improve nutrient preservation during ensiling and inhibit the growth of undesirable microbes. For instance, despite the well-documented potential to use tannins to reduce protein degradation during ensiling, practical application of this strategy is exceptionally low.

Further, additives should be applied using irrigation systems or remote-controlled devices like drones fitted with sensors that monitor the application rate and coverage areas sprayed, and that adjust both of the latter as needed to ensure uniformity. Furthermore, applicators that can apply more than one product simultaneously will be an advantage.

Silage covering

Effective sealing is essential to lower DM losses during ensiling. The quality of plastic seal is a crucial factor determining the silage quality because of longer duration of silage storage under farm conditions. The primary characteristics of a plastic film for providing effective seal include high mechanical strength and low oxygen permeability (Borreani et al., 2018). Low density polyethylene films were used commonly until the early 2000's, some of which involved thicker plastic sheets. More recently, multi-layer co-extrusion technology has been exploited to reduce oxygen permeability to levels achieved by 2000 µm thick low density polyethylene films (Borreani et al., 2018). These oxygen barrier films result in less aerobic deterioration, lower DM losses, and less labor for removing and discarding the top-layer silage in silos.

Despite advances in the sealing technology, the disposal of polyethylene and oxygen barrier films represent an environmental concern because of their non-biodegradable and difficult to recycle nature (Kyrikou and Briassoulis, 2007). Efforts have now been focused on replacing petroleum-based plastic covers with biodegradable bioplastics made with renewable sources. However, the challenge with using biodegradable plastic is that silage cannot be stored greater than 5 months under lab conditions or 3 months under farm conditions (Tabacco et al., 2020). Hence, using biodegradable plastic cover is not recommended for long-term storage of silage. Therefore, future silage technologies should use covers that are oxygen impermeable, resilient to inclement weather, pest and weather resistant as well as either renewable / reusable, and or edible. Furthermore, the labor intensity of covering silage with plastic should be avoided by using automated systems that extend the cover over the silage mass after compaction and that recoil it progressively during feed out.

Aerobic stability and feed out

The aerobic deterioration of silages conserved in horizontal, drive-over bale silos is influenced by feed-out rate from the silo, the silage density, the covering, and the weighing down of shoulder and top sheets, the use of additives, and the ambient temperature (Borreani et al., 2018). The risk of including spoiled silage in dairy cattle diets can be lowered by achieving the correct feed-out rate. The feed-out rate is generally measured as the mean linear progress of the unloading of a silo face because of the daily consumption of silage (m/wk.). When this index is used to predict the risk of the aerobic spoilage of farm silages, different results emerge according to the silage densities or environmental temperatures (Holmes and Muck, 2007; Borreani et al., 2018). De Oliveira et al. (2018) proposed an innovative approach to integrate the linear feed-out rate (LFR) and density in one indicator that is easily measurable on a farm, and which expresses the daily feed-out silage amount taken from a square meter of silo face. This method is fast and easy to use as it reduces the difficulties involved in measuring silage densities and the mean daily or weekly LFR however, it only provides static and nondynamic measurements. Another method to detect silage spoilage is related with the temperature of the central core compared with temperature measured in other locations of the silo face which are more prone to aerobic deterioration (Borreani and Tabacco, 2010). However, under certain circumstances, excessive heat accumulation in the core sample due to plant respiration during silo filling, can lead to problems with result interpretation. The ideal situation would be to detect all the spoiled and spoiling silage and avoid including it in the feed ration. While visibly moldy silage can easily be located, non-visibly moldy or spoiled silage is more challenge to detect. Yet it is just as risky, due to direct deleterious effects of spoilage yeasts and molds and potential hazards from mycotoxin contamination on animal and human health. These factors emphasize the importance of developing sensors for detecting non-visibly spoiled silage in silos.

It is currently impossible to accurately and timely evaluate the microbial and chemical quality of the whole working face in a cost-efficient way or in a routine manner on a farm during the feed-out phase (Davies et al., 2018). Hence, methods that enable a forage producer to assess the silage quality and the extent of aerobic deterioration quickly and accurately at the silo face are essential. Thermal imagery of the area on and behind the silo face should be integrated with silage density and microbiome estimates to better predict the aerobic stability of the silage on and behind the face. This information about the aerobic stability, yeast and mold growth should be delivered to intelligent face shavers that will alter the feed out rate as needed to minimize spoilage. Further, such information coupled with that from sniffing

sensors should be integrated in automated silo face additive applicators that spray preservatives on the silo face to minimize spoilage.

Silage feeding management

The physical properties of silage including particle size, along with fermentation characteristics of starch and fiber, and fermentation end products may influence feeding behavior, intake potential, and subsequently milk production in dairy cows fed silage-based diets (Grant and Ferraretto, 2018). Research in past several years has been focused on managing physical aspects of silage for optimizing intake, rumination, and resting behavior and observing its subsequent impact on diet digestibility, and production of milk and milk components. Jensen et al. (2016) observed a negative linear relationship between chewing index (min. of chewing per kg DM) for silage-based diets and dry matter intake (DMI). The chewing index decreased with greater forage neutral detergent fiber (NDF) digestibility, short particle size, and lower NDF content and these parameters have subsequent effects on DMI in ruminants. Similarly, greater fiber degradability is associated with changes in feeding behavior and meal patterns that improve DMI (Miron et al., 2007; Jiang et al., 2017). Feeding higher-forage diets, lower NDF digestibility forages, and longer particle size changes feeding behavior by increasing time needed to consume feeds and this comes at expense of resting time. Greater chewing time may be a constraint for increasing DMI for high producing dairy cows. Future strategies are needed that optimize intake of silage by addressing these factors.

Diets formulated with high starch content or ruminal starch digestibility may influence DMI by increasing the proportion of ruminal propionate (Allen et al., 2009). Studies evaluating the effects of greater starch content or ruminal starch digestibility on DMI, and meal patterns were conducted using non-forage feed sources (Ferraretto et al., 2013). Most of these studies reported lower feed consumption in response to greater ruminal starch degradability with no effects observed on milk production. Future studies that evaluate strategies for improving starch degradability and feeding behavior concurrently are needed. These studies are important considering that strategies used for improving starch degradability in silage may influence physical characteristics of forage fiber (kernel processing in corn silage).

Improving food safety

Recent research showing that silage inoculants can be used to bind aflatoxin (Ma et al.,2017), inhibit the growth of pathogens like E. coli O15H7 (Pedroso et al., 2010) and prevent aflatoxin accumulation in diseased plants (Queiroz et al. 2012), reveal that silage can be strategically used to inhibit pathogen recycling on farms or toxin transmission in food systems. Further expansion of this area of research is important because cattle are among the main pathogen reservoirs on farms and because of increasing public awareness and concerns about food safety/foodborne diseases.

Potential environmental concerns

The environmental impact of silage production will become increasingly monitored. This will necessitate regular monitoring of greenhouse gases from silage production and development of management strategies and technologies including additives, for their mitigation. In addition, volatile organic compounds (VOC) emitted from silage have been blamed for poor air quality in regions of concentrated agriculture (Howard et al., 2010). These VOC are precursors of ozone (Bonifacio et al., 2017) and in presence of sunlight, they can react with oxides of N to form ground-level ozone. Considering their impact on air quality,

measurement of VOC will become increasingly important in future. Future research should develop technologies that allow online monitoring of greenhouse gases and VOC concentrations in silage using face shavers.

Conclusions

Silage will continue to be an integral component of ruminant diets in Western countries and its production and use will increase considerably in low- and middle-income countries in the future. Recent silage research efforts in the past few decades were focused on enhancing fermentation characteristics and improving the nutritive value and aerobic stability of conserved forages. In addition to further enhancing and refining the latter, future research will need to be multidisciplinary, including experts from agronomy, microbiology, animal sciences, as well as agricultural engineering and artificial intelligence to ensure proper leveraging of opportunities to improve all aspects of silage production using novel analytical, monitoring, and measurement techniques, as well as smart, profitable yet resilient and sustainable approaches.

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Effects of a microbial silage inoculant on fermentation of nitrate-free grass silage ensiled in round bales at low and high dry matter level

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Introduction

Nitrate inhibits clostridia at the initial stages of fermentation by being decomposed into nitrite and NOx gases (Spoelstra 1983). Grass and grass-legume mixtures, containing less than 1 g nitrate per kg of dry matter (DM), can be classified as "nitrate-free" (Kaiser et al., 1997). If the count of lactic acid bacteria (LAB) is less than 10^5 per g of fresh matter, and the nitrate content is less than 0.5 g/kg DM, silage containing butyric acid must be expected despite a fermentability coefficient (FC) that is far above 35 (Weissbach and Honig, 1996). The level of dry matter content was not taken into account in the mentioned study. Therefore, this trial aimed to investigate the effects of a microbial silage inoculant on fermentation of nitrate-free grass silage ensiled in round bales at low and high dry matter levels.

Materials and Methods

The herbage originated from two parts of one field located northwest of Germany. The dominant species of the meadow were perennial ryegrass (*Lolium perenne*), timothy grass (*Phleum pratense* 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 of May 30, 2021. One part of grass was picked up with a round baler after approximately 18 hours of wilting (WET). Another part of the grass was picked up after a wilting period of approximately 36 hours (DRY). Six bales per treatment were prepared. The control bales (CON) were treated with water. The inoculated bales (INO) were treated with SiloSolve® MC, containing *Lactobacillus plantarum* (DSM26571), *Enterococcus faecium* (DSM22502) and *Lactococcus lactis* (NCIMB30117) at a target application dose of 150 000 total CFU/g of forage. Bales were wrapped with 8 layers of plastic film and placed on their flat ends on plastic pallets. DRY Bales were sampled for proximate analysis, forage hygiene, and fermentation profile after 65 days of storage. WET bales weres sampled after 92 days of storage. One bale per dry matter level and treatment was used in a pilot feeding study investigating the eating behaviour of heifers offered both treatments at the same time. One pen from the research and training centre LVZ Futterkamp was used for the test. Because of animal rotation on the farm and the different dates of bales sampling, animals of different ages were located in the pen during feeding of DRY and later WET silage. The test was done with 18 heifers (average weight 405 kg) for WET and with 13 heifers (average weight 460 kg) for DRY silage. The feeding table was divided into equal parts with the same number of feeding places. All the animals in the pen had free access to both silages at the same time. The feed fences were opened about one hour after all the silage had been completely presented. The feed was regularly pushed up to the feeding fence. Each test lasted 24 hours and ended by closing the feeding fences. The volume of silage at the start and the silage residuals after 24 hours were weighed. To exclude the effect of the feeding place (to the left or the right of the front of the pen) the position of the CON and TRT silages was switched over by feeding DRY and WET silages.

Data were analysed as a completely randomized design using PROC GLM procedure (SAS 9.4) with treatment as a fixed effect separately for each dry matter level because of the different storage periods for WET and DRY silages. The effects were considered statistically significant when $P < 0.05$. No statistical analysis was performed for the feeding behaviour test because of the absence of repetitions per dry matter level and treatment. This part of the study is only to be understood as a supplement to get an impression of the preferences of the animals for differently treated silages.

Results and Discussion

The weight of bales ranged from 835 to 961 kg for WET, and from 546 to 709 kg for DRY grass. Dry matter content, nitrate content, and FC coefficient (Schmidt et al., 1971) were 20.8 and 46.0%, 0.3 and 0.2 g/kg, and 46.1 and 72.9, for WET and DRY grass, respectively.

Table 1 Characteristics of grass silages prepared at low (WET) and high (DRY) dry matter levels without inoculation (CON) or inoculated (INO)

Wilting level			**WET**			**DRY**		
Variable	**Unit**	**N**	**CON**	**INO**	**SEM**	**CON**	**INO**	**SEM**
Fresh weight loss	%	6	2.12[a]	0.73[b]	0.08	1.89[a]	1.33[b]	0.08
DM loss	%	6	9.50[a]	3.46[b]	0.32	4.14[a]	2.87[b]	0.18
DM	%	6	20.7	20.8	0.44	44.7	46.3	2.00
Crude ash	% DM	6	8.14[a]	6.89[b]	0.29	7.51	7.35	0.07
Crude protein	% DM	6	13.9[a]	15.0[b]	0.35	13.5	13.7	0.22
WSC[1]	% DM	6	0.76[a]	4.56[b]	0.56	12.01	12.82	0.56
$aNDF_{OM}$[2]	% DM	6	50.6[a]	47.7[b]	0.63	52.2	51.6	1.30
ADF_{OM}[3]	% DM	6	32.5[a]	29.7[b]	0.46	31.6	31.3	0.68
pH		6	4.70[a]	3.93[b]	0.05	5.08[a]	4.53[b]	0.10
Lactic acid	% DM	6	3.89[a]	10.82[b]	0.36	2.36[a]	4.91[b]	0.34
Acetic acid	% DM	6	1.24	0.77	0.09	0.92[a]	0.42[b]	0.08
n-Butyric acid	% DM	6	4.64[a]	1.04[b]	0.19	0.35[a]	0.10[b]	0.04
Ammonia-N	% total N	6	14.97[a]	8.67[b]	0.63	6.15[a]	4.71[b]	0.23
Ethanol	% DM	6	1.80[a]	1.19[b]	0.09	1.59[a]	1.07[b]	0.11
LAB	$\log_{10}$ CFU/g	6	7.25	6.94	0.10	7.45[a]	6.63[b]	0.29
Yeasts	$\log_{10}$ CFU/g	6	2.33[a]	4.99[b]	0.26	2.55	2.33	0.23
Moulds	$\log_{10}$ CFU/g	6	Nd[4]	Nd	-	2.16	Nd	0.12
Voluntary silage intake[5]	kg FM/pen	1	259	396	-	102	167	

[1]WSC = water-soluble carbohydrates as sugar + fructans; [2]$aNDF_{OM}$ = neutral detergent fibre; [3]ADF_{OM} = acid detergent fibre; [4]Nd = value below the limit of detection (< 2 $\log_{10}$ CFU/g); [5]No statistical analysis performed, because only one pen used for the observation.
Means with different superscripts within dry matter levels differed significantly at $P < 0.05$.

The grass could be considered “nitrate-free” and “easy to ensile”. The count of epiphytic lactic acid bacteria was 4.9 Log_{10} CFU/g FM in WET and 4.1 Log_{10} CFU/g FM in DRY grass. Very low nitrate content and epiphytic LAB count below 5.0 Log_{10} CFU/g FM indicate a high risk for butyric acid fermentation by clostridia according to Weissbach and Honig (1996). The silage characteristics are presented in Table 1. The bacterial inoculant

significantly reduced the fresh weight loss and dry matter loss at both dry matter levels. The concentration of 8.67 % of total N for ammonia-N and of 1.04 % DM for butyric acid in the INO WET silage were in an acceptable range for grass silage (Kung et al., 2018). The presence of butyric acid in the INO WET silage may come from the initial stage of fermentation in nitrate-free grasses where clostridia act as competitors of LAB (Kaiser et al., 1997). A significantly higher concentration of butyric acid and ammonia-N in the CON WET silage and a very low concentration of lactic acid indicate that the fermentation was dominated by clostridia (McDonald et al., 1991). The higher degree of wilting could not completely inhibit the growth and activity of clostridia in the CON DRY silage. INO DRY silages had significantly lower concentrations of butyric acid and ammonia-N compared to CON DRY silages.

Although the WET and DRY silages had completely different characteristics, and the differences between CON and INO DRY silages were smaller in contrast to WET silages, heifers preferred INO silages in both cases. The WET CON silage could be classified as spoiled by clostridia, but the animals did not refuse it completely, despite free access to the better INO silage (according to the analysis) at the same time. To gain reliable information on the palatability of differently fermented silages, a longer trial period including more pens would be required.

Conclusions

Inoculation of nitrate-free pasture with the strains producing mainly lactic acid is required to reduce the risk of silage spoiling by clostridia. Increased level of dry matter through intensive wilting reduces the activity of clostridia. The combination of intensive wilting with bacterial inoculation can be recommended as the best technique for nitrate-free grass silage prepared in bales.

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Effects of innovative inoculants on fermentation quality of grass silage

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Introduction

Due to the fast development of livestock sector, the demand for high-quality feeds throughout the year is greatly increasing worldwide in order to properly feed ruminants. Ensiling can reduce shortage of green biomass for ruminants in countries with prominent climate seasonality, and a successful silage production plays an important role in dairy farm's economy. Preservation of silage is based on lactic acid production by anaerobic lactic acid bacteria (LAB; McDonald *et al*., 1991). If ensiling is conducted carefully, pH of ensiled feed reduces to around 4 due to the presence of undissociated organic acids (lactic, acetic, propionic and other volatile fatty acids [VFAs]), which are produced during fermentation and prevent the activity of undesirable microbes. Development and use of various silage inoculants aims at modulating fermentation pattern in silage to ensure a better quality. Although the feed preservation industry has developed considerably in the past few decades, efforts to improve the quality of preserved feeds still require additional knowledge and tools. In this project new developmental products were tested. New inoculants might improve silage fermentation quality and, consequently, improve the profitability and sustainability of the animal production systems. The objective of the present study was to produce silages in laboratory scale to find out the effects of different innovative silage inoculants on fermentation quality, aerobic stability and microbial quality of grass silage.

Materials and Methods

This experiment was conducted in the facilities of the Natural Resources Institute Finland (Luke) in Jokioinen, Finland (60°48'N, 23°29'E). Timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) grass was harvested from a regrowth ley in early September 2021, chopped and transported to the laboratory facilities without any additive. A representative raw material sample was immediately taken for chemical composition analyses. To evaluate the efficiency of innovative silage inoculants to preserve grass silage, seven treatments with different strains of *Lactobacillus plantarum* and *Pediococcus pentosaceus* were applied in a dose of 1×10^6 cfu/g fresh forage, including five replicates of 10kg each per treatment as follows: control, without inoculant; *L. plantarum* IMI 507026 and *P. pentosaceus* IMI 507024 in a ratio 50:50 (Inoc 1); *L. plantarum* IMI 507027 / *P. pentosaceus* IMI 507025 in a ratio 75:25 (Inoc 2); *L. plantarum* IMI 507026 / *P. pentosaceus* IMI 507025 in a ratio 50:50 (Inoc 3); *L. plantarum* IMI 507026 / *P. pentosaceus* IMI 507024 in a ratio 75:25 + 25 mg/kg fresh forage of $MnSO_4$ (Inoc 4); *L. plantarum* IMI 507026 / *P. pentosaceus* IMI 507025 in a ratio 75:25 + 25 mg/kg fresh forage of $MnSO_4$ (Inoc 5); *L. plantarum* IMI 507028 / *P. pentosaceus* IMI 507025 in a ratio 50:50 + 25 mg/kg fresh forage of $MnSO_4$ (Inoc 6). Silos were opened after an ensiling period of 3 months. Deteriorated parts were discarded and silage was carefully mixed and samples were taken and analysed for chemical composition, fermentation quality, aerobic stability and microbial quality. Data was analysed using the MIXED procedure of SAS 9.4. Contrasts were used to evaluate the effect of $MnSO_4$ addition

Table 1 Chemical composition, fermentation quality, ensiling losses and microbial quality of grass silages treated with different inoculants

Item	Treatments[1]							SEM[2]	P-value[3]			
	Control	Inoc 1	Inoc 2	Inoc 3	Inoc 4	Inoc 5	Inoc 6		Treat	C vs I	$MnSO_4$	Strain
Dry matter (DM), g/kg	194	194	193	194	194	196	196	1.1	0.401	0.770	0.060	0.804
pH	3.87^{ab}	3.84^{b}	3.86^{ab}	3.85^{ab}	3.83^{b}	3.84^{b}	3.90^{a}	0.012	0.006	0.298	0.381	0.044
Ammonia N, g/kg N	60.8^{a}	42.6^{cd}	45.3^{bc}	44.9^{bcd}	40.0^{d}	43.5^{bcd}	48.3^{b}	1.10	<0.001	<0.001	0.704	0.015
Ethanol, g/kg DM	9.74^{abc}	11.92^{a}	8.67^{bc}	8.78^{bc}	9.59^{abc}	7.89^{c}	10.91^{ab}	0.596	0.001	0.856	0.509	0.001
Water soluble carbohydrates, g/kg DM	38.3^{b}	78.2^{a}	71.6^{a}	74.1^{a}	74.5^{a}	76.2^{a}	77.0^{a}	3.26	<0.001	<0.001	0.638	0.390
Fermentation acids, g/kg DM												
Lactic (LA)	96	104	104	99	108	109	103	3.2	0.075	0.021	0.091	0.059
Acetic (AA)	26.2^{a}	11.7^{b}	14.0^{b}	13.8^{b}	11.9^{b}	13.3^{b}	13.2^{b}	0.81	<0.001	<0.001	0.537	0.808
Propionic	0.330	0.250	0.280	0.288	0.270	0.256	0.308	0.0299	0.507	0.103	0.829	0.590
Butyric	0.05	0.06	0.11	0.08	0.08	0.07	0.05	0.023	0.515	0.318	0.366	0.214
Total volatile fatty acids	26.6^{a}	12.0^{b}	14.4^{b}	14.2^{b}	12.3^{b}	13.6^{b}	13.6^{b}	0.82	<0.001	<0.001	0.590	0.768
Total fermentation acids	122.4	116.1	118.1	112.7	120.6	122.7	116.4	2.78	0.154	0.134	0.074	0.026
Total fermentation products	132	128	127	122	130	131	127	2.6	0.131	0.097	0.073	0.101
LA/AA ratio	3.83^{d}	8.88^{ab}	7.40^{c}	7.16^{c}	9.14^{a}	8.22^{abc}	7.82^{bc}	0.267	<0.001	<0.001	0.014	0.182
Aerobic stability, 2 hours	51.4^{a}	32.7^{c}	41.5^{b}	38.6^{bc}	37.4^{bc}	38.7^{bc}	34.8^{bc}	1.51	<0.001	<0.001	0.600	0.005
Aerobic stability, 3 hours	57.8^{a}	35.7^{c}	45.5^{b}	42.8^{bc}	41.3^{bc}	43.0^{bc}	37.8^{c}	1.63	<0.001	<0.001	0.622	0.003
Density, kg/m^3	728	753	711	727	758	749	753	15.1	0.265	0.420	0.076	0.680
Discarded, % of fresh matter	5.51	4.54	4.77	5.11	4.67	4.50	5.31	0.510	0.723	0.223	0.962	0.421
Visual mould	1.80^{a}	0.95^{bc}	0.70^{c}	1.80^{a}	1.50^{ab}	1.60^{ab}	1.40^{abc}	0.164	<0.001	0.013	0.015	0.392
Ensiling losses, g/kg of initial DM	12.6^{a}	10.8^{b}	10.9^{ab}	10.8^{b}	10.8^{b}	10.3^{b}	10.1^{b}	0.36	0.002	<0.001	0.141	0.634
Microbial quality, cfu/g												
Yeasts	8.0×10^{1}	1.8×10^{2}	2.3×10^{2}	1.1×10^{2}	1.7×10^{2}	1.1×10^{2}	1.1×10^{2}	6.7×10^{1}	0.719	0.336	0.443	0.516
Moulds	3.7×10^{2}	1.8×10^{2}	1.4×10^{2}	1.3×10^{3}	1.4×10^{2}	1.4×10^{2}	8.0×10^{1}	4.4×10^{2}	0.499	0.922	0.267	0.316
Lactic acid bacteria	9.9×10^{7a}	5.5×10^{4b}	4.5×10^{4b}	1.9×10^{4b}	1.0×10^{5b}	7.4×10^{4b}	9.8×10^{4b}	1.1×10^{7}	<0.001	<0.001	0.995	0.999
Clostridia	3.46	6.36	2.10	2.64	3.40	2.64	2.10	1.641	0.571	0.888	0.469	0.469

[1]Treatments: Control, without inoculant; Inoc 1: *L. plantarum* IMI 507026 and *P. pentosaceus* IMI 507024 in a ratio 50:50; Inoc 2: *L. plantarum* IMI 507027 / *P. pentosaceus* IMI 507025 in a ratio 75:25; Inoc 3: *L. plantarum* IMI 507026 / *P. pentosaceus* IMI 507025 in a ratio 50:50; Inoc 4: *L. plantarum* IMI 507026 / *P. pentosaceus* IMI 507024 in a ratio 75:25 + 25 mg/kg fresh forage of $MnSO_4$; Inoc 5: *L. plantarum* IMI 507026 / *P. pentosaceus* IMI 507025 in a ratio 75:25 + 25 mg/kg fresh forage of $MnSO_4$; Inoc 6: *L. plantarum* IMI 507028 / *P. pentosaceus* IMI 507025 in a ratio 50:50 + 25 mg/kg fresh forage of $MnSO_4$. [2]SEM, Standard Error of the Mean. [3]Treat: effect of treatment; C vs I: control versus inoculant treated silages; $MnSO_4$: effect of $MnSO_4$ addition in the silages; Strain: 50:50 versus 75:25 strain ratio. Values with different superscript letter in a row are significantly different at 5% Tukey test, while values without superscript letter are not significantly different at 5% Tukey test.

and ratio of strains (0.50:0.50 versus 0.75:0.25) although the effects were not perfectly ortoghonal across treatments.

Results and Discussion

Although the dry matter content of the grass was low (214 g/kg), the water soluble carbohydrate concentration was relatively high (125 g/kg dry matter) to provide a high fermentation coefficient (55) when combined to a low buffering capacity (2.97 g lactic acid/100 g). Ash content of grass before ensiling was 88 g/kg dry matter, while crude protein and neutral detergent fibre were 133 and 501 g/kg dry matter, respectively.

Silages treated with inoculants showed higher residual water soluble carbohydrates after fermentation than the control silage (Table 1), but no differences were identified among inoculants. There was no effect of silage inoculants on lactic acid production. Consequently, only minor differences were found on pH. The effect of strain ratio was found on pH, which was lower for treatments with strains mixed in the ratio 75:25 than 50:50. Control silage had a higher concentration of ammonia than the inoculant treated silages. According to Wilkinson (1990), grass silage with ammonia N within a range of 50-100 g/kg total N is regarded as well fermented, showing that even if ammonia N in control was higher than inoculated silages, it was still satisfactorily preserved. Additionally, grass silage having ammonia N below 50 g/kg total N is considered a very well fermented silage, which was the case of all silages treated with inoculants. For both ammonia and ethanol, lower concentrations were found for treatments with strains mixed in the ratio 75:25. Acetic acid production was the highest in control and no differences were found between inoculated silages. Total fermentation acids were higher for treatments with strains mixed in the ratio 75:25. Treatments with inclusion of $MnSO_4$ resulted in higher lactic-to-acetic acid ratio than treatments without it. Inoculant treated silages resulted in aerobic stability of approximately 2 days, and minor differences were observed among the different inoculants. The aerobic stability was longer for treatments with strains mixed in the ratio 75:25. The longer aerobic stability of control was probably due to excessive production of acetic acid, which could be produced by a "wild-type" fermentation. This fermentation may lead to reduced feed intake, decreased milk quality and unexpected losses. In general, this impairs the fermentation quality of silage, but could be avoided by the use of silage inoculants.

Conclusions

Inoculant treated silages had an improved fermentation quality. Moreover, the use of $MnSO_4$ had only minor effects on silage quality. On the other hand, there were indications that the strains mixed in the proportion 75:25 resulted in lower pH, lower concentrations of ammonia N and ethanol, higher concentration of total fermentation acids, and longer aerobic stability than 50:50 strains. The use of the innovative silage inoculants evaluated in the current experiment supported good fermentation of the silage and ensured its nutritional and hygienic quality for animal feeding.

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Effect of wilting and application rate of silage additive restricting fermentation on estimated grass silage protein value

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Introduction

Grass silage is the main ingredient in the diet and the main dietary protein source of dairy cows in the Nordic countries. However, high quality feed protein of soybean and rapeseed origin is used to stimulate milk production of high yielding cows. These protein feeds are to a large extent imported, and there is an ongoing search for alternative domestically produced feed protein sources (e.g. Sharma et al. 2018). As the protein from forage constitute the major part of dietary protein, even small improvent of the protein quality of forage, and of particularly grass silage, may be beneficial. In previous studies, it has been demonstrated that the estimated protein value of grass silage increases with the wilting rate of the herbage pre-ensiling (Edmunds et al., 2014; Johansen et al., 2018) and by use of acid based silage additives that restrict the fermentation (Jaakkola et al., 2006; Bakken et al., 2017). The increase in silage metabolizable protein content by restricting the fermentation has been attributed to an increase in both microbial protein synthesis and of rumen-undegradable protein (Jaakkola et al., 2014; Johansen et al. 2017). The objective of the current study was to test the combined effect of crop wilting and dosing rate of a formic acid-based additive on silage fermentation and protein value.

Materials and Methods

Plant material from the first cut of a pure *Lolium perenne* (mixture of 6 cutivars) ley was harvested 8th of June, 2020, at Fureneset research station, Norway. The herbage was wilted by force air at 30°C to the target dry matter levels 25, 35 or 45% DM. The wilted material was chopped and 350 g (on DM basis) was ensiled with a formic acid-based additive (GrasAAT® Lacto, ADDCON Nordic AS, Porsgrunn, Norway) in evacuated sealed polyethylene bags at the dosing rates 0, 2, 4 or 6 ml/kg herbage material, which are 0, 50, 100 and 150% of the recommended application rates, respectively. Freeze dried samples of fresh herbage, wilted herbage and silages were analysed for ash, crude protein, and buffer soluble crude protein (sCP) according to NorFor standards (Volden et al., 2011), ash free neutral detergent fibre (NDFom) according to Mertens (2002), and water-soluble carbohydrates (WSC) as described by Randby et al. (2011). Silage samples were analysed for pH, and the content of organic acids and ethanol according to Ericsson and André (2010). Concentration of indigestible NDFom (iNDF) in silage was determined by in situ incubation, and organic matter digestibility was calculated from iNDF and NDFom concentrations (Huhtanen et al., 2013). Metabolizable protein (AAT_{20}) and protein balance in the rumen (PBV_{20}) were calculated according to NorFor at daily intake of 20 kg DM (Volden, 2011). The constituents in silage were modelled (regression) using the procedure GLM in SAS (SAS, 2014) with silage DM content (measured) and additive dosing rate and their interaction as continuous fixed effects.

Results and Discussion

The target herbage wilting levels of 10%-point difference were reached (Table 1). Wilting increased the content of soluble CP and decreased the content of WSC. Silage pH increased with wilting rate with no effect of additive dosing rate (Table 2). Organic matter digestibility, and the contents of NDFom and CP (figures not shown) were not affected by treatments, but the content of soluble CP increased with wilting rate (Table 2). The effects of wilting and additive dosing rate on the silage contents of WSC and organic acids appeared as expected (Edmunds et al., 2014; Jaakkola et al., 2006); wilting increased the content of WSC and decreased the total content of fermentation acids, while increasing additive dosing rate increased WSC content and decreased the content of total acids. For the total content of acids there was a wilting by additive interaction effect, i.e. the effect of silage additive dosing rate diminished with wilting rate and was low for the strongest wilting rate. The effect of wilting rate on WSC content tended (P=0.053) to diminish with additive dosing rate. The estimated AAT_{20}-values increased with both wilting and additive dosing rate, and the silage additive effect diminished with increasing wilting rate. The effects were linear. Thus, restricting the fermentation and conserving the WSC by wilting or adding a formic acid-based additive improved the estimated protein value.

Table 1 Content of dry matter (DM), ash, neutral detergent fibre (NDFom), crude protein (CP), soluble CP (sCP) and water soluble carbohydrates (WSC) in fresh and wilted herbage pre-ensiling (n=3)

Herbage	DM, g/kg	Ash, g/kg DM	NDFom, g/kg DM	CP, g/kg DM	sCP, g/kg DM	WSC, g/kg DM
Fresh	154^{d}	76	501	144	62^{c}	176^{ab}
Wilted, 25%	242^{c}	73	504	137	73^{b}	189^{a}
Wilted, 35%	342^{b}	74	510	139	81^{ab}	148^{b}
Wilted, 45%	440^{a}	76	513	146	84^{a}	154^{ab}
SEM	6.6	1.8	4.3	3.3	2.1	9.3
P-value	<0.001	0.737	0.257	0.288	<0.001	0.043

SEM is standard error of the mean; Data were analysed with target DM level as fixed effect in a mixed model and field replicate as random effect (SAS, 2014). Means in a column without a common superscript differ (P<0.05, Tukey's multiple comparison).

Table 2 Estimates of the relationship between crop wilting rate (DM, %) and dosing rate of the silage additive GrasAAT Lacto (Add, ml/kg fresh crop) and the silage pH and content of soluble CP, water soluble carbohydrates (WSC), sum of silage organic acids (TA) and amino acid absorbed in the intestine (AAT_{20})

Item		Intercept	DM	Add	DM×Add	R^2	RMSE
pH	Est.	3.64***	0.016***	0.070^{ns}	0.003^{ns}	0.976	0.032
	S.E.	0.050	0.001	0.090	0.003		
sCP, g/kg CP	Est.	747***	1.65*	-0.701^{ns}	-0.246^{ns}	0.845	11.3
	S.E.	22.4	0.658	6.002	0.175		
WSC, g/kg DM	Est.	-37.2***	1.84**	28.3***	-0.30^{ns}	0.974	8.4
	S.E.	16.71	0.491	4.48	0.131		
TA, g/kg DM	Est.	199***	-3.55***	-18.0***	0.36**	0.978	4.7
	S.E.	9.3	0.273	2.49	0.073		
AAT_{20}, g/kg DM	Est.	69.9***	0.23***	1.87***	-0.033**	0.956	0.6
	S.E.	1.12	0.03	0.30	0.009		

Est. is estimates; S.E. is standard error of estimates; RMSE is root square error of estimates;
Significance ^{ns}: P>0.05, *: P<0.05, **: P<0.01, ***: P<0.001, respectively.

Conclusions

The estimated protein value of grass silage, expressed as the estimated AAT_{20}-value, improved with restricting the silage fermentation, either by wilting the herbage or adding a formic acid based silage additive pre-ensiling. The effects were linear but diminished with increasing level of the other factor. The results should be verified *in vivo.*

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Biochar addition at ensiling – effects on silage characteristics

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Introduction

Biochar is a potential carbon sink and soil enhancer. A possible strategy for introducing it into the feed chain of ruminant livestock farms is addition to green chop during ensiling (Pereira et al., 2014). The silage characteristics may then be impacted in several ways. Two experiments were performed that yielded data regarding this: i) a laboratory scale experiment with pure stands of timothy and red clover, respectively, that were ensiled at two different dry matter levels with the addition of 0, 2, 4 or 6% wooden biochar on a dry matter basis and ii) full scale ensiling for a feeding trial with a precision-chopped grass-clover crop in round bales with the addition of 0, 1.5 or 3.0% wooden biochar on a DM basis.

Materials and Methods

For the laboratory scale experiment, the primary growth from pure stands of timothy (11 June, early heading stage) and red clover (15 June, pre-budding stage), respectively, were harvested 2020 in the Uppsala region (Table 1). The crops were mowed and one portion was immediately prepared for ensiling, while another portion was wilted overnight for a target DM of 40%. Prior to ensiling, the crops were chopped with a compost grinder to mimic a precision chopper with 2 cm theoretical cutting length. The crops were then ensiled in triplicates in laboratory scale silos with 4.5-L capacity with the addition of 0, 2, 4 or 6% wooden biochar on a DM basis. Target densities were 160 and 200 kg DM/m^3 for unwilted and wilted material, respectively. The biochar was from spruce/pine (70/30) sawdust pellets and was ground in a food blender, oven dried and maintained at 40°C until the biochar amount for one silo was promptly weighed and mixed with the corresponding green chop amount. The silos were then stored at 20°C for 325 d before opening and sampling for analysis with routine wet chemistry methods described by Eriksson and Rustas (2014).

Table 1 Composition of ley crops used for ensiling with incremental doses of biochar

Crop	Silo	DM, g/kg	Ash, g/kg DM	NDF, g/kg DM	WSC, g/kg DM	CP, g/kg DM
Timothy, unwilted	Minisilo	226 ± 0.3	76 ± 1.2	521 ± 7	116 ± 1.5	130 ± 1.4
Timothy, wilted	Minisilo	408 ± 1.5	75 ± 2.0	532 ± 4	111 ± 2.5	128 ± 3.8
Red clover, unwilted	Minisilo	206 ± 1.0	96 ± 0.9	266 ± 10	83 ± 1.9	186 ± 1.3
Red clover, wilted	Minisilo	408 ± 3.9	97 ± 0.2	275 ± 5	96 ± 1.0	180 ± 3.6
Mixed ley	Bales	253 ± 7.5	79 ± 2.2	522 ± 30	65 ± 2.0	188 ± 1.6

DM = dry matter; NDF = neutral detergent fibre; WSC = water soluble carbohydrates, CP = crude protein

Silage for the feeding trial was prepared from a third cut of a mixed ley with timothy, perennial ryegrass, festololium, red clover and white clover, harvested near Laholm, S. Sweden on 22 August 2021 (Table 1). The ley was cut with a 10-m mower-conditioner, wilted overnight in swaths for a target DM of 27% and chopped with a precision chopper (Krone Big X 580). The green chop was mixed with biochar and a silage inoculant (Xtrasil Bio Ultra, Lantmännen, at > 210000 cfu/g fresh matter) in 5-ton batches in a mixer wagon

(Kverneland Siloking Duo 20 m^3) and then baled and wrapped with 12 layers of plastic with a stationary round baler/wrapper (Orkel 2000 Compactor).The biochar was from spruce pyrolysed at 650°C (Obio Fórkull, Oplandske Bioenergi, Biri, NO) and milled by the

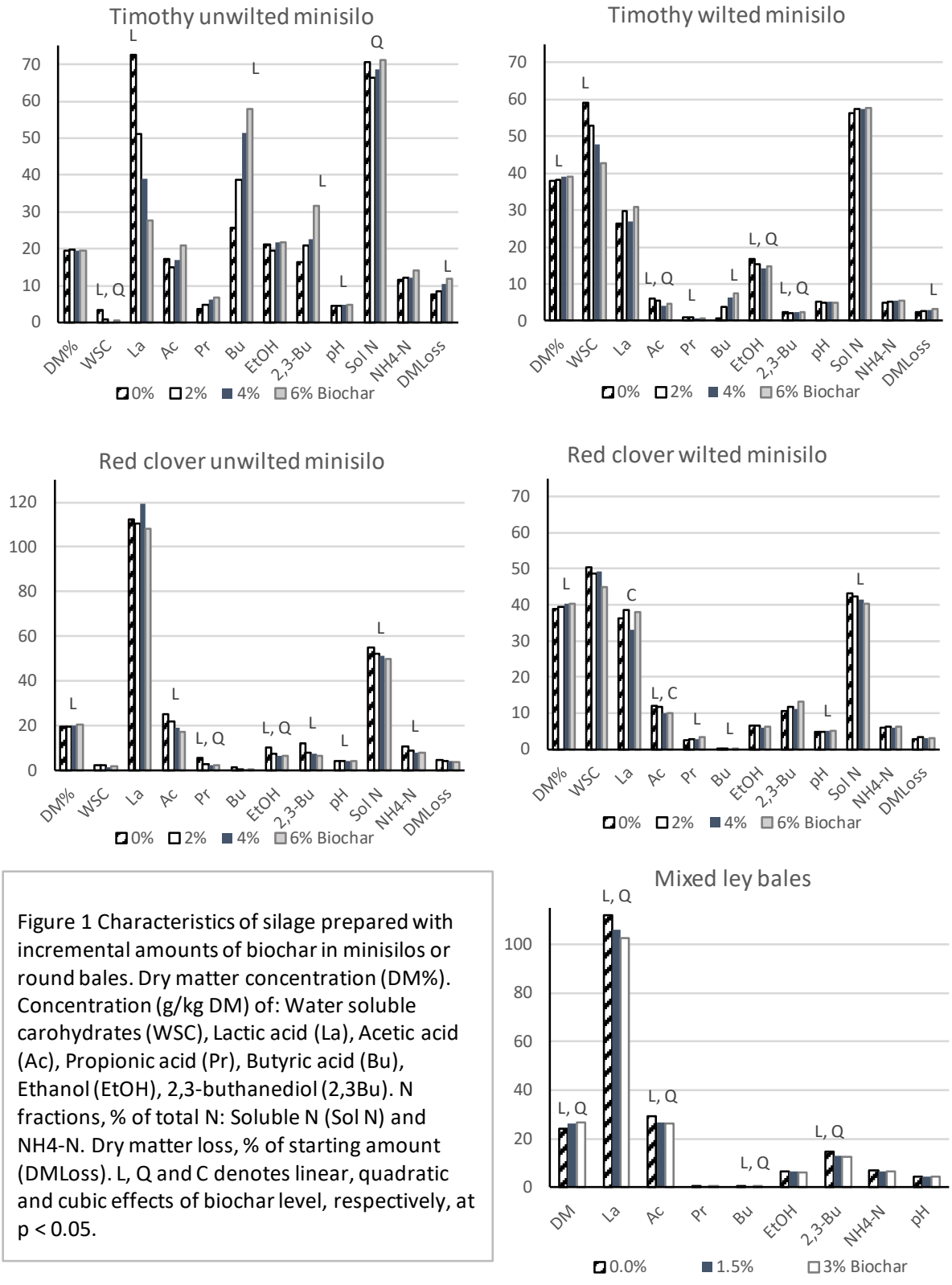

Figure 1 Characteristics of silage prepared with incremental amounts of biochar in minisilos or round bales. Dry matter concentration (DM%). Concentration (g/kg DM) of: Water soluble carohydrates (WSC), Lactic acid (La), Acetic acid (Ac), Propionic acid (Pr), Butyric acid (Bu), Ethanol (EtOH), 2,3-buthanediol (2,3Bu). N fractions, % of total N: Soluble N (Sol N) and NH4-N. Dry matter loss, % of starting amount (DMLoss). L, Q and C denotes linear, quadratic and cubic effects of biochar level, respectively, at $p < 0.05$.

manufacturer to have 53% passing a 1 mm screen and 77% a 2 mm screen. The biochar was dried to approx. 95% DM and precautions were taken to avoid moisture uptake prior to use. Added biochar amounts corresponded to 0, 1.5 and 3.0% of green chop DM. The bales were opened and sampled during the course of a feeding trial running with five 2-w periods from 17 January to 25 March 2022 (147 to 214 d after ensiling). Samples from each period were subjected to the same wet chemistry analyses as in the laboratory scale experiment.

Data were analysed with Proc GLM of SAS 9.4 with biochar level as a class variable, with linear, quadratic and cubic (minisilos only) contrasts < 0.05 being reported.

Results and Discussion

General signs of fermentation intensity for different DM levels (Figure 1) were logical, with higher DM being associated with more remaining WSC, lower protein solubility, less fermentation products and higher pH. Silage DM concentration increased with incremental biochar addition except for unwilted timothy, but increase was smaller than added biochar DM proportions. Butyric acid concentration was very high in unwilted timothy silage at zero biochar addition, and then together with 2,3-buthane-diol increased in a linear fashion for incremental biochar dose while lactic acid at the same time declined. A similar pattern was visible when timothy had been wilted to 40% DM, but butyric acid levels then did not exceed limits for ”acceptable quality” (Spörndly, 2003). Red clover silage and mixed ley ensiled in bales had low concentrations of butyric acid. Timothy crop was probably contamined with *Clostridia* spores. Aerobic stability was very good for unwilted timothy, temperature increase was less than 2°C after 282 h in 25°C. Wilted control timothy, reached 3°C increase after 65 h. Red clover silage reached 3°C increase after >140 h, with endpoint temperature being negatively linear to biochar dose. Bale silage reached 3°C increase at >188 h with no effect of biochar dose. There were minor effects on protein quality with soluble fraction decreasing linearily in red clover with increased biochar dose and a small quadratic effect in unwilted timothy with least solubility for 2% biochar.

Conclusions

Addition of biochar to ley crops resulted in silage of good quality, except for when butyric acid fermentation occurred in the control silage and then increased with increasing biochar dose. The addition caused a minor reduction of protein solubility in red clover.

Acknowledgments

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Preservation quality of crimped barley grains ensiled at variable moisture contents and using different silage additives

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Introduction

Cereal grains harvested under humid Nordic conditions need to be artificially dried in order to secure a proper preservation during storage. However, it involves extra costs and energy requirement to the feed production system. Crimping grain and ensiling it under anaerobic conditions overcomes this problem and ensures proper lactic acid fermentation that preserves the grain in an adequate way (Franco *et al*., 2019). The crimping process breaks and flattens the grain, which exposes the kernel's endosperm and contributes for greater compaction, creating a more anaerobic environment inside the silo. Crimping grain offers farmers an additional advantage of harvesting cereals with less dependence on weather conditions, and at wider range of ripening stage than when conventionally harvested for drying.

Nowadays the ensiling of crimped grain is common, even though there is a high risk of losses when preservatives are not used. Although the feed preservation industry has developed considerably in the past few decades, efforts to improve the quality of preserved feeds still require additional knowledge. Therefore, the objective of this study was to evaluate the fermentation quality, aerobic stability, microbial quality and ensiling losses of crimped barley ensiled under different management conditions, such as additives used and moisture content (MC) levels of the grains.

Materials and Methods

This experiment was conducted in the experimental facilities of the Natural Resources Institute Finland (Luke) in Jokioinen, Finland (60°48'N, 23°29'E). Barley grain was combine harvested and crimped using farm scale machinery, and transported to the laboratory without any additive. A representative raw material sample was immediately taken for chemical composition analyses, and the MC concentration of it was 199 g/kg. The MC of crimped grain was manipulated with water addition resulting in three levels: 228 (MC1), 287 (MC2) and 345 (MC3) g/kg fresh matter. In order to evaluate the efficiency of additives to preserve the crimped barley under different MC, four additive treatments were tested, including: 1. control, without additive (C); 2. formic and propionic acid based additive (Acid; AIV Ässä Na, Eastman Chemical Company, Oulu, Finland at 5 l/t); 3. inoculation with homolactic and heterolactic acid bacteria strains (LAB; Kofasil Duo, ADDCON, Bitterfeld-Wolfen, Germany at 1 g/t); 4. salt based additive (Salt; Safesil Pro, Salinity AB, Göteborg, Sweden at 4 l/t). Crimped barley was ensiled into 1.5 l glass jars using three replicates per treatment. The top of the jar was covered with plastic film, closed airtight, weighed and stored at room temperature in the dark. Silos were opened after 81 days of ensiling period and samples were taken for chemical composition, fermentation quality, aerobic stability, and microbial quality analyses. Ensiling losses were calculated according to Knicky and Spörndly (2015). Data was analysed using a MIXED procedure of SAS 9.4. with silage additive and MC as fixed effects.

Table 1 Chemical composition, fermentation quality, ensiling losses and microbial quality of crimped barley treated with additives under different moisture content levels

Moisture content[1]	MC1				MC2				MC3				SEM[3]	P-value[4]		
Additive[2]	C	Acid	LAB	Salt	C	Acid	LAB	Salt	C	Acid	LAB	Salt		MC lin	MC quad	C vs Add
Moisture content, g/kg	238^{f}	235^{f}	238^{f}	238^{f}	298^{d}	291^{e}	292^{e}	295de	357^{a}	350^{bc}	347^{c}	354^{ab}	0.9	<0.001	0.116	<0.001
pH	6.16^{a}	4.88^{bc}	5.97^{a}	6.24^{a}	5.25^{b}	4.54^{cd}	4.25^{de}	5.29^{b}	4.56^{cd}	4.19^{de}	4.03^{e}	4.34^{de}	0.084	<0.001	<0.001	<0.001
Ammonia N, g/kg N	6.77^{g}	5.34^{g}	6.41^{g}	5.34^{g}	16.39^{cd}	9.26^{f}	17.45^{c}	14.60^{de}	38.29^{a}	14.25^{e}	29.21^{b}	28.14^{b}	0.354	<0.001	<0.001	<0.001
Ethanol, g/kg dry matter (DM)	6.45^{b}	0.05^{f}	6.45^{b}	3.80^{d}	9.48^{a}	0.40^{f}	5.80^{bc}	4.89^{c}	9.64^{a}	2.02^{e}	5.61^{bc}	6.15^{b}	0.186	<0.001	0.304	<0.001
Acids, g/kg DM																
Lactic (LA)	0.57^{e}	0.01^{e}	1.27^{de}	0.26^{e}	6.16^{c}	1.06^{de}	19.09^{b}	5.07^{cd}	16.80^{b}	6.42^{c}	27.28^{a}	17.52^{b}	0.864	<0.001	0.096	0.169
Acetic (AA)	0.91^{e}	0.82^{e}	1.10^{de}	0.96^{e}	2.11^{d}	1.42^{de}	4.38^{bc}	2.11^{d}	4.17^{c}	3.48^{c}	5.29^{ab}	5.63^{a}	0.198	<0.001	0.025	0.006
Propionic[5]	0.12^{a}	0.07^{a}	0.11^{a}	0.12^{a}	0.13^{a}	0^{b}	0.12^{a}	0.11^{a}	0.12^{a}	0^{b}	0.12^{a}	0.12^{a}	0.012	0.052	0.199	<0.001
Butyric	0.02^{b}	0^{b}	0.01^{b}	0^{b}	0.03^{b}	0^{b}	0.01^{b}	0^{b}	0.44^{a}	0.01^{b}	0.07^{b}	0.01^{b}	0.026	<0.001	<0.001	<0.001
Total volatile fatty acids	1.1^{f}	0.9^{f}	1.3^{f}	1.1^{f}	2.3^{e}	1.4^{ef}	4.5^{c}	2.2^{e}	4.8^{bc}	3.5^{d}	5.6^{ab}	5.8^{a}	0.18	<0.001	0.002	0.074
Total fermentation acids	1.6^{e}	0.9^{e}	2.5^{de}	1.3^{e}	8.4^{c}	2.5^{de}	23.6^{b}	7.3^{cd}	21.6^{b}	10.0^{c}	32.9^{a}	23.3^{b}	1.04	<0.001	0.050	0.144
Total fermentation products	8.1^{def}	1.0^{g}	9.0^{de}	5.1^{efg}	17.9^{c}	2.9^{fg}	29.4^{b}	12.2^{cd}	31.2^{b}	12.0^{cd}	38.5^{a}	29.5^{b}	1.17	<0.001	0.108	0.000
LA/AA ratio	0.62^{ghi}	0.02^{i}	1.16^{fg}	0.27^{hi}	2.83^{cd}	0.72^{gh}	4.36^{b}	2.39^{de}	4.04^{b}	1.83^{ef}	5.15^{a}	3.11^{c}	0.134	<0.001	<0.001	<0.001
Aerobic stability, 2 °C	45^{c}	265^{ab}	43^{c}	74^{c}	58^{c}	269^{ab}	198^{abc}	149^{bc}	206^{abc}	360^{a}	151^{bc}	349^{a}	34.2	<0.001	0.394	<0.001
Ensiling losses, g/kg of initial DM	7.0^{bc}	0.7^{e}	7.0^{bc}	4.8^{cd}	10.0^{ab}	2.6^{de}	7.7^{bc}	5.7^{cd}	12.3^{a}	2.8^{de}	7.4^{bc}	6.9^{bc}	0.63	<0.001	0.338	<0.001
Enterobacteria, cfu/g	3×10^{4a}	1×10^{5a}	5×10^{4a}	7×10^{2a}	7×10^{1a}	1×10^{1a}	1×10^{1a}	1×10^{1a}	1×10^{1a}	1×10^{1a}	5×10^{1a}	1×10^{1a}	3.2×10^{4}	0.053	0.251	0.692
Moulds, cfu/g	2×10^{6b}	1×10^{2c}	4×10^{6a}	6×10^{4c}	1×10^{6bc}	1×10^{2c}	6×10^{4c}	1×10^{3c}	9×10^{4c}	1×10^{2c}	10×10^{4c}	1×10^{2c}	2.3×10^{5}	<0.001	<0.001	<0.001
Yeasts, cfu/g	4×10^{2b}	1×10^{6a}	3×10^{2b}	3×10^{3b}	2×10^{2b}	4×10^{3b}	2×10^{2b}	1×10^{4b}	5×10^{2b}	4×10^{2b}	1×10^{2b}	2×10^{4b}	1.6×10^{5}	0.034	0.196	0.269

Moisture contents, MC1: 228 g/kg; MC2: 287 g/kg; MC3: 345 g/kg. Additives, C: control; Acid: formic and propionic acid based additive; LAB: inoculation with homolactic and heterolactic acid bacteria strains; Salt: salt based additive. [3]SEM, Standard Error of the Mean. [4]MC lin: linear effect of moisture content; MC quad: quadratic effect of moisture content; C vs Add: control versus additive treated silages. [5]Propionic acid was corrected for its amount added via additive. Values with different superscript letter in a row are significantly different at 5% Tukey test.

Results and Discussion

Protein, starch, ash and neutral detergent fibre of crimped barley was 117, 542, 33 and 215 g/kg dry matter, respectively. The microbial quality of the crimped barley before ensiling was 1.8×10^6, 1.9×10^5 and 1.3×10^6 for yeasts, moulds and enterobacteria, respectively.

There was a quadratic effect of the incremental MC on pH values where greater decline happened at MC2, although MC3 also decreased it, but in smaller intensity (Table 1). The pH levels for MC1, MC2 and MC3 were 5.81, 4.83 and 4.28, respectively. Ammonia-N concentration increased quadratically with increasing levels of MC, with greater intensity from MC2 to MC3 (5.97, 14.43 and 27.47 g/kg N for MC1, MC2 and MC3 respectively. There was a positive linear effect of MC on ethanol, lactic acid and acetic acid concentrations. According to Kung Jr. (2010), low MC inhibits fermentation resulting in low amounts of lactic acid and volatile fatty acids, and subsequenty, in higher pH. Aerobic stability linearly improved from 107 h at MC1 to 267 h on MC3, which can be explained by increased concentrations of fermentation end products with antimicrobial activities.

Using additives was effective in reducing pH of crimped ensiled grain and also in reducing the concentrations of ammonia-N and ethanol (Table 1). Although all additives were efficient in reducing the concentrations of ammonia-N and ethanol, Acid resulted in the most significant reductions in both parameters. Silage additives were effective in decreasing the butyric acid concentration and ensiling losses in ensiled crimped barley grain when compared to control. All additives improved aerobic stability, but Acid was the only additive that was effective also at the lowest MC level. The average aerobic stabilities for the additives Control, Acid, LAB and Salt were 103, 298, 131 and 190 h, respectively. As stated by Wilkinson and Davies (2013), the aerobic stability of feeds is the main factor to secure that the feed is properly preserved and harmless in terms of least possible presence of moulds before offering it to ruminants. The effects of additives on the extent of fermentation were rather typical for silages, in a way that LAB boosted the fermentation of the ensiled crimped barley grain, while Acid restricted it, and Salt had no effect on it.

Conclusions

The moisture content of crimped barley has a great impact on the extension of the fermentation of ensiled grains, as greater moisture content resulted in more intensive fermentation during the anaerobic storage. Silage additives successfully improved the fermentation quality of crimped barley grains, with lower pH and longer aerobic stability.

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Chemical composition and in vivo digestibility of biorefined pulp from fresh and ensiled grass-clover forage

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Introduction

Grasslands play a vital role in ensuring a sustainable future for global agriculture both as feed for livestock and to diminish carbon dioxide and nitrous oxide emissions to the atmosphere by being carbon sinks (Poeplau, 2020). Biorefinery of grasslands diversifies the utilization of grasslands by producing a protein-rich press juice and a fibre-rich pulp by mechanical pressing (Savonen et al., 2020). Use of both ensiled and fresh forage gives all-year round use of the biorefinery but ensiling of the forage can affect the quality of biorefined products. The aim of this study was to evaluate the feed value of the pulp from fresh compared to ensiled timothy-red clover forage.

Materials and Methods

Forage consisting of 62% timothy (*Phleum pretense* L.) and 38% red clover (*Trifolium pratense* L.) of dry matter (DM) was mowed, pre-wilted and chopped at 28% DM from one field at Sötåsen Agricultural High School, Töreboda, Sweden (N 58° 41', E 14° 8',) in the first cut on June 3, 2020. The maturity stage of timothy varied form stem elongation to heading stage whereas the maturity stage of red clover varied from leaf-to-stem elongation stage. One part of the fresh forage was kept intact (F) and another part was refined to pulp, which was ensiled in hard-pressed roundbales (FP). A third part of the fresh forage was ensiled in hard-pressed roundbales (S) and the final silage was biorefined to pulp (SP). Both fresh forage and silage were refined in a screw press (Cir-Tech, Skærbæk, Denmark) at 1.5 tons/h at a speed of 70% of its full capacity and the amperage was set at 25, which provided a pressing power of 3000 watts.

The four treatments were fed to eight wethers at SLU Götala Beef and Lamb Research Centre, Skara, Sweden in a duplicated 4 × 4 Latin square. The wethers were crosses of Suffolk, Texel or Swedish Finewool, were 7 months old, weighed 60 (SD 6.4) kg and had a body condition score of 3.3 (SD 0.22) at start of the experiment. Each of the four periods was 4 weeks long, starting with an adaptation period of 14 days before 7 days of registration of *ad libitum* intake, when the wethers were housed in individual pens. During the last 7 days, the wethers were fed individually at 80% of *ad libitum* intake in metabolic cages. After a 3-day adaptation to the restricted feeding, total daily collection of faeces occurred during 4 days. Composited daily samples of feed, orts and faeces from each period were analysed for nutrient contents according to conventional methods (Sousa et al., 2022). Crude protein (CP) fractions (A, B_1, B_2, B_3 and C) based on degradability characteristics according to the Cornell Net Carbohydrate and Protein System (Sniffen *et al*., 1992) were determined according to Licitra *et al*. (1996). Feed quality data were analysed by analysis of variance for a randomized block design using the mixed procedure of SAS version 9.3, including fixed effects of ensiling, refining and ensiling × refining and random effect of block, which equaled period in Latin square). Data on feed intake and *in vivo* apparent digestibility were analysed for a duplicated 4 × 4 Latin square using the same procedure and fixed effects as for the feed

quality data with addition of periodand random effects of animal within square and square. Significant differences between least-square (LS) means were done with Tukey-Kramer adjustment at $P \leq 0.05$ and tendency to significance at $0.05 \leq P \leq 0.10$.

Results and Discussion

Pressing of forages in a biorefinery leaves much of the soluble nutrients in the juice fraction, resulting in increased concentrations of DM, neutral detergent fibre (NDF) and acid detergent fibre (ADF), in FP and SP compared to F and S, respectively, with a greater increase in SP compared to FP (Table 1). The water-soluble carbohydrate (WSC) concentration was lower in FP compared to F ($P = 0.025$) and part of this difference was caused by fermentation of WSC during ensiling of FP in addition to the WSC extraction to the press juice. No difference in WSC content was found between S and SP, because the WSC already had been fermented mainly to lactic acid in S (data not shown). Some of the nonprotein nitrogen (NPN; fraction A) in S was apparently extracted to the press juice, resulting in a smaller proportion of the NPN in the SP compared to S. However, SP contained larger proportions of fraction B_2, which mostly is rumen degradable and fraction C, which is the ADF-bound protein and is considered to be indigestible, compared to S. No such differences were found between F and FP (Table 1).

Table 1 Chemical composition of forage (F), forage pulp (FP), silage (S) and silage pulp (SP), n=4

	Treatment					*P* - value		
	F	FP	S	SP	SEM	Ensiling (E)	Refining (R)	E × R
DM, g/kg	271^{d}	349^{b}	285^{c}	464^{a}	2.6	<0.001	<0.001	<0.001
Ash, g/kg DM	68^{b}	64^{c}	71^{a}	48^{d}	0.6	<0.001	<0.001	<0.001
aNDFom, g/kg DM	471^{c}	542^{b}	461^{c}	629^{a}	7.1	<0.001	<0.001	<0.001
ADFom, g/kg DM	263^{d}	320^{b}	297^{c}	383^{a}	6.8	<0.001	<0.001	0.018
ADL, g/kg DM	26	39	30	36	2.5	0.913	0.002	0.168
iNDF, g/kg NDF	183^{a}	154^{b}	143^{bc}	136^{c}	4.4	<0.001	<0.001	0.011
IVOMD, g/kg OM	792^{b}	803^{b}	851^{a}	770^{b}	18.9	0.226	0.008	0.002
WSC, g/kg DM	$192^{(a)}$	$89^{(b)}$	$88^{(b)}$	$68^{(b)}$	22.5	0.013	0.015	0.070
CP, g/kg DM	142	135	130	100	10.8	0.046	0.099	0.284
Fraction A, % of CP	42.4^{b}	44.0^{b}	61.2^{a}	40.3^{b}	3.69	0.053	0.019	0.009
Fraction B_1, % of CP	3.1	1.6	2.7	1.7	0.72	0.785	0.074	0.624
Fraction B_2, % of CP	35.3^{ab}	27.4^{bc}	26.3^{c}	37.9^{a}	2.04	0.703	0.374	<0.001
Fraction B_3, % of CP	15.9	22.4	6.2	12.8	1.07	<0.001	<0.001	0.968
Fraction C, % of CP	3.3^{b}	4.6^{b}	3.6^{b}	7.3^{a}	0.44	<0.001	<0.001	0.003

^{a,b,c,d}LS means in a row with different superscripts differ significantly at $P \leq 0.05$. $^{(a),(b)}$LS means in a row with different superscripts tends to differ at $0.05 \leq P \leq 0.10$.
aNDFom=ash-free neutral detergent fibre with addition of amylase to the detergent solution, ADFom=ash-free acid detergent fibre, ADL=acid detergent lignin, iNDF=*in vitro* indigestible NDF at 240 h incubation, IVOMD=*in vitro* organic matter (OM) digestibility, WSC=water soluble carbohydrates, CP=crude protein; A=NPN, B_1=buffer-soluble protein, B_2=neutral detergent-soluble protein, B_3=acid detergent-soluble protein, C = acid-detergent insoluble protein.

Intakes of DM and OM by wethers fed FP and SP were lower compared to wethers fed F and S, which are related to the higher NDF concentrations of the pulps compared to their original forages (Tables 1 and 2). The NDF intake was lower for the pulps than for the intact forages, which shows that rumen fill limited intake. According to Allen (2000), forage NDF concentration is the main factor limiting intake in forage-based diets because of its slow passage rate. Furthermore, *in viv*o digestibility of NDF and ADF was not affected by the mechanical pressing of F and S. *In vivo* apparent digestibility of CP were lower for FP and SP

compared to F and S and this difference was driven by SP. Thus, the decreased *in vivo* and *in vitro* OM digestibility of SP compared to S is to a large extent related to the decreased CP digestibility of SP (Tables 1 and 2).

Table 2 Intake and *in vivo* apparent digestibility of forage (F), forage pulp (FP), silage (S) and silage pulp (SP) fed to wethers, n=8

	Treatment					*P* - value		
	F	FP	S	SP	SEM	Ensiling (E)	Refining (R)	E × R
Body weight (BW), kg	64.0	62.1	62.7	63.1	4.90	0.872	0.408	0.243
Intake								
DM, kg/day	1.71	1.33	1.50	1.06	0.184	0.011	<0.001	0.788
DM, % of BW	2.63	2.13	2.38	1.67	0.132	0.003	<0.001	0.316
OM, kg/day	1.60	1.24	1.39	1.01	0.172	0.013	<0.001	0.883
NDF, kg/day	0.81	0.74	0.76	0.66	0.092	0.072	0.026	0.611
NDF, % of BW	1.23	1.19	1.21	1.04	0.069	0.106	0.067	0.229
CP, g/day	201	171	187	114	22.7	0.011	0.007	0.103
Digestibility, %								
DM	72.8[a]	69.9[a]	72.8[a]	65.2[b]	1.11	0.045	<0.001	0.044
OM	74.3[a]	71.6[a]	74.5[a]	67.1[b]	1.01	0.046	<0.001	0.030
NDF	68.3	69.2	66.3	66.0	1.39	0.070	0.835	0.663
ADF	62.9	64.9	65.9	64.8	1.78	0.414	0.791	0.393
CP	64.3	59.6	59.7	49.1	2.07	0.001	0.001	0.170

[a,b]LS means in a row with different superscripts differ significantly at $P \leq 0.05$.
See footnote for table 1 for definitions of abbreviations.

Conclusions

Biorefinery of fresh and ensiled forage affects the chemical composition and, consequently, in *vivo* apparent OM digestibility of the pulps differently.

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Effects of silage additives on the ensiling characteristics of pulp from bio-refining

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Introduction

Fractionation in bio-refineries of green biomasses such as grass yields a fibrous pulp which can be fed to cows (Damborg *et al.*, 2019). During fractionation, green juice and thereby a large amount of soluble nutrients are extracted and can therefore not be used for microbial fermentation in the pulp during ensiling. If the amount of substrate is insufficient, a formation of undesired fermentation patterns or high losses may occur. Application of silage additives may counteract this, if the additive ensures that substrate is abundant or a specific microbial population is favoured, e.g. homofermentative lactic acid bacteria. After heat precipitation of protein from the green juice, a residual liquid termed brown juice remains, which, among other compounds, has high concentrations of carbohydrates and inorganic minerals (Jørgensen *et al.*, 2021). Transfer of the brown juice back to the pulp may ensure sufficient amounts of substrate for the microbial fermentation. The fermentation process can be monitored by investigating the fermentation weight loss (Samarasinghe *et al.*, 2019), which is a measure of losses through CO_2. In the current experiment, the effect on ensiling characteristics of pulp by applying different silage additives were investigated.

Materials and Methods

Grass (*Lolium perenne*) was harvested late summer 2019 at Aarhus University – Research center Foulum (Denmark) after 35 or 44 days of regrowth, corresponding to an early (**ERL**) and a late (**LAT**) developmental stage, respectively. Within each developmental stage, grass was harvested and fractionated immediately using a screw press (5 t h^{-1}). The pulp remaining after the first fractionation was rehydrated and fractionated a second time to extract additional protein. Within each developmental stage, pulp remaining after the second fractionation was sampled at two occasions during the production and then used as true replicates for the ensiling experiment. The pulp was treated in one of 6 ways: no addition of additives (**CON**), addition of sugar (**SUG**), addition of lactic acid bacteria (**LAB**), addition of both sugar and lactic acid bacteria (**S+L**), addition of raw brown juice (**RBJ**), and addition of concentrated brown juice (**CBJ**). For all treatments, 5 kg (fresh weight) of pulp was weighed into polyamide-polyethylene plastic bags and respective compounds were then added as followed. A 60 % solution of sugar (sucrose) mixed in water was added to the pulp using a spray bottle (167 g of the solution equal to 100 g sugar) when making SUG. A solution was made from mixing 10 g of homofermentative lactic acid bacteria (Feedtech™ Silage 10, Delaval) in 1 L of deionized water, and 5 g of the solution was added to pulp when making LAB. The same procedure was followed when making S+L. A total of 3.5 kg of brown juice recovered after extraction of protein from the first fractionation was mixed into the pulp when making RBJ. At each developmental stage, some raw brown juice was upconcentrated 12 and 33 times by membrane filtration, and therefore 298 and 107 g of concentrated brown juice was added to pulp at ERL and LAT, respectively, when making the CBJ. All silage additives were added while mixing the pulp by hand, after which the bags were sealed and weighed. Fermentation weight loss (**FWL**) was measured by weighing all bags 0, 1, 7, 14, 30, and 60 days after ensiling. Silages were frozen after weighing on d 60 to stop the ensiling process. Thawed samples were analysed for DM (60 °C for 48 h) and the fermentation pattern. Extracts of

thawed samples were analysed for pH, short-chained fatty acids, L-lactate, glucose, and NH_3-N. Data was analysed using PROC MIXED in SAS, and FWL was analysed using a mixed model including treatment (n = 6), maturity stage (n = 2), and day of ensiling (n = 6) as the main effects, as well as their two-way interactions. Samples measured on the 6 different days of ensiling were considered repeated measurements with autoregressive covariance structure. When analysing other response variables, day of ensiling was removed from the model.

Results and Discussion

The FWL on d 1, 7, 14, 30, and 60 was expressed as the total loss (g) as a proportion of the initial weight of fresh matter on d 0 of ensiling and represents the loss of gaseous compounds (Table 1). The FWL was higher for treatments where sugar was applied (SUG and S+L).

Table 1 Effect of treatment, maturity stage, and day of ensiling of pulp on the fermentation weight loss (g/kg fresh matter)

	Treatment[1]						SEM[2]	*P*-value[3]					
Day	CON	SUG	LAB	S+L	RBJ	CBJ		T	M	D	T×M	M×D	T×D
							0.422	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
Early maturity stage													
1	2.44	3.87	3.69	3.48	2.82	3.48							
7	3.64	8.13	4.79	8.03	3.70	4.60							
14	4.15	9.58	5.49	9.09	4.35	5.35							
30	4.95	10.4	6.29	9.96	4.94	6.30							
60	6.36	12.0	7.59	11.6	6.70	7.62							
Late maturity stage													
1	1.50	1.54	1.46	1.88	1.10	1.55							
7	3.37	7.15	3.50	7.25	2.47	3.43							
14	4.36	9.75	4.49	9.94	3.06	4.31							
30	5.35	11.1	5.69	11.3	4.06	5.68							
60	6.66	12.5	6.78	12.7	5.30	7.24							

[1]CON = Control silage (no addition of additives), SUG = addition of sugar, LAB = addition of homofermentative lactic acid bacteria, S+L = addition of both sugar and homofermentative lactic acid bacteria, RBJ = addition of raw brown juice, CBJ = addition of concentrated brown juice. [2]Standard error of mean. [3]T = Treatment, M = Maturity stage, D = Day of ensiling.

However, large amounts of raw brown juice was applied in RBJ, resulting in lower DM concentration on d 60 of ensiling compared to all other treatments (Table 2). Hence, the loss expressed per kg of DM ensiled on d 0 would probably be higher for RBJ compared to CON, LAB, and CBJ. The higher increase in FWL for SUG and S+L compared to CON indicated that the amount of substrate for microbial fermentation was limited in CON. Although pH was lower for SUG compared to CON, no other differences were found in the concentration of fermentation acids, when comparing CON to SUG and S+L. The lower pH for SUG and higher increase in FWL for SUG and S+L suggested that the initial formation of L-lactate occurred faster in these treatments compared to CON. Further, a potentially faster production of L-lactate causing a concomitantly rapid reduction in pH in the initial phase of ensiling could be the reason why NH_3-N concentration was lower in SUG and S+L compared to CON. No differences in FWL or the fermentation characteristics were found between LAB and CON, indicating that either abundant amounts of microbes were present in CON or that substrate for microbial fermentation was the limiting factor. However, combining application of both substrate and homofermentative bacteria (S+L) did not show any additive effect on fermentation characteristics compared to SUG or LAB, indicating that substrate for fermentation was probably the main limiting factor. With the low DM concentration in RBJ,

a larger amount of especially L-lactate was required to reduce pH to levels similar to CON. However, L-lactate concentration was lower and pH higher in RBJ compared to both CON and CBJ. Interestingly, concentrations of acetate, propionate, and caproate were higher in RBJ compared to CON and CBJ. The specific composition of raw and concentrated brown juice was unknown in the current experiment, but they were both chosen as additives because the liquid is easily transferred back to pulp in practice and because the liquid is a potential source of readily fermentable substrate. However, both RBJ and CBJ had a distinct foul odour when bags were opened after ensiling, and based on the present study this approach therefore cannot be recommended on practical farms. In general, CON ensiled well compared to the corresponding chopped silage (Hansen *et al.*, 2020), mainly due to its lower buffer capacity.

Table 2 Effect of treatment and maturity stage pulp on the fermentation characteristics after 60 days of ensiling

Analyte	Maturity stage	Treatment[1]						SEM[2]	*P*-value[3]		
		CON	SUG	LAB	S+L	RBJ	CBJ		T	M	T×M
DM,	Early	393^{a}	397^{a}	387^{a}	397^{a}	235^{b}	392^{a}	14.3	<0.01	0.01	0.96
g kg^{-1}	Late	365^{a}	358^{a}	366^{a}	363^{a}	219^{b}	362^{a}				
pH	Early	3.98^{bc}	3.83^{d}	3.98^{b}	3.89^{cd}	4.30^{a}	4.05^{b}	0.030	<0.01	0.26	0.07
	Late	4.02^{bc}	3.91^{d}	4.08^{b}	3.92^{cd}	4.21^{a}	4.03^{b}				
L-Lactate,	Early	31.1^{a}	34.1^{a}	32.1^{a}	34.2^{a}	16.7^{b}	30.2^{a}	2.31	<0.01	0.37	0.19
g kg^{-1} DM	Late	31.9^{a}	31.4^{a}	30.9^{a}	33.3^{a}	26.4^{b}	32.1^{a}				
Acetate,	Early	20.8^{C}	20.4^{C}	21.7^{C}	20.1^{C}	40.7^{A}	23.1^{C}	1.10	<0.01	0.04	0.03
g kg^{-1} DM	Late	20.7^{C}	20.8^{C}	20.9^{C}	20.9^{C}	33.3^{B}	21.6^{C}				
Propionate,	Early	3.26^{b}	1.56^{b}	3.33^{b}	1.53^{b}	8.82^{a}	3.37^{b}	0.679	<0.01	0.11	0.24
g kg^{-1} DM	Late	3.77^{b}	3.54^{b}	3.78^{b}	3.55^{b}	7.61^{a}	3.67^{b}				
Caproate,	Early	0.258^{b}	0.256^{b}	0.264^{b}	0.250^{b}	0.428^{a}	0.269^{b}	0.0125	<0.01	0.12	0.12
g kg^{-1} DM	Late	0.284^{b}	0.279^{b}	0.285^{b}	0.284^{b}	0.389^{a}	0.277^{b}				
Glucose,	Early	0.372	0.348	0.351	0.464	0.334	0.35	0.146	0.80	0.14	0.97
g kg^{-1} DM	Late	0.460	0.437	0.474	0.662	0.601	0.38				
NH_3-N,	Early	0.819^{a}	0.628^{c}	0.782^{ab}	0.641^{bc}	0.908^{a}	0.929^{a}	0.0502	<0.01	<0.01	0.42
g kg^{-1} DM	Late	1.04^{a}	0.790^{c}	1.01^{ab}	0.855^{bc}	1.08^{a}	0.961^{a}				

[1]CON = Control silage (no addition of additives), SUG = addition of sugar, LAB = addition of homofermentative lactic acid bacteria, S+L = addition of both sugar and homofermentative lactic acid bacteria, RBJ = addition of raw brown juice, CBJ = addition of concentrated brown juice. [2]Standard error of mean. [3]T = Treatment, M = Maturity stage.$^{a-d}$Values within same analyte with different lowercase superscripts differ between treatments ($P < 0.05$). $^{A-C}$Values within same analyte (across maturity stage) with different uppercase superscripts differ due to a significant T×M interaction ($P < 0.05$).

Conclusions

Pulp of grass fractionated twice in a screw-press ensiled well. Addition of substrate seemed to increase fermentation weigth loss and decrease the pH. Addition of homofermentative lactic acid bacteria did not affect the fermentation pattern, indicating that substrate for microbial fermentation was the limiting factor for ensiling. Application of raw or concentrated brown juice cannot be recommended for use on practical farms.

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Ensiling of common reed

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Introduction

In Sweden, common reed (*Phragmites australis*) is the dominant vegetation in bays and other sheltered areas. Reed is an important species in aquatic ecosystems but expansion of reed, caused by eutrophication and decreased grazing, may result in too dense and homogeneous reed belts which reduces biodiversity and aquatic ecosystem quality (Pitkänen et al., 2013). Harvesting of reed can be a way of restoring aquatic ecosystems. It also removes nutrients from aquatic environments for recirculation to crop production systems.

Reed is expected to have relatively low digestibility and moderately crude protein content (Spörndly, 2003). It can therefore be a suitable feed for ruminants and horses with low energy demand. Ensiling of reed may be impaired by low content of water-soluble carbohydrates (WSC) and low concentrations of lactic acid bacteria (LAB) on the crop (Asano *et al*., 2018). The aim of this research was to evaluate the effect of additives on ensiling of common reed.

Materials and Methods

Reed was sampled at three sites in June and August 2019. In June, sampling was made on land adjecent to Lake Mälaren, a large fresh water lake close to Uppsala (N 59°48´; E 17°36´) (Sample 1). In august, samples were collected on land at a ditch near Lake Mälaren (Sample 2) and in the water at Gräsö, an island in the Baltic sea with brackish water at the coast of Uppsala county (Sample 3). Sample 3 was cut above the water surface.

Shortly after collection, samples were frozen and, later, after thawing, analysed for content of ash, water soluble carbohudrates (WSC), crude protein (CP), neutral detergent fibre (NDF) and 96-h in vitro organic matter digestibility (IVOMD) and microbial content with routine methods (Table 1). Thawed samples were chopped and 1500-1700 g were ensiled in duplicates in air-tight laboratory scale silos with 1.7-L capacity fitted with waterlocks. The following five treatments were applied: A. no additive (Control), B. 5 g/kg fresh weight (FW) of Promyr (ProMyr NT 570, Perstorp AB, Malmö, Sweden, containing formic acid 35-40%, sodium formate 10-20%, propionic acid 15-25%), C. 50 g/kg FW of molasses, D. 2.1×10^5 cfu/g FW of LAB (Xtrasil bio ultra, Konsil Scandinavia AB, Tvååker, Sweden, containing Lactobacillus Plantarum, Lactobacillus Paracasei, Lactobacillus Brevis) and E. LAB and molasses (LAB+M) at same inclusion rates as treatment 3 and 4. All treatments were supplemented with 36 g water.

Silos were opened after 80 days of storage in room temperature silages analysed for pH, NH_4, volatile fatty acids (VFA) and microbial content with routine methods described by Eriksson and Rustas (2014). Aerobic stability was determined by monitoring the increase of silage temperature during 18 days of aerobic exposure at 20°C ambient temperature. Weight loss was determined when silos were opened.

Data from the ensiling experiment was averaged over silo duplicates and analysed in GLM (Minitab 18.1, 2017) including treatment and crop as fixed factors.

Table 1 Chemical composition and microbial content of reed

	Sample 1	Sample 2	Sample 3
Harvest environment	On land	On land	In water
Harvest time	June	August	August
Dry matter, g/kg fresh weight (FW)	308	420	402
Ash, g/kg DM	84	102	61
WSC, g/kg DM	64	74	54
CP, g/kg DM	166	127	71
NDF, g/kg DM	616	598	693
IVOMD, g/kg OM	637	593	537
LAB, log CFU/g FW	2.0	4.3	3.4
Enterobacter, log CFU/g FW	<1.7	5.8	5.5
Moulds, log CFU/g FW	2.6	3.6	3.7

WSC=water souluble carbohydrates, CP=crude protein, NDF=neutral detergent fibre, IVOMD= in vitro organic matter digestibility, LAB=lactic acid becteria.

Results and Discussion

Content of water soluble carbohydrates (WSC) was below (Sample1), close (Sample 2) and slightly above (Sample 3) the suggested lower level of 2 % of fresh weight (FW) that should be needed to avoid poor quality silage (Gordon *et al*., 1964). Likewise, content of LAB did not reach up to to the minum level (≥5 log CFU/g FW) that is required to produce good quality silage, according to McDonald *et al*. (1991). As the samples in this study were frozen before microbial analyses the results might not be relevant to fresh crop.

pH tended ($P<0.1$) to be affected by treatment with Control treatment being higher than the threshold of 4.2, under which activity of unwanted bacteria and fungi is limited (McDonald *et al*., 1973) (Table 2). Lactic acid concentration was higher ($P<0.05$) for LAB and LAB+M than for Promyr, reflecting restricted fermentation with acid treatment. Acetic acid concentration was greater ($P<0.05$) in Control, molasses, LAB+M treatments compared to the Promyr treatment. Aerobic stability, days until silage temperature increased ≥3°C, tended to be affected by treatment with LAB and LAB+M being stable for longest time.

Molasses was used as an additive because of low WSC content in the crops. Asano *et al*. (2018) used glucose for the same reason but, unlike our results, the glucose additive did not results in good silage quality. Similarly, LAB, as the only additive, was not sufficient in the study buy Asano et al. (2018) to support a good ensiling process, contrary to our results. One reason why all additives promoting fermentation in this study resulted in acceptable silage quality could be related to the relatively high DM content. Another reason could be the composition of LAB on the crop. Asano et al. (2018) conlcluded that not only the amount of LAB is important for for the ensiling process, they should also be of the right species. As little information exist on what number and species of LAB that inhabit common reed, future investigations are encouraged to include those analyses.

Conclusions

Results from this this study indicate that ensiling of reed can result in acceptable silage quality, even without the use of additives. Ensiling additives may improve silage quality.

Table 2 Chemical characteristics and microbial content of reed silage produced with different additives

	Control	Promyr	Molasses	LAB	LAB+M	SED	*P*-value
pH	4.5	4.4	4.1	3.9	3.8	0.26	0.097
NH_4, g/kg DM	2.3	0.7	1.2	1.5	0.8	0.61	0.17
Lactic acid, g/kg DM	18.4	9.1	34.9	40.4	45.8	8.25	0.01
Acetic acid, g/kg DM	9.3	2.6	10.8	7.5	10.2	1.43	0.003
Butyric acid, g/kg DM	2.2	0.2	0.2	0.2	0.2	1.24	0.46
Ethanol, g/kg DM	7.8	3.8	6.8	6.2	5.8	2.75	0.68
Yeast, log CFU/g FW	2.5	1.3	2.7	0.9	2.6	0.78	0.13
Moulds, log CFU/g FW	1.0	1.0	0.9	1.2	0.9	0.27	0.71
Aerobic stability, days until + 3°C	6.8	7.9	7.9	10.0	10.1	1.17	0.086
Weight loss, g/kg DM	28.9	11.1	18.8	16.2	14.5	7.83	0.29

LAB= lactic acid bacteria, M= molasses.

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Technologies for improving fiber utilization

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Introduction

Dietary Importance and Roles of Fiber

Fiber plays a fundamentally important role in ruminant livestock production, health, and welfare. In addition to being an important energy source, it stimulates chewing and salivation, rumination, gut motility, and health, buffers ruminal acidosis, regulates feed intake, produces milk fat precursors and is the structural basis of the scaffolding of the ruminal raft, which is vital for digestion of solid feed particles in the rumen. Cellulose and hemicellulose – the main components of fiber – are intrinsically ruminally digestible but are rendered partly indigestible by their close association with lignin and hydroxycinnamic acid in the plant cell wall. This paper describes different technologies for improving fiber digestion and their merits and demerits. It is a summary of Adesogan et al. (2019) which gives more details on the subject.

Importance of increasing fiber digestion

It is critically important to increase fiber digestion for productivity, profitability and environmental reasons. Incomplete fiber digestion reduces profitability of dairy production by limiting intake and hence, animal productivity, and increasing manure production. A second reason to increase fiber digestion is to increase energy supply from fibrous feeds that are not consumed by humans. A third reason to increase fiber digestion is that compared to that of starch, ruminal fermentation of fiber-derived hexoses generates more hydrogen ions that reduce carbon dioxide to methane. Consequently, fibrous feed fermentation results in greater production of methane and less energy supply than concentrate feeds (Adesogan et al., 2019). Consequently, it is of paramount importance to increase forage fiber digestion to enhance animal productivity and environmental stewardship of livestock farming.

Strategies to Increase Forage Fiber Digestion

Mechanical Processing

Several studies have examined influence of mechanical processing on particle size measures, typically chop length of hay, straw, or silage or particle size distribution of diets for lactating cows.

<u>Chopping</u>

Although particle size can be manipulated to enhance fiber digestibility, research findings have been inconsistent and outcome is not related to alterations in chemical composition of forage fiber. A meta-analysis of published studies (Ferraretto and Shaver, 2012b) reported that digestibility of dietary neutral detergent fiber (NDF) was not altered by chop length of corn silage. This should not be surprising as fiber digestion is influenced by many factors and the combination of the benefits of long or short forage particles may be countered by disadvantages. For instance, short forage particles have greater surface area for bacterial attachment, which may enhance forage digestibility despite their faster passage rate (Johnson et al., 1999). In contrast, coarse particles are retained for longer periods in the rumen and

require more chewing leading to higher ruminal pH (Allen, 1997), which is conducive for cellulolytic bacteria and forage digestion in general.

Shredding

A recently developed form of silage, called corn shredlage, is produced when corn forage is harvested with a self-propelled forage harvester fitted with cross-grooved crop-processing rolls set at approximately 20% greater roll speed differential and a chopped at greater theoretical length of cut (22 to 26 mm) than the norm. Despite longer chop length used, the shredding process causes greater damage to coarse stover particles and kernels than conventional harvesting. Compared to conventionally processed silage, yields of fat-corrected and actual milk yields were increased by 1.0 and 1.5 kg/cow/day when whole-plant corn was harvested as corn shredlage using either conventional (Ferraretto and Shaver, 2012a) or BMR (Vanderwerff et al., 2015) hybrids, respectively, though NDF digestibility may not be increased. Further research to understand ruminal in vivo NDF digestion kinetics of corn shredlage is warranted.

Pelleting

Pelleting may enhance handling, storage and transportation (Bonfante et al., 2016), and it enhances use of certain bulky forage or crop residues as livestock feeds (Mani et al., 2006). Caution is warranted when forages or TMR are fed as pellets to due to risk of acidosis from reduced saliva production and resulting reduction in ruminal acid buffering caused by pelleting, but the effect may depend on the production stage of the cows. Pelleting crop residues may become an important practice (Mani et al., 2006) in the future, therefore future research should determine the best conditions for feeding forage and TMR pellets.

Steam Treatment

Steam heating or steam pressure and explosion have been used to pre-treat forages to increase fiber digestibility. Steam pressure and explosion increased energy availability from rice straw, sugarcane bagasse and sugarcane field trash by hydrolyzing cellulose and hemicellulose or by eliminating crosslinks between these digestible fractions and lignin (Hart et al., 1980). In addition, lambs fed steam-treated corn stover had greater intake, presumably driven by greater cellulose digestibility (Oji & Mowat, 1978). Costs and equipment required have limited adoption of the technology. Future research should develop cost effective strategies for steam treatment of crop residues as well as forages and by-products.

Genetic Improvement

Brown-midrib mutants

Forage fiber digestion has been reliably improved genetic control of the lignification process or selection for naturally occurring mutations (Porter et al., 1978). For example, brown-midrib (BMR) mutant forages consistently have lower lignin concentrations compared to conventional forages (Sattler et al., 2010), resulting in greater milk production when BMR forages are fed. In a meta-analysis of published studies, Ferraretto & Shaver (2015) reported increases in total tract NDF digestibility (44.8 vs. 42.3% of intake), DM intake (24.9 vs. 24.0 kg/d), yields of milk (38.7 vs. 37.2 kg/d) and protein (1.18 vs. 1.13) for cows fed BMR versus conventional corn silage diets. BMR sorghum and millet hybrids are also more digestible than conventional hybrids but may be prone to lodging, necessitating timely harvests to avoid the problem.

Ferulate ester mutation

Corn hybrids with seedling-leaf-ferulate-ester mutation (SFE) can have greater NDF digestibility than the wild type. Jung and Phillips (2010) reported that corn hybrids with SFE had reduced formation of ferulate ether cross-links and thus greater in vitro cell wall digestibility. Furthermore, Jung et al. (2011) reported that feeding cows SFE-corn silage instead of isogenic CCS increased dry matter intake (DMI) and milk and 3.5% fat corrected milk yields. More studies on SFE mutants are needed to enable proper exploitation of this promising technology.

Reduced-lignin alfalfa, grasses and corn

Feeding reduced-lignin alfalfa to dairy cows has become topical in recent years. Guo et al. (2001) examined lignin concentration and in vitro NDF digestibility (NDFD) of six independent transgenic alfalfa lines with reduced lignin concentration compared to control lines (non-transgenic) and reported a range from 13 to 29% in lignin concentration. Furthermore, they observed an increase of 8% in NDFD for one of these transgenic lines compared to its isogenic counterpart. Mertens & McCaslin (2008) fed transgenic alfalfa hay with reduced lignin concentration (5.3 vs. 5.8% of DM) to young lambs and observed greater NDF intake (1.6 vs. 1.42% of body weight/d) and digestibility (57.5 vs. 49.1% of NDF intake) compared to a non-transgenic line. Further studies evaluating responses of dairy cows are warranted.

Transgenic enzyme-producing plants

Transgenic manipulation of plants to express fibrolytic enzymes may decrease cost of production of exogenous fibrolytic enzymes and is also an efficient method to increase hydrolysis or saccharification of forage biomass (Ransom et al.,2007; Taylor et al., 2008; Furukawa et al., 2014). This technology has been researched for biofuel production primarily and its application to forages is yet to be exploited.

Alkali Treatment

Alkali treatments are effective at breaking hemicellulose-lignin and lignocellulose bonds, hydrolyzing uronic and acetic acid esters, and disrupting cellulose crystallinity by inducing cellulose swelling (Jung and Deetz, 1993). These processes increase cell wall degradability and enable rumen microorganisms to attack structural carbohydrates and lignin and increase degradation of hemicellulose and cellulose (Jung and Deetz, 1993; Sun et al., 1995). Additionally, alkali treatment has potential to degrade lignin, thereby increasing its water solubility and allowing it to be removed from the cell wall (Chesson, 1988). Various alkalis including ammonia, sodium hydroxide (NaOH), calcium oxide (CaO) and calcium hydroxide (Ca(OH)2) have been successfully used to increase fiber digestion and hence nutritive value of low quality forages, particularly crop residues (Singh & Klopfenstein, 1998). Their widespread adoption for this purpose has been limited by their hazardous and caustic nature and high prices.

Acid Treatment

Use of acid hydrolysis for hydrolysis of lignocellulosic materials is well established (Kumar et al., 2009) as the process is effective at hydrolyzing hemicellulose, decreasing cellulose crystallinity, and increasing porosity of treated biomass (Sun and Cheng, 2005). To foster ease of handling and cost-effectiveness, dilute acid treatment is preferred. While acid pre-

treatment is effective at improving nutritive value of low quality feed, it is not widely used for livestock production because of cost, health hazards and corrosive nature of the acids.

Exogenous Fibrolytic Enzymes (EFE)

Effects of EFE in ruminant diets can be classified as pre-ingestive, ruminal, and post-ruminal (McAllister et al, 2001). When EFE are applied to fibrous substrates before feeding, fiber hydrolysis can be observed as partial solubilization of NDF and acid detergent fiber (ADF) and release of sugars and free or monomeric hydroxycinnamic acids (Krueger et al., 2008; Romero et al., 2015c), which may contribute to improvements in in vitro fiber digestibility (Romero et al., 2015a) and microbial growth (Forsberg et al., 2000).

Application of EFE often increases ruminal and or post-ruminal fiber hydrolysis and NDF digestibility of forages, which partially explains their ability to improve animal performance. A recent meta-analysis of published studies reported that EFE application to dairy cow diets resulted in an increase in milk yield (0.83 kg/d) and this was attributed to a tendency for EFE to improve NDF and DM digestibility (Arriola et al., 2017). This meta-analysis also reported that application to TMR instead of concentrate or forage tended to improve milk protein concentration. Another recent meta-analysis reported an increase in milk yield (1.9 kg/d) when cows were fed enzyme-treated diets containing high forage to concentrate ratios (≥ 50%), but no increase occurred when diets with low forage to concentrate ratios (< 50%) were fed (Tirado-Gonzalez et al., 2017). The latter study reported also that cellulose-xylanase enzyme treatment of high forage legume based diets increased milk production by cows (2.3 kg/d) and xylanase treatment of high forage grass based diets improved milk yield (3.1 kg/d). Nevertheless, the results of individual studies have been variable due to various factors described by Adesogan et al. (2019). Recent approaches like proteomics, metagenomics and metatranscriptomics are providing a better understanding of the structure, interaction and functions of the ruminal microbial community (Meale et al., 2014) and the enzyme activities missing from the rumen that are critical for increased fiber digestion (Dai et al., 2015).

Ferulic and p-coumaric acid esterases have been used recently to increase the potency of EFE in ruminant diets (Beauchemin et al., 2003; Krueger et al., 2008). However, the ester portion of the ferulic acid bridge is not available to enzymes since the lignin polymer is in such close proximity, impeding substrate attachment. This may partly explain the variable results observed when ferulic acid esterase is applied to forages (Krueger et al., 2008). Etherase enzymes are required to hydrolyze ether linkages and release ether-linked ferulic acid esterase (FAE) from cell walls but they are produced rarely by fungi and not in the rumen.

Expansin Treatment

Expansins and expansin-like proteins are a recently discovered group of non-hydrolytic proteins with the unique ability to induce cell-wall relaxation or loosening (Cosgrove, 2000, Georgelis et al., 2011). Plant expansins can be divided in two major families, α-expansins (EXPA) and β-expansins (EXPB). However, only the EXPA family has cell wall relaxing or loosening activity under acidic conditions (Cosgrove, 2015). Perhaps the most remarkable characteristic of expansins and expansin-like proteins is their ability synergize with EFE to increases hydrolysis of cellulose and hemicellulose (Kim et al., 2009; Bunterngsook et al., 2015; Liu et al., 2015). Previous studies have demonstrated that synergistic effects between BsEXLX1 and EFE increased hydrolysis of cellulose and hemicellulose more than 5-fold compared to EFE alone (Kim et al., 2009; Bunterngsook et al., 2015; Liu et al., 2015).

Efficient methods of producing these proteins are required to supply sufficient quantities for testing in cattle diets.

Yeast Supplementation

Various studies have shown that yeast products improved fiber utilization and animal performance (Marden et al., 2008; Ferraretto et al., 2012; Jiang et al., 2017a) but others have not (Ouellet and Chiquette, 2016) (Ferraretto et al., 2012; Bayat et al., 2015). A meta-analysis by Desnoyers et al. (2009) showed that supplementing live yeast increased OM digestibility by 0.8 percentage units, DMI by 0.44 kg/d, and milk yield by 1.2 kg/d by dairy cows. Similarly, in a meta-analysis by Poppy et al. (2012), yeast culture supplementation increased milk and milk fat and protein yields by 1.18, 0.06, and 0.03 kg/d. Future studies also should aim to optimize yeast products to achieve consistent results on fiber digestion and animal performance over a wide range of conditions including diet type, lactation stage, stress conditions, etc.

White and Brown-Rot Fungi

White-rot fungi

The white-rot fungi achieve lignin depolymerization through activity of their ligninolytic enzymes. In addition, white-rot fungi also use extracellular reactive oxygen species, which may initiate lignocellulose decay, as lignocellulose-degrading enzymes are too large to penetrate an intact cell wall (Srebotnik et al., 1988, Blanchette et al., 1997). In addition to ligninolytic enzymes, certain white-rot fungi also produce cellulose degrading enzymes (β-glucosidase, cellobiohydrolase, and β-xylosidase) (Vrsanska et al., 2016), resulting in simultaneous degradation of lignin and cellulose components by several strains (*Trametes versicolor, Heterobasidium annosum* and *Irpex lacteus*). Consequently, white-rot fungi can improve digestibility and nutritive value of low quality forages such as wheat straw and bermudagrass (Akin et al., 1993, Nayan et al., 2018). Tuyen et al. (2012) reported that 9 of 11 species of white-rot fungi increased NDF and ADF degradability of wheat straw. However, excessive carbohydrate degradation is one of the main drawbacks to using some strains of white-rot fungi to improve utilization of fiber by ruminants (Wong, 2009, Sarnklong et al., 2010). Fortunately, some strains (*Ceriporiopsis subvermispora, Phellimus pini, Phlebia spp., Lentinula edodes, Hericium clathroides* and *Pleurotus spp*.) selectively degrade lignin alone or preferentially, probably due to a lack of a complete cellulolytic enzymatic complex (Sethuraman et al., 1998, Guerra et al., 2003; Wong, 2009, van Kuijk et al., 2015). Such strains are clearly more likely to improve fiber digestion and animal performance without adverse effects on carbohydrate utilization.

Few studies have involved feeding white rot fungi to animals. A notable exception is the study of Fazaeli et al. (2004) in which treatment of wheat straw with a lignin-selective strain, *Pleurotus ostreatus*, increased DMI (12.2 vs. 10.6 kg/d, DM digestibility (58.8 vs 52.3 %), NDF digestibility (42.3 vs. 34.3 %), milk yield (9 vs. 7.5 kg/d), and body weight gain (743 vs. 272 g/d) of dairy cattle in late lactation, when the untreated and treated straw were fed as 30% of a TMR.

White-rot fungi are not widely used for ruminant fiber digestion. This is due to partly to the long pre-treatment time required (van Kuijk et al., 2015) and more importantly, risk of degradation of cellulose and xylan, thus reducing the absolute nutrient content of the residual forage. Careful strain selection can be used to minimize the latter but additional challenges

with white rot fungi treatment are that laccase, is a potential inhibitor of cellulase activity (Moreno et al., 2012; Yingjie et al., 2018) and fungal delignification is an aerobic process (van Kuijk et al., 2015) that does not occur in the anerobic rumen.

Brown-rot fungi

Brown-rot fungi can degrade lignocellulose polysaccharides by supposedly modifying rather than removing lignin (Highley, 1991) and producing enzymes that selectively depolymerize cellulose and hemicellulose, leaving a brown-coloured rot (Cowling, 1961, Gao et al., 2012). Modifications to lignin include demethylation, hydroxylation, and side chain oxidation (Arantes et al., 2012; Martinez et al. 2011; Yelle et al., 2011). Brown-rot fungi metabolize amorphous cellulose associated with lignin, leaving crystalline cellulose (Eriksson et al., 1990, Kleman-Leyer et al., 1992). The brown rot process involves an initial non-selective oxidation of lignocellulose components via an extracellular Fenton reaction in which reduced iron (Fe2+) is oxidized by hydrogen peroxide (H2O2), yielding reactive oxygen species such as hydroxyl radicals (·OH). Activities of reactive oxygen species result in electron transfer from the lignocellulose complex, causing structural changes and depolymerization (Kerem et al., 1999, Arantes et al., 2012, Kaffenberger & Schilling, 2015). The destructive activity also increases porosity of the lignocellulose matrix, making the polysaccharides more accessible to enzymatic actions (Flournoy et al., 1991).

Several studies have used brown-rot fungi to pretreat biomass for biofuel production, but few animal nutrition studies have used them to increase fiber utilization in ruminant diets. Gao et al. (2012), pre-treated corn stover with different strains of white- and brown-rot fungi and reported that the greatest conversion of cellulose to glucose occurred with a strain of brown-rot fungi, *G. trabeum* (KU-41), after 20 days of pre-treatment. These authors reported 32.0% and 31.4% conversion of xylan to xylose with two strains of *G. trabeum*, KU-41 and NBRC6430, respectively, compared to 11.2% for the control treatment after 48 h of enzymatic hydrolysis. Agosin et al. (1989) reported also that lignin modification by brown-rot fungi, such as its oxidation, can increase its hydrophilic groups, which could increase its solubility, and thus, overall lignin loss.

Only a few studies have examined effects of brown-rot fungi treatment on digestibility of forage or by-product fiber or other nutrients. El-Banna et al. (2010) reported that in vivo digestibility of crude protein (CP), NDF, ADF, hemicellulose and cellulose of sugarcane bagasse (SCB) treated with the brown rot fungi, *Trichoderma reesei* (F-418), were increased compared to the untreated control, when fed to sheep. Furthermore, in vivo NDF digestibility was increased when these authors (El-Banna et al., 2010) fed brown-rot fungi-treated bean straw to sheep. However, Nurjana et al. (2016) reported that a different *T. reesei* strain, QM6a, decreased NDF and ADF concentrations of Napier grass but did not affect NDFD.

That only few in vitro and in vivo studies have been conducted to examine digestibility improvements by brown-rot fungi are attributable to the long pre-treatment time, the need for aerobic conditions for the treatment, and the fact that certain strains of brown-rot fungi degrade desirable polysaccharides, which could reduce residual nutrient content of treated forages. More research is needed to identify strains that remove or modify lignin in ways that increase accessibility to cellulose and hemicellulose, without degrading these beneficial polysaccharides.

Conclusions

Using brown midrib hybrids has been among the most consistent, cost effective and adopted strategies to increase forage fiber digestion and milk production by dairy cows. In this context, more research is needed to examine and validate efficacy and cost effectiveness of other genetic technologies like low-lignin alfalfa or grasses, seedling-ferulate ester mutants, and transgenic fibrolytic-enzyme secreting forages. Mechanical treatment methods that reduce forage particle size vary in effects on fiber digestibility depending on the particle size achieved. A balance between maintaining physical effectiveness of the fiber and reducing particle size is critical for such approaches even when they increase intake and facilitate handling and transport of feeds. Chemical treatment methods of improving fiber digestibility are consistent and effective, but their widespread adoption has been limited by their caustic nature and cost. Among biological treatment techniques, some (yeast products, enzymes and inoculants) have increased fiber digestion and milk production by dairy cows in recent meta-analysis though responses in individual studies have varied. Omic technologies should be exploited to make such products more potent and consistently effective. Other biological treatments (brown and white rot fungi) have considerable potential to improve fiber utilization provided selective strains are used that avoid or minimize carbohydrate degradation.

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Appendix 1. Expansins and expansin-like proteins previously reported in the literature

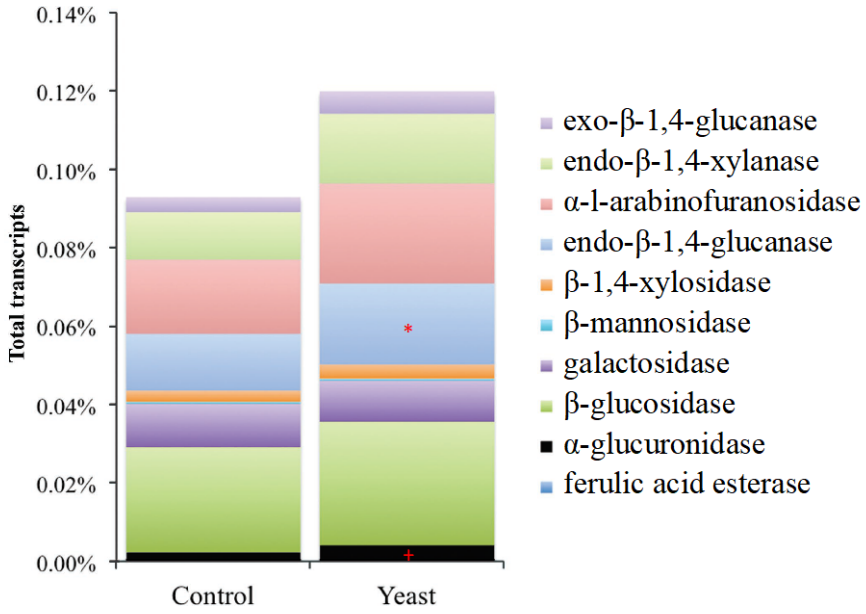

Figure 1 Effect of active dry yeast on abundance of cellulase and hemicellulase enzymes (Adapted from AlZahal et al., 2017). $^{*}P = 0.02$, $^{+}P = 0.10$.

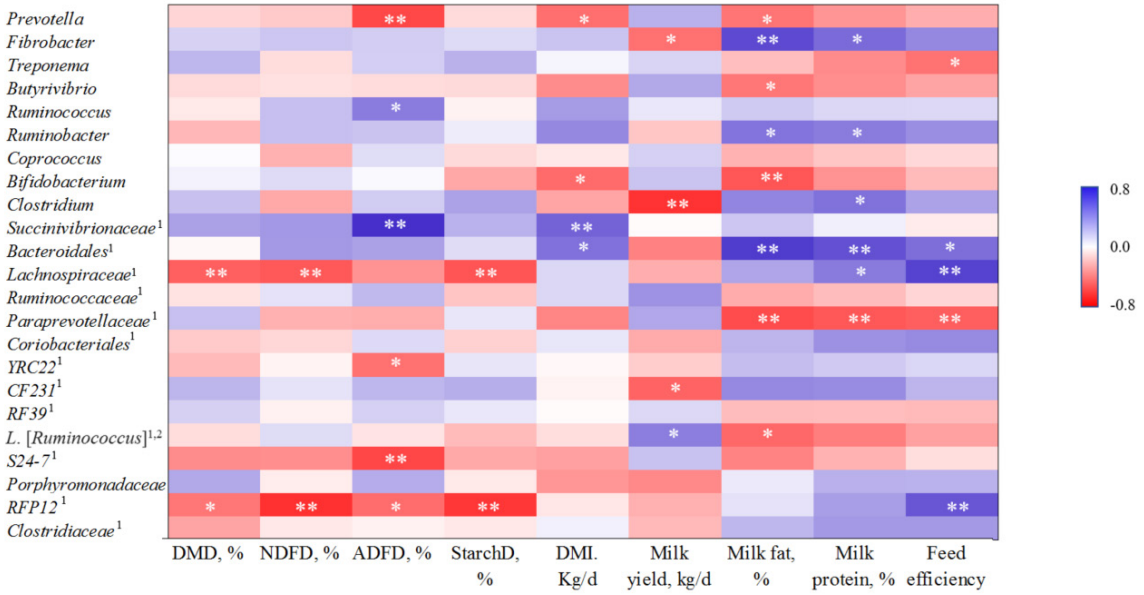

Figure 2 Pearson correlation between animal performance measurements and relative abundance of dominant bacteria (> 0.5% of the bacterial population) in the solid fraction of rumen contents. [1]Unknown genera in the respective family/order. [2] The genus in parenthesis is a candidate taxon. Feed efficiency was calculated dividing 3.5% fat-corrected milk by dry matter intake. **Significant Pearson correlation coefficient ($P \leq 0.05$). *Pearson correlation coefficient tended to be significant ($0.05 < P \leq 0.10$).

Table 1 Expansins and expansin-like proteins previously reported in the literature

Specie	Protein name	Synergism with	Magnitude of Improvement	Optimum Conditions	Substrate	Host strain	Reference
C. sativus	cEX29/30	Cellulases	2-fold	pH 4.5 / 50 °C	FP, Avicel	-	Cosgrove (2001)
Z. Mays	zEXP	Cellulases	2-fold	pH 6.8 / 50 °C	FP, Avicel	-	Cosgrove (2001)
L. esculentum	LeEXP2	Endoglucanases	1.4 to 9.8-fold	pH 4.8 / 50 °C	FP	*P. pastoris*	Liu et al. (2014)
O. Sativa	OsEXP	Cellulases	1 to 3-fold	pH 4 / 50 °C	Crystalline cellulose	*E. coli*	Seki et al. (2015)
B. subtilis	BsEXLX1	Cellulases	2 to 5.7-fold	pH 4.8 / 50 °C	FP, Avicel, Cotton	*E. coli and P. pastoris*	Kim et al. (2009), Wang et al. (2014)
B. pumilis	BpEX	Cellulases, Hemicellulases	2 to 4-fold	pH 5 / 50°C	Avicel, Arabinoxylan	*E. coli*	Bunterngsook et al. (2014; 2015)
P. caratovorum	PcEXL	Cellulases, Hemicellulases	2 to 4-fold	pH 5 / 50 °C	Avicel, Arabinoxylan	*E. coli*	Bunterngsook et al. (2014; 2015)
S. aurantiaca	SaEX	Cellulases, Hemicellulases	2 to 4-fold	pH 5 / 50 °C	Avicel, Arabinoxylan	*E. coli*	Bunterngsook et al. (2014; 2015)
C. michiganensis	CmEX	Cellulases	No effects	pH 4.8 / 50 °C	FP	*E. coli*	Georgelis et al. (2014)
M. aurantiaca	MaEX	Cellulases, Hemicellulases	4 to 4-fold	pH 5 / 50 °C	Avicel, Arabinoxylan	*E. coli*	Bunterngsook et al. (2014; 2015)
H. chejuensis	HcEXLX2	Xylanases and Cellulases	3.1-fold	pH 7 / 30 °C	Xylan	*E. coli*	Lee et al. (2013)
X. campestris	xEXP	Cellulases	No effects	pH 4.8 / 50 °C	FP	*E. coli*	Georgelis et al. (2014)
R. solanacearum	rEXP	Cellulases	No effects	pH 4.8 / 50 °C	FP	*E. coli*	Georgelis et al. (2014)
B. sp AY8	Cms	Cellulases	4-fold at least	pH 4.8 / 45 °C	Cotton	*E. coli*	Haque et al. (2015)
T. reesei	SWO1[1]	Endoglucanases	No tested	pH 5 / 45 °C	Cotton, FP	*S. cerevisiae*	Saloheimo et al. (2002)
B. adusta	LOO1[2]	Cellulase, Hemicellulases	2-fold	pH 5 / 50 °C	Cotton, agave leaves	*S. cerevisiae*	Quiroz-Castañeda et al. (2011)
P. oxalicum	POSWO1[1]	Cellulases	50 % more	pH 4.8 / 50 °C	Avicel	*T. reesei*	Kang et al. (2013)
A. fumigatus	$AfSWO1_1$	Endoglucanases	1.2-fold	pH 5 / 50 °C	Avicel	*A.oryzae*	Chen et al. (2010)

[1]Swollenin protein, [2]loosening protein, FP = filter paper, Cotton = cotton fibers

Reducing carbon footprint of milk using rapeseed and NorFor in Danish dairy herds

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Introduction

A newly established Green Deal in the Danish Parliament obliges Danish dairy farmers to reduce greenhouse gas (GHG) emissions by 0.17 and 1.0 million tons of CO2e in 2025 and 2030, respectively. The first 2025 target corresponding approximately to 7% reduction of enteric methane being emitted from dairy cows in Denmark is to be reached by more fat in the ration. The second 2030 target corresponding approximately to 40% reduction of enteric methane is to be reached by feed additives. There are two main sources of fat that can be used in practice: 1) palm fat which is already widely used although not in the amounts needed to achieve above mentioned target, 2) home grown rapeseed that contains 49% crude fat (norfor.info). However, palm fat has a much higher carbon footprint than rape seed (5364 vs. 1031 g CO2e/kg DM; norfor.info) so in order to produce sustainable milk rapeseed is needed in the rations. The aim of the test presented here was to implement mills that can crush the rapeseed and to test the effect of whole crushed rapeseed on milk production, feed efficiency and carbon footprint of milk under practical conditions.

Materials and Methods

The criteria for farms to participate in the project were: 1) able to mill or roll whole rapeseed; 2) able to feed with the same silages for the whole period, i.e. no shifts in batch/silos; 3) willing to register daily amounts of feed stuff being mixed and fed out to the cows and the amount of left overs on a daily basis; 4) participating in the herd yield recording system; 5) increasing the fatty acids (FA) level in the diet 6) willing to register daily amounts of milk that was discarded, used for calves or sold on a daily basis. Ten farms were included in the investigation, 7 conventional and 3 organic, that was carried out from November 2020 to June 2021. Of the 7 conventional farms, 5 were using palm fat in the diet, that was replaced with whole crushed rapeseed (WCR). Also, the level of FA was increased whether palm fat was a part of the initial diet or not.

The effect of WCR was assessed using a changeover design where the herd was fed their normal diet referred to as 'Control' in period 1. In period 2, the herd was fed a diet with WCR and then the herd was put back on the 'Control' diet in period 3. Each period lasted for approximately 4 weeks. Feed intake was registered on herd level daily by scales on the mixerwagon and registration of left overs. Milk yield was recorded on herd level via bulk tank milk every second day including analysis of milk composition. Feed analysis based on NIR were done on raw materials, compound feeds, silages and TMRs/PMRs in each herd using Kvægbrugets ForsøgsLaboratorium (Kristensen, 2021). Faeces was collected in 7 of the 10 herds and analyzed individually by NIR from 12 cows that was approximately 100 DIM in each period. Enteric methane emission and carbon footprint were estimated by NorFor (FRC revision 2.11).

Data on DMI, milk yield, milk composition, carbon footprint and fecal measures were analysed using PROC MIXED in SAS. In all statistical calculations, farm was used as experimental unit and effects were considered as statistically significant when $P < 0.05$.

Results and Discussion

Table 1 gives an overview of average nutrients and energy concentration in the control- and WCR-diets used across the dairy farms. In average 750 g DM of WCR was fed to the cows per day which increased fatty acids in the diet from 32 to 41 g/kg DM. WCR typically replaced palm fat supplements grain, and protein feedstuffs. NDF, crude protein and concentrate share were kept constant while starch decreased and energy concentration increased when WCR was included in the diet.

Table 1 Average rapeseed allowances and diet composition in 10 Danish dairy herds fed a diet with and without whole crushed rapeseed

	Unit	Control	Rapeseed	P-value
Rapeseed	g DM/cow/day	50	750	P<0.01
Fatty acids	g/kg DM	32	41	P<0.01
Starch	g/kg DM	192	186	P<0.01
NDF	g/kg DM	299	300	NS
Crude protein	g/kg DM	167	166	NS
Concentrate share	% of DM	43	44	NS
Net energy	MJ/kg DM	6.66	6.73	P<0.01

Table 2 shows that feeding WCR increased milk yield by 1.6 kg and lowered milk fat and milk protein by 0.20 and 0.11 percentage units, respectively. Especially herds that was not feeding any fat supplements in the control diet increased ECM production when WRC was included in the diet (data not shown), indicating that cows responded better to fat addition at lower fatty acids levels. All herds experienced reduced fat content in milk when feeding WCR but especially herds where WCR replaced palm fat the reduction in milk fat was marked. This is in contrast to other trials (Brask et al., 2013; Stergiadis et al., 2014) where no significant changes in milk yield and milk composition was detected when feeding WCR. No significant changes were detected in our investigation for DMI and feed efficiency.

Table 2 Feed intake, milk yield, milk composition and feed efficiency in 10 Danish dairy herds fed a diet with and without whole crushed rapeseed

	Unit	Control	Rapeseed	P-value
Feed intake	kg DM/cow/d	23.4	23.3	NS
Milk yield	kg/cow/d	31.4	32.8	P<0.05
Fat content	%	4.61	4.41	P<0.01
Protein content	%	3.75	3.64	P<0.01
ECM	kg/cow/d	34.0	34.5	NS
Feed efficiency	kg ECM/kg DM	1.46	1.49	NS

Table 3 shows that feeding WCR increased the content of crude fat in feces while no changes were seen NDF and starch content. The higher crude fat content was expected as the intestine seem to have limited capacity to absorb fatty acids (Weisbjerg et al., 1992). Surprisingly the

content of NDF was not increased which could have been expected due to the indigestible rapeseed hulls.

Table 3 Characteristics of faeces in 7 Danish dairy herds fed a diet with and without whole crushed rapeseed

	Unit	Control	Rapeseed	P-value
DM	g/kg	134	130	NS
NDF	g/kg DM	455	433	NS
Starch	g/kg DM	7	7	NS
Crude fat	g/kg DM	48	61	P<0.01

Table 4 shows that feeding WCR decreased enteric methane by 6% and the carbon footprint of milk by 4%.

Table 4 Methane emission and carbon footprint of milk estimated in NorFor for 10 Danish dairy herds fed a diet with and without whole crushed rapeseed

	Unit	Control	Rapeseed	P-value
Methane	g/cow/day	457	431	P<0.01
Carbon footprint[1]	g CO_2e/kg ECM	700	671	P<0.05

[1]Carbon footprint includes enteric methane, emissions from feedstuffs, carbon sequestration in the soil and greenhouse gas emissions from manure.

Conclusions

It is possible to mill rapeseed on farm in different ways and feed it to dairy cows without affecting feed intake. WCR increased milk yield but decreased milk fat and milk protein content, while ECM was unaffected. Crude fat from WCR is not 100% digested and absorbed as 27% more crude fat was found in faeces. Calculations done in NorFor for the 10 herds showed that feeding WCR decreased enteric methane by 6% and the carbon footprint of milk by 4%.

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Feeding value of processed birch measured *in vitro*, *in sacco* and *in vivo*

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Introduction

Based on the drought of 2018 summer in the Nordic countries, alternative feeds for ruminants have been studied. In 2019, results of using sawdust from aspen tree *(Populus tremula)* as emergency feed were reported (Prestløkken and Harstad, 2019). The conclusion in that report was: "Aspen sawdust can replace substantial part of grass silage in diets for lactating cows without hampering milk production and milk composition and quality. A minimum requirement of grass silage, or other feed giving structure to the diet appears to be needed. Further studies on aspen sawdust, or other wood products are needed". This manuscript summarizes results of using birch (*Betula pubescense*) as alternative feed resource for ruminants. Birch was chosen for its abundance and, in contrast to aspen, it grows all over Norway. Birch was processed using various methods, including steam explosion and alkali treatments. Feeding value was evaluated using in vitro gas production, in sacco rumen degradation in dairy cows, and digestibility in sheep.

Materials and Methods

Birch sawdust was obtained in two forms; (A) steam exploded and (B) fresh. These two forms were further treated using chemicals: (1) not treated, (2) ammonia at 4% of dry weight, and (3) sodium hydroxide at 4% dry weight simulating commercial treatment of straw for ruminants. This produced a 2x3 factorial combination (Tabel 1).

Table 1 Description and chemical composition (g/kg DM, unless mentioned) of sawdust used for in vitro

	Description	DM, g/g	Ash	NDF	CP	HC	ResCHO
A1	Steam exploded, not treated	963	3.6	569	18.5	19.0	409
A2	Steam exploded NH3 treated	925	3.7	589	168	19.5	388
A3	Steam exploded NaOH treated	953	24.2	565	16.7	13.0	394
B1	Fresh sawdust, not treated	987	3.5	937	12.0	242	47
B2	Fresh sawdust NH3 treated	950	3.2	930	134	196	54
B3	Fresh sawdust NaOH treated	989	24.2	921	9.5	232	46

HC= hemicellulose; ResCHO = residual carbohydrate adjusted for NH_3-N according to NorFor (2011).

In vitro gas production was carried out using Ankom RF GP System. Approximately 1.0 g DM of sawdust (milled to pass a 1.0-mm screen) was incubated in triplicates for 72 h in two batches. Blanks (buffer + rumen fluid without substrate) and an internal standard feed were included. Samples were incubated in 100 mL of buffered (Goering and Van Soest, 1970) rumen fluid (67 mL buffer and 33 mL rumen fluid) using 250 mL glass bottles. Cumulative gas production, in vitro dry matter degradation (inDMD) and pH of the incubated media (pH_{72}), after 72 h of incubation, were registered. The model of Groot et.al., (1996) was fitted to the gas production profiles, assuming a single fermentation pool (i.e. $GP = A/(1+ B^t/t^C)$; where GP is cumulative gas volume at time t; A is asymptotic gas volume (mL/g DM), B is time (h) taken to produce half of A, and C (unitless) is shape parameter of the curve. The *in vitro* GP data was compared among the treatments using Proc GLM in SAS (version 9.4).

Rumen degradability of DM was determined in sacco for sawdust prepared as A1, B1 and B2 (Table 1), according to Åkerlind et al. (2011). Untreated and NH_3 treated wheat straw was

added for comparison. Rumen degradation profiles were fitted to an exponential function using the NLIN procedure of SAS (version 9.4). Effective rumen DM degradability was estimated according to Ørskov and McDonald (1979), using a passage rate of 0.05 h^{-1}.

In vivo digestibility of steam exploded birch was determined using three adult castrated male sheep fed at maintenance. Early harvested grass silage was used as sole feed, with untreated wheat straw used for comparison of feeding value. On DM basis the diets consisted of (1) grass silage only, 1211 g/d; (2) grass silage and straw, 732 and 481 g/d; and (3) grass silage and birch, 732 and 467 g/d. Digestibility of nutrients in straw and steam exploded birch was calculated by difference. Energy value is given as feed units milk (FEm) (Ekern, et al. 1991).

Results and Discussion

For in vitro data (Table 2), steam explosion improved the fermentation quality of sawdust, but with a significant interaction effects of steam explosion by chemical treatment. As such, chemical treatment improved inDMD, GP72 and asymptotic gas volume to a greater extent in the fresh than in the steam exploded sawdust. But this improvement was subtle in the steam exploded sawdust. Treatment of fresh sawdust with NH_3 showed greater improvement in the above parameters than treatment with NaOH. This could be due to the volatility and penetration of NH_3 in the sawdust along with enriched nitrogen with NH_3 treatment.

Table 2 In vitro gas production data (GP_{72} = gas volume at 72h, mL/g DM, A= asymptotic gas volume, mL/g DM incubated; B= time in hours taken to produce half of the asymptotic gas volume; and C is shape parameter)

	Description	GP_{72}	A	B	C	pH_{72}	inDMD, %
A1	Steam exploded, not treated	202^{e}	212^{c}	11.0^{a}	2.03^{c}	6.04^{a}	67.3^{d}
A2	Steam exploded NH_3 treated	189^{d}	205^{c}	16.8^{a}	2.24^{c}	6.29^{b}	65.3^{d}
A3	Steam exploded NaOH treated	190^{de}	196^{c}	12.1^{a}	2.29^{c}	6.21^{b}	65.4^{d}
B1	Fresh sawdust, not treated	33.2^{a}	40.1^{a}	14.5^{a}	1.41^{b}	6.76^{c}	5.85^{a}
B2	Fresh sawdust NH_3 treated	123^{c}	218^{c}	56.9^{b}	1.60^{b}	6.57^{c}	37.2^{c}
B3	Fresh sawdust NaOH treated	63.5^{b}	102^{b}	45.0^{b}	1.14^{a}	6.69^{c}	18.7^{b}
	RootMSE	8.63	41.8	12.6	0.21	0.089	3.37
Statistics (effect of)							
	Physical treatment	***	***	***	***	***	***
	Chemical treatment	***	***	***	ns	ns	***
	*Interaction (Physical*Chemical)*	***	***	*	*	***	***

inDMD = in vitro dry matter digestibility; RootMSE = root mean square error; Statistics: * = $P < 0.05$; *** = $0.01 \leq P < 0.001$; ns= not significant; Means in a column with different superscripts are different at $P < 0.05$.

Tabel 3 gives effective rumen degradability (ED), fractional rate of degradation (k_d) of Pd, soluble (S), potential degradable (Pd), and undegraded dry matter of birch sawdust and wheat straw. Steam exploded birch showed a rumen degradation of DM higher than NH_3 treated straw, whereas untreated fresh birch was not degraded at all. Ammonia treatment of birch increased rumen degradation of DM, but to a much lesser extent than steam explosion.

Table 3 Effective rumen DM degradability (EDMD), fractional rate of DM degradation (k_{DM}), soluble DM (S), potential degradable DM (PdDM) and undegraded DM in sawdust from birch and wheat straw

	ED, %	k_D, %/h	S, %	Pd, %	Undegraded %
Fresh sawdust, not treated	0	0	0	0	100
Fresh sawdust, NH3 treated.	10	1.08	0	53	47
Steam exploded, not treated	50	3.47	26	59	15
Untreated straw	24	2.75	2	63	35
NH3 treated straw	40	3.44	8	78	14

Figure 1 gives digestibility of organic matter (OMD), neutral detergent fiber (NDFD) and FEm. The OMD, NDFD, and FEm of steam exploded birch was higher than that of untread

straw. The FEm of untreated and ammonia treated straw in the old Norwegian feed table (STIL, 1992) was 0.30 and 0.68 respectively. Thus, the energy value of steam exploded birch was higher than that of untreated straw and almost matches that of ammonia treated straw.

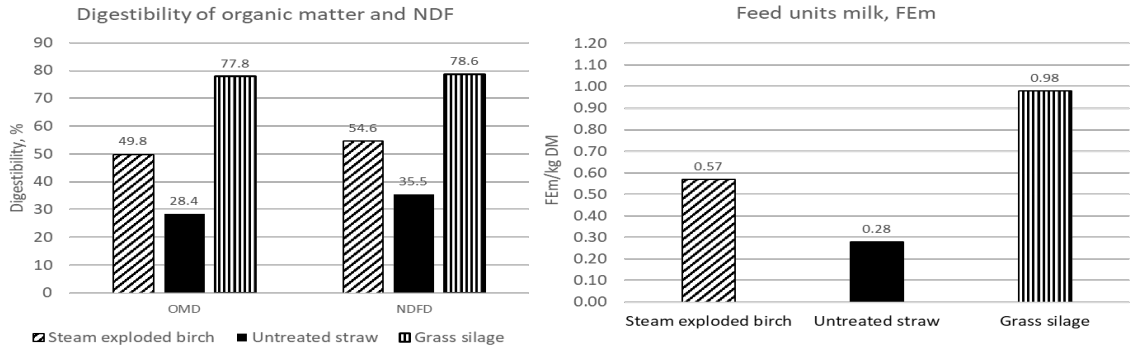

Figure 1 Digestibility of organic matter (OMD), neutral detergent fiber (NDFD) and energy value (FEm) of steam exploded birch, untreated wheat straw and early harvest grass silage.

Conclusions

Feeding value of birch sawdust is highly dependent on processing method. Steam exploded birch has a feeding value close to ammonia treated straw and could serve as an alternative feed ingredient if the production of forage feeds is limited by climatic conditions.

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Effects of decreasing silage particle size by extrusion on chewing behaviour and rumen pH in dairy cows

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Introduction

Increased use of ley crops (grasses and legumes) in cattle production is desirable as they offer several environmental benefits and may also be economically beneficial for farmers. Forage intake, and hence the use of ley crops, can be enhanced by the reduction of particle size before feeding. According to a meta-analysis by Nasrollahi *et al.* (2015), decreased silage chop length from 10 to 6 mm increased daily dry matter (DM) intake by 0.5 kg. More extensive particle size reduction, such as pelleting (Minson, 1963), may increase the intake even more but particle size reduction might be negative for ruminants due to impaired rumen function. Eating and rumination times increase with increased forage particle size (Nasrollahi et al., 2016) and stimulate saliva production, thereby supplying rumen with liquid and buffering capacity (Allen, 1997). This paper aimed to evaluate the effect of extensive silage particle size reduction on chewing behaviour and rumen characteristics in dairy cows.

Materials and Methods

A grass-dominated (70% timothy) ley crop was harvested at a first cut on June 13 (Harvest 1) and 10 days later (Harvest 2) at Lövsta research centre, Uppsala, Sweden (Table 1). The maturity stage at Harvest 1 was estimated according to Pomerleau-Lacasse *et al.* (2017) with 22% of timothy being at elongation stages E4-E5 and 78% at the reproductive stage (42% R0, 19% R1, 17% R2-R3). After wilting to 45-50% DM, the grass was round baled and wrapped with plastic film. At feed out, bales were mixed in a feed mixer (SILOKING TrailedLine Classic Premium 14, Kverneland, Klepp, Norge). Half of each mixed bale was processed in an extruder (Bio-Extruder MSZ-B15e, LEHMANN Maschinenbau GmbH), where screws sheared the material under pressure, for particle size reduction and cell wall break up. Four rumen fistulated cows (Swedish Red, 143 ± 38 DIM) were used in an experiment with a Latin square design, including 4 periods (21 days long) and 4 treatments; silage from Harvest 1 or Harvest 2 that was fed as is or extruded, in a 2 × 2 factorial arrangement. Silage was fed separately *ad libitum* twice a day. Concentrates, 2 kg soybean meal, 6 kg compound feed (Komplett Norm 180, Lantmännen, Sweden) and 120 g mineral mix (Mixa Optimal, Lantmännen, Sweden), were fed four times per day.

Feeds were analysed for the contents of ash, crude protein (CP), neutral detergent fibre (aNDFom) and 96-h *in vitro* organic matter digestibility (IVOMD, only silages) with routine methods (Eriksson and Rustas, 2014). A sieve (Penn State Particle Separator, Nasco, Fort Atkinson, WI, USA) with four levels and openings of 19, 8, 4 and 0 mm (bottom pan) at each level, was used to determine particle size distribution in silages.

Rumen liquid samples were collected on 20 occasions during days 15-19 in each experimental period, representing each hour from 6 until 22 and every other hour from 22 until 6. Samples were strained immediately after collection, pH was measured and samples were frozen at -20°C. Thawed samples were pooled by period and analysed for VFA by HPLC (Ericson and André, 2010).

Video recordings (24 h) on day 21 of each collection period were scanned at 5-minute intervals and 1 min of each 5-minute interval was observed. Observed activities were assumed to persist for the entire 5-min period. Based on observations and these assumptions, eating and rumination times were calculated (Beauchemin et al., 2003).

Statistical analysis was performed on means from each period in GLM (Minitab 18.1, 2017) for all variables, except for rumen pH. The model included period, cow, harvest time (Harvest 1 or 2), silage processing (mixed or mixed and extruded) and the interaction between harvest time and processing, all factors were considered fixed effects. Rumen pH analysis was based on all observations within each period and performed in Proc Mixed (SAS, 2018; version 9.4) with a model that included the factors described above and sampling hour, as a repeated measure, and interactions between hours, harvest and processing.

Table 1 Composition of silages and extruded silages from Harvest 1 and Harvest 2

	H1S	H1E	H2S	H2E
DM, g/kg	458	477	505	524
Ash, g/kg ts	80	80	71	69
CP, g/kg ts	125	124	106	105
NDF, g/kg ts	548	549	551	552
IVOMD, g/kg OM	799	801	713	722
PSD, % of DM				
19 mm	33	0	32	0
8 mm	40	53	37	50
4 mm	16	27	15	28
0 mm	11	20	16	22

PSD=particle size distribution on sieves, H1S=Harvest 1 silage, H1E=Harvest 1 extruded, H2S=Harvest 2 silage, H2E=Harvest 2 extruded.

Results and Discussion

Both eating and rumination times, reflecting chewing activity (Allen, 1997), decreased with decreased particle size by extrusion but were not affected by forage maturity (Table 2). Rumen pH decreased by extrusion but was not affected by harvest date. There was no interaction between silage processing and sampling hour for the pH. Total VFA concentrations increased by earlier harvest (6.7 mM) and particle size reduction (5.0 mM), presumably due to improved digestibility at earlier harvest and greater intake by extrusion

Conclusion

Decreasing particle size by extrusion caused reduced chewing time and rumen pH in dairy cows.

Table 2 Responses from cows fed silage and extruded silage from Harvest 1 and Harvest 2

						P-value		
	H1S	H1E	H2S	H2E	SED	harvest	process	H x P
Intake								
Silage DM, kg/d	18.0	19.2	17.2	19.3	0.33	0.21	< 0.001	0.08
Total DM, kg /d	25.0	26.0	24.2	26.2	0.27	0.28	< 0.001	0.09
Rumen liquid								
pH	6.09	5.99	6.11	6.01	0.024	0.21	<0.001	0.93
Acetate, mM	82.6	86.3	77.7	81.7	1.97	0.014	0.032	0.92
Propionate, mM	21.8	22.2	20.2	21.6	0.55	0.031	0.058	0.25
Butyrate, mM	13.8	14.3	13.3	13.7	0.41	0.094	0.18	0.71
Valerate, mM	4.3	4.1	4.0	3.9	0.02	0.022	0.052	0.42
Tot VFA, mM	122	127	115	121	2.8	0.015	0.044	0.78
Chewing behaviour								
Eating (E), min/d	267	228	279	245	16.7	0.22	0.006	0.83
Rumination (R), min/d	605	465	580	485	18.0	0.85	< 0.001	0.084
E and R, min/d	906	731	896	772	23.0	0.34	< 0.001	0.12

H1S=Harvest 1 silage, H1E=Harvest 1 extruded, H2S=Harvest 2 silage, H2E=Harvest 2 extruded.

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Effects of forage and grain legume-based silages supplemented with faba bean seed or rapeseed expeller on milk production and composition in dairy cows

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Introduction

Forage legumes, such as red clover (RC; *Trifolium pratense*), have gained interest in grassland cultivation due to their ability to fix nitrogen and increase carbon sequestration in the soil. Grain legumes, such as faba bean (FB; *Vicia faba*), can be harvested both as seed and whole-crop silage. In Northern conditions this offers a possibility not only to include FB as a locally grown protein source in dairy cow diets but also to use it as a forage after an unfavourable growing season (Palmio et al., 2022). Use of legume feeds decrease the reliance on synthetic nitrogen fertilizers and imported protein concentrates, thereby improving the economics and sustainability of dairy farms (Stoddard et al., 2009). Inclusion of RC in dairy cow rations has shown to alter milk yield, composition and fatty acid profile compared with grass silage (Vanhatalo et al., 2009; Halmemies-Beacheut-Filleau et al., 2014). Faba bean seed has previously been studied as an alternative for rapeseed, but data is scarce and inconsistent. The objective of this experiment was to investigate the effect of forage type (RC vs. FB rich silages) and concentrate type (FB vs. rapeseed expeller; RE) on milk production and composition in dairy cows.

Materials and Methods

A total of eight multiparous Nordic Red cows were used in a replicated 4 × 4 Latin square experiment with a 2 × 2 factorial arrangement of treatments. Each experimental period lasted for three weeks. The experimental diets were: 1) RC–grass silage (RCG) with RE, i.e. RCG-RE treatment, 2) RCG with FB, i.e. RCG-FB treatment, 3) FB-rich silage (2/3 of FB and 1/3 of grass silage; FBG) with RE, i.e. FBG-RE treatment, and 4) FBG with FB, i.e. FBG-FB treatment. Inclusion rate of rapeseed expeller and FB was isonitrogenous. All diets included oats, barley and a mineral mix, and were fed ad libitum as TMR. The RCG silage was prepared from secondary growth of mixed RC (51%), grass (49% mix of timothy, meadow fescue, Italian ryegrasss) sward harvested at a late growth stage, wilted for 24 h and ensiled into round bales, and contained (DM-basis): 42.2% NDF and 13.5% CP. The FB silage was prepared from green FB crop when pods were mainly filled. It was wilted for 48 h and harvested into round bales, and contained (DM-basis): 43.7% NDF and 16.9% CP. The grass silage was first cut timothy-meadow fescue grass stored in a bunker silo, and contained (DM-basis): 51.6% NDF and 16.5% CP. All silages were preserved with a preservative AIV2 Plus Na targeted at 6 L/1000 kg. The composition of the experimental feeds is presented in Table 1. Dry matter intake and milk yield were recorded daily. Milk samples were analysed for milk fat, CP, lactose, and urea, and milk fatty acid composition.

Table 1 Chemical composition of feed ingredients used in the experimental diets

Item	Faba beansilage	Grass silage	Red clover-grass silage	Faba bean meal	Rapeseed expeller	Barley and oats mix
DM, %	27.8	28.4	32.0	85.5	89.8	86.3
Chemical composition, % of DM						
Ash	7.59	10.3	8.78	3.80	6.71	2.79
NDF	43.7	51.6	42.2	14.2	26.2	22.1
Starch	3.06	0.120	1.46	33.2	2.85	45.0
Crude fat	0.861	2.49	2.70	1.35	12.3	3.68
OM	92.4	89.7	91.2	96.2	93.3	97.2
CP	16.9	16.5	13.5	31.8	34.5	13.7

Results and Discussion

Overall, there were no interactions between forage and concentrate types. Dry matter intake did not differ across diets averaging 26.7 kg/d. Milk yield was greater for RCG vs. FBG silage (36.1 vs. 35.1 kg/d; P=0.05) and was lower for FB vs. RC (34.5 vs. 36.7 kg/d; P<0.001). Milk protein and milk urea concentrations (Figure 1) decreased (P<0.001) and milk fat concentration tended to decrease (P=0.09) for cows fed RCG compared with cows fed FBG. Milk urea concentration was increased (P<0.001; Figure 1) by FB vs. RE. Further, milk protein yield tended to be lower (P=0.08) for cows fed RCG vs. FBG and was lower (P=0.002) for FB vs. RE (Figure 2).

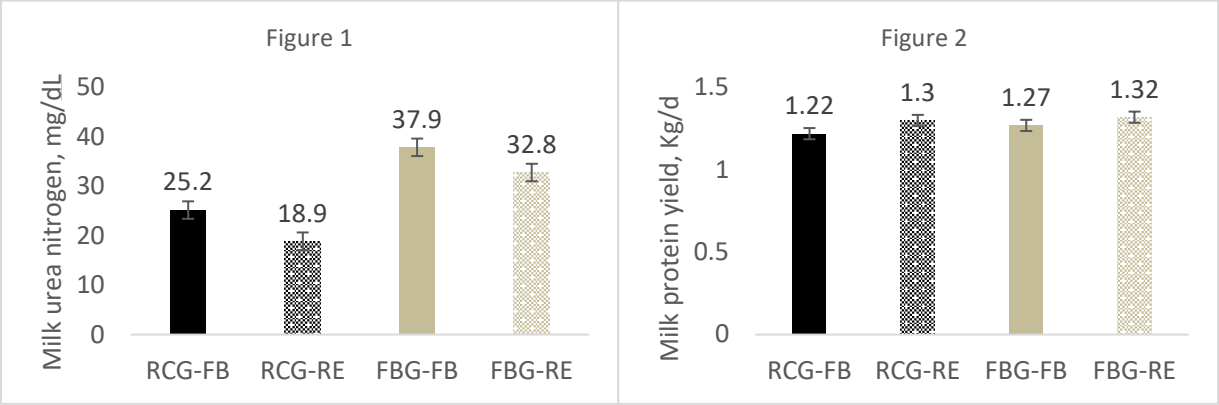

Figure 1 & 2 Milk urea nitrogen concentration (mg/dL) and milk protein yield (kg/d) in mid-lactation dairy cows receiving two types of forage (red clover-rich silage or faba bean-rich silage; RCG or FBG) and two types of protein concentrates (faba bean meal or rapeseed expller; FB or RE).

Of milk fatty acids (Table 2), short-chain fatty acids (SFA) were decreased by RCG vs. FBG and increased by FB vs. RE (P<0.001), whereas concentration of monounsaturated fatty acids (MUFA) was increased (P=0.04) by RCG vs. FBG, and lower (P<0.001) for FB vs. RE. In particular, 18:1n-9 concentration was lower (P<0.001) for FB vs. RE. Poly-unsaturated fatty acids were also greater (P<0.001) for RCG vs. FBG. Further, 18:2n-6 and 18:3n-3 were greater (P<0.001) for RCG vs. FBG, and 18:2n-6 greater whereas 18:3n-3 was lower (P≤0.05) for FB vs. RE. In addition, cis-9,trans-11 CLA was lower (P<0.001) for FB compared with RE.

Table 2. Effect of forage or concentrate type on milk fatty acid composition in mid-lactation cows

	Treatment					P-value		
Fatty acid	RCG-FB	RCG-RE	FBG-FB	FBG-RE	SEM	Forage type	Concentrate type	Interaction
SFA	78.1	72.7	79.6	73.8	0.66	<0.001	<0.001	0.54
MUFA	17.7	22.9	16.8	22.5	0.53	0.04	<0.001	0.34
PUFA	2.96	3.06	2.44	2.41	0.120	<0.001	0.42	0.10
18:1n-9	0.090	0.173	0.084	0.175	0.0064	0.43	<0.001	0.17
18:2n-6	1.51	1.47	1.36	1.26	0.059	<0.001	0.02	0.25
18:3n-3	0.719	0.769	0.396	0.403	0.0250	<0.001	0.05	0.11
cis-9trans,trans 11 CLA	0.268	0.366	0.251	0.346	0.0210	0.12	<0.001	0.87

Conclusions

Red clover silage resulted in greater milk yield compared with FBG, while RE resulted in greater milk and milk protein yields of the two protein concentrates. Milk fatty acid profile was altered by both forage and concentrate type, reflecting the differences in fatty acid content and composition of the different feeds. Red clover silage resulted in lower SFA concentrations and greater 18:2n-6 and 18:3n-3 concentrations compared with FBG. Furthermore, cows fed FB had a greater SFA and lower 18:1n-9 and cis-9,trans-11 CLA concentrations compared with cows fed RE.

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Effect of harvesting frequency and grassland species on estimated silage protein value

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Introduction

As silage is the main dietary ingredient and protein source to dairy cows in the Nordic countries, improving the protein quality of silage may reduce the need for high quality protein feeds. The objective of the current study was to test harvest frequency and grassland species on silage protein value.

Materials and Methods

Silages were made from all cuts of three different leys; T= pure timothy (*Phleum pratense* L. cv. 'Liljeros'), T+RC= timothy and red clover (*Trifolium pratense* L. cv. 'Gandalf') mixture and PRG=pure perennial ryegrass (*Lolium perenne* L. cv. 'Figgjo') in their first production year. The herbage was harvested from a field trial with three replicated blocks at Fureneset research station, Norway, in 2020, with harvest system (two or three cuts per year) on main plots and the grassland species on the sub-plots. The grass plots received in total 260 and 280 kg N ha^{-1} per year in the two and three cut system, respectively, while the T+RC received 50% N of the grass. Herbage samples from each plot were wilted by force air at 30°C to the target dry matter levels 22.5 or 37.5% DM. The wilted material was chopped and ensiled with a formic acid-based additive (GrasAAT® Lacto, ADDCON Nordic AS, Porsgrunn) in evacuated sealed polyethylene bags at the dosing rates 0 or 4 ml/kg herbage material, respectively. Freeze dried samples of fresh and wilted herbage and of silages were analysed for ash, crude protein, and buffer soluble crude protein (sCP) according to NorFor standards (Volden et al., 2011), ash free neutral detergent fibre (NDFom) according to Mertens (2002), and water-soluble carbohydrates (WSC) as described by Randby et al. (2011). Frozen silage samples were analysed for pH, and organic acid contents according to Ericsson and André (2010). Concentration of indigestible NDFom (iNDF) in silage freeze dried samples was determined by in situ incubation, and organic matter digestibility was calculated from iNDF and NDFom concentrations (Huhtanen et al., 2013). Metabolizable protein (AAT_{20}) content was calculated according to NorFor at daily intake of 20 kg DM (Volden, 2011). The yield and silage constituents were modelled using the MIXED procedure in SAS 9.4 with harvest number (1 to 5) and grassland mixture (T, T+RC or PRG) and their interactions as fixed effects. The effects of wilting and silage additive is not presented in this paper. Planned contrasts were used to test difference between the harvest systems, between the grass species and between timothy and the timothy red clover mixture.

Results and Discussion

The PRG ley yielded on average 16 and 27% more DM than T and T+RC, respectively, and two cuts per season yielded on average 7% more than three cuts (Table 1). However, total DM yield of PRG cut three times was 4% higher than two cuts, while T and T+RC cut three times yielded 10 and 14% less than two cuts, respectively. The herbage clover proportion in the T+RC was 0.01 and 0.28 g/g DM in the first and second cut of the two cut system,

resepectively, while in the three cuts system it was 0.06, 0.12 and 0.44 g/g DM in the first, second and third cut, respectively.

Table 1 Effect of species (T=timothy, T+RC=timothy+red clover, or PRG=perennial ryegrass) and harvest system (3 or 2 cuts per season) on DM yield, silage organic matter digestibility (OMD), pH and silage content of crude protein (CP), soluble CP (sCP), water soluble carbohydrates (WSC), sum of fermentation acids (TA), and amino acid absorbed in the intestine (AAT_{20}) (n=4)

Item	Cut	T		T+RC		PRG		SEM	Effects					
		3cut	2cut	3cut	2cut	3cut	2cut		C	M	C×M	C1	C2	C3
Yield, g DM/m^2	1st	465	729	458	740	602	837	21.7	***	***	*	***	***	**
	2nd	370	419	263	331	452	404							
	3rd	198		196		240								
DM, g/kg	1st	311	352	319	373	293	348	47	ns	ns	ns	ns	ns	ns
	2nd	343	353	362	327	331	344							
	3rd	310		288		299								
OMD, %	1st	75.3	65.8	75.9	66.2	77.3	66.1	0.46	***	***	***	***	ns	***
	2nd	73.7	68.1	75.3	70.1	67.9	69.0							
	3rd	75.5		76.3		77.9								
CP, g/kg DM	1st	147	103	126	89	121	95	1.7	***	***	***	***	***	**
	2nd	141	106	144	109	124	113							
	3rd	113		158		112								
sCP, g/kg CP	1st	790	580	755	557	781	721	24.9	***	***	*	**	***	**
	2nd	582	596	538	486	704	660							
	3rd	510		459		664								
WSC, g/kg DM	1st	38	60	46	58	92	84	21.8	ns	ns	ns	ns	ns	ns
	2nd	42	57	56	52	65	78							
	3rd	113		51		77								
pH	1st	4.46	4.22	4.50	4.41	4.24	4.31	0.192	***	ns	ns	***	ns	ns
	2nd	4.04	4.80	4.21	4.98	4.06	5.21							
	3rd	4.08		4.19		4.16								
TA, g/kg DM	1st	58	37	60	32	73	42	15.1	**	ns	ns	***	ns	ns
	2nd	52	29	59	40	54	34							
	3rd	63		98		73								
AAT_{20}, g/kg DM	1st	82	77	82	78	80	74	1.5	***	***	ns	***	***	ns
	2nd	84	80	85	81	75	78							
	3rd	84		84		81								

SEM is standard error of the mean; P-values: C=effect of cut, M=effect of ley mixture, 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.

The target DM was achieved with no differences between species or cuts (Table 1). The fermentation in silages made from the three cuts system was more intensive than in the silages made from two cuts, as indicated by higher concentrations of fermentation acids and lower pH in the silages from three cuts. The concentration of WSC in wilted, pre-ensiled

herbage was on average higher in the two than the three cut system (figures not shown). Thus, more intensive fermentation in the three cut system was likely due to higher OMD (Table 1) and not WSC. Silages produced from three cuts had on average higher CP content and proportion of sCP than two cuts. T silages had on average higher content of CP but lower proportion of sCP than PRG, while T+RC silages had on average higher CP content than the T silages in the three cut system and on average 15% lower proportion of sCP than the grass silages. The reason for lower content of CP in PRG than in T is likely due to deluting effect of high DM production, while the general lower sCP proportion in T+RC was likely due to polyphenol oxidase activity in RC protecting the proportion from being protolyzed during the ensiling process (Lee et al., 2008). The protein value, expressed as the AAT_{20}-value, was higher in silages from three cuts per season than two. This effect is mainly explained by higher herbage CP content and OMD with three than two cuts. Higher silage protein value of T than PRG silages can also be explained by lower sCP in addition to higher CP content and OMD, while protein value of T+RC grass silages is mainly due to higher OMD and lower sCP.

Conclusions

Three cuts per season gave higher silage protein value than two cuts, and timoty and timothy red clover mixtures had similar protein value while timothy had higher protein value than perennial ryegrass.

Acknowledgements

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Preliminary study. Effect of cultivated Red Seaweed on ruminal methane production and degradation in vitro

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Introduction

Methane is an important greenhouse gas with a contribution to global warming only second to that of carbon dioxide. Atmospheric concentrations of methane have steadily increased over the past decades, partly as a result of massive increase in global ruminant production (IPCC, 2013). Ruminants produce methane in the process of enteric fermentation in which fibrous feed components are digested by microbes. Methanogens, a type of archaea that thrive in the rumen environment, produce methane by reducing CO2 with hydrogen (Patra et al., 2017). It is estimated that enteric methane contributes to 30% of global anthropogenic methane emissions. Moreover, methane has a high Global Warming Potential (GWP-100) of 28, meaning that methane is 28 times more potent than carbon dioxide in heating up the planet on a 100-year scale despite its atmospheric concentration being small. Red macroalgae Asparagopsis spp. has recently been researched extensively, and several published studies demonstrate that supplementation with Asparagopsis in ruminant diets is a promising methane mitigation strategy (Kinley et al., 2020, Roque et al., 2021). Remarkable reduction in enteric methane production, up to 98%, has been demonstrated in short- and-long term in vivo studies, when Asparagopsis has been included in the basal diet of ruminants at a concentration as low as 0.2% of feed dry matter (Kinley et al., 2020). Asparagopsis naturally synthesizes and stores bromoform, which is the main bioactive compound accounting for the methane-reducing effect (Machado et al., 2016). Bromoform reduces methane production by inhibiting the function of enzymes coenzyme M methyltransferase and methyl coenzyme M reductase in the methane production pathway of methanogenic archaea (Glasson et al., 2022). Several studies have been conducted using in vitro fermentation with rumen inoculum to determine effect of Asparagopsis on enteric methane production and diet degradation. One of them (Kinley et al., 2016) reported that at dose levels ≥2% of substrate organic matter, methane production was eliminated and at dose levels ≤5% of substrate organic matter, there was no negative impact on substrate digestibility. Total volatile fatty acids were not significantly affected with a 2% inclusion and acetate levels were reduced in favour of increased propionate and to some extent butyrate. Noteworthy is that throughout the years, better processing and cultivation conditions of this seaweed has resulted in reduced inclusion rates while still maintaining high or total methane reduction, both in vitro and in vivo. Land-based systems enable a high level of control over cultivation parameters which translates in continuous production with a high and sustained product quality that is difficult for ocean-based, traditional seaweed cultivation.

Materials and Methods

Volta Greentech produces Asparagopsis sp. in tank-based systems and in a controlled environment in the coastal city of Lysekil, Sweden. The seaweed is harvested and centrifuged to eliminate excess water. Thereafter Asparagopsis is freeze-dried and milled into a powder which makes the final product.

The preliminary experiment consisted of two *in vitro* runs with multiple replicates within run (different cultivation batches of Asparagopsis). Rumen content for inoculum was collected from three cows at different stages of lactation receiving a standard diet based on grass silage and concentrate (50:50) and filtered through two layers of cheese cloth to create inoculums. In the *in vitro* system, standard diet based on grass silage and concentrate (60:40) was used. Red seaweed was included at the level of 0.6 % of dry matter (DM). The positive control, bromoform, was included at six levels (0.01, 0.02, 0.025, 0.04, 0.05, and 0.1 mg/g DM diet). The *in vitro* incubations were carried out in 250 ml glass flasks in a water bath equipped with constant shaking at 39 °C. There were two *in vitro* runs with four flasks with the same treatment within each run. Two of the flasks were used for total gas production measurements using the Ankom modules. Remaining two flasks were used for total gas collection for measurements of methane concentrations and for diet degradation. In brief, to measure methane concentration, the Ankom module was replaced with a rubber plug with a silicone hose, and gas produced during incubation was collected into air-tight gas collection bags. The total gas was sampled at 6 and 24 h for methane concentration measurements. In the flask for total gas measurement, 0.5g of sample DM was incubated in 60 ml of buffer and 20 ml of inoculum for 24 h. To increase weight of the residue for degradability measurements after fermentation, 1g of DM samples with 120 ml of buffer and 40 ml of inoculum were incubated for 24 h in the flasks for methane measurements. To measure diet degradability, residues from flasks for methane measurements after 24 h were quantitatively transferred to small Ankom fibre analysing bags, dried, and analysed. Therefore, this study was conducted to evaluate effect of in-land cultivated red seaweed on ruminal methane production and degradation *in vitro*.

Results and Discussion

Data presented in this report are part of a larger *in vitro* preliminary study on the effect of red seaweed on rumen fermentation. The inclusion of red seaweed at 0.6 % of diet DM completely blocked methane production at 6 and 24 h. The positive control, bromoform (Figure 1), blocked methane production at 6 h at the dose of 0.04 mg/g diet and at 24 h reduced the methane production up to 98 %, compared to control. Inclusion of bromoform at 0.05 mg/g diet completely blocked the methane production. This preliminary data on cultivated Asparagopsis is similar to previously published *in vitro* and *in vivo* data on the effect of Asparagopsis on enteric methane production (Kinley et al., 2020, Kinley et al., 2016).

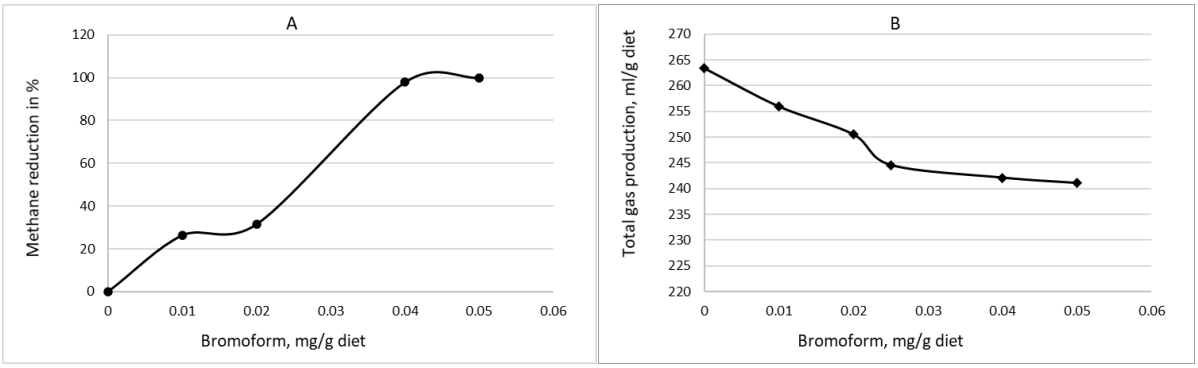

Figure 1 The effect of bromoform on: A) methane reduction and B) total gas production at 24 h.

Similarly, to Kinley et al. (2016) our preliminary data also do not show any negative effect of cultivated Asparagopsis supplementation on degradation of basal diet. Total gas production was reduced by on average 5 %, and hydrogen production increased from almost zero in control treatment to on average 12 mg/g DM diet.

Conclusions

The data indicate that inclusion of cultivated read seaweed at approximately 0.04 mg/g DM diet of active ingredient will completely block methane production with no negative effect on degradation. The in-land base cultivation system of Asparagopsis can produce high quality Asparagopsis with constant high level of active ingredient. This preliminary data indicates that supplementation of ruminant diets with in-land cultivated Asparagopsis is a promising methane mitigation strategy. Red seaweed meal (including Asparagopsis) is registered as a category 7 feed material ID 008818-EN on the EU Feed Materials Register. The practical usability of this feed as a methane mitigation method within EU would require an additional feed additive authorization by the European Commission.

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Supplementation of *Asparagopsis taxiformis* for mitigation of enteric methane emissions in dairy cows: Feed Intake and milk yield responses

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Introduction

Globally, livestock is estimated to contribute 30 % of total anthropogenic methane (CH_4) emissions (Gerber et al., 2013), thus having a significant impact on global warming. Several different mitigation strategies have been worldwide evaluated aiming to decrease enteric CH_4 emission by ruminants (McCauley et al., 2020). In this context, *Asparagopsis taxiformis* (AT) has shown high efficiency in CH_4 mitigation in ruminants (Stefenoni et al., 2021). However, despite of the aforementioned effects of AT supplementation on CH_4 production and rumen fermentation stoichiometry, its concomitant effects on productive performance and efficiency of dairy cows must be also taken into account, in order to assure that a higher sustainability level may achieved, yet keeping adequate aspects of productivity and profitability. Hence, our objective was to evaluate the effects of AT inclusion in the diet on voluntary intake, milk yield, CH_4 and hydrogen production in lactating Swedish Red dairy cows.

Materials and Methods

The study was performed at the Swedish University of Agricultural Sciences, Röbäcksdalen Livestock Research Center, Umeå, Sweden (63°45′N; 20°17′E) from January to April 2022. The study was designed according to the rules and guidelines proposed by the “Swedish University of Agricultural Sciences Animal Care and Use Committee" and the “National Animal Research Authority” (Dnr: 6-2021). In total, 9 primiparous and 21 multiparous Swedish Red dairy cows that were 61 ± 25.3 (mean ± std) days in milk (DIM) at the start of the experiment were enrolled in the trial. The cows were blocked according to parity and DIM and then randomly assigned to one of three treatment groups with different inclusion levels of AT in % of organic matter: a control group with no inclusion of AT (CON), a group with 0.15% AT (LOW) and a group with 0.3% AT (HIGH). The study included 2 weeks of adaptation to groups, system and diet followed by one week , week 0, as pre-treatment and then followed by 12 weeks where treatments were applied. The cows were fed a TMR mix based on feeds commonly used in Swedish dairy production, consisting of 50:50 silage and concentrate on DM basis. The cows were fed the TMR ad libitum, and the diets were mixed using a TMR mixer (Nolan, Viborg, Denmark) and delivered in feed troughs three times/d (05:00,13:00, and 18:00 h) by an automatic feeding wagon.
Cows were milked twice daily with start at 05:30 and 16:45 h in a 2 × 8 milking parlor. Milk yield was recorded throughout the trial with gravimetric milk recorders (SAC; S.A. Christensen and Co Ltd. Kolding, Denmark). Feed intake was recorded individually on a daily basis throughout the trial in roughage intake control feeders (Insentec B.V. Marknesse, The Netherlands). Methane emissions and hydrogen level through the breath were measured by the GreenFeed system (C-Lock Inc. Rapid City, SD USA) as described by Huhtanen et al. (2015). The equipment was programmed to allow each cow in the experiment to visit at minimum 5-h intervals. During each visit, the cows were given 8 drops of 50 g concentrate

every 40 s. Calibrations were performed once every two weeks with span gas (CO_2, CH_4 and O_2 mixture) and zero gas (N_2). CO_2 recovery tests were conducted once per month. Airflow rates and gas concentrations were also measured continuously. Data were analyzed by the MIXED procedure of SAS 9.3. The model included the fixed effect of treatments and the random effect of blocks. The values taken during the pre-treatment week were used as covariate. The measurements across the experiment weeks were considered as repeated measurements. The best structure for the (co)variance matrix was based on Akaike Information Criterion with correction. Denominator degrees of freedom were obtained by the Kenward-Roger method. When necessary, means were compared using the Tukey-Kramer test. Significances were declared at $P < 0.05$.

Results and Discussion

There was no interaction ($P \geq 0.05$) between treatments and lactation weeks for dry matter intake and milk yield. On average, voluntary intake was lower ($P<0.05$) in HIGH treatment compared to LOW and CON, which did not differ from each other ($P>0.05$; Table 1). No difference was observed for milk yield. On the other hand CH_4 production exhibited an interaction ($P<0.05$) between treatments and lactation week. The average values across weeks indicated that a decrease in CH_4 production ($P<0.05$) was only observed when the HIGH treatment was applied to the cows. The interaction effect on CH_4 production was caused by a highly prominent difference during the first weeks of the experiment (Figure 1). However, that difference became gradually lower up to the sixth week. From that moment, the inhibition in CH_4 production observed in the HIGH treatment tended to be lower and with a stable pattern. However, for the last week the inhibition was very low. If this is related to an adaptation of rumen over time or an effect of reduced algae quality due to storage time, as also seen by Stefenoni et al. (2021), we aim to further investigate. The decreased CH_4 production is a direct effect of the halogenated CH_4 analogue components present in AT, which decrease the intensity of the Wolfe-cycle performed by ruminal archaea (Glasson et al, 2022). From this, a lower amount of CO2 is reduced to CH_4 in the rumen, which also influences the stoichiometric partitioning of the hydrogen that originated from volatile fatty acid production. In fact, the CH_4 mitigation caused by the AT was reinforced by a concomitant increase in H_2 production (Figure 1). However, the absence of effect on CH_4 in the treatment LOW makes evident that the effect of AT as a CH_4-mitigating additive is dose dependent, which deserves further investigations.

Table 1 Effect of different levels of supplementation of *Asparagopsis taxiformis* (AT; CON 0%, LOW 0.15% and HIGH 0.3% of organic matter) on dry matter intake (DMI), milk production parameters and ruminal production of methane (CH_4)

Items per cow and day	Treatment			SEM	P-value	
	CON	LOW	HIGH		Treatment* week	Treatment
Total DMI, kg/day	24.9[a]	24.7[a]	23.0[b]	0.41	0.573	<0.001
Milk yield, kg/day	37.2	37.6	36.1	0.52	0.054	0.126
CH_4, g/day	433 [a]	401[a]	281[b]	17.5	<0.0001	<0.0001

SEM = standard error of the mean; different letter superscripts denote significant differences ($P<0.05$) between treatment groups.

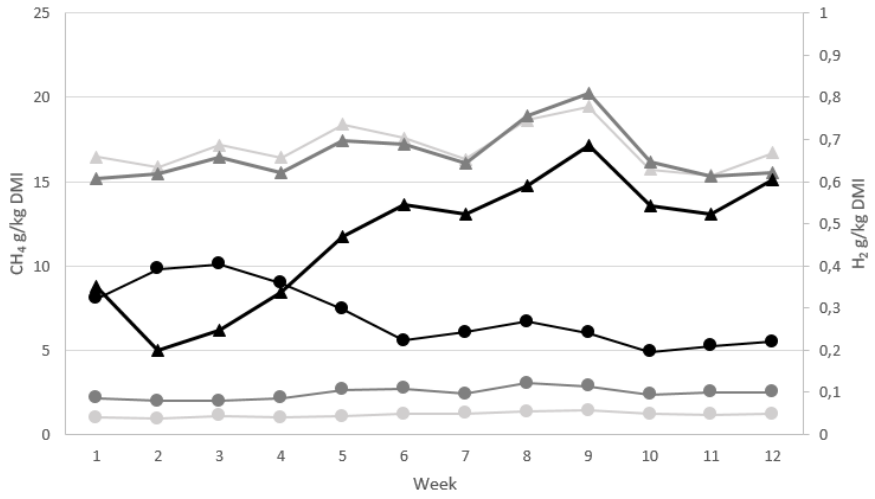

Figure 1 Methane (CH_4), and hydrogen (H_2) production in grams per kilo dry matter intake (DMI) within the three different groups supplemented with *Asparagopsis taxiformis* (AT; CON 0%, LOW 0.15% and HIGH 0.3% of organic matter). The average values from 12 weeks are presented as least squares means per group and week. Methane is represented by triangles according to values on the y-axis to the left ,while hydrogen is represented by circles according to values on the y-axis to the right. The treatments are represented by different colors: CON (light grey), LOW (grey) and HIGH (black).

Conclusions

The inclusion of *Asparagopsis taxiformis* at 0.30% of the diet (organic matter basis) is able to mitigate enteric CH_4 emissions in lactating dairy cows. However, a concomitant negative effect on voluntary intake is also observed. Further investigations on *Asparagopsis taxiformis* as a CH_4-mitigating additive are necessary, as its effects are dose dependent and do not remain constant after start being fed to the animals.

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Does feeding *Asparagopsis taxiformis* to dairy cows reduce methane emission from their faeces?

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Introduction

The dairy sector is one of the most contributors to methane (CH_4) emissions from livestock, even though science and companies have been making great efforts to reduce the greenhouse gas (GHG) impacts from ruminants over the decades. Most CH_4 produced by a dairy cow is from enteric fermentation, but part of the emissions are originated from cows' faeces decomposition. The manure stored in the cool-temperate European climate is estimated to be approximately 12% of total CH_4 emissions from the dairy system (Hindrichsen *et al*., 2015). Many factors can influence the extent of CH_4 emission from the faeces, such as volatile solid content and the diet composition fed to the cows. For instance, incubated faeces from cows fed with or without rapeseed oil produced 3.45 vs. 3.85 L of CH_4 per kg of dry matter of incubated material, respectively (Ramin *et al.,* 2021). Only a few studies in the literature present strategies to decrease GHG from manure. On the other hand, several strategies have been reported to decrease enteric CH_4 emissions. Recently, a red macro algae native to tropical areas, *Asparagopsis taxiformis* (AT)*,* was identified as a natural enteric CH_4 mitigator. The most exploited halogenated compound found in AT is the bromoform and it mitigates CH_4 emission in the rumen by impairing the final step in methanogenesis (Duin *et al.,* 2016).

We hypothesized that faeces from cows supplemented with AT would produce less CH_4 than cows not supplemented with AT and that the addition of AT to incubated cows' faeces might impair methanogenesis during faeces decomposition, decreasing even further CH_4 emmision. Thus, we aimed to estimate the CH_4 emissions from stored cows' faeces by adding or not adding AT to the faeces of dairy cows previously supplemented with or without AT in their diets.

Materials and Methods

We collected faecal samples in connection to a feeding trial conducted at Röbäcksdalen experimental farm (63°45′ N, 20°17′ E) that aimed to evaluate the effect of supplementing AT to a grass-based forage diet fed to dairy cows on enteric CH_4 emission. The samples were provided from animals registered and cared for according to guidelines approved by the Swedish University of Agricultural Sciences Animal Care and Use Committee and the National Animal Research Authority (Number Dnr. A17/2016 + A33/16). The pooled faecal samples were collected at 2 different periods from 2 different groups of cows supplemented with or without AT in their diets at a level of 0.5% of the organic matter (OM) intake. A 2 × 2 factorial design was set in the laboratory in which cows that were supplemented with AT (2 cows) were further added AT in their faeces (0.5% OM of the incubated material). The collected faecal samples from the other 2 cows that were not supplemented with AT in their diet were added with AT too. This made a total of 4 treatments with 2 replicates each. The same procedure was repeated at period 2 with different cows. Four hundred grams of pooled fresh faecal samples were incubated in 1-litre serum bottles for 9 weeks at 39°C water bath. Methane and total gas production were measured at day 1, 4, 7 and then every second week

until the end of the incubation. Bottles were gently shaken 3 times per week. Cumulative CH_4 emissions were then computed. Methane emissions at each time point were calculated as:

Methane (mL) = $HSCH_4 \times 600$ + Bag $CH_4 \times$ Total gas produced (mL),
Where, $HSCH_4$ is the headspace CH_4 concentration; Bag CH_4 is the CH_4 concentration in the sampling bag; and 600 is the headspace volume in the serum bottles. The concentration of CH_4 in gas samples was determined by injecting 0.2 mL of gas withdrawn from the headspace/sampling bag into a gas chromatograph using a gas tight syringe,
We used the following model using the statistical package of SAS (9.4):
$Y_{ijk} = \mu + F_i + A_j + (FA)_{ij} + \varepsilon_{ijk}$,
where μ = the overall mean, Fi = the effect of level i of factor F fed AT to diet or not, Aj = the effect of level j of factor A, Added AT to faeces or not, (FA)ij = the effect of the interaction of F with A and εijk = random error.
Subsamples of 15g from faeces of cows fed with AT and not fed were collected from the sampling described above, frozen at -80°C and sent for microbial analylsis.
The extracted community DNA from the faeces was subjected to 16S based metabarcoding sequencing and the raw sequencing reads were processed using default QIIME2 pipeline (Boylen *et al.,* 2019). The observed OTUs were then annotated against SSUrRNA database of SILVA (version 138.1) and the final image was rendered in MEGAN-LR (Huson *et al*., 2018).

Results and Discussion

The results indicated that CH_4 emission was numerically ($P = 0.61$) lower in dairy cows faeces that were supplemented with AT in their diet (Table 1). Adding AT to the faeces of dairy cows significantly reduced ($P < 0.01$) CH_4 emission in the faeces by almost 50% compared to the cows faeces that was not added with AT in the faeces (2.83 vs. 5.03 L/kg DM, Table 1).

Table 1 Methane (CH_4) emission from stored faeces of cows fed with and without *Asparagopsis taxiformis* (AT) and added or not added AT to faeces

Item	Feeding of AT (F)		Adding AT to faeces (A)		SEM	*P values*		
	With	Without	Added	Not added		F	A	F * A
CH_4, L/kg DM	3.73	4.13	2.83	5.03	0.53	0.61	0.01	0.50
Total gas, L/Kg DM	20.2	20.2	16.3	24.1	2.36	0.98	0.04	0.86
CH_4/total gas	0.18	0.22	0.18	0.21	0.017	0.23	0.40	0.95

The most reported CH_4 active compound in AT is bromoform, and it acts by inhibiting the enzyme methyl-coenzyme M reductase (MCR) which catalyzes the final step in methanogenesis in rumen archaea (Duin *et al.,* 2016). The AT supplementation in dairy cow diets has been shown in literature as an enteric CH_4 mitigator (Stefenoni *et al*., 2021). Our results showed that the inhibitory effect when using AT in their diet was milder on cows faeces CH_4 emission when compared to enteric CH_4 emission reported by Stefenoni *et al*. (2021). No significant variation was observed in the bacterial and archaeal community profiles of faeces samples from cows fed with AT and non-AT diets. However, *Firmicutes* and *Bacteroidetes* were the most dominant bacterial phyla, and *Euryarchaeota* was the most dominant archaeal phyla observed in both AT and non-AT fed samples (Figure 1).

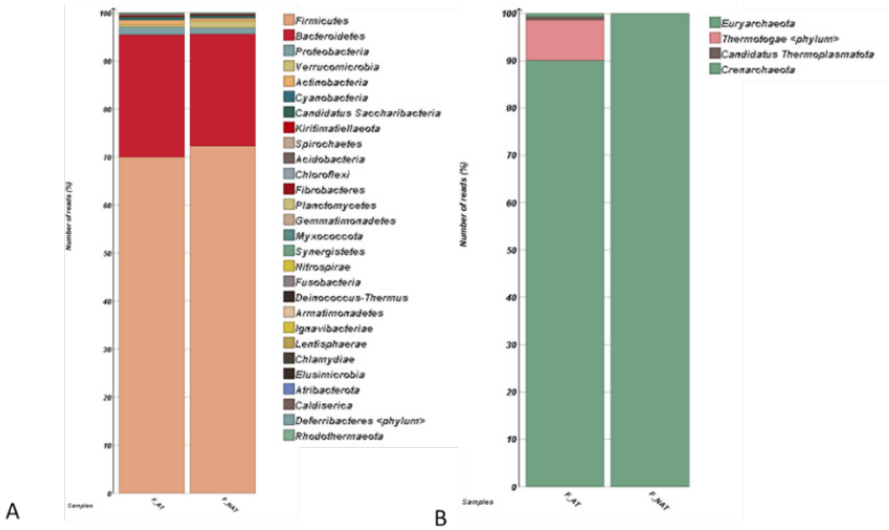

Figure 1 Bar-chart represents percent distribution of A) bacterial B) Archaeal communities at phylum level in AT (n=4) and NAT (n=4) samples. AT: faeces from cows supplemented with *Asparagopsis taxiformis* in their diet; NAT: faeces from cows not supplemented with *Asparagopsis taxiformis* in their diet.

Our results revealed that faeces from cows supplemented with AT in their diet did not emit less CH_4 compared to faeces of cows not supplemented with AT in their diet (Table 1). Nonetheless, more studies should be conducted to elucidate the interactions between ATs halogenated compounds and faeces microbiome.

Conclusions

The current study could not detect any differences in CH_4 emission from cows faeces previously summplemeted with AT in their diet compared to not supplemented with AT in their diet. However, adding AT directly to the feceas decresed CH_4 emisison by about 50%. The changes in the distribution of bacterial and archaeal community was not obvious between the cows faeces fed or not fed with AT in their diet.

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Effects on rumen microbiome and milk quality of dairy cows supplemented the macroalgae *Asparagopsis taxiformis* in a grass silage-based diet

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Introduction

The globally increasing cattle population contribute to the total antrophogenic greenhouse gas emissions. Methane (CH_4) emission from cattle is part of a cycle, but contributes to global heating because of the much greater heating effect of CH_4 compared to carbon dioxide. However, when CH_4 composes to carbon dioxide in the atmosphere it suggestively does not contribute to further increase because it will be bound to vegetation in near future. This implies that reduced CH_4 emissions will lead to a drop in average global temperature (Sterner and Johansson, 2017). The red seaweed *Asparagopsis taxiformis* (AT) has been shown to be a strong natural inhibitor of ruminal CH_4 formation (Machado et al., 2016). Stefenoni et al. (2021) decreased CH_4 emissions in lactating dairy cows by 80% by inclusion of AT at 0.5% of DM intake. Important factors to consider when using algea supplemented feed is the rumen microbiome and the milk quality, which were not assessed in the previous studies. The objective of this study was thus to assess these parameters combined with level of CH_4 reduction and feed digestibility after the addition of ATto diets of dairy cows.

Material and methods

Six Nordic Red cows at 122 ± 13.7 (mean ± SD) days in milk, parity 2.7 ± 0.52 and producing 36 ± 2.5 kg milk/d at the start of the trial were blocked by milk yield, and assigned to an extra period Latin square change-over design (Lucas, 1957) comprising two dietary treatments. The dietary treatments were either a diet consisting of grass silage and a commercial concentrate mixture (50:50) not supplemented or supplemented with 0.5% of *Asparagopsis taxiformis* (AT) on OM intake basis. Recordings of CH_4 and hydrogen production with the GreenFeed system (C-Lock Inc., Rapid City, SD, USA), feed intake and milk yield were made the last week of every 3 week period. Milk samples were collected and subjected to sensory, composition and milk fatty acid analysis. Diet digestibility was measured by faecal spot sampling with indigestible NDF as an internal marker. Rumen fluid samples were collected for microbiome and volatile fatty acid composition analysis. Milk samples, rumen fluid and faecal samples were also analyzed for bromoform content, and milk samples additionally iodine and bromine content.

Bacterial library preparation and sequencing was performed by Novogene with total DNA extracted using TIAN amp Stool DNA Kit. For archaeal library, DNA was extracted with the FastDNA™ Spin kit for soil (MP Biomedicals, Irvine, USA) according to Singh (2020). Archaeal amplicon sequencing was performed by the SNP&SEQ Platform. Illumina MiSeq sequencing with v3 chemistry and paired end reads (2x 300 bp) was used for both bacterial and archaeal amplicon libraries.

Experimental data were subjected to analysis of variance using the GLM procedure in SAS (SAS Inc. 2002-2003, Release 9.4 SAS Inst. Inc., Cary, NC, USA) by applying a model correcting for effect of period, cow within square and experimental dietary treatment. Potential carry-over effects on all traits were evaluated by including a residual effect in the model described above. There were no significant ($P \geq 0.22$) residual effects for any traits evaluated, and the effect was not included in the final analysis.

Results and Discussion

AT supplementation decreased CH_4 production on average by 60%, dry matter intake by 2.8 kg/d, and ECM by 1.9 kg/d compared to cows fed the non-supplemented control diet (Table 1). The decrease in ECM was caused by lower milk fat yield for cows fed AT, and was in agreement with the shift in rumen fermentation from acetate to propionate. Except for the observed effect on milk fat yield, these results are in agreement with a previous study investigating an AT supplemented diet fed to dairy cows (Stefenoni et al. 2021). Moreover, also in line with Stefenoni et al. (2021), the present study observed an increase in milk iodine and bromine, and no change in bromoform in milk. Sensory test results did not reveal any quality changes and there were only minor changes in milk fatty acid composition when diets were supplemented with AT (results not presented).

Table 1 Effect of supplementation of *Asparagopsis taxiformis* (AT) compared to a grass-silage based diet (Control) on ruminal production of methane (CH_4) and hydrogen (H_2), dry matter intake (DMI), energy corrected milk (ECM), milk quality parameters and digestion

Item[1]	Control	AT	SEM	P-value
CH_4, g/d	383	154	30.2	<0.01
CH_4, g/kg DMI	19.0	9.2	1.47	<0.01
CH_4, g/kg ECM	11.6	5.1	0.79	<0.01
H_2, g/d	0.9	5.0	0.44	<0.01
DMI, kg/d	20.3	17.5	0.42	<0.01
Organic matter digestibility, g/kg	764	760	4.0	0.51
Neutral detergent fibre digestibility, g/kg	656	646	4.8	0.17
ECM, kg/d	33.3	31.4	0.58	0.05
Milk fat, g/d	1399	1280	32.8	0.03
Milk bromine, mg/L	5.1	43.2	2.70	<0.01
Milk iodine, µg/L	139	2105	146.2	<0.01
Milk bromoform, µg/kg	4.09	4.92	0.712	0.44
Rumen acetate, mmol/mol	62.9	55.2	0.83	<0.01
Rumen propionate, mmol/mol	20.4	25.9	0.68	<0.01
Rumen butyrate, mmol/mol	12.0	14.2	0.28	<0.01

The archaeal community was mainly represented by two different phyla (Figure 1a). The relative abundance of Methanobacteriota was lower (P=0.05) in diets with AT than in Control (29.9 vs. 58,8 %, respectively). Methanobrevibacter is dominating in Methanobacteriota and has been linked to ruminal production of CH_4. The relative abundance of Thermoplasmatota was numerically higher (P=0.08) in diets with AT than in Control (66.2 vs. 40.3%, respectively). Themoplasmatota was mainly represented by a genus belonging to the family

Methanomethylophilaceae. Not much is known about this archaeal group but members within this family use methyl groups, instead of H_2, for CH_4 production and these methylotrophs have been associated with reduced CH_4 emissions from the rumen (Poulsen et al., 2013). The bacterial population was repesented by 44 different phyla, with 17 phyla found across all samples (Figure 1b).

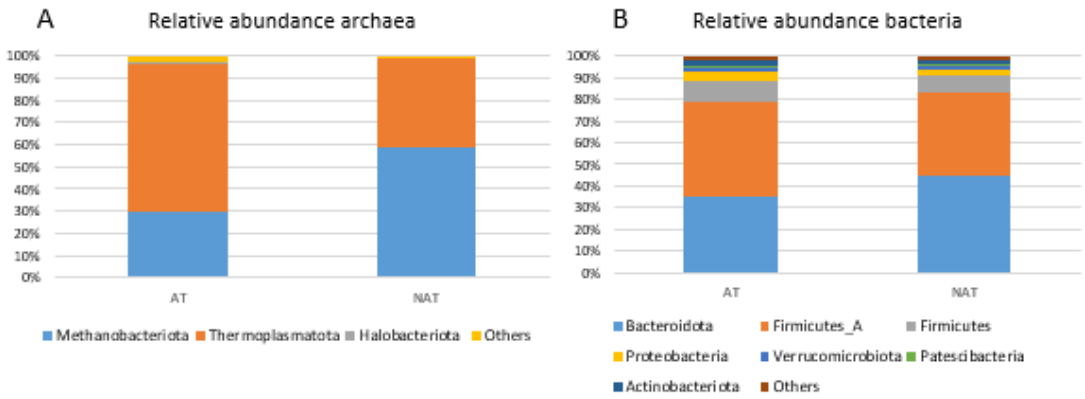

Figure 1. Relative abundance of all sequences at phyla level for archaea (A) and bacteria (B) in cows fed diets with inclusion of *Asparogopsis taxiformis* (AT) or without (Control).

Conclusions

Milk energy output was decreased by 5.7% and CH_4 production by 60 % when cows were fed AT. The AT treatment had a tendency to effect the archaeal population with a decrease in relative abundance of Methanobacteriota. The most prominent change in milk quality were the increases in bromine and iodine when the diets were supplemented with AT.

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Metagenomics analysis suggests disparity in rumen microbiome profiles of dairy cows fed with forest by-products

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Introduction

In the event of unavailability of traditional forages, alternative fibrous feed such as forest by-products, in particular aspen tree residues with substantial amounts of carbohydrates, can serve as potential feed substitutes for ruminants (Fritschel et al., 1976; Seymour and Kamstra, 1979). To utilize plant biomass, ruminants have evolved an exceedingly complex and remarkable organ, "rumen," which harbors a diverse and unique microbial population capable of utilizing such products (Yeoman & White, 2014). Among the key microbial inhabitants of the rumen, bacteria constitute majority of the rumen microbial population and perform a vital role in the breakdown of recalcitrant plant polysaccharides and fermentation (Pope et al., 2010; Pitta et al., 2014). Previous studies to evaluate the microbial composition of rumen microbiota have relied primarily on culture-dependent methods and studies using a culture-independent approach to represent variation in microbial community composition are limited (Hungate, 1969). Some studies have highlighted the importance of members within the family *Prevotellaceae* and *Paraprevotellaceae* in adaptations to changes in diet using a culture-dependent approach (Henderson et al., 2015). Since only a handful of studies have applied metagenomics to delineate the microbial diversity and functional aspects in rumen nutrition studies, our study aims to discern the effects on rumen microbiota and its associated function when aspen by-products are included in dairy cows' regular diet using DNA and RNA-based sequencing.

Materials and Methods

Three rumen cannulated, lactating dairy cows of the Swedish red breed were used in a 3×3 Latin square design with three 21 days periods. The experiment was conducted at the Swedish livestock research center, Lövsta, Swedish university of agricultural research, Uppsala, Sweden, with prior ethical approval from Uppsala ethics committee (no: 5.8.18-18643/2020003488). The cows were consuming either 12.2 kg dry matter (DM) grass silage (control diet), 6.9 kg DM grass silage with 3.6 kg DM aspen wood chips (wood diet), or 6.9 kg DM grass silage with 3.6 kg DM aspen bark (bark diet). All diets were supplemented with (as fed) 7 kg grain based concentrate and 3.5 kg protein concentrate (Komplett Xtra 205 and Konkret Mega 28, respectively, Lantmännen, Stockholm).

Rumen fractions were collected after two weeks of dietary adaptation, i.e. on day 14 and day 21 in each period, at 13:00 hrs. Rumen fraction collection and storage until further processing was as per recommendations of Gruninger et al. (2018). DNA and RNA were extracted using the QIAamp DNA stool mini kit (Qiagen, USA) and RNeasy PowerMicrobiome kit (Qiagen, USA), respectively, as per the manufacturer's instruction. Sequencing of DNA (meta-barcoding) and RNA (meta-transcriptomics) was performed using Illumina (Hi-Seq and NovaSeq) platforms. The raw reads from meta-barcoding were processed using the QIIME pipeline (Caporaso et al., 2010) and visualized with R software (version 2.15.3), while meta-transcriptomics reads were processed using an online Galaxy server (Afgan et al., 2018).

Results and Discussion

In total, 24 rumen samples were processed including 6 pre and post control samples, resulting in on average ~2250 operational taxonomic units (OTUs) bacterial targeted meta-barcoding sequencing. From meta-transcriptomics sequencing, on average, ~17 Gb of raw data was generated per sample. The overall rumen bacterial population showed a high abundance of *Bacteroidota* (45.2%)*, Firmicutes* (29.5%)*, Proteobacteria* (11.4%)*, Fibrobacterota* (5.4%), and *Spirochaetota* (3.7%) in all samples. While at the genus level, *Prevotella* (~27%) was most dominant, followed by *Fibrobacter* (~5.3%). Interestingly, phylum *Bacteroidota* increased from ~41 to ~45 % (average mean abundance) during period 1 to period 3, while a decline in *Firmicutes* was observed from ~53% to ~37% and ~57% to 40 %, respectively, in wood and bark diets fed samples from period 1 to 3 (Figure 1). Since members of *Bacteroidota* are known degraders of polysaccharides and plant biomass, most of this activity is achieved by carbohydrate-active enzymes (CAZyme), which explains the relatively higher abundance of *Bacteroidota* in wood and bark diet samples (McKee et al., 2021).

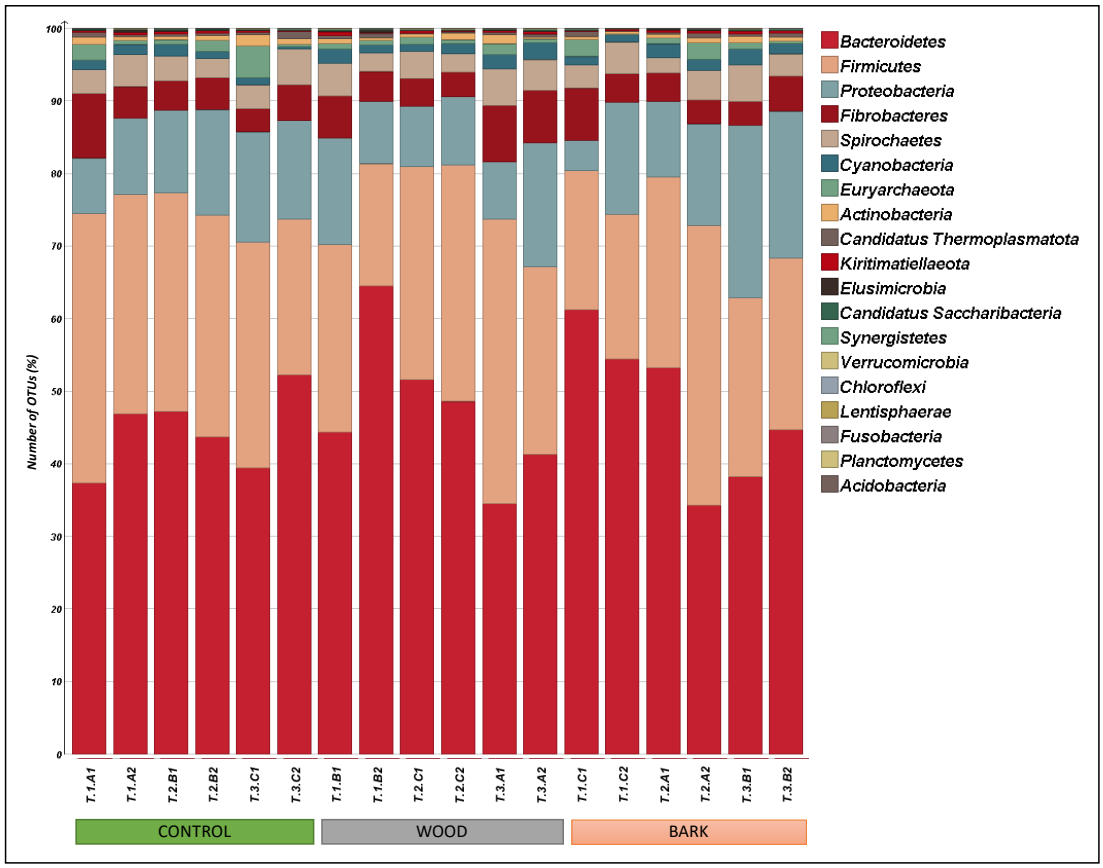

Figure 1 Bar chart representing the relative abundance of phyla in rumen samples from lactating cows fed regular diet, aspen wood, and aspen bark during three 21 d periods (T1 – T3). Samples are from cows A – C with samples A1 – C1 taken during day 14 in each period and samples A2 – C2 taken day 21. Legends represent phylum abundance in decreasing order.

The gene expression levels among samples were measured based on fragments per kilobase of transcript per million mapped reads (FPKM). Overall, greater gene expression values were observed in wood (T_BCA) and bark (T_CAB) diet groups compared to the control (T_ABC) group. Compared to control samples, a noticeable representation of hydrolase

activity and Gene Ontology (GO) terms was observed in wood and bark diet groups. We speculate that the complex structure of lignocellulosic biomass may also favor the enrichment of microbial communities capable of producing hydrolyzing enzymes to facilitate greater utilization of complex forest feed products.

Conclusions

The ability of ruminants to utilize fibrous feed can be utilized to explore feed alternatives or roughage extenders in the form of forest by-products. A comparative multi-omics analysis highlighted dominant microbial communities in the rumen and their related functions when a regular diet is mixed with aspen by-products. Primary data suggests that phylum Bacteroidota and Firmicutes primarily constitute rumen bacterial community and the relative abundance of *Bacteroidota* was higher in wood and bark diets. At the lower taxonomic level of the genus, *Prevotella* is most dominant and plays an important role in the degradation of fibrous feed using different enzymatic systems, particularly esterases and hydrolases.

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Aspen wood or aspen bark as substitution for grass silage in dairy cow diets

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Introduction

The 2018 forage shortage spurred interest for alternative forage sources and their possibilities to supply energy and structure to ruminant diets. Aspen wood and bark from the Swedish matchstick industry is among those sources. Aspen has in previous research been among the most digestible wood materials (Mellenberger et al., 1971) and it has recently been consumed by dairy cows (4.5 kg DM /cow/d) when partially substituting grass silage (Prestløkken & Harstad, 2019). During 2020, a trial was performed at the Swedish University of Agricultural Sciences with three lactating cows where aspen wood or aspen bark partially replaced grass silage. The main purpose was to study changes in rumen microbiota composition and function, whereas data on production, intake and digestibility from the experiment are presented here.

Materials and Methods

The experiment was performed as a 3×3 Latin square with three ruminally cannulated lactating Swedish Red cows (804 ± 72 kg of BW; 156 ± 15 DIM; 34.5 ± 1.1 kg milk/d at experimental onset), three diets and three 21-d periods, where measurements were carried out the last eight days. A 21-d preparation period when cows were introduced to the novel feeds preceeded the experiment. The cows were kept in a separate section of a loose house barn with a common water bowl, a common concentrate feeding station and two forage feeding troughs per animal (BioControl, Rakkestad, Norway). Cows were manually moved to an adjacent AMS for milking at 06:00 and 18:00 h. The Control diet (Table 1) contained grass silage, a basal concentrate and a protein supplement (Komplett Xtra 205 and Konkret Mega 28, respectively, both from Lantmännen, Stockholm). For the diets Wood and Bark, silage was partially replaced by aspen wood and aspen bark, respectively. Silage, wood and bark were supplied in individual forage troughs. Concentrates were supplied in a concentrate station and in the AMS, except for 4.3 kg DM basal concentrate that was mixed with the wood/bark allowance at feeding (1:1 on a DM basis). The same proportion was assumed for orts from the mixture. Aspen material was from Swedish Match, Vetlanda and consisted of bark from rotor debarking and wood chips from the log cores. The aspen products were stored frozen until milling on a hammermill to pass a 8-mm screen shortly before feeding. Weighing of orts and provision of new feeds were performed daily at 09:00 h. All feeds were accessible until daily allowance (silage and concentrates only) was reached.

Sampling, analyses and calculations followed routine procedures described by Eriksson & Rustas (2014). Cows were test milked on Day 17-18; on Day 17-21 feeds were sampled, feces were spot sampled for digestibility assessment with acid insoluble ash and ruminal liquid was sampled during the interval 05:00 – 19:00 . Total rumen evacuations were performed at 13:00 h on Day 14 and 21. Data were analysed by Proc Mixed of SAS 9.4 with diet and period as fixed variables and cow as random variable. Results are presented as least square means with standard error of difference and probability for diet effect. Because of few observations, most of the response variables differed only numerically between treatments

(P>0.05) and the results should be regarded as descriptive for this experiment rather than as generally applicable research findings.

Results and Discussion

Table 1 Daily feed allowance and composition of feeds

	Grass silage	Aspen wood	Aspen bark	Basal concentrate	Protein supplement
Control diet, kg dry matter (DM) allowed	12.29			6.08	3.05
Wood diet, kg DM allowed	6.76	4.58		6.08	3.05
Bark diet, kg DM allowed	6.76		4.13	6.08	3.05
Composition of feeds					
Dry matter, g/kg	307 ± 2.6	571 ± 9.8	516 ± 3.9	869 ± 3.4	873 ± 7.1
Ash	104 ± 1.5	6.3 ± 1.9	26.2 ± 3.2	70 ± 2.3	88 ± 3.6
Crude protein (CP), g/kg DM	164 ± 0.1	9.3 ± 4.4	20 ± 2.3	205 ± 1.6	265 ± 1.6
Neutral detergent fiber (NDF), g/kg DM	605 ± 48	880 ± 17	716 ± 9.8	199 ± 12	233 ± 19
In vitro OM digestibility 96 h, %	81.2 ± 0.2	16.5 ± 5.1	20.8 ± 2.8	-	-

Table 2 Intake, production, digestibility and rumen parameters in dairy cows offered aspen wood or aspen bark as a partial replacement for grass silage in a Latin square (n = 3)

	Control	Wood	Bark	SED	P
Silage dry matter intake (DMI), kg/d	12.18	6.88	6.88	-	-
Wood/Bark DMI, kg/d	-	3.57	3.60	0.045	0.55
Concentrate DMI, kg/d	9.10	8.16	8.56	0.36	0.22
Total DMI, kg/d	21.29	18.61	19.04	0.81	0.14
Total organic matter intake, kg/d	19.33	17.25	17.58	0.77	0.19
Total NDF intake, kg/d	9.30	9.02	8.53	0.27	0.19
Total CP intake, kg/d	4.05	3.04	3.14	0.11	0.02
Milk yield, kg/d	29.42	24.92	26.23	1.84	0.24
Energy corrected milk yield, kg/d	29.61	25.63	26.55	1.98	0.31
Rumen fresh weight, kg	84.60	84.70	77.80	4.30	0.37
Rumen DM, kg	12.17	13.92	12.43	0.45	0.10
Rumen OM, kg	11.11	12.65	11.29	0.56	0.18
Rumen NDF, kg	7.11	8.08	7.13	0.26	0.10
Rumen average pH (0500 -1900 hrs)	6.24	6.31	6.28	0.06	0.61
Rumen DM concentration, %	14.44	16.55	16.15	0.37	0.05
DM digestibility	0.67	0.65	0.63	0.02	0.37
OM digestibility	0.68	0.66	0.64	0.02	0.37
NDF digestibility	0.64	0.60	0.56	0.03	0.24
CP digestibility	0.70	0.71	0.69	0.009	0.35
Fecal DM concentration, %	15.1	15.5	17.0	0.26	0.03

SED= Standard error of difference

The whole silage and concentrate allowances were consumed when wood or bark was not mixed in (Table 2). For the Wood and Bark diets, 2.0 and 1.1 DM, respectively, of the aspen/concentrate mix was not eaten. This resulted in equal DM intake of wood and bark, because they were supplied on an as fed basis with moderately different DM concentration. Dietary crude protein concentration was 163 – 165 g/kg DM with Wood/Bark diets, compared to 190 g/kg DM with the control. The daily ME intake with the control diet (249 MJ) corresponded to feeding standards for the pre-experimental yield of approx. 35 kg ECM (Spörndly, 2003). The silage and concentrate intake for Wood and Bark diets was sufficient for 23 and 24 kg ECM, respectively, not including possible energy contribution from consumed wood/bark. The actual ECM yields recorded, although compromised by large variation and possible mobilization/deposition effects, then corresponds to an oversupply of ME for the control diet and to a contribution of about 3.3 MJ ME/kg DM from the wood/bark eaten. Applying the ME equation for straw (Spörndly, 2003) to the 96 h *in vitro* digestibilities (Table 1) resulted in 2.3 and 2.8 MJ ME/kg DM for wood and bark, respectively. However, the *in vitro* digestibility for these wood/bark samples was relatively low compared to literature reports (Mellenberger et al., 1971; Baker et al., 1975).

Rumen average pH was similar among diets, although the control diet had a larger diurnal range (5.9 – 6.5) compared to wood and bark diets (6.1 – 6.5). Rumen pools of DM and NDF tended to be largest with the wood diet, as well as DM concentration of rumen contents. Acid insoluble ash (AIA) based digestibility measures only differed numerically but consistently declined in the order control-wood-bark. Together with rumen pools and the larger fecal DM concentration with bark, this suggests less retention time for the bark than for the wood.

Conclusions

Aspen wood and aspen bark were both accepted by lactating cows achieving intakes of 3.6 kg DM/cow/d in the experiment. Data from rumen evacuations and digestibility measurements suggests lower *in vivo* digestibility for bark than wood because of shorter rumen retention time of bark in the experiment.

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Effects of expander and extruder processed concentrate pellets with specific physical functional properties on *in sacco* rumen degradation

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Introduction

Ruminants are fed concentrate feedstuffs to meet their nutrient demands for milk or meat production. To ease handling and reduce segregation, concentrate feeds usually are pelletized. Most commonly, conventional steam pelleting is used. Expander pelleting is an alternative where the meal is processed under additional temperature and pressure before pelleting. The third alternative, extruder cooking, is rarely used in ruminant feed processing but predominate in fish feed industry to obtain pellets with specific physical functional properties like water stability and sinking velocities (Sørensen, 2012; Welker et al., 2018). In ruminants, rumen fluid stability and specific density of pellets influence rumen degradation by altering the rate of degradation and rate of passage simultaneously (Khan, 2021). In this regard, feed pellets with optimal density may escape rumen and thus provide more starch and protein for small intestine digestion. To maintain density, fluid stability of pellets in the rumen is essential which also affects rate of degradation. A pellet with high fluid stability will have slow rate of degradation and vice versa. Conventionally produced pellets typically have high density but low water stability (Larsen and Raun, 2018). Producing pellets with expander or extruder processing are alternatives, but scarcely studied with respect to physical functional properties in ruminants. Rumen degradation is usually determined *in sacco* as described by Åkerlind et al. (2011). In this technique, pellets are ground, probably altering their physical properties in the rumen. The objective of this experiment was to evaluate the effect of processing and grinding on *in sacco* rumen degradation of feed pellets varying in physical properties.

Materials and Methods

Feed processing was carried out at Center for Feed Technology (FôrTek) at NMBU. The feed was composed of 70% barley and 30% solvent-extracted soybean meal (SBM). Barley and SBM were ground using a hammer mill (E-22115 TF, Muench-Wuppertal, Germany) and a 2- mm screen. After mixing (Forberg AS, Larvik, Norway), the feed was subjected to three treatments. One treatment (high-density expanded; HDexp) was processed using an expander (Kahl OE 23, Reinbek, Germany; 110 °C) prior to pelleting. Two other treatments were extruded (Twin Screw BCTG 62 Extruder, Bühler, Uzwil, Switzerland; 5 sections; 3-mm die) using two distinct processing settings. The low-density treatment (LDext) was produced using 275 rpm screw speed and injection of steam, whereas the high-density treatment (HDext) was produced using 210 rpm screw speed and cooling at the last section of the extruder barrel.

Physical properties (Table 1) analyzed were pellet durability index (PDI), bulk density (BD), specific density (SD), sinking velocity (SV), and fluid stability index (FSI). Holmen tester (NHP 200, TekPro Ltd., UK) was used to determine PDI. Bulk density was determined by measuring pellets' weight in a 1 L steel cylinder. The SD, SV, and FSI were analyzed as described by Khan (2021). In short, SD was determined by measuring weight of 5 pellets and then pellets' volume by a volumetric displacement method using 0.5-mm glass beads in a

tapped density analyzer (AUTOTAP, Quantachrome Instruments, Florida, USA). The SV was determined by measuring the time taken by a pellet to pass 220 mm distance in a transparent glass cylinder (310 mm high and 35 mm inner diameter) filled with rumen fluid at 39°C. The FSI of pellets was determined by measuring the dry matter (DM) remained in ball-shaped baskets (2-mm mesh) after incubation for 90 min in rumen fluid at 39°C.

Table 1 Physical properties of extruder and expander treated[1] concentrates to ruminants

Item [2]	LDext	HDext	HDexp
PDI (%)	95.8	99.0	92.0
BD (g/L)	412	625	650
SV (mm/sec)	0.00	102	154
SDdp (g/mL)	0.70	1.03	1.07
SDwp (g/mL)	0.93	1.14	-
FSI (g/kg DM)	725	846	131

[1] LDext= Low-density extruded; HDext= High-density extruded; HDexp= High-density expanded pellets.
[2] PDI= Pellet durability index; BD= Bulk density; SV= Sinking velocity; SDdp= Specific density in dry state; SDwp = Specific density after soaking pellets in rumen fluid for 30 min. For HDexp, not possible due to quick pellet disintegration; FSI= fluid stability index, determined after incubation for 90 min in rumen fluid at 39°C.

In sacco DM degradability was determined as described by Åkerlind et al. (2011) incubating both ground and intact pellets in 3 rumen fistulated cows. Rumen degradation was estimated using the NLIN procedure of SAS (SAS, 2013). *In sacco* data was fitted to an exponential function and effective rumen DM degradability (EDMD) was estimated using passage rate of 0.05 h^{-1} (Ørskov and McDonald, 1979). Data was analysed using a GLM procedure of SAS with models having either Treatment (Trt), Cow, Type (ground/intact pellets) and Trt × Type or Trt and Cow as fixed effects. Contrasts tested were: LDext *versus* HDext+HDexp treatment, denoted as LD *vs* HD and LDext+HDext *versus* HDexp, denoted as Ext *vs* Exp. Least square (LS) means were judged different using the PDIFF statement and $P \leq 0.05$.

Results and Discussion

Within both ground and intact pellets, all *in sacco* parameters differed among treatments ($P_{Trt} \leq 0.003$; Table 2) and between pelleting technique ($P_{Ext\ vs\ Exp} \leq 0.016$). Comparing ground and intact pellets (Type), all *in sacco* parameters differed ($P_{Type} \leq 0.008$), except the D fraction.

In general, *in sacco* parameters increased with grinding, but *kd* of HDexp decreased ($P_{Trt \times Type} < 0.001$). With intact pellets, EDMD was lower for HDext than LDext and HDexp, but it was opposite with ground pellets ($P_{Trt \times Type} = 0.003$). *A* fraction was lower in extruded pellets than expanded pellets. However, *Pd* fraction for extruded pellets increased, which was probably the reason for their higher *kd* than expanded pellets. Extruded pellets were expected to have a low *kd* especially in pellet form due to their high fluid stability. Despite this, a lower EDMD of HDext intact pellets was probably due to low *A* fraction than HDexp. The degradation curves for intact pellets (Figure 2), indicates that HDext will have a lower degradation rate than HDexp up to 4 hours after entering rumen, which is an important time interval for rumen escape.

Conclusions

Physical properties of feed pellets affected *in sacco* rumen degradation. Extruded pellets with high fluid stability had lower soluble fraction and EDMD than expander treated pellets. To

reflect their intended functional properties in the rumen, it is recommended that pellets with specific physical properties should not be ground before *in sacco* incubation.

Table 2 *In sacco* rumen degradation (LS means) of extruder and expander treated[1] concentrates to ruminants

					P values[4]		
Item[2]	LDext	HDext	HDexp	SEM[3]	Trt	LD *vs* HD	Ext *vs* Exp
Ground pellets							
A	17.9[c]	18.7[b]	42.7[a]	0.14	<0.001	<0.001	<0.001
Pd	66.4[a]	67.4[a]	50.9[b]	0.29	<0.001	<0.001	<0.001
Kd	34.5[a]	33.9[a]	10.8[b]	0.82	<.0001	<0.001	<0.001
D	84.4[c]	86.1[b]	93.6[a]	0.39	<.0001	<0.001	<0.001
$EDMD_5$	75.9[b]	77.5[a]	77.4[a]	0.15	0.003	0.001	0.016
Intact pellets							
A	7.50[b]	7.32[b]	26.7[a]	0.35	<0.001	<0.001	<0.001
Pd	77.4[b]	80.8[a]	64.0[c]	0.76	<0.001	0.006	<0.001
Kd	30.0[a]	20.8[b]	16.0[c]	1.14	0.002	0.001	0.003
D	84.9[c]	88.1[b]	90.7[a]	0.41	0.002	0.001	0.001
$EDMD_5$	73.6[b]	72.0[c]	75.2[a]	0.13	0.001	0.729	<0.001

[1] LDext= Low-density extruded, HDext= High-density extruded, HDexp = High-density expanded pellets.[2] *A*= Soluble fraction (%), *Pd*= Potentially degradable fraction (%), *kd*=rate of degradation of *Pd* (%), *D*= total degradation (%), EDMD: Effective dry matter degradability calculated using a passage rate (*kp*) of 0.05 h^{-1} ($EDMD_5$). [3] Standard error of the mean for n=3. [4] Contrasts LD vs. HD, and extruded vs. expanded pellets

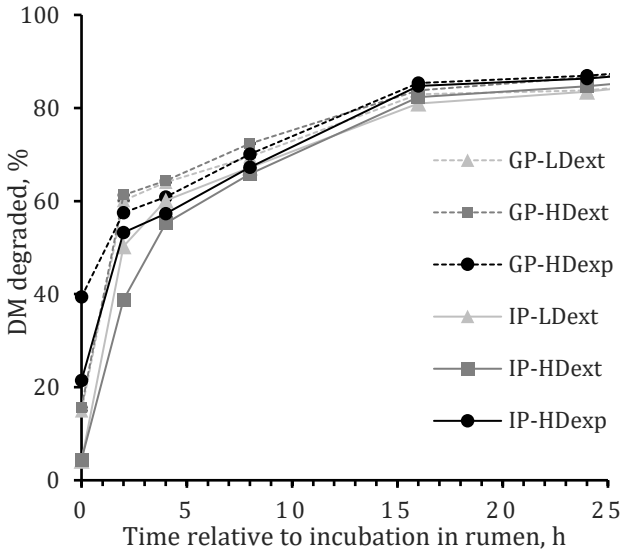

Figure 1 *In sacco* dry matter (DM) degradation of treatments (LDext= Low-density extruded, HDext= High-density extruded, HDexp = High-density expanded) using either ground pellets (GP) or intact pellets (IP).

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Evaluation of *in vitro* digestibility of fungal biomass as a potential ruminant feed supplement

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Introduction

Fungal protein (single-cell protein) is a sustainable alternative protein source that can be conveyed through bioconversion of low-value residues (Spalvins et al., 2018, Uwineza et al., 2021). Fungal biomass can partially or fully replace ruminant feed protein fractions based on its digestibility and amino acid profile. Therefore, this research project aimed to optimize the cultivation of fungal biomass on volatile fatty acids derived from acidogenic fermentation of organic residues and to evaluate the extent and rate of degradability of fungal biomass in rumen in vitro systems.

Materials and Methods

Aspergillus oryzae biomass, grown on glucose and volatile fatty acid (VFA) effluents recovered from anaerobic digestion of organic waste, was investigated for in vitro behaviour compared to silage and rapeseed meal. VFA effluents used to cultivate fungal biomass were produced and recovered through anaerobic digestion of fermented potato protein liquor, food waste and chicken manure using a submerged microfiltration membrane bioreactor. Before cultivation, all media preparations were performed under septic conditions. After medium and reactor sterilization, 4-L bubble column reactors were inoculated with the prepared *Aspergillus oryzae* spore solution. Cultivation was completed without any nutrient addition, at 35°C with initial pH of 6-6.2 and adjusted every 24 h. After fungal cultivation, the biomass was dried and stored for further analysis.

The next step of the experiment was performed by evaluating the rumen in vitro digestibility of the produced fungal protein biomass compared to rapeseed meal (ExPro) and silage. The in vitro assays were performed using McDougall buffer (McDougall, 1948) and planned as follows: 1. In vitro dry matter digestibility analysed according to Tilly and Terry (1963) and modified using 750 mg fungal biomass, rapeseed meal and silage and pepsin after 48 h. 2. In vitro gas production by monitoring fermentation products (gas, VFAs, and ammonia nitrogen) for 48 h. 3. In vitro gas production, fermentation products and dry matter disappearance when the biomass and controls were placed in Ankom F57 nylon bags for 48 h in glass bottles.

Results and Discussion

The produced fungal biomass contained 41-49% crude protein, 41-55% neutral detergent fiber (NDF) while the silage and rapeseed used as control contained 18% and 39% crude protein respectively. The residues of various biomasses after digestion were used to evaluate in vitro dry matter digestibility (IVDMD) in all three methods (Table 1). It was observed that the dry matter digestibily of rapeseed meal and silage ranged between 50-70% and 65-69% respectively in which they were similar to other studies using different methods, where the digestibility of rapeseed meal and silage ranged from 60% to 70% and 65 to 70%, respectively (Aerts, et al., 1977, Südekum, et al., 1997, Adesogan, et al., 2005). The digestibility of the produced fungal biomass was approximately 10-20% higher than the references feeds.

Table 1 In vitro dry matter digestibility (IVDMD)[1]

	Item	FWCKM A. o	PPL A. o	Syn. Gluc. A. o	Silage	Rap. ExPro.
TT method	IVDMD %	84.12±0.22	82.33±3.05	87.29±4.76	65.02±0.81	70.25±1.42
GP method	IVDMD%	71.77±2.3	81.44±2.21	84.60±3.09	69.84±3.52	50.90±2.25
Bag method	IVDMD%	69.69±2.07	76.94±3.03	78.17±0.24	66.64±2.17	57.02±1.37

[1]For abbreviations, see Fig. 1.

Furthermore, the fungal biomass contained 41-49% crude protein and 41-55% neutral detergent fiber (NDF) of dry matter. There is a possible grouping according to the rate of DM degradation and protein sources. The substrates with high protein contents had fairly high DMD and high NDF content.. According to an earlier report, feed containing 50 to 60% crude protein had a constant NDF degradation rate of 0.048 to 0.054 h^{-1}. Additionally, it was reported that brewer's grains and corn gluten feed with 25 to 30% crude protein had rapid NDF degradation rates in the higher range (0.065 to 0.072 h^{-1}) as well (Varga, et al., 1983). In previous studies, *Aspergillus oryzae* was added to feed ingredients as a supplement but in this trial, *Aspergillus oryzae* was considered as an independent feed ingredient.

The in vitro total gas produced when samples where placed in bags showed rather different trend compared to samples suspended in rumen fluids (Figure 1). In bags with fungal biomass, the total gas produced ranged between 20-36 NmL, which was slightly higher compared to rapeseed meal and silage 29 NmL and 20 NmL respectively. The higher total gas volume from fungal biomass correlated with the obtained IVDMD (Table 1).

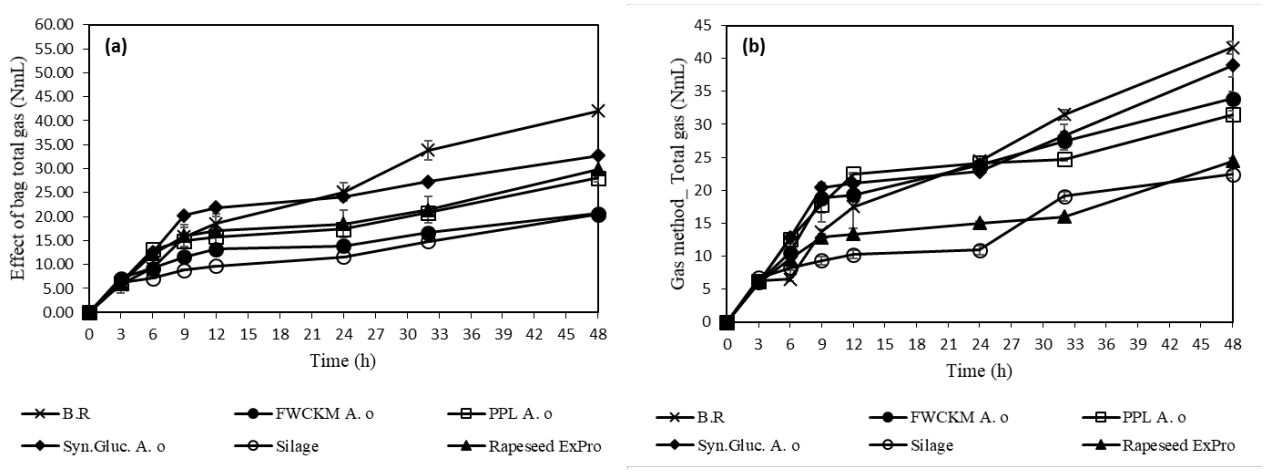

Figure 1 Gas production during in vitro total gas produced (a) samples placed in bags and (b) when samples were suspended in rumen fluid. (B.R: rumen fluid and buffer, FWCKMA.o: biomass grown on VFA effuents from food waste and chicken manure at 1:1 ratio, PPLA.o: biomass grown on VFA effluent from popato protein liquor, Dry.Gluc.A.o: Biomass grown on synthetic glucose, Rap.Expro: Rapeseed meal used as control).

There were no difference in the composition of VFAs from fungal biomass compared to rapeseed meal and silage (Table 2). The produced VFAs were dominated by acetic acid (2.67-3.37 g/L) and propionic acid (1.53-2.22 g/L), resulting in an acetate to proponate ratio (A/P) of 1.27-2.21. Luthfi, et al. (2018) reported that A/P ratio indicate the availability of energy supply for microbial growth and that generally A/P ratios safe for animal can vary from 1-5.

Table 2 Volatile fatty acids produced after 48 h during gas production methods when samples was in direct contact with rumen microorganism and effect of sample in bag[1]

	B.R	F.W.CKMA.o	PPLA.o	Dry Gluc.A.o	Silage	Rap. ExPro.
VFAs (g/L) produced during gas methods after 48h						
Acetic acid	1.55±0.07	3.22±0.0	3.37±0.19	3.15±0.46	2.82±0.02	2.67±0.46
Propionic acid	0.38±0.02	1.64±0.0	1.53±0.1	1.75±0.30	2.22±0.05	1.90±0.45
Butyric acid	0.32±0.02	0.25±0.00	0.78±0.04	0.78±0.14	0.56±0.04	0.58±0.14
TVFAs	2.26±0.03	5.3±0	5.99±0.07	5.92±0.17	5.6±0.19	5.17±0.22
A/P ratio	4.05±0.05	1.96±0	2.21±0.15	1.80±0.36	1.27±0.04	1.40±0.45
VFAs (g/L)produced during gas methods (substrates in bag) after 48h						
Acetic acid	1.7±0.11	3.01±0.3	3.63±0.14	3.91±0.09	2.95±0.02	2.99±0.14
Propionic acid	0.44±0.04	1.43±0.15	1.64±0.07	1.78±0.02	2.09±0.05	1.97±0.09
Butyric acid	0.38±0.03	0.78±0.02	0.92±0.03	0.99±0.01	1.25±0.08	0.87±0.02
TVFas	2.52±0.04	5.51±0.11	6.47±0.05	7.09±0.03	6.37±0.03	5.99±0.06
A/P ratio	3.86±0.07	2.11±0.15	2.22±0.07	2.19±0.06	1.41±0.03	1.52±0.09

[1]For abbreviations, see Fig. 1.

Conclusions

The in vitro dry matter digestibility of *Aspergillus oryzae* fungal biomass were successful compared with rapeseed meal and silage. It has a high potential and to be considered as a alternative high quality protein source. The promising results obtained need to be validated and extended by other in vitro methods in future studies.

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Predicting daily dry matter intake using milk mid-infrared spectra data with different multivariate regression approaches in Swedish dairy cattle

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Introduction

In general, dry matter intake (DMI) in dairy cow is important in relation to feed utilization, feed formulation, methane production and many other attributes that contribute to measuring the performance or efficiency of an animal. Measuring feed intake are for i.e. crucial to ensure that the energy intake is sufficient, especially during the first stage of lactation (Coffey et al., 2002). But, measuring feed intake, especially forage intake, are usually not possible on commercial farms. In dairy cows, the availability of milk mid-infrared spectroscopy (MIRS) data for the milk composition information are today available on many commercial farms. McParland et al. (2014) documented that the milk MIRS data could be used to estimate the feed intake in dairy cows. Therefore, this would be a good opportunity for the farmers to utilize the availability of milk MIRS data for estimation of DMI. The objective of this study was to determine and evaluate the performance of three different multivariate regression approaches in predicting daily DMI from milk MIRS in Swedish dairy cows.

Materials and Methods

Data from cows kept in the research herd at Swedish Livestock Research Center from the beginning of the year 2017 until the end of the year 2021 was used. Milk volume was recorded with an automatic milking system (AMS) every time the cows were milked. Milk samples were taken fortnightly and analyzed using MIRS (CombiScope FTIR 300 HP, Delta Instruments B. V., Drachten, the Netherlands). Each full MIR spectra data consisted of absorbance data from 935 wavenumbers ranging from 397.307 to 4000.071 cm^{-1}. Total raw feed intake was recorded with BioControl's System for Controlling and Recording Feed Intake V 2.0 (CRFI) for roughages and concentrate dispensers (FSC400, DeLaval International AB). Samples were taken for dry matter (DM) analysis five days per week and combined into one sample per two weeks for analysis according to NorFor (Volden, 2011). Prior to further coupling with MIRS data, the DMI data were preprocessed by removing intake data with more than 40 kg DMI per day per cow. The daily DMI was averaged over three days before the date of milk MIR spectra data. The 7 days average of milk yield (kg/day) and concentrate DMI (kg/day) were included in the models as additional variables. The development of DMI prediction models was performed with three different approaches; partial least square (PLS) regression v2.8-0 package (Wehrens and Mevik, 2007), support vector machine (SVM) regression e1071 package version 1.7-9 (Karatzoglou et al., 2006) and random forest (RF) regression package version 4.6-14 (Breiman, 2001). In total, the dataset with 1323 data lines from the year 2017 to 2020 was used to train the models with different predictors. To validate the prediction models, we used 471 data lines originating from the year 2021. The model's performances were evaluated by the values of the R^2 or coefficient of determination, root mean squared error of prediction (RMSEP) and mean absolute error (MAE).

Results and Discussion

Table 1 present the summary of each prediction model's evaluation with the R^2, RMSEP and MAE values of the three approaches for 3-180 DIM. It can be seen that when we added more variables to the model the MAE was reduced. The best model with the highest prediction accuracy and lowest error was when the full milk MIRS data were used together with MY and concentrate DMI regardless of any approach. Using full milk MIRS alone had low prediction accuracy compared to when adding MY in the models.

Table 1 Prediction accuracy evaluation for the validation dataset (471 data lines) PLS, SVM and RF regression analyses for different predictors at 3-180 DIM with the coefficient of determination (R^2), RMSEP (kg/day) and MAE (kg/day) between predicted and actual observations of DMI kg/day

Predictors	R^2 (validation)			RMSEP (kg/day)			MAE (kg/day)		
	PLS	SVM	RF	PLS	SVM	RF	PLS	SVM	RF
MIRS	0.19	0.16	0.18	3.67	3.80	3.75	2.96	3.05	3.05
MY	0.31	0.25	0.23	5.03	4.90	5.18	4.19	4.02	4.16
MIRS + MY	0.43	0.34	0.33	3.19	3.66	3.82	2.48	2.91	3.00
MIRS + MY + Conc	0.62	0.52	0.61	2.71	2.88	2.78	2.13	2.26	2.17

PLS: Partial Least Square; SVM: Support vector machine; RF: Random Forest; MIRS: full milk mid-infrared spectra data (935 wavenumbers); MY: average daily milk yield (kg/day); Conc: concentrate DMI; R^2: Coefficient of determination; RMSEP: root mean square error of prediction; MAE: mean absolute error

In dairy cattle, Vérité and Delaby (2000), Brun-Lafleur et al. (2010) reported that the milk composition i.e. fat, protein, lactose and solid could be determined by the diet composition especially energy and protein. Some studies reported that the metabolizable energy and protein composition in the diet influenced the DMI (Ipharraguerre and Clark, 2005, Brun-Lafleur et al., 2010). Therefore, the relationship between MIRS analysis and the DMI could be used to estimate the daily DMI in dairy cows. In 2014, it was first reported the possibility to predict feed intake using milk MIRS (McParland et al., 2014). Later on from 2017 until 2021, several efforts were conducted to predict DMI in dairy cows with different strategies. Shetty et al. (2017) have had the highest coefficient of determination with R^2=0.82 when MY and body weight were included together with MIRS. In another study, Wallén et al. (2018) had a different strategy when they compared 2 different multivariate approaches and found PLS with 8 factors to be the best model to predict net energy intake. In a similar study, Lahart et al. (2019) added more variables such as milk fat percentage, milk protein percentage, MY and stage of lactation in the model and the best coefficient of determination was with all the factors added in the model (R^2=0.78). Tedde et al. (2021) stated the importance of increasing the variability of the animals to make the models more versatile to be used in different conditions. The present study shows that the inclusion of MY and concentrate DMI in the models could improve the prediction accuracy consequently and reduced the random error in the predictions. The milk composition were derived from the MIRS data itself, therefore, to avoid redundant information, the milk composition (fat, protein and lactose) or energy corrected milk were not included in the prediction model.

Conclusions

In a nutshell, the DMI could be best estimated using the partial least square regression approach, including the milk MIRS with milk yield and concentrate intake in the model. The usage of the full milk MIRS data together with MY and concentrate intake has a quite good

coefficient of determination to predict the DMI for Swedish dairy cattle when using the other two approaches which are the non-linear multivariate regression (support vector machine and random forest regression).

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A proposed structure of a model for optimizing forage harvest according to energy requirements of sheep in a forage dominant feeding system

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Introduction

The goal with the proposed model, based on conditions in Iceland, was to optimize forage harvest to enable the conserved forage to potentially fulfill energy requirements of sheep fed indoors, essentially on forage ad libitum, through the dry period and pregnancy and in the first 1-2 weeks of lactation. Using forage energy value as a key parameter for this optimization does not replace more detailed models for predicting dry matter intake (DMI) and making feed plans when the forage has been harvested and analyzed.

The animal part of the model

From data reported by Ólafsdóttir (2012) and Sveinbjörnsson (2020) an average intake of 0.0263 EV kg BW^{-1} through the dry period and most of the pregnancy was estimated; where energy value (EV) of the feed is measured in milk feed units per kg dry matter, FE_m kg DM^{-1}(Van Es, 1978). Proportional deviations from this simple relationship are expressed by the DMI factor for with important exceptions from the value of 1 caused by lower intake in the last 2 weeks before lambing, and an increase of intake in the first two weeks after lambing, and some increase after shearing (Ólafsdóttir, 2012; Sveinbjörnsson, 2020).

Table 1 Feed plan for ewes of the Icelandic sheep breed during the indoor feeding period, with practical concentrate levels and aiming on two lambs born and reared per adult ewe and one lamb per ewe lamb.

	Starts	Initial BW*), kg	BW gain*) kg d^{-1}	En.req. FE_m d^{-1}	DMI factor	Forage DMI kg d^{-1}	For. EV FE_m kg DM^{-1}	FE_m d^{-1} forage	Forage class
Adult ewes									
Autumn & mating	Nov-15	65.0	0.100	1.28	1.10	1.58	0.81	1.28	C
Untill 90 days pregnant	Dec-27	69.2	0.050	0.95	1.00	1.33	0.71	0.95	D
Day 91-115 of pregnancy	Mar-15	73.1	0.075	1.41	1.10	1.74	0.81	1.41	C
Day 116-130 of pregnancy	Apr-9	75.0	0.050	1.34	1.05	1.67	0.80	1.34	C
Day 131-144 of pregnancy	Apr-24	75.7	0.000	1.27	0.90	1.51	0.84	1.27	B
First 2 weeks of lactation	May-8	75.7	0.000	2.48	1.20	2.33	0.90	2.10	A
Ewe lambs (1st prod. year)									
Autumn till start of mating	Oct-15	40.0	0.110	1.11	1.10	1.12	0.90	1.01	A
Until 75 days pregnant	Dec-5	45.6	0.090	1.00	1.00	1.08	0.83	0.90	B
Day 75 -115 of pregnancy	Feb-26	53.1	0.100	1.26	1.13	1.38	0.84	1.16	B
Day 116-130 of pregnancy	Apr-7	57.1	0.100	1.34	1.05	1.35	0.85	1.14	B
Day 131-144 of pregnancy	Apr-22	58.6	0.050	1.22	0.92	1.21	0.85	1.02	B
First 2 weeks of lactation	May-6	59.3	0.000	1.68	1.20	1.66	0.89	1.48	A

*)excluding the effects of pregnancy on BW

The total energy requirements reported in Table 1 account for maintenance and effects of shearing, planned body weight (BW) gain and requirements for pregnancy and lactation. The contribution of concentrate is zero for adult ewes in all periods except 0.20 FE_m d^{-1} in the two first weeks after lambing; only in that period some mobilization of body reserves (0.18 FE_m d^{-1}) is planned. For ewe lambs the contribution of concentrates is 0.10 FE_m d^{-1} for the majority of the indoor feeding period but is raised to 0.20 FE_m d^{-1} at day 115 of pregnancy. As energy available to the animal is the product of DMI (kg DM d^{-1}) and feed energy value (EV, FE_m kg DM^{-1}), and the relationship between feed quality and feed intake explained above also includes EV, the forage EV required to fulfill the energy requirements demanded from forage is:

$$\frac{FEm}{kg\ DM} = \sqrt{\left(\frac{(FEm\ req - FEm\ conc)}{(BW \times DMI\ factor \times 0.0263)}\right)}$$

The required forage energy values found by this equation are reported in Table 1 for each feeding period. In the last column of the table the forage is classified according to these energy values. Table 2 summarises forage requirement for a sheep farm with 400 adult ewes and 100 ewe lambs.

Table 2 Demand for forage of different classes for a farm with 400 adult ewes and 100 ewe lambs according to the feed plan in Table 1

			EV, FE_m kg DM^{-1}		
Forage class	Kg DM $year^{-1}$	% of demand	Target (average)	Upper limits	Lower limits
A	21104	15	0.90		0.875
B	26622	19	0.85	0.875	0.825
C	53902	38	0.80	0.825	0.775
D	41638	29	0.75	0.775	0.700

The plant part of the model

Leys used for harvest of winter feed on Icelandic sheep farms often get old, as the conditions for re-cultivation are variable. However, too little re-cultivation results in limited possibilities of producing the higher quality forage. Table 3 shows possible yields of the different forage classes A-D from three different life stages of the leys, for which different goals are relevant with respect to production of the four forage classes, as a result of variation in initial digestibility and rate of decrease in digestibility in the dominant grass species (Thorvaldsson et al., 2007). The shadowed cells in Table 3 are choices excluded because of impractical yields. Only the youngest ley produces Class A, but more options exist for producing the other forage classes.

Table 3 Leys of different age – suitable harvesting dates for forage of differerent classes in the 1st cut, and the possible yields in tonnes DM ha^{-1}. See text above for details

						Forage class in 1st cut ; date and **tn DM**			
Ley	Dom. species	Date *) "green"	Growth kg DM d^{-1}	EV when green	Decr. EV**) d^{-1}	A (0,90)	B (0,85)	C (0,80)	D (0,75)
Young	Timothy	15-5	130	1.02	0.0049	8-6 **3.4**	19-6 **4.6**	29-6 **5.8**	9-7 **7.0**
Adult	Knt. blueg.	20-5	90	0.96	0.0037	5-6 **1.8**	18-6 **2.9**	2-7 **4.0**	15-7 **5.1**
Old	Native	25-5	100	0.86	0.0029	0	28-5 **0.8**	15-6 **2.4**	2-7 **4.0**

*)Date when field is fully green, yield ~ 500 kg DM ha^{-1} **)EV=FE_m kg DM^{-1}

Although the energy value starts at a lower level in the 2nd than the 1st growth, it decreases at a slower rate and the forage harvested has higher leaf/stem ratio than the first cut. A limitation was set that 2nd cut should not be taken later than 20th of August, as there is normally also a great demand for aftermath for autumn grazing. The shaded cells in Table 4 are choices that are not relevant based on the chosen conditions.

Table 4 Cutting dates and yields for forage classes B and C in 2nd cut, for leys of different age and date of 1st cut

Ley -forage type in 1st cut	Growth rate 2nd cut kg DM d-1	EV 15 days after 1st cut	Decrease EV d-1	Forage class in 2nd cut; date and tn DM	
				B (0.85)	C (0.80)
Young -A	55	0.90	0.0015	27-7 **2.7**	29-8 **4.5**
Young -B	45	0.90	0.0015	6-8 **2.2**	8-9 **3.7**
Young -C	35	0.90	0.0015	16-8 **1.7**	18-9 **2.8**
Adult -B	55	0.90	0.0015	5-8 **2.7**	8-9 4.5
Adult -C	45	0.90	0.0015	19-8 **2.2**	21-9 **3.7**
Adult -D	35	0.90	0.0015	1-9 **1.7**	5-10 **2.8**
Old-C	45	0.80	0.0015	----------	2-8 **2.2**
Old-D	35	0.80	0.0015	----------	19-8 **1.7**

Optimisation of harvest

When the demand for forage is known (Table 2) as the conditions given for the forage harvest (Table 3 and 4), harvest can be planned in order to meet the forage demand. The Solver tool (Fylstra et al., 1998) in Microsoft® Excel was used for this purpose. All harvest choices, according to Tables 3 and 4, were summarized and compared to the demand for the different forage classes (A-D). A ley was defined "young" for four years and then "adult" for another four years; after that, "old". The goal set in the optimization was to minimize the squared sum of differences between the demand and production of different forage classes. The changing variables were a)total active ley in ha, b)the proportion re-cultivated per year and c)the ratio of each ley type (young, adult, old) cut for the production of different forage classes in the 1st cut. This was subject to the constraints that 1) production of each forage class should be ≥ demand and 2) the ratio within each ley type used for different forage classes sums up to 1. The results of the optimization were as follows: The total active leys needed were 25.9 ha; yearly 1.6 ha are recultivated. All the young leys are harvested for Class A forage in the 1st cut. Roughly 5, 90 and 5 % of the adult leys are harvested in the 1st cut for B, C and D forage classes. About 24% of the old leys are harvested for forage class C and 76% for forage class D in the 1st cut. There was about 15% overproduction of forage class B, but the differences between production and demand were less than 3% for the other classes.

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Modelling the effects of feed distribution on the supply of animal products at national level in Finland

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Introduction

Ecological and economic instabilities such as climate change, disease outbreaks, political tensions, land degradation, price variations etc. could lead to fluctuations in the operability of the world food system. It is therefore vital to prepare for impending uncertainties that could result from future crises to ensure the security of food supply. Livestock production in most countries relies on the import of inputs such as protein feeds for improved production. Livestock's ability in converting a wide array of biomass into high biologically valuable human-edible food is of great significance in global food production (Wilkinson, 2011). However, efficient allocation and management of available feed resources are imperative to achieve a steady feed supply and a resilient agricultural industry. This study aims to optimize human-edible energy (HEE) and human-edible protein (HEP) from livestock products by simulating variations in quantity and availability of feed resources while considering the feed conversion efficiency of various livestock.

Materials and Methods

Using Finland's livestock production sector in 2021 as a case study, data regarding the population, feed intake, and HEE and HEP output for each livestock category were considered. In this report, we focused on the dairy, beef, pork, chicken, turkey, egg and fish production systems. Feed types were categorised as forage, grain, protein feed and other feeds (by-products, minerals, vitamins, trace elements). Feed intake was estimated on a dry matter (DM) basis considering total feed intake from birth to slaughter for meat-producing animals as well as the feed consumed by the breeders at all production stages and later weighted throughout all animal sub-categories. The feed intake of lactating and dry dairy cows, as well as replacement heifers, was calculated according to the feed consumption statistics (ProAgria 2021; ProAgria Association of ProAgria Centers, Tuija Huhtamäki, personal communication) and the Finnish nutrient requirements (Luke, 2022a, https://maatalousinfo.luke.fi/). Milk ingested by suckling animals was accounted for by the feed consumed by the dam. The annual livestock population and animal products were obtained from Luke (2022b; https://statdb.luke.fi/), while the HEE and HEP concentrations for the products were collected from Fineli (2021, https://fineli.fi/fineli/fi/index; Table 1). Edible meat and eggs were calculated by considering the bone and shell percentages in the overall carcass and egg weight, respectively.
Scenarios explored include the current production situation (Scenario 1), reducing the amount of grain available by 25% while keeping the quantity of protein and other feed constant (Scenario 2), channelling all grain from the dairy and beef production system into the monogastric production system while taking into account the production responses of the ruminant systems (Scenario 3), as well as channelling all grain from the dairy and beef production systems into the monogastric production system and replacing the grain deduction with 25% more forages while considering the production responses of the ruminant (Scenario 4). The production responses for the milk and beef cattle were modelled according to the

predicted equations for Finnish cattle production (Huhtanen and Nousiainen, 2012; Huuskonen and Huhtanen, 2015).

Optimization was carried out using Microsoft Excel® Solver maximizing HEE and HEP output when livestock population was set as changing variables using available feed and feed conversion ratio of the livestock as constraints. All scenarios were addressed and the corresponding HEE and HEP produced as well as the livestock population to utilize the available feed input were reported.

Table 1 Population (thousands) and output (million kg) from various livestock as well as concentrations of human-edible energy (HEE) and protein (HEP) in animal products for the Finnish livestock sector in 2021

Livestock group	Population	Product	Output	HEE (KJ/kg)	HEP (g/kg)
Dairy system					
Dairy cows	254	Milk	2206	3234	35.6
Replacement heifers	93				
Beef system					
Suckler cows	72				
Heifers	99	Beef	86	5990	169
Bulls	235				
Calves	290				
Swine	3160	Pork	176	8170	189
Broilers	89917	Chicken	139	7880	202
Layers	3729	Eggs	78	4940	111
Turkey	1165	Turkey meat	9	5740	220
Farmed fish	7527	Fish	15	6380	168

Results and Discussion

In the current situation, feed available for livestock production is 2338, 1861, 342 and 353 million kg DM of forage, cereal grain, protein feed and other feed types, respectively. Dairy cows (1.05) and non-ruminants (1 - 3) are more efficient converters of feed (in kg DM) into livestock products compared to beef cattle (20.7). Globally, the feed conversion ratio of ruminants (meat-producing) has been calculated to be about 20:1 while for non-ruminants, it is 3.8:1 (Galloway et al., 2007).

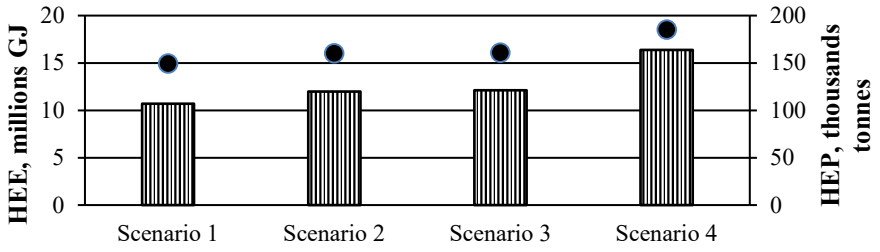

Figure 1 Optimized human-edible energy (HEE; bars) and human-edible protein (HEP; black markers) obtained from simulating variations in feed quantity available for animal output production.

With a 25% decrease in grain availability (Scenario 2), the population of fish and dairy cows increased compared to Scenario 1 (Table 2) resulting in a 7% and 12% rise in HEP and HEE production, respectively (Figure 1). However, in contrast to Scenario 2, Scenario 3 promoted both egg and milk production with a decline in or no production of other livestock while

slightly increasing overall HEP and HEE. Dairy cow production improved in all scenarios due to the low feed input required to produce every unit of milk product. Despite the low HEE and HEP concentration in milk compared to other livestock products, the quantity of HEE and HEP derived from milk is relatively high. Although there was a 13% increase in forage use in Scenario 4, it proved to be the best strategy for producing HEP and HEE relative to the other scenarios. In generating this output, non-ruminants and beef cattle production except for layers were immensely lower than in the baseline situation. This suggests that increasing low-opportunity cost feeds in ruminants' diets, especially dairy cows, in the absence of grain availability could improve the food system (van Zanten et al., 2019) as well as boost the output of human-edible livestock products.

Table 2 Projected livestock population (thousands) according to optimized human-edible energy and protein output under the 4 scenarios

Livestock	Dairy cows	Beef cattle	Swine	Broilers	Layers	Turkey	Farmed Fish
Scenario 1	347	696	3160	89917	3729	1165	7527
Scenario 2	484	362	2600	766	-	129	63791
Scenario 3	481	368	3015	-	9540	-	638
Scenario 4	776	37	-	-	4254	-	-

Conclusions

The highest human-edible energy and protein outputs were produced in Scenario 4 when forage availability was increased for both dairy cows and beef cattle. Harnessing livestock's potential in converting human inedible feed resources into valuable human food and manure will considerably benefit the human food supply, efficient use of available grasslands and sustainability. Non-ruminants, which are more dependent on the quality of feed material (e.g. amino acid profile) could be raised on available grains if there is a shortage in cereal production. This modelling exercise provides possibilities to evaluate different scenarios. In general, scenarios with changes in livestock numbers include time lags in changing the population size. Further, actions depend on the decisions of individual farmers as well as demand from consumers, but knowledge from such simulations could provide support for policymakers in case of feed supply shortage.

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Simulating the yield and nutritive value of whole crop barley

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Introduction

Whole crop silage making, including grain and vegetative fractions of barley or other small grain cereals before full maturity, is a common preservation practice in northern Europe (Rustas *et al.*, 2011). Effects of weather, soil and management practices on growth, biomass yield and chemical composition of barley and other cereal crops can be simulated using crop simulation models and integrated in decision support systems. The BASic GRAssland (BASGRA) model has previously been developed to simulate the yield and nutritive value of timothy (Höglind *et al.,* 2020). Here, we adapt this model to simulate yield and nutritive value of whole crop barley as a function of weather, soil and management factors.

Materials and Methods

The BASGRA model was adapted to simulate dry matter yield, crude protein and fibre content and dry matter digestibility in whole crop barley. To simulate carbon and nitrogen dynamics in whole crop barley, a spike pool, equations that set the allocation of newly photosynthesized carbohydrates to spikes, and equations that set the C and N translocation from stems and leaves to spikes, were added to complement stem, leaf, root and reserve pool. A grain filling stage, which sets the period when carbon and nitrogen can be allocated to the spike pool, was also defined. Moreover, equations that determine fibre content and digestibility of the spike pool were introduced.

Parameters in the modified model version were calibrated against observations of dry matter (DM) yield, protein and fibre content, and DM digestibility from a number of different spring barley varieties. These observations were obtained from field experiments carried out from 2002 to 2020 under a range of soil and climate conditions at several locations in Sweden. Dry matter and nutritive value observations were made from development stage 59 to 89, according to Zadoks *et al.* (1974), and varied among experiments. Bayesian calibration techniques, where prior distributions for model parameters are updated to form posterior distributions based on observed data, followed previous applications of the BASGRA model (Höglind *et al.*, 2020). Here, the parameter prior distributions were either obtained from a previous calibration against field trial data from northern Europe and Canada (Persson *et al.*, 2019) or set, based on previous knowlegde about barley physiology. In the first calibration, data from the whole country were included. Further calibrations, using separate data from the northern and southern regions in Sweden, were carried out to identify regional variation in calibration accuracy. The most likely parameter values that were identified in the calibrations were subsequently tested against randomly selected independent data from the same regions that had been excluded from the calibration data. Daily input data on minimum and maximum air temperature, precipitation, relative air humidity, wind speed and global radiation for the model calibrations and tests were obtained from weather stations within the networks of the Swedish Meteorological and Hydrological Institute (SMHI) and Lantmet,

located nearby the experimental fields. Information on soil texture and water holding capacity, that was also input to the model calibrations and test, was based on field specific soil sampling.

The ability of the model to predict DM biomass, crude protein (CP) and neutral detergent fibre (NDF) content, and DM digestibility was evaluated using the Root Mean Square Error (RMSE), which was normalized by dividing it by the mean value of the observations. The relative mean bias error (rMBE), which provides a measure of the relative magnitude of the misprediction (over- or underprediction), and Willmott's index of agreement, which delivers a value between 0 (no agreement between observed and simulated values) and 1 (complete agreement) were also applied.

Results and Discussion

The prediction, as evaluated by the normalized RMSE was better for NDF (6.6 % and 10.7 %) and DM digestibility (12. 8 % and 10.6 %) than for DM biomass (20.8 % and 27.9 %) and CP content (26.5 % and 17.6 %) in the validation of the model calibrated for the whole Sweden and northern Sweden, respectively. In validation of the model calibrated for southern Sweden, prediction was better for DM biomass (3.5 %) than for the other output variables using the same metric. NDF and dry matter digestibility were also overpredicted in all three validations. DM biomass and crude protein were underpredicted in the whole Sweden validation and overpredicted in validation for northern Sweden. In the validation for southern Sweden, DM biomass was also overpredicted, whereas crude protein was underpredicted after the same calibration (Figure 1). Prediction accuracy of nutritive value attributes tended to be higher across a range of calibrations with a variation in the number of included cultivars, which could be related to a lower variability in nutritive value composition than in biomass among cultivars, and weather, soil and management conditions. Moreover, in many of the datasets, there was a greater variance within biomass observations than within the nutritive value attributes at single sampling events suggesting that the former observations are associated with a greater uncertainty.

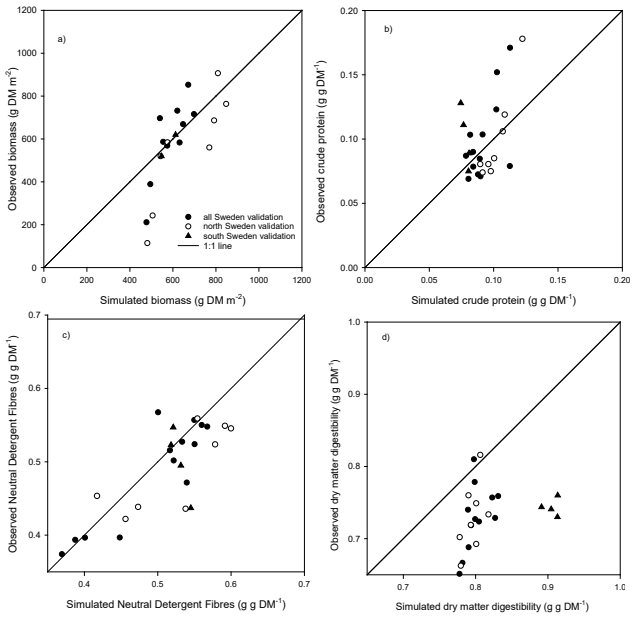

Figure 1 Observed versus simulated biomass a), crude protein content b), Neutral Detergent Fibre content c) and dry matter digestibility d) in the validation dataset.

Lack of clear differences in prediction accuracy after calibration against data from the whole country on the one hand, and calibrations against data from northern and southern Sweden on the other hand, indicates that mispredictions of the observations were caused by factors that were not associated with regional variation in production conditions between these two regions.

Conclusions

The model predicted NDF content with reasonably high accuracy. However, predictions of crude protein content, DM biomass and DM digestibility were poorer. NDF content and DM digestibility were overpredicted both after calibrations against data from the whole country and data from southern and northern regions of Sweden, respectively, whereas there were neither any over- or underpredictions of crude protein content and DM biomass. More data from field experiments in Norway and Sweden from 2021 and 2022 will be used in further model development to improve prediction accuracy.

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Screening of energy content of compound feeds using the new energy evaluation model in NorFor

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Introduction

During the last edition of the Nordic Feed Science Conference (2019), NorFor presented a new method for estimating net energy content (NEL_{20}) of compound feeds based on analysis of chemical composition and in vitro analysis of organic matter digestibility (Álvarez et al., 2019; 2021). Before this model, NEL_{20} of compound feeds could only be calculated by knowing their ingredients. However, during October 2021 NorFor updated its digestion model, which affects directly the NEL_{20} values used for reference for the model (https://www.norfor.info/news/). Therefore, a model rerun was necessary to keep the NEL_{20}-model updated.

Among the several purposes this model can serve, it can be used to compare actual products with the declared values in product sheets or in the NorFor Feedstuff Table (FST). An accurate energy value of feeds is necessary to precisely match animal requirements to achieve a production goal with minimum losses. Thus, NorFor requires that feed companies introduce accurate energy content of their compound feeds in the Feedstuff Table. The objective of this project was to collect compound feed samples from the 4 country partners and use the model to evaluate the NEL_{20} values stated by the feed companies in the FST.

Materials and Methods

The NEL_{20} reference value of the original 75 compound samples were used to develop the first model. Then, models were rerun and reevaluated using the same procedure as in the first model (see detailed methods in Alvarez et al. 2021).

Screening: 146 samples were collected between January 2021 to March 2022 from Sweden (23), Iceland (15), Norway (24), and Denmark (84). Samples were taken directly from mill plants or in farms. Swedish, Norwegian and Icelandic samples were sent to the laboratory Eurofins Agro Testing and chemically analyzed for DM, ash, crude protein (CP), crude fat (CFat), NDF, and organic matter digestibility (by EFOS, % of organic matter). Danish samples were analyzed for the sample parameters by NIRS at the laboratory Kvægbrugets forsøgslaboratorium (SEGES, Denmark), as these samples were part of the Feed Unit screening methodology. Digestible organic matter (DOM_{EDOM}, % of DM) was calculated as EFOS*(100-Ash). From the FST, registered values by feed companies were extracted to compare NEL_{20} and chemical content.

Tolerance level of ±5%, set by EU, was used as criteria to determine significant differences between FST and analyzed values for energy and chemical composition (European Commission, 2010).

Results and Discussion

Model rerun: After rerunning the models with new NEL_{20} values, the best model was: 5.523 - 0.0524 NDF + 0.0992 CFat + 0.0251 DOM_{EDOM} + 0.0146 CP_{corr} - 0.0327 ash, where all variables are in % of DM. The present model had lower RMSE than the previous model

(RMSE = 0.132 vs 0.149 MJ / kg DM, corresponding to mean prediction errors of 1.85 and 2.08%, respectively). In addition, the new model includes ash as a new parameter as compared to the previous model.

Table 1 Summary of composition of sampled compound feed (% of DM) and net energy content (MJ/kg DM). The lowest and highest values are presented within brackets

Origin of sample	Sweden	Iceland	Norway	Denmark
N samples	23	15	24	84
DM	877 (855 – 901)	878 (870 – 889)	869 (852 – 900)	876 (855 – 897)
Ash	7.3 (6.0 – 10.1)	8.2 (3.7 – 9.8)	7.3 (5.7 – 17.0)	5.8 (2.2 – 9.9)
CP	25.2 (16.5 – 42.3)	18.2 (11.2 – 22.2)	18.2 (9.5 – 24.4)	23.4 (10.9 – 41.6)
CPcorr	24.9 (16.5 - 42.3)	18.2 (11.2 - 22.2)	17.6 (9.5 - 21.4)	23.0 (10.9 - 41.6)
NDF	26.1 (20.2 – 39.7)	18.0 (9.6 – 23.6)	21.0 (15.8 – 37.0)	24.4 (13.4 – 37.0)
CFat	6.3 (3.5 – 10.9)	3.6 (1.8 – 5.7)	4.4 (2.2 – 6.3)	6.4 (3.0 – 11.9)
DOM_{EDOM}	81.9 (75.3 – 86.1)	87.4 (85.4 – 93.8)	84.1 (70.6 – 90.9)	85.9 (78.7 – 93.4)
NEL_{20} FST	7.31 (6.39 – 7.98)	7.33 (6.95 – 8.22)	7.10 (5.89 – 7.80)	7.33 (6.31 – 8.66)
NEL_{20} model	6.96 (6.16 – 7.44)	7.13 (6.83 – 7.92)	6.98 (6.08 – 7.59)	7.18 (6.3 – 8.17)

[1]DOM_{EDOM}: digestible OM by enzymatic digestibility of OM method; CP_{corr}: CP corrected by urea content in compound feeds; CFat: crude fat; ST: starch; NEL_{20} reference: net energy of lactation at 20 kg of DMI/d. NEL_{20} FST: registered by feed companies in the NorFor Feedstuff Table.NEL_{20} model: predicted using the model.

Screening: The comparison between NEL_{20} values estimated by the model and registered in FST by feed companies can be seen in Figure 1. From 146 samples, 35 (24%) were considered outliers. All but 2 of the outliers laid in the upper tolerance limit, meaning NEL_{20} values registered in FST were 5% or more higher than the estimated. The Swedish screening showed the highest proportion of outliers (46%), followed by Norway (33%), Denmark (14%) and Iceland (13%). The yearly screening of energy and chemical composition for the Feed Unit method in Denmark, which is also a net energy measurement, could explain the low proportion of outliers in that country, despite having the highest number of samples in this screening.

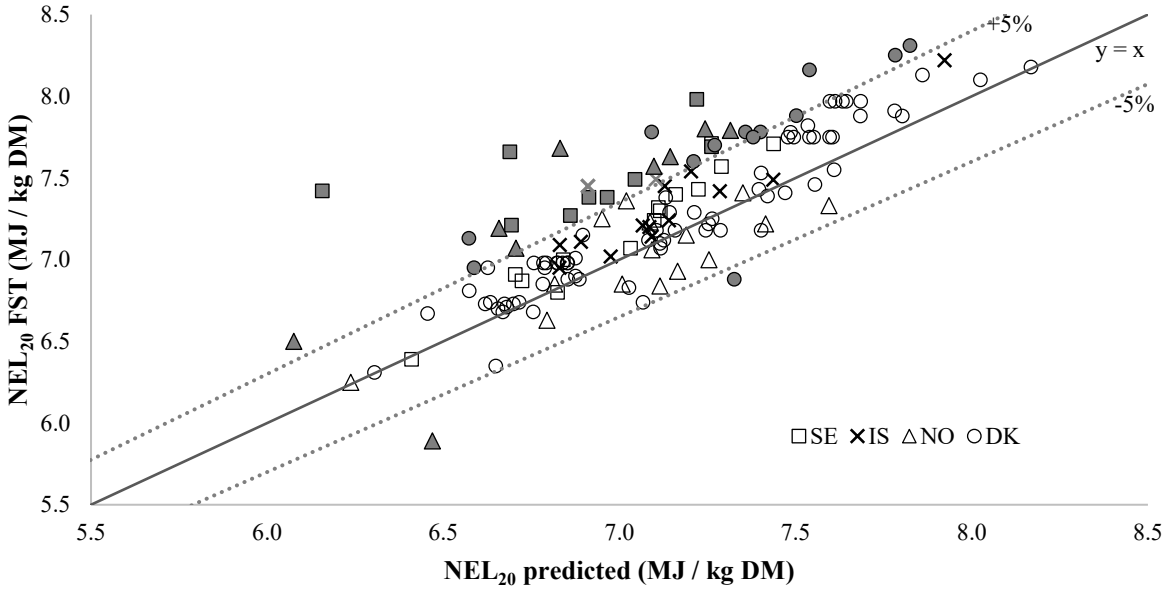

Figure 1 Energy content predicted by the model (x-axis) compared to the energy content registered by feed companies in FST (y-axis). Outliers according to EU tolerance levels (±5%) are coloured in grey.

When evaluating the relationship between NEL_{20} residuals and the parameters used in the model, NDF showed the highest relationship. This means that the higher the difference between NDF analyzed and FST values, the higher was the difference in NEL_{20} value between FST and model (Figure 2). This confirms the importance of NDF in this model, as it was selected (by stepwise AIC selection) first to be included in the model. Moreover, this demonstrates the importance of this parameter to determine energy content in the NorFor system.

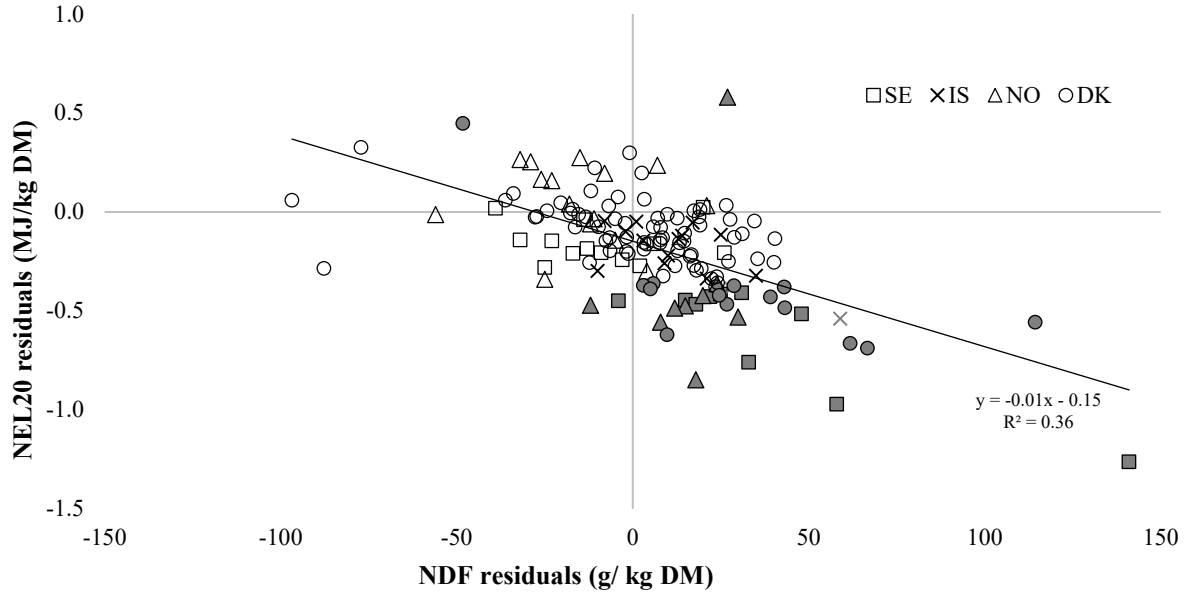

Figure 2 Regression between NDF residuals (analyzed-FST, x-axis) and NEL_{20} residuals (model-FST; y-axis). NEL_{20} outliers according to EU tolerance levels (±5%) are colored in grey.

Although compound feeds could keep the same recipe, the raw materials vary due to the yearly production. Thus, the FST needs regular update when companies alter their products. However, 50% of the Swedish and Danish outliers had not been updated for more than one year when samples were collected.

Conclusions

The new model allowed screening of compound feeds to compare with values registered by companies in FST. Out of 146 samples, 24% were considered outliers and differences between NDF analyzed and FST values explained these outliers. Updating chemical composition regularly in the FST could help to reduce the number of outliers.

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Is the random variation among animals relevant to estimate *in situ* degradation rate of ruminant feeds?

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Introduction

The *in situ* methods are widely used to assess ruminal degradation kinetic characteristics (i.e., rate and extent of degradation). However, some results have demonstrated that there are animal influences on *in situ* degradation parameters (Sampaio et al., 2014), which would indicate that an evaluation of the *in situ* degradation profile obtained with a single animal may bias the degradation rate estimates. Although diet is considered to have the greatest impact on rumen microbial diversity, there is an intrinsic effect of the host animal on microbial population structures (Weimer et al., 2010). Such a statement supports the use of a group of animals rather than a single one to assess a likely rumen degradation profile using *in situ* methods. Nevertheless, information is still lacking regarding on how animal effects can influence on the estimates of degradation rate and on overall quality adjustment of non-linear models to describe the *in situ* degradation pattern of feeds for ruminants.

Materials and Methods

Three forage and four concentrate samples were evaluated as follow: fresh sugarcane, corn silage, Tifton 85 hay, corn grain, soybean hulls, cottonseed meal, and soybean meal. Fresh forages were oven-dried (55°C). Then, all samples were ground in a knife mill to pass through a 2-mm screen sieve for use in the *in situ* procedures.

Five rumen-cannulated Nellore heifers (328±9.8 kg BW) were used. The heifers were housed in individual pens with concrete floor and equipped with individual feeders and drinkers with free access to a complete mineral mixture and fresh water. The basal diet (120 g crude protein/kg dry matter) consisted of Tifton 85 hay and a commercial concentrate in the proportions of 80:20 on a dry matter basis.

Three sequential incubations were performed, each containing a group of feeds. In each run/group, the samples were incubated in all animals. The incubation bags were 8 × 15 cm and made of nylon textile (50 μm of porosity, Sefar Nitex, Sefar, Switzerland). Aliquots of 6.0 g of ground samples were added to the nylon bags (20 mg dry matter/cm² of surface). The set of incubation times for forage samples encompassed 16 points (from 0 to 240 h) for forages and 13 points (from 0 to 144 h) for concentrates. The incubation times were arranged in the rumen in a reverse order so that all bags were removed at the same time. At the end of incubation, bags were removed and washed in running water to remove the excess of residues from outer bag, and then washed in a washing machine for 5 cycles of 1 min each (Vanzant et al., 1998). The bags were then oven-dried (55°C) and weighed. The forage incubation residues were analyzed for dry matter (DM) and neutral detergent fiber (NDF) contents, whereas the concentrate residues were analyzed for DM and crude protein (CP).

A first-order exponential model (Ørskov & MacDonald, 1979) was chosen to describe the DM and CP degradation profiles, and a gamma-2 time-dependent model (Van Milgen et al., 1991) was chosen to describe NDF degradation. To include animal influence, both models

were adapted to a mixed model approach by including one representing the random variability among animals on the degradation rate. The NLIN and NLMIXED procedures of SAS 9.4 were used to adjust the models according to the fixed or mixed (including animal random variance) modelling approaches, respectively.

Results and Discussion

The evaluation of variability among animals was based on the degradation rate assuming that undegradable and potentially degradable fractions are intrinsic characteristics of the feeds. In spite of the peculiarities associated with each modelling approach, there were no differences ($P \geq 0.34$, data not shown) in the degradation rates estimates obtained by the fixed or mixed models. This pattern seems to be logical, as the mathematical expectations of the parameters are the same for both approaches.

Table 1. Characteristics of estimation process of the parameters associated with degradation rate (kd, h^{-1}) in different feeds and feed components using fixed or mixed modelling approaches.

	Fixed Model[1]		Mixed Model[1]				
Feed	kd[2]	σ^2_ε	kd[2]	σ^2_a	P value	σ^2_ε	Δ(%)
			Dry Matter				
Sugarcane	0.017±0.0009	1.9	0.017±0.0017	3.6×10^{-6}	0.513	1.5	21.6
Corn silage	0.026±0.0012	4.6	0.026±0.0015	4.6×10^{-6}	0.235	3.8	18.7
Tifton hay	0.027±0.0017	8.7	0.026±0.0017	9.6×10^{-6}	0.154	6.2	29.4
Soybean meal	0.049±0.0044	48.8	0.051±0.0061	1.3×10^{-4}	0.313	34.5	29.2
Corn grain	0.049±0.0027	19.7	0.048±0.0039	7.2×10^{-5}	0.219	10.5	46.7
Soybean hulls	0.028±0.0020	27.1	0.028±0.0020	6.1×10^{-6}	0.214	23.2	14.5
Cottonseed meal	0.030±0.0023	16.9	0.030±0.0025	6.9×10^{-6}	0.330	14.9	11.9
			Crude Protein				
Soybean meal	0.042±0.0039	88.3	0.042±0.0041	2.9×10^{-5}	0.408	73.6	16.6
Corn grain	0.034±0.0034	25.7	0.036±0.0045	4.6×10^{-5}	0.355	19.8	23.1
Soybean hulls	0.036±0.0040	19.6	0.036±0.0032	0	---	19.6	---
Cottonseed meal	0.079±0.0058	32.7	0.083±0.0078	1.3×10^{-4}	0.399	27.1	17.1
			Neutral Detergent Fiber				
Sugarcane	0.044±0.0016	8.5	0.044±0.0017	1.4×10^{-5}	0.078	6.6	22.4
Corn silage	0.052±0.0019	18.5	0.052±0.0026	2.9×10^{-5}	0.248	13.6	26.5
Tifton hay	0.059±0.0020	12.9	0.059±0.0032	5.2×10^{-5}	0.143	7.9	38.6

[1] σ^2_a, variance component associated with variation among animals on kd; P value, significance associated with H_0: $\sigma^2_a = 0$; σ^2_ε, residual variance; Δ(%), contribution of the variability among animals on the residual variance based on the fixed model adjustment. This value was calculated as the percentage of decrease in σ^2_ε when the mixed model approach was applied instead of the fixed model. [2] Estimate ± asymptotic standard error.

For all the degradation profiles studied, except for CP from soybean hulls, we obtained adjustments with estimates of variance among animals on the degradation rate numerically greater than zero (Table 1). However, only one degradation profile provided a variance component different from zero ($P<0.08$, sugarcane NDF). At first glance, such a pattern

seems to indicate that there is no variation among animals regarding degradation rate. However, we must emphasize an intrinsic limitation of the method we used here to point out the significance of the variance components. Actually, we applied an asymptotic approach to the Student's t distribution, whose statistical diagnostics could be compromised by the restricted sample size and degrees of freedom in the test denominator (d.f. = 4).

However, the variation among animals can be indirectly inferred from the size of residual variance (i.e., error), which quantifies the variation caused by any source of variation that is not covered by the model structure. When we considered variation among animals on degradation rate, we observed decreased values of residual variance for all adjusted degradation profiles (Table 1). According to our results, the variation among animals corresponded to 11.9-46.7% (averaging 24.3%) of the total random variability of the degradation profiles. Conversely, the decrease in residual variance associated with the mixed model approach could be partially attributed to the consideration of one additional parameter in the models. Then, residual variance was weighed according to the number of parameters in the model by using the Akaike Information Criterion (AIC). In this sense, the inclusion of one more parameter to account for animal influence on degradation rate showed to be efficient in terms of improving the precision of degradation profiles, as the AIC decreased, on average, by 17.2% (range 10.0-34.3%) when mixed modelling approach was used.

Considering the lack of influence on the degradation rate estimates, the obvious advantage for using a mixed model approach relies on residual variance reduction. Thus, any effective practical benefit from using non-linear mixed models would be observed if the incubation procedures were linked to an experimental design in which the main objectives relies on statistical comparison between feeds or diets. Otherwise, fixed model approach would provide a huge operational advantage, as it is less complex and converges faster through iterative algorithms, which are also less sensitive to the use of non-optimal starting values.

Conclusions

Taking the animal random variation into account does not influence the *in situ* estimates of degradation rate, but improves the precision of the adjusted models.

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Profitable dairy production in turbulent times from a European perspective

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Introduction

Due to the turbulent market situation, costs for feed are currently in particular focus for the European Dairy Farmers (EDF). EDF is a club of farmers producing yearly benchmarks on costs of production and other key figures. From the benchmarks some focus areas has been identified. Costs for feed, labour and buildings, and a high production output are all crucial parts for profitability in Nordic EDF farms.

European Dairy Farmers

European Dairy Farmers is a club of progressive and visionary dairy farmers looking for inspiration. It serves as a platform for exchange of ideas, experiences and knowledge on an international level. EDF wants to connect open-minded farmers and other players of the dairy chain across Europe. EDF is a non-political, impartial and independent club from farmers for farmers (EDF, 2022).

Every year data is collected from more than 300 member farms. From the Nordic countries, data is collected from approximately 80 farms in Sweden, Finland and Denmark. The data includes economic results and important key figures from the production system. All farm data is processed, using a standardized method to make the farms comparable and gives a unique benchmarking database. In all its benchmarking activities, EDF is supported by the Scientific Team for Analysis and Research (EDF STAR). EDF STAR are scientists, advisors and dairy experts from the EDF countries. All data presented from EDF is only representative for the farms in the network and should not be seen as a statistic sample for the countries.

Focus areas from EDF benchmarking

Within EDF, we have recognised some important focus areas of for the Nordic farms in the network. These are:

1. Costs for feed

Feeding cows and heifers often stands for about 50% of the total costs on a dairy farm. To keep feeding costs under control is crucial for profitability in dairy farming. This includes all parts of the feeding chain, from an efficient crop management to keeping an optimised feed ration.

2. Costs for labour and buildings

Labour and building costs are sometimes connected to each other, where investments in high-tech building equipment can be made in order to cut down the need of labour force. However, on many EDF farms, the technical systems do not repay fully compared to the investment cost. We also see that the Nordic farms in the EDF network have higher costs for labour per hour, and buildings are more expensive, compared to the average EDF farm.

3. High production output

The higher costs for buildings, labour and machinery in the Nordic EDF farms than other EDF farms are to some extend compensated for by subsidies, but also by a high output system. This is managed by having high-yielding cows so fixed costs can be diluted, which

seems to be crucial for profitability on these farms. Feeding is an important part of gaining high yields, along with animal health and welfare.

Feed market development

For the last couple of years, the feed market has been turbulent in many ways. There are several factors going on in the world that has huge impacts on feed and food markets: climate change causing extreme weathers and fires, cutting down rainforests on greenhouse gas emissions, the Covid pandemic and of course the invasion of Ukraine.

The future development is hard to foresee for these factors, and given that they affect each other, predicting the future market effects is impossible. Turbulent times on the world market are likely to continue, which is driving the interest in home grown feed on many EDF farms. Farmers who can become more self-sufficient on feed will probably have a competitive advantage during the coming years. This requires efficient crop production and storage on individual farms or with farm cooperation. Access to and price of fertilizer and other inputs can also become a challenge.

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Defining a minimum time design to evaluate *in situ* dry matter degradation of tropical feeds

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Introduction

The *in situ* method has been extensively applied to assess ruminal degradation kinetics of forages and concentrates. However, in literature there is no agreement on the set of incubation times that should be adopted for *in situ* procedures. It is known that the number and order of different incubation times can affect the estimation of the model parameters in a degradation profile (Mertens, 2005). Even though there are suggestions regarding the number and order of incubation times in non-tropical regions (e.g., Mertens, 2005; NRC, 2001); to the best of our knowledge, no studies have been carried out with such direct objective for feeds produced under tropical conditions. Due to differences concerning plant species and climate, different degradation patterns may be expected in terms of rate and extent of rumen degradation, requiring different times designs for an adequate *in situ* assay in the tropics.

Materials and Methods

Three forage and four concentrate samples were evaluated as follow: fresh sugarcane, corn silage, Tifton 85 hay, corn grain, soybean hulls, cottonseed meal, and soybean meal. Fresh forages were oven-dried (55°C). Then, all samples were ground in a knife mill to pass through a 2-mm screen sieve for use in the *in situ* procedures.

Five rumen-cannulated Nellore heifers (328±9.8 kg BW) were used. The heifers were housed in individual pens with concrete floor and equipped with individual feeders and drinkers with free access to a complete mineral mixture and fresh water. The basal diet (120 g crude protein/kg dry matter) consisted of Tifton 85 hay and a commercial concentrate in the proportion of 80:20 on a dry matter (DM) basis.

Three sequential incubations were performed, each containing a group of feeds. In each run/group, the samples were incubated in all animals. The incubation bags were 8 × 15 cm and made of nylon textile (50 μm of porosity,Sefar Nitex, Sefar, Switzerland). Aliquots of 6.0 g of ground samples were added to the nylon bags (20 mg dry matter/cm² of surface). The set of incubation times for forage samples encompassed 16 points (from 0 to 240 h) for forages and 13 points (from 0 to 144 h) for concentrates (Table 1). The incubation times were arranged in the rumen in a reverse order so that all bags were removed at the same time. At the end of incubation, bags were removed and washed in running water to remove the excess of residues from outer bag, and then washed in a washing machine for 5 cycles of 1 min each. The bags were then oven-dried (55°C) and weighed. The forage incubation residues were analyzed for DM using Karl Fischer titration.

A first-order exponential model (Ørskov & MacDonald, 1979) was chosen to describe the DM degradation profiles. The evaluations to establish a minimum time design for *in situ* incubations were performed according to the following procedures. First, the degradation profiles were adjusted considering all the incubation times (16 and 13 times for forages and

concentrates). From this adjustment, asymptotic confidence intervals (1-α = 0.95) were estimated for the parameters (A, B, and kd; Table 2).

Our first objective was to establish the optimal value for the final incubation time. Decreasing the final incubation time will decrease the time spent with the whole incubation procedure. In this way, the times were gradually withdrawn, one by one, going back in relation to the last time used in this study (240 h for forages and 144 h for concentrates) and a new adjustment was performed after each time withdrawal. This procedure was repeated until at least one of the parameters estimates (A, B, or kd) obtained with the reduced design has been found outside its respective asymptotic confidence interval estimated with the total set of incubation times. Thus, this point was kept in the design, as its removal would cause a significant bias in the estimates of one or more parameter. After defining the incubation end point, intermediate time points were removed from the profile aiming to increase the time interval among incubation times. An incubation time was considered unnecessary when its removal did not compromise the estimates of the parameters A, B, and k according to asymptotic confidence intervals obtained with the total set of incubation times.

When all minimum time designs were established, their residual variances were compared with those obtained with the total set of incubation points by the Snedecor-Fisher test (α = 0.05). The NLIN procedure of SAS 9.4 was used to adjust the non-linear models.

Results and Discussion

Nine incubation time points were defined as the minimum design to evaluate the DM degradation profiles (Table 1). We highlight that, within both forages and concentrates, there was a convergence in the incubation times, which evidences the adequacy and robustness of our proposal protocols. The minimum designs produced similar estimates for DM degradation parameters (P>0.05) when compared to the whole set of incubation times, while not affecting the residual variance estimates (P≥0.11, Table 2). This shows similarity in both accuracy and precision.

Table 1 Description of selected incubation times to establish minimum time designs for *in situ* degradation studies of forage and concentrate dry matter

Feed	Incubation times (h)[1]															
	0	3	6	9	12	18	24	30	36	48	72	96	120	144	168	240
Forages	×	×	×	-	×	-	×	×	-	×	×	-	×	-	-	-
Concentrates	×	×	×	n	×	-	×	×	-	×	×	×	-	-	n	n

[1] (×), times selected; (-), omitted times. (n), not evaluated.

Using a minimum time design based on nine incubation times converges to the recommendation of Mertens (2005), who affirmed that the estimability of degradation profiles could be assured using at least three incubation times for each parameter in the model. The model used to define the minimum incubation schemes consisted of only three parameters. Thus, based on those statements, if the applied model is composed for more than three parameters, the minimum time designs suggested here could be altered by adding more incubation times.

The main difference among the minimum time designs proposed in this study and those highlighted in the literature for non-tropical conditions relies on the value of the longest incubation time. In our work, 96 and 120 h were recommended for studying the DM degradation of concentrates and forages, respectively (Table 1).

The NRC (2001) standard procedures for CP degradation studies are based on maximum incubation times of 48 and 72 h for concentrates and forages, respectively. Moreover, the time designs proposed by Mertens (2005) for rapidly and slowly digesting feed components are based on maximum incubation times of 64 and 96 h, respectively. If applied to the feeds here evaluated, both proposals would produce biased degradation profiles.

Taking proper information at the end of the degradation process is critical to accurately estimate the extension of degradation (Mertens, 2005). In this sense, using very short times may lead to the overestimation of undegradable fraction. Feeds produced under tropical and non-tropical conditions are likely to differ in terms of ruminal degradation dynamics. This difference seems to support the need for longer incubations periods for the feeds produced in the tropics.

Table 2 Estimates of the degradation parameters of the dry matter, crude protein, and neutral detergent fiber of the different feeds considering the complete set of incubation times and the minimum time designs

	Complete time set				Minimal time design[1]				P value[2]
Feed	A	B	kd	$\sigma^2\varepsilon$	A	B	kd	$\sigma^2\varepsilon$	
Sugarcane	48.5	26.1	0.017	1.9	48.7	26.9	0.016	2.5	0.151
Corn silage	47.3	39.2	0.026	4.6	47.7	38.4	0.026	5.6	0.224
Tifton hay	21.7	54.2	0.027	8.7	22.4	54.2	0.026	11.9	0.118
Soybean meal	32.1	69.1	0.049	48.8	31.9	72.7	0.044	42.2	0.690
Corn grain	28.5	71.2	0.049	19.7	28.3	73.3	0.046	23.3	0.263
Soybean hulls	22.8	76.3	0.028	27.1	23.4	78.6	0.026	26.1	0.544
Cottonseed meal	33.5	53.9	0.030	16.9	32.2	55.3	0.031	15.2	0.642

[1]A, soluble fraction (g/100 g); B, insoluble and potentially degradable fraction (g/100 g); kd, degradation rate of the insoluble and potentially degradable fraction (h^{-1}); $\sigma^2\varepsilon$, residual variance. [2] P value for the comparison between residual variances obtained with the complete and minimum designs.

Conclusions

The minimal time designs to study *in situ* DM degradation in tropical feeds are: 0, 3, 6, 12, 24, 30, 48, 72, and 96 h for concentrates, and 0, 3, 6, 12, 24, 30, 48, 72, and 120 h for forages.

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Variations in the procedures for quantification of crude ash in animal feeds

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Introduction

The term crude ash refers to the inorganic residue after complete oxidation of organic matter (OM) in a sample. Sometimes, that residue should be more properly named as a residue on ignition (Thiex et al., 2012) when oxidation is mostly provided by burning. Crude ash plays an important role in the nutritional interpretation of animal feeds by allowing the indirect estimation of total OM as well as feed components quantified by difference, such as non-fiber carbohydrates. The ashing procedure can be simplifiedly described as the submission of a sample to a physical binomy based on temperature and time. However, sometimes a small part of the sample OM seems to be refractory. For this reason, ashing aids have been incorportated to the methods aiming at optmizing the refractory OM elimination. However, despite of all the current theoretical knowledge on the dry ashing process, the recommendations regarding laboratory procedures are still highly variable. Possibly, a "perfect" standard procedure cannot be achieved for all feed materials. Notwithstanding, the efficiency of variations in the procedures must be checked in order to assure adequate levels of method robustness while keeping an optimal capacity to discriminate between samples with different chemical characteristics.

Materials and Methods

Eight different feeds were chosen aiming to compose a representative set regarding diets offered to beef and dairy cattle: corn silage, fresh sugarcane, sugarcane silage, Tifton-85 hay, soybean meal, corn grain, wheat bran, and dried distillers grain (DDG). For each feed, three different field samples were used, totalling 24 feed samples. The high-moisture samples were oven-dried (55°C) and, along with the other samples, were ground in a knife mill to pass through a 1-mm screen. The samples were then individually analyzed for dry matter (DM) content (dried overnight at 105°C).

The so-called control ashing procedure herein was based on the official method M-001/2, the standard procedure of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2021) where samples are ignited in a furnace at 550°C for 3 h. The following variations in the basic procedure were evaluated: 1. increasing ignition temperature to 600°C; 2. increasing ashing time to 6 h; 3. using fresh air as an ashing aid between two ignition cycles of 3 h each (550°C); 4. using fresh air and water as ashing aids between two ignition cycles of 3 h each (550°C); 5. using fresh air and hydrogen peroxide as ashing aids between two ignition cycles of 3 h each (550°C). Each ashing run contained all 24 feed samples. We performed three ashing runs for each method, totaling the evaluation of 432 aliquots (i.e., test portions).

The crude ash contents were analyzed through a model including the fixed effect of feed, the random effect of sample nested to each feed, the fixed effect of method variation k, the fixed effect of interaction between feed and method variation, and the random error. Means were grouped using the Fisher's multiple comparison procedure. After the first analysis of

variance, data was analyzed again in an independent way for each method variation using a model that included the random effect of feed, the random effect of sample nested to feed, and the random error. From the second analysis, we estimated the repeatability inherent to each method variation. All statistical evaluations were performed by using the GLIMMIX procedure of SAS 9.4. Statistical significances were declared at $P < 0.05$.

Results and Discussion

There was an interaction ($P<0.01$) between feeds and method variations on the crude ash contents. The slicing of this effect indicated that for most feeds (corn grain, DDG, wheat bran, grass hay, and fresh sugarcane) the method did not interfere ($P\geq0.23$) on the values of residues on ignition. However, the crude ash estimates differed according to the method variation ($P<0.01$) for soybean meal, corn silage, and sugarcane silage (data not shown). On average, the overall mean comparisons indicated that increasing either temperature or ashing time caused a consistent decrease in crude ash content when compared to the control method ($P>0.05$, Table 1). In terms of ashing aids, the simple introduction of fresh air was not enough to decrease ($P>0.05$) crude ash content in comparison to the simple increase in either temperature or time. However, using liquid ashing aids caused an additional decrease ($P<0.05$) in ash contents compared to the other method variations.

Table 1 Overall least square means for the crude ash contents and repeatability (i.e., within-laboratory variation) according to the variation in the laboratory procedures (values were averaged from obtained from eight feeds, three field samples per feed, and three test portions per sample; n = 72/method variation)

		Method							
	Temp. (°C)	550	600	550	550	550	550		
	Time (h)	3	3	6	3+3	3+3	3+3		
Feed	Ashing aid	-	-	-	Air	Air+H_2O	Air+H_2O_2	SEM	P value
Crude ash (% DM)[a]		4.24a	4.21b	4.20b	4.18b	4.15c	4.15c	0.228	<0.01
Repeatability (%)		2.13	2.73	2.80	2.12	1.50	1.24	-	-

[a] Means followed by different letters differ at $P<0.05$.

The results here obtained brought evidence that the physical binomial 550°C × 3 h is not the best option for estimating the crude ash content in feed samples. An ideal ignition temperature should be as low as possible intending to reduce volatile compound losses, yet high enough to ensure total carbon loss. A recurrent issue associated with excessive temperatures is the loss of minerals by volatilization. From this, it could be speculated that increasing the temperature to 600°C could have decreased ash content because of increased volatilization. However, by considering that the simple extension of either time or temperature led to the same average decrease in crude ash, the most probable cause was an improvement in the elimination of some refractory OM, rather than an increased volatilization. Once more, the inadequacy of the control procedure is evidenced.

The simple fresh air supply was not enough to bring down crude ash contents at the same levels observed when liquid aids were applied. It has been stated that a fresh air supply

between two ignition cycles could renew the oxygen supply inside the muffle furnace and, consequently, improve the releasing of carbon that might remain in the sample after the first ignition cycle (Thiex et al., 2012). However, our results did not support that statement.

Further decreases in crude ash estimates were obtained only when liquid ashing aids were added between the two ignition cycles. During the ashing procedure, a heavy layer might be formed on the top of the sample interfering with carbon release (Liu, 2019). Then, adding liquid aids between ignition cycles might improve OM decomposition by a mechanical act, crushing the crust eventually formed at the previous ignition cycle and improving the degradation of refractory compounds in the second ignition cycle. On the other hand, hydrogen peroxide could accelerate OM combustion in dry ashing methods by providing reactive free radicals (Pojić et al., 2015). However, we did not observe differences between using water and hydrogen peroxide as ashing aids, indicating that their effects were similar and most likely associated with the physical breaking of the heavy layer in the top of the sample. That breaking further increased organic matter elimination from bottom layer during the second ignition cycle. Considering the similarity of both liquid ashing aids, water is recommended, mainly because of its lower cost.

A desirable method for analyses should be both precise and accurate. Particularly, precision in our study was represented by repeatability (i.e., as within-laboratory variation). All method variations exhibited adequate repeatabilities. However, repeatability was further improved by using liquid ashing aids (Table 1). Such a pattern reinforces our previous discussion. Besides overestimating crude ash contents, the amount of organic matter retained in the bottom layer of the samples seems to be variable among test portions. Thus, the action of the liquid ashing aids also improves the precision of the procedures by decreasing random variation among replicates.

Conclusions

The method to obtain residues on ignition in animal feeds based on the binomial 550°C × 3 h has not enough robustness and may overestimate crude ash contents. Adequacies in either ashing time or temperature can improve crude ash estimates, but the best results are obtained by using liquid ashing aids between two ignition cycles. The method recommended here is based on using 550°C and two ignition cycles of 3 h with water addition over the sample between them.

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Nitrite degradation during early silage fermentation

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Introduction

Sodium nitrite has proven to be an effective silage additive, inhibiting certain bacteria during the initial phase of the ensiling process. The nitrite degradation process can take several paths depending on various parameters. The bacterial respiratory denitrification process, creating nitrous oxide (N_2O) (Simon, 2002), is a common path, although it is active at higher pH values than are usually found in silage fermentation. If enterobacteria are present and the pH is above 4.5, dissimilatory reduction is possible, converting nitrite to ammonia (NH_4^+) (Simon 2002). If the pH is lowered below 6, a chemical reaction is possible, converting nitrite into nitrite oxide (NO) gas (Spoelstra, 1985). These possible processes are well known, although not fully analysed in the early stages of silage preparation. Among others, Wieringa (1966) and Kaiser & Weiß (2007) showed that the nitrate level in the crop will affect the silage fermentation, as confirmed by Knicky et al. (2017), although very few researchers (e.g., Knicky & Spörndly, 2009) have presented results concerning nitrite content and nitrate and ammonia development in early fermentation and whether there are any relevant interactions. This study suggests a method and analytical practice for future experiments to identify the various processes that nitrite and nitrate act on and to analyse the levels remaining after various ensiling processes.

Materials and Methods

Fresh forage was cut with a forage harvester to a theoretical cut length of 2–3 cm in western Sweden on 1 June 2021. The crop consisted of 90% meadow fescue and 10% red clover. The crop was dried for 15 h, reaching 24% dry matter (DM) with 155 g/kg DM in water-soluble carbohydrates (WSC) and 191 g/kg DM in protein. The forage was packed in 2.2-L glass jars (silos), each with a water trap, prepared with aeration holes at bottom and top. Aeration was conducted every second week during the fermentation process. The trial consisted of three treatments with seven replicates each. Four replicates were opened on days 2, 6, 13, and 23, while the three last were opened on day 230 (data not shown). The treatments were: i) untreated control ("Control"), ii) 1893 ppm nitrite (NO_2^-) ("Nitrite"), and iii) positive control based on chemical additives with 1893 ppm nitrite (NO_2^-) and other conserving additives as sorbate, benzoate and propionate ("Salt").

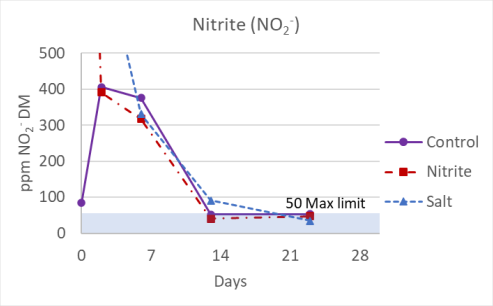
According to Spörndly et al. (2016), heat-drying the samples during sample preparation can result in 90% nitrite loss and 36% nitrate loss. It is therefore very important to prepare the samples without heating or drying. A method for measuring nitrate and nitrite directly from the press liquid was used. Press liquid is prepared by adding 100 g of distilled water to 100 g of silage, resting the mixture for 10 h in a fridge, and extracting the liquid with a hydraulic press at 4 bars.

Nitrite and nitrate in the press liquid are measured spectrophotometrically (Hach DR 1900). According to Bedwell et al. (1995), spectrophotometric and the ion-selective electrode methods are advantageous. Diazotization is the spectrophotometric method for nitrite determination, while the 2.6-dimethylphenol method is used for nitrate. Ammonia is also measured spectrophotometrically (Hach DR 1900). At pH 12.6, ammonium ions react with

hypochlorite and salicylate ions, with nitroprusside sodium as the catalyst for indophenol blue, which is then measured photometrically. Statistical analyses were performed using R-package. Differences between treatments were calculated with t-test ($p<0.05$).

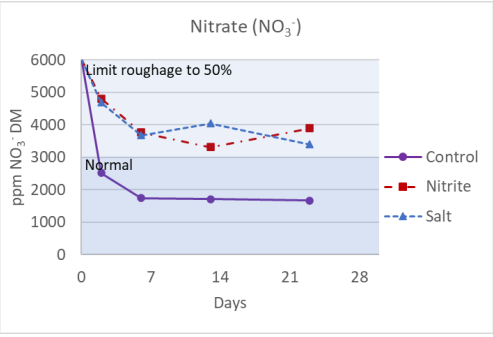
Results and Discussion

Endogenous nitrite was 85.4 ppm DM at start of trial. After addition of 1893 ppm DM nitrite, the nitrite level declined rapidly, reaching the same level as in "Control" after 2 days for treatment "Nitrite" and after 6 days for treatment "Salt" (see Fig. 1). After 23 days, the nitrite levels were below 50 ppm DM in "Nitrite" and "Salt" and at 53 ppm DM in "Control" (see Table 1). Endogenous nitrate was high at 6019 ppm DM. It rapidly declined in "Control," indicating that nitrate is active in the initial fermentation process. In contrast, for treatments "Nitrite" and "Salt" the nitrate did not decrease as quickly, indicating that nitrite is directly active in the reduction process, making nitrate breakdown less active, see figure 2 and table 2.



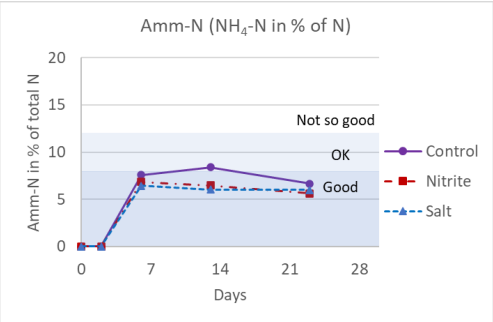
Day:	0	2	6	13	23
Control	85,4	406a	376a	52,1a	52,9a
Nitrite	1978	391a	317a	40,4b	46,8a
Salt	1978	803b	331a	90,7c	34,1a
SED**		100	32	21,7	8,2

Figure 1 Nitrite development during early fermentation. **Table 1** Nitrite (ppm NO_2^- DM) in silage.*



Day:	0	2	6	13	23
Control	6 019	2 522a	1 738a	1 714a	1 670a
Nitrite	6 019	4 805b	3 769b	3 317b	3 888b
Salt	6 019	4 693b	3 675b	4 046b	3 395b
SED**		477	632	712	864

Figure 2 Nitrate development during early fermentation **Table 2** Nitrate (ppm NO_3^- DM) in silage.*



Day:	0	2	6	13	23
Control	0,02	0,03	7,60a	8,39a	6,67a
Nitrite	0,02	0,03	6,87a	6,50ab	5,65a
Salt	0,02	0,03	6,43a	6,06b	5,99a
SED**			0,9	0,5	1,1

Figure 3 Ammonia development during early fermentation. **Table 3** Ammonia-N (% NH_4^+-N of Total N) in silage.*
*Values within columns with different letters are significantly different at $P < 0.05$.
**SED = Standard error of difference.

Ammonia was more or less absent in the first days of fermentation (see also Shao et al. 2002), but rapidly increased to about 7% NH_4^+-N of total N after 6 days. No apparent differences in ammonia levels could be seen between "Control" and the "Nitrite" and "Salt" treatments after 23 days see figure 3. DM weight losses were highest in "Control" for the first 2 days (see Fig. 4), but after that were very similar in all treatments. The same applies to the pH development: starting at 6.5, pH reached 4.3–4.5 after 2 days and thereafter declined only slightly, indicating that in silage at 23% DM, most fermentation occurs in the first 2 days. As ammonia was produced only after 2 days, the initial degradation of both nitrite and nitrate could not have been caused by the dissimilatory reduction of nitrate or nitrite. The degradation must instead have been due to other biological processes or, in the case of nitrite, a chemical reaction. After 2 days, there was still some nitrate degradation as well as ammonia production, which could indicate dissimilatory reduction. To explore this further, future trials must also take account of the presence of enterobacteria.

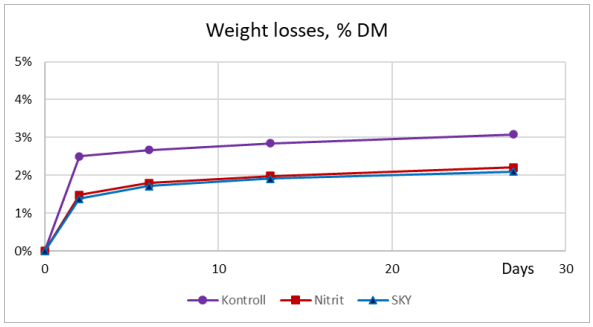


Figure 4 Dry matter losses (%).

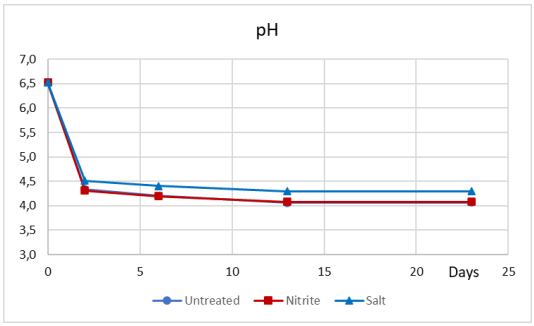


Figure 5 pH development.

Conclusions

The method clearly reveals the development of nitrite, nitrate, and ammonia during early silage fermentation. In low-DM crops, the analyses must be conducted earlier, after days 1, 2, and 4 days, and be complemented with enterobacteria counts as well as WSC, lactic acid, and acetic acid determinations to fully capture changes in substrates and end products. The final nitrate levels are below the threshold. To obtain full information about the nitrite levels before intake, analyses should be conducted after a TMR is mixed, as the TMR ingredients and oxygen level will affect the nitrite remaining after the fermentation process. Furthermore, differences between silo and bale fermentation could also be analysed.

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Method of measuring stability and losses in a total mixed ration

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Introduction

In warm summer months, total mixed rations (TMRs), are exposed to high temperatures causing silage and other feed materials rapidly becoming degraded. The degradation process usually begins with an increase in the presence of yeast, which primarily consumes sugars aerobically to form carbon dioxide and water. This is often noticed as an increase in temperature. Losses in dry matter (DM) can be significant in a short time.

In a series of three experiments, we have tested a method for measuring TMR durability and DM losses in order to estimate the aerobic stability and total DM loss during roughage production. To examine differences in heating and DM losses, experiments were conducted with several treatments comparing untreated TMR "Control" to TMR treated with the chemical additive "Salt" and with additives based on acid in solution "Acid" (Trials 1&2) and as a dry product "Acid-powder" (Trial 3).

Materials and Methods

TMRs were obtained from farmers in western and southern Sweden. The mix in Trials 1 and 2 consisted of about 50% grass silage mixed with corn silage and beet pulp on wet matter basis. The DM content of the TMR was 37,9% and the initial pH was 4.1. Trial 3 consisted of 60% grass silage (DM 55%), 7% rolled grain, and the rest a mixture of protein concentrates and minerals blended with 29% water. The DM content of the TMR was 39,1% and the initial pH for Trial 3 was 5.5.

Each TMR was mixed thoroughly in the lab for each trial and divided into three or four equal parts. The additives were sprayed onto the mix in a large plastic bag and mixed thoroughly. The dosage was 2 L/metric ton FM (L/mt) of mixed feed for "Salt" and 4 L/mt for "Acid" according to the manufacturer's recommendation; water (4 L/mt) was added to the "Control". Each TMR treatment was performed in two replicates.

Two kg of each replicate was packed loosely in a 9-L thermos allowing air passage at the top and bottom to let carbon dioxide out and oxygen in. The thermoses were placed in polystyrene blocks to ensure correct temperature measurements. Temperature sensors were placed in the middle of the thermos to log the temperature for 5 days. The temperature probes, Dallas PRO, were calibrated from start and the temperatures were logged every minute. The criteria for aerobic stability was an increase in temperature of 3^{o}C. Analyses were done from start, day 0, and the thermoses were opened after days 1, 2, and 3 and a small amount (30 g) of TMR was taken out to determine the yeast count and pH. After 5 days, the experiment was terminated and the thermoses were weighed and measured to determine DM content, pH and yeast count. The DM content was measured by oven drying for 18 h at 65°C (not shown). The pH was measured after mixing 30 g of silage with 270 g of water and allowing the mixture to stand for 2 h (data not shown). Weight DM loss was measured continuously on a KERN scale with a precision of 0.5 g. Yeast was measured on agar plates using malt extract with penicillin G 13752-1G-F (30 mg/L agar) and streptomycine sulphate S6501-5G (30 mg/L agar) (Sigma-Aldrich). For low-pH TMRs, lactic acid was added to the

agar plate until the pH was 3.8; for higher-pH TMRs, no adjustment was made on the agar plate.

To determine the yeast count, 30 g of silage was mixed with 270 g of distilled water and mixed for 15 min. The concentration on each plate was chosen so that the outcome was expected to be 30–300 cfu/g. The plates were cultured at about 24–26°C for about 3 days aerobically. To calculate the yeast count on plates, methods described in ISO 21527-1.

Results and Discussion

Trials 1 and 2 developed almost similar, so figures for Trial 2 are shown only. In Trial 1 aerobic stability lasted for 10, 41 and over 120 h and in Trial 2 for 21, 37 and over 120 h for "Control", "Acid", and "Salt", respectively (Fig. 1a). In Trial 3, aerobic stability lasted for 14, 16, 22, and 24 h for "Control", "Acid-powder", "Acid" and "Salt", respectively (Fig. 1b). In Trial 2 the yeast count was 5.3 log cfu/g at start and the "Acid" treatment immediately lowered the yeast count to 3.5 and "Salt" lowered it to 4.9 log cfu/g (Fig. 2a). In both "Control" and "Acid", the yeast count increased from days 0 to 3, while in "Salt" it decreased. In Trial 3, the yeast count increased in all treatments from days 0 to 3 (Fig. 2b), increasing the most in "Control" and the least in "Salt".

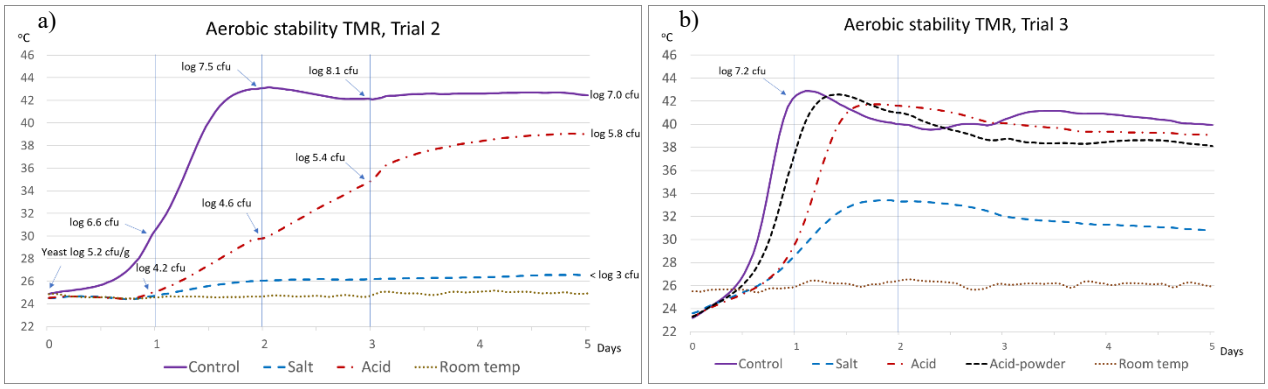

Figure 1 Aerobic stability TMR, temperature, and yeast count vs. days: a) Trial 2, b) Trial 3.

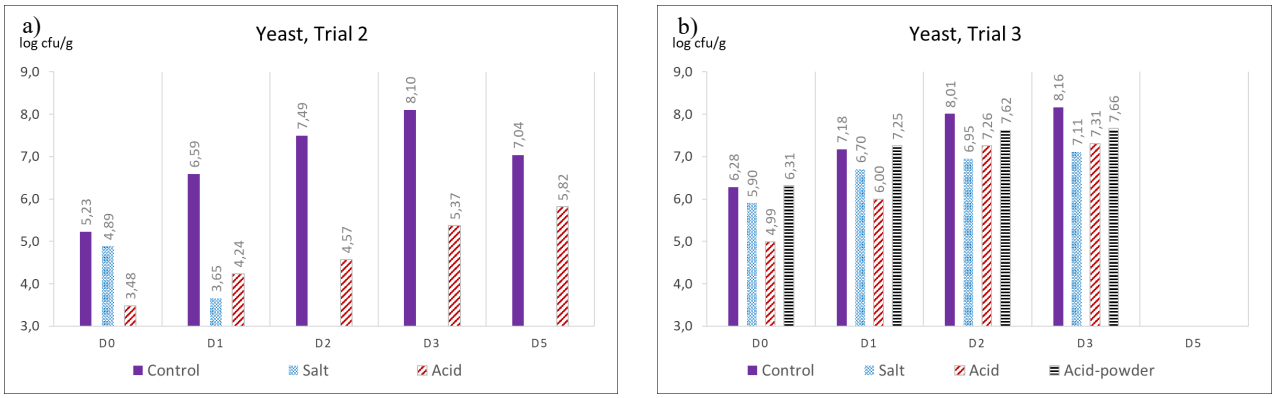

Figure 2 Aerobic stability TMR, yeast count: a) Trial 2, b) Trial 3.

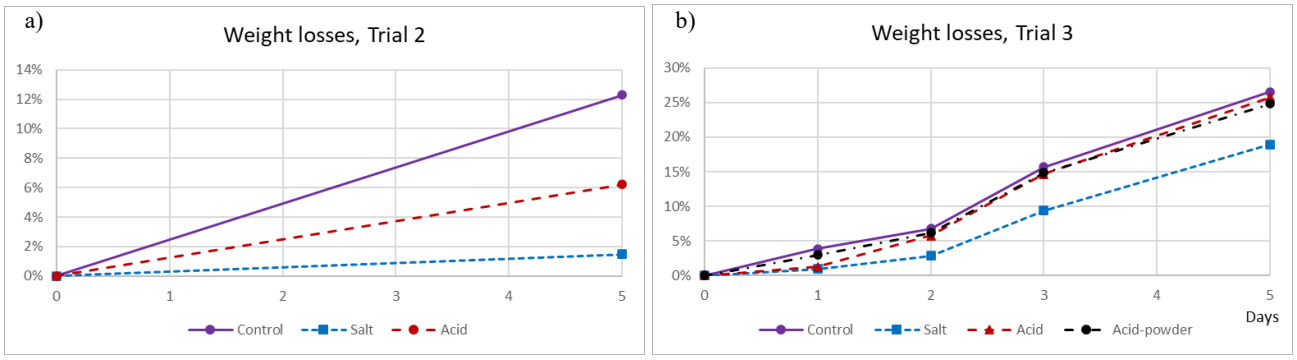

Figure 3 Dry matter losses: a) Trial 2, b) Trial 3.

In Trial 2, the DM losses were only measured after 5 days, where "Control" had over 12% DM loss, "Acid" 6%, and "Salt" below 2%. In Trial 3, the DM losses after 5 days were 26.5, 25.8, 24.8, and 18.9% in "Control", "Acid", "Acid-powder" and "Salt", respectively. The losses after 2 days were considerably lower, between 2.9% for "Salt" and 6.8% for "Control".

Conclusions

The temperature increase was the parameter that most clearly indicated TMR degradation, and the yeast count was also a good indicator. The degradation after 1 day in "Control" resulted in a bad smell and the yeast count was 6.6 and 7.2 log cfu/g in Trial 2 and 3. In the "Acid" treatment, the yeast count was 4.6 log cfu/g after 2 days and 5.4 log cfu/g after 3 days in Trial 2. Thus, the yeast count was not as clear an indicator as was temperature, although, it was still useful. The pH changed more slowly (data not shown) and was not as clear an indicator, though it is important to consider in order to monitor the degree of degradation. The DM loss was also a valuable parameter indicating the decomposition of the mix.

For further trials, temperature should of course be measured continuously, as it is the parameter that first indicates degradation. We recommend a trial length of 3 days, as most feed is consumed after 1–2 days, with DM losses, pH, and yeast counts being carefully measured on days 1, 2, and 3. If silage is well fermented (low pH) and/or high in sugar, measurement after 0.5 day would be interesting. For nutritional purposes, it would be interesting to perform a full chemical analysis after 1 day, to detect changes in protein, ammonia, fatty acid, and energy.

It should be considered that no statistical analyses were performed to justify the significances of the results in this experiment.

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ISO 21527-1. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0.95.

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Simplify the analysis of feed and food quality – the role of Gas Endeavour® system

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Introduction

For the purpose of feed and food evaluation, *in vitro* digestion and fermentation methods, which are often used as screening method prior to *in vivo* methods, are faster and less expensive than *in vivo* techniques. A good example of an *in vitro* fermentation method is the *in vitro* gas production technique, in which the gas evolved as a result of fermentation is used as the primary measurement. The method utilises the relationship between feedstock degradation and fermentative gas production to evaluate the nutritional parameters of the feed. The benefits of *in vitro* gas production techniques for digestibility evaluation is the ability to run large batches simultaneously at a low cost, the ability to measure fermentation kinetics of soluble and insoluble fractions of feeds or foods, and the ability to easily make relative comparisons among samples.

Gas Endeavour® for *in vitro* digestibility assays

The Gas Endeavour® (Figure 1) is a premier automatic instrument from BPC Instrument AB, Lund, Sweden (formerly Bioprocess Control) for continuous monitoring of fermentation gas (i.e., hydrogen, methane, carbon dioxide) released in *in vitro* ruminant digestive models and *in vitro* monogastric hindgut digestive model for monogastric animals and humans. The Gas Endeavour® is a novel platform for analysing low gas volumes and flows whenever there is a demand for high accuracy and precise measurements. In comparison with the other *in vitro* methods measuring the transformation of fermentable substrate, the Gas Endeavour® allows highly accurate analysis for a large number of samples in a short time. In addition, the automated *in vitro* protocol based on the Gas Endeavour® significantly reduces the workload compared with manual analyses.

Figure 1 An example of Gas Endeavour® system configuration with 15 parallel test lines.

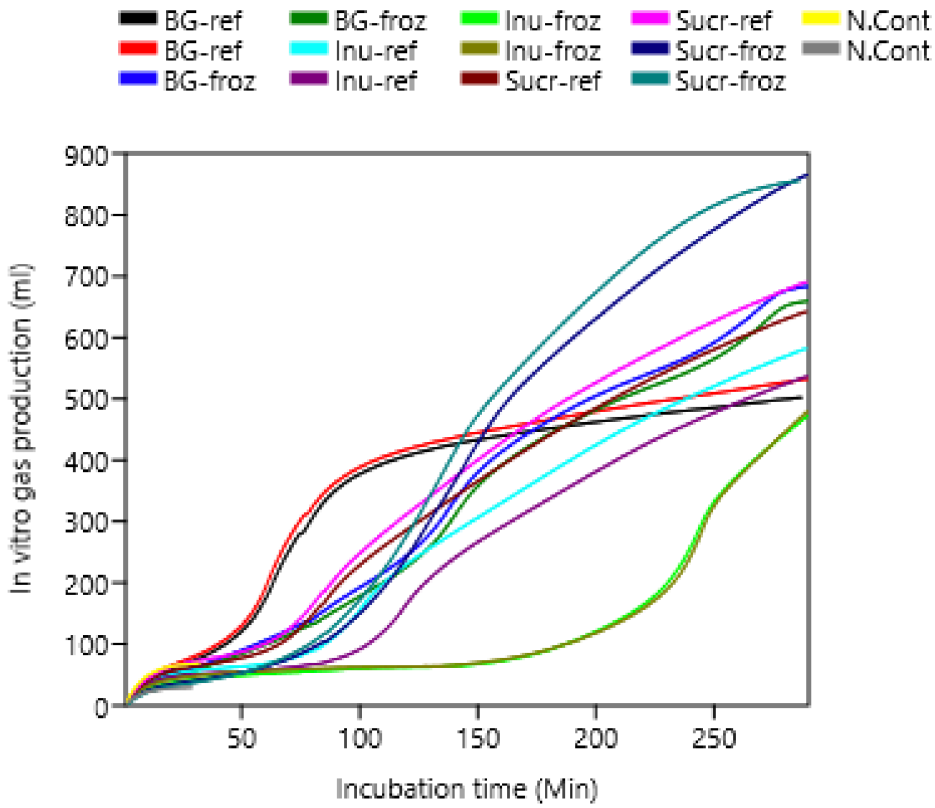

Figure 2 An example of gas production curves monitored by Gas Endeavour® data processing unit: cumulative gas production profiles (ml) within time (min) from an *in vitro* trial during 24 hours of incubation period (Muragijeyezu, 2020).

Gas Endeavour® has been considered in different studies as an automated gas measuring system. It has been used as an *in vitro* digestibility assay to perform various batch fermentation analysis. Hindgut fermentation in monogastric animals, ruminal digestion (Muragijeyezu, 2020; Matteo, 2020; Elgemark, 2019) and fermentation in human fecal cultures (Adlercreutz et al 2018) are few examples of area, in which Gas Endeavour® has been successfully applied for continuous monitoring of gas production.

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In vitro digestibility analysis **made easier** with the Gas Endeavour

The Gas Endeavour allows users to measure low gas volume and flow, whenever there is a demand for accurate and precise measurments. This smart analytical insturment can be used for both research and industrial applications in animal nutrition studies, reducing labour demands and making *in vitro* digestibility analysis easier.

Visit us at our stand to see the Gas Endeavour in action!

A smart instrument for continuous and simultaneous gas volume, flow, and composition analysis.

- Real-time gas volume monitoring with high accuracy and precision
- A cost and time efficient solution for the simultaneous analysis of multiple sample

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