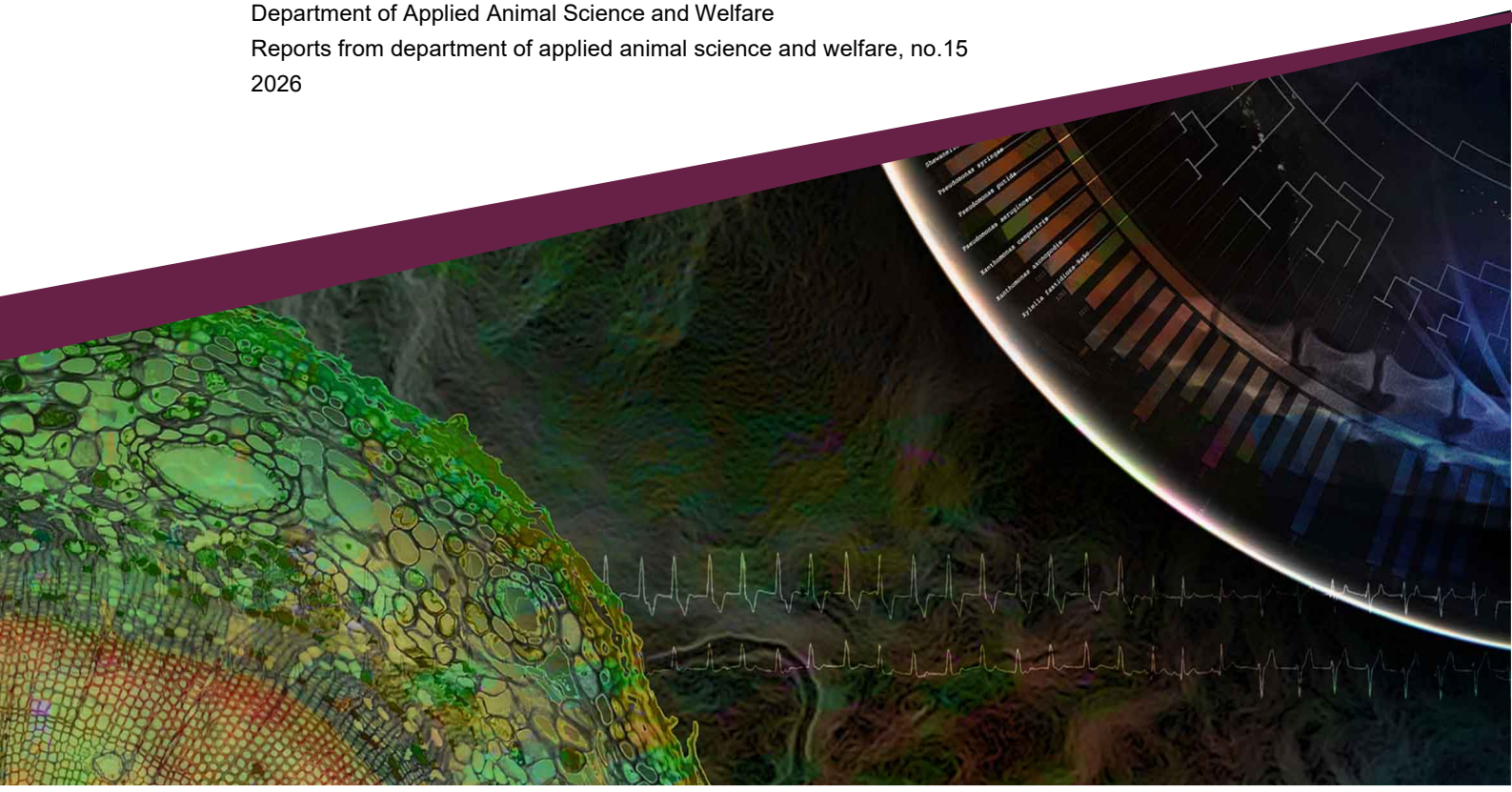
Proceedings of the 13th Nordic Feed Science Conference, Uppsala, Sweden

Nordic Feed Science Conference

June 9 - 10, 2026

www.slu.se/nordicfeedscienceconference

Swedish University of Agricultural Sciences, SLU
Department of Applied Animal Science and Welfare
Reports from department of applied animal science and welfare, no.15
2026



Proceedings of the 13th Nordic Feed Science Conference, Uppsala, Sweden

Proceedings of the 13th Nordic Feed Science Conference, Uppsala, Sweden

Editor

Egil Prestløkken

Reviewers in addition to Organising Committee

Karl Heinz Südekum
Ingunn Schei
Johanna Karlsson
Ghulam Qasim Khan
Mogens Larsen
Alemayehu Kidane
Mikaela Lindberg
Ingjerd Dønnem
Cecilia Müller

Organising Committee

Rasmus Bovbjerg Jensen
Meelis Ots
Egil Prestløkken
Marketta Rinne
Bengt Ove Rustas
Martin Weisbjerg

Publisher:	Swedish University of Agricultural Sciences, Department of Applied Animal Science and Welfare
Year of publication:	2026
Place of publication:	Uppsala
Title of series:	Reports from Department of Applied Animal Science and Welfare
Part number:	15
ISSN (Print):	2004-9803
ISSN (Online):	2004-934X
ISBN (Print):	978-91-8124-317-8
ISBN (Online):	978-91-8124-318-5
DOI:	https://doi.org/10.54612/a.f10r94e3pp
Keywords:	Nordic Feed Science Conference

© 2026 SLU

This publication is licensed under CC BY NC 4.0, other licences or copyright may apply to illustrations.

Foreword

We are proud of the fact that we can get together for the 13th Nordic Feed Science Conference. This year we have several contributions concerning nutrition of and feed for horses. We are glad for that and hope this will continue in coming conferences.

In total we have received 47 written contributions this year with the following distribution: 6 on Feed evaluation systems for horses and ruminants, 10 on Ruminant and horse nutrition, 13 on Environmental impact, 7 on Forage quality and conservation, and 11 on Novel feeds and miscellaneous.

Feed evaluation concerns researchers as well as practitioners and three of the invited speakers will address this topic. Rasmus Bovbjerg Jensen, Associate Professor at Aarhus University, will give a presentation on energy and protein evaluation of horse feeds in the Nordic countries. Nicolaj Ingemann Nielsen, Chief Consultant at SEGES Innovation, will talk about status and future of NorFor, the Nordic feed evaluation system. Marketta Rinne, Research professor at Natural Resources Institute Finland (Luke), will present the Finnish feed evaluation system and how it serves the livestock sector. Another important area of research is the activity of rumen microbes, affecting efficiency of livestock and their environmental footprint. Velma Tea Essi Aho, Researcher at the Norwegian University of Life Sciences, will give a talk on how rumen metabolism can be studied through microbiomes and meta-omics.

The organising committee this year included representatives from the Nordic countries and Estonia. This has been fruitful and hopefully it will continue in coming conferences.

A special thank this year goes to Egil Prestløkken, who has carried a heavy load to put the conference proceedings together.

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/nordicfeedscienceconference-proceedings

Uppsala 2026-06-02

Bengt Ove Rustas

List of contents

Session I - Feed evaluation systems for horses and ruminants

Energy and protein evaluation of horse feeds in the Nordic countries 6
R.B. Jensen & C.E. Müller

NorFor – status and future development 9
N. I. Nielsen, M. Åkerlind & J. Karlsson.

The Finnish feed evaluation system serves the livestock sector 14
M. Rinne, J. Vattulainen & K. Kuoppala.

A three-stage procedure for determining metabolisable energy of feeds for dairy cattle 21
Karl-Heinz Südekum

Update of NorFor values for beets and beet silage 24
E. M. V. Hvas, M. Larsen, N. I. Nielsen, H. Martinussen & M. R. Weisbjerg

The effect of processing protein feedstuffs to increase AAT in dairy cow diets in the NorFor system– a meta study focusing on milk production responses 27
I. Schei, Sommerseth, J.K., Åkerlind, M., Kjeldsen, A.M., M., H. Volden &N.I Nielsen

Session II - Ruminant and horse nutrition

New windows into rumen metabolism through microbiomes and meta-omics 30
V. T. E. Aho, A. O. Maynez-Perez, C. M. Kobel, A. Schwarm & P. B. Pope

Risk potential of different diets for rumen and hindgut acidosis in dairy cows 36
Matthias Schilde & Wilfried van Straalen

Effects of reduced concentrate feeding on rumen fermentation and methane production in early lactation dairy cows 39
T. Kokkone, N. Rummukainen, S. Salin, P. Rissanen, M. Kemppainen, S.E. Räisänen, M. Hovinen, A. Halmemies-Beauchet-Filleau, M. Lamminen & A. Vanhatalo

Dietary AAT calculated in NorFor and its effects on responses in ECM- and protein yield in dairy cows 42
J. K. Sommerseth, I. Schei, M. Åkerlind, A. M. H. Kjeldsen, H. Volden & N. I. Nielsen

Reed (Phragmites australis) silage for Icelandic horses- intake and digestibility 47
W. Ashworth, M. Connysson, Ö. Östman & C. E. Müller

Individual and group level production responses to rapeseed meal supply 50
T. Karhula, S. Kajava & A. Sairanen

Rumen fermentation pattern of dairy cows fed grass-clover silage or grazing on pasture 53
D. W. Olijhoek, N. P. Hansen, M. R. Weisbjerg & M. Larsen

Effects of silage digestibility and diet concentrate level on performance of finishing bulls 56
K. Manni & A. Huuskonen

Effects of decreasing silage particle size by a screw press on rumen passage rate and digestion in dairy cows 59
B.O. Rustas, M. Managos & T. Eriksson

Effect of harvest time of ensiled reed on intake and digestibility in dry dairy cows 62
W. Ashworth, Ö. Östman & B.O. Rustas

Session III - Environmental impact

Excretion calculations of horses in Finland 65
J. Vattulainen, H. Högel & M. Saastamoinen

Enteric methane emission in dairy cows on early vs late summer pasture and effect of 3-nitrooxypropanol 68
E. Økland, T. Undheim, A. Kidane, A. Schwarm, A. Perez & E. Prestløkken

CO2 prediction model based on an international dataset with lactating dairy cows, intended for usage in on-farm settings 71
M. Kjeldsen, M. Johansen, M.R. Weisbjerg, A.L.F. Hellwing, et al.

Cow efficiency and diet effects on milk production and methane emissions 75
M. Vadroňová, P. Fant, K. Kozicka, P. Huhtanen & M. Ramin

Evaluating equations predicting enteric methane emissions from lactating dairy cows fed grass silage-based diets 78
A. R. Bayat, T. Kokkonen, A. Sairanen & S. Hietala

Predicting enteric methane from growing cattle 81
J. Karlsson, M. Åkerlind, F. Hahn Lau-Jensen & N. I. Nielsen

Comparison of enteric methane emission from rumen cannulated and intact cows 86
D. W. Olijhoek, N. P. Hansen, M. Maigaard, M. R. Weisbjerg, P. Lund & M. Larsen

Methods to estimate daily enteric methane from cows given pulse doses of short-lived methane inhibitors using the GreenFeed system 89
E. Økland, A. Kidane, A. Schwarm & E. Prestløkken

Evaluation of high-purity tribromoethanol (TBE) and rumen fluid from efficient or inefficient cows on in vitro methane production and fermentation pattern 92
A. Alipour, M. Vadroňová & M. Ramin

Evaporation of ethanol from the feed bunk when including sugar beet silage 95
E. M. V. Hvas, J. R. Thyssen & M. Larsen

Tracking atmospheric methane over Swedish dairy farms using multi-year TROPOMI observations 98
K. Kozicka, J. Oliveira, J. Karlsson & M. Ramin

Research on sustainable feed for livestock in Norway: a knowledge summary 101
I. Dønnem, T. Persson, H. Steinshamn, A.K. Uhlen, E. Prestløkken, A.K. Bakken, E. Byers, M. Höglind, L.T. Mydland, Å. Ergon, V. Kvakkestad & H.H. Simonhjell.

Effect of different methane mitigation strategies on milk vitamins B2 and B12 concentration 104
M. H. Kjeldsen, M. Thorsteinsson, C. F. Børsting, M. R. Weisbjerg & S. Lashkari

Session IV - Forage quality and conservation

Harvested forages for horses from a feeding perspective – a systematic literature review 107
S. Ringmark, M. Connysson, K. A. Segerkvist & C. E. Müller

Germination of Echinochloa crus-galli seeds could be reduced by ensiling 110
M. Rinne, I. Leino, M. Franco, P. Ruuttunen & T. Hyvänen

In vitro total gas and methane production of emerging forage species for ruminants 113
N. Ayanfe, B. Micke, D. Parsons & P. Fant

Predicting microbial community in sugar beet silage based on fermentation patterns 116
E. M. V. Hvas, M. Larsen & M. R. Weisbjerg

Possibility of using foam in forage conservation 119
M. Knicky & P. Melin

Ensiling characteristics of intermediate wheatgrass (IWG), Kernza ®: effects of harvest timing and silage additives 122
K. Lätt, F. Dahlström, D.-A. Danielsson, V. Picasso ,O. Hallin & K. Arvidsson Segerkvist

Assessing the energy and protein value of timothy hay harvested at different maturities for horses 125
R. B. Jensen & C. Müller

Session V - Novel feeds and miscellaneous

Beet types high in dry matter have higher sugar proportions of organic matter, diluting other nutrients 128
E. M. V. Hvas, M. Larsen, L. Andersen, U. Bedenk & M. R. Weisbjerg

Determination of water concentration in forage by Karl Fischer titration 131
N. B. Kristensen

Horse preference for silage press cake from green biorefinery compared to lucerne - preliminary studies 135
C. E. Müller, E. Brodin, F. Karlsson Lindgren & J. Yngvesson

Interaction between breed and feeding 138
M. Kargo, C. Rosman & S. Agenäs

In situ and in vitro degradation of concentrates for dairy-beef calves 141
M. R. Weisbjerg, N. P. Hansen, H. Martinussen, K. S. Jensen, C. B. Geven, N. B. Kristensen & M. Vestergaard

Production of grass protein paste for monogastric livestock feeding 144
N. Ayanfe, Q. Wang, T. Stefański, A. Halmemies-Beauchet-Filleau, M. Franco, S. Perttilä, T. Kokkonen, A. Vanhatalo & M. Rinne

Evaluating the feed value of oat varieties using in vitro methods 147
T. Undheim, E. Prestløkken & A. Kidane

Rumen degradation kinetics of grass-clover pulp silage evaluated by in sacco and in vitro methods 150
G. Q. Khan, J. K. Sommerseth & S. Adler

EUnetHorse improves the resilience of horse farms – including grassland management 154
M. Rinne, A. Sironen, A. Yli-Viikari, R. Isomäki, E. Uusivuori, J. Vattulainen, M. Saastamoinen & L. Rantamäki-Lahtinen

Potential of sea buckthorn residues as ruminant feed: rumen degradability and fermentation profiles 157
D. Malenica, M. Ramin, R. Bhat, Meelis Ots, R. Leming, A. Olt & M. Kass

Positive effect of sprouted grains on the fatty acid profile of intramuscular fat in bull meat 160
E. Meškinytė, Ž. T. V. Jukna & O. Ilina

Energy and protein evaluation of horse feeds in the Nordic countries

R.B. Jensen[1] & C.E. Müller[2]
[1]*Aarhus University (AU), Department of Animal and Veterinary Sciences, Blichers Allé 20, 8830 Tjele, Denmark*
[2]*Swedish University of Agricultural Sciences (SLU), Department of Applied Animal Science and Welfare, Box 7024, 75007 Uppsala, Sweden.*
Correspondence: rbj@anivet.au.dk

History

Several feed evaluation systems for horses are currently in use worldwide, many of which integrate both feed evaluation and energy and nutrient requirement systems, thereby providing a coherent framework for balancing dietary supply against requirements. These can broadly be divided into systems such as the American defined by the National Research Council (NRC, 2007), the French system (INRA, 2015), the Dutch system (CVB, 2023), and the German system (GfE, 2014). In 1992, a Nordic working group under "Nordisk Jordbrugsforskning" (NJF), entitled "Feed evaluation and nutrient recommendations for horses", was established with the objective of developing a harmonised feed evaluation system across the Nordic countries (NJF, 1996). The group evaluated existing international systems, including the German, American, and French models. In its 1996 report, the working group recommended adoption of the French energy evaluation system for horses, supplemented with methodological modifications developed in the Netherlands. This system describes energy utilisation from gross energy (GE) to net energy (NE) and expresses energy supply in Feed Units for Horses (FU_{Horse}). Although the Nordic countries had previously applied different national energy units, the recommendation provided a common methodological framework for energy evaluation. For protein evaluation digestible crude protein (DCP) was however retained rather than adopting the French horse digestible crude protein (MADC) system (Austbø, 2004). Despite the fact that approximately 30 years have passed since the NJF recommendations were published, there is still a lack of harmonisation and transparency in feed evaluation for horses in the Nordic countries. This situation is further complicated by the concurrent use of different systems both between and within countries, posing challenges for researchers, advisors, feed manufacturers, and horse owners. These challenges have been highlighted repeatedly over the years. In response, a Nordic network funded by the Nordic Joint Committee for Agricultural and Food Research (NKJ) was established in 2018 to assess recent developments and industry needs, with the aim of identifying pathways towards a common and updated Nordic feed evaluation system for horses. This NKJ network is ending by the end of 2026, with a recommendation of new Nordic feeding recommendations for horses.

Energy

The energy value of horse feeds is expressed as digestible energy (DE) in the American system (NRC, 2007), metabolizable energy (ME) in the German system (GfE, 2014), or as net energy (NE) expressed as feed units for horses (FU_{Horse}) in the French (INRA, 2015) and Dutch (CVB, 2023) systems. Traditionally, FU_{Horse} has been used in Iceland, Norway, and Denmark, whereas ME has been used in Sweden and Finland, as stated in the NJF report from 1996 (NKJ, 1996; Austbø, 2010). In principle, the coexistence of these systems should not pose any major problems, as conversion equations are provided in the NJF report and the systems are described

in the French (INRA, 2015) and Dutch (CVB, 2023) references. However, practical application is challenged by methodological differences. Furthermore, in the French system (also used in the Nordic countries), barley is used as the reference feed when expressing NE as FU_{Horse}, whereas oats are used in the Dutch system. This difference complicates direct comparisons of FU_{Horse} between systems. Furthermore, field-based observations indicate that FU_{Horse} are applied differently both within and between Nordic countries. This variability appears to be linked to the complexity and theoretical nature of the conversion from ME to NE, as well as limited transparency regarding how different stakeholders calculate FU_{Horse} values in practice. From a theoretical perspective, NE should represent the most precise approach for describing both the energy requirements of the horse and the energy content of feeds in the ration. Nevertheless, the current lack of transparency and the use of differing calculation methods for estimating FU_{Horse} highlight the need to reassess and update the recommendations based on the NJF report from 1996. Sweden and Finland have continued to use ME, which may reflect that ME is conceptually easier to understand and less complex to estimate than FU_{Horse}. A recent update in the German system has improved the estimation of ME of feeds by accounting for digestible organic matter, increasing the complexity of the model but also improving biological accuracy (GfE, 2025).

Protein

In horses, amino acids (AA) are almost completely derived from protein digested in the small intestine, and protein evaluation of feeds for horses should ideally be based on small intestinal protein and AA digestibility. However, as with energy evaluation, different protein evaluation systems are currently used worldwide. In the American system, the protein value of feeds is expressed simply as crude protein (CP) (NRC, 2007), whereas the French system expresses the protein value as horse digestible crude protein (MADC) (INRA, 2015), and the Dutch system as digestible crude protein (dCP) (CVB, 2023), thereby accounting for variations in protein digestibility among feeds. The German system represents the most recently updated approach and attempts to account for equine digestive physiology by expressing protein value as pre-caecal digestible crude protein (pcdCP) (GfE, 2015). In the Nordic countries, it was decided in the NJF report from 1996 not to implement the French MADC system, but to use dCP like in the Netherlands (Austbø, 2010). However, transparency regarding this system has been limited, and easily accessible reference material has been lacking. In Sweden, the document “Feeding recommendations for horses” has been used as a practical reference (Jansson, 2011), although the equations used do not fully comply with those provided in the NJF report (NJF, 1996). This has further contributed to inconsistency in protein evaluation and practical feeding recommendations across the Nordic countries.

Future recommendations

Reaching consensus across Europe as well as other parts of the world may be challenging; however, there is a clear need for a common reference system within the Nordic countries if the horse industry is to move beyond the 30-year-old NJF recommendations (NJF, 1996). Such a system must be transparent, biologically relevant, and relatively easy to implement for the different stakeholders in the horse sector. Based on both historical experience and current scientific understanding, it is recommended to move towards a system aligned with the German recommendations, where energy and protein contents of feeds, as well as nutrient requirements of horses, are expressed as ME and pcdCP, respectively. During a transition period, dCP values

could continue to be provided to facilitate implementation and communication. Successful implementation will require support and coordination among key stakeholders, including researchers, advisors, feed manufacturers, and industry organisations. In a research field with limited funding compared to other livestock species, a common system would also facilitate targeted research efforts and improve knowledge transfer. Future research should focus on validation and standardisation of analytical methods for describing the energy (ME) and protein (pcdCP) value of feeds, with particular emphasis on Nordic feedstuffs and feeding practices, where forage traditionally accounts for a large part of the horse's diet. Additional research areas include the effects of feeding level, the associative effects of combining different feedstuffs, the contribution of hindgut fermentation on energy supply, and improved understanding of energy and protein requirements in different categories of horses, including their relationship to health, disease, and feeding management. Importantly, the new Nordic feed evaluation system should be flexible and adaptive, remaining open to new scientific evidence and allowing for continuous updating and further development in the future.

Conclusions

It is recommended to continue and finalise the development of new Nordic feeding recommendations initiated in 2018, resulting in a harmonised, scientifically sound, and practically applicable system for energy and protein evaluation of horse feeds in the Nordic countries, building on the principles of the German energy and protein evaluation system while remaining open to future scientific developments.

References

Austbø, D. 2004. The Scandinavian adaptation of the French UFC system. In: Nutrition of the performance horse - EAAP publication No. 111. (eds. V. Julliand and W. Martin-Rosset), Wageningen Academic Publishers, Wageningen, The Netherlands, p. 69-77.

Austbø, D., 2010. Energy and protein requirements for horses in the Nordic countries. In: Proc. 1st Nordic Feed Sci. Conf., Uppsala, Sweden, p. 47-50.

CVB, 2023. Chemical composition and nutritional values of feedstuffs. Stichting CVB, the Netherlands.

GfE, 2014. Empfehlungen zur energie- und nährstoffversorgung von pferden. DLG-Verlag , Frankfurt am Main, Germany.

GfE, 2025. The three-step procedure for determining the metabolisable energy of feeds for horses. In: Proc. Soc. Nutr. Physiol. 34, 153-167.

INRA, 2015. Equine nutrition. Wageningen Academic Publishers, Wageningen, The Netherlands

Jansson, A., 2011. Utfodringsrekommendationer för hästar. Report 289. Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala.

NJF, 1996. Feed evaluation and feeding recommendations for horses. Report from NJF-working group (in Norwegian).

NRC, 2007. The nutrient requirements of horses. 6th edition. National Academy Press, Washington DC.

NorFor – status and future development

N. I. Nielsen[1], M. Åkerlind[2] & J. Karlsson[2]
[1]SEGES Innovation, 8200 Aarhus N, Denmark. [2]Växa Sverige, Box 288, 751 05 Uppsala, Sweden.
Correspondence: ncn@seges.dk

Introduction

NorFor is a feed evaluation system and ration formulation model for dairy and beef cattle developed in the Nordics. It combines scientifically based biological knowledge of digestion and metabolism with practical ration optimisation in IT-tools, supporting efficient, profitable, and climate-smart feeding strategies.

Organisation and usage

NorFor is jointly owned by farmer-based organisations in four Nordic countries:

- Växa Sverige (Advisory service/R&D, Sweden)
- TINE (Dairy company Norway)
- Landbrug & Fødevarer (Agricultural Industry Association, Denmark)
- Ráðgjafarmiðstöð landbúnaðarins (RML) (Advisory service/R&D, Iceland)

The system was developed through Nordic collaboration starting in the early 2000s. The purpose was to create a common Nordic feed evaluation system to maximize the benefit for dairy farmers. The overall aim of NorFor was to create an identical, common feed evaluation system for the Nordic countries to facilitate communication between farmers, consultants, feed industry and researchers.

NorFor is fully implemented in the four owner countries, where it is used through national advisory software systems (e.g. FoderOpti in Sweden, DMS in Denmark, OptiFôr in Norway and Iceland) for optimizing and evaluating feed rations. These tools integrate the NorFor model into daily advisory work and farm-level decision support.

In terms of scale, NorFor is used on the majority of dairy farms that use feed planning and on many beef farms in Denmark, Norway, Sweden and Iceland. Beyond the owner countries, NorFor is also used internationally, in countries such as Estonia, Latvia, Lithuania, Germany, Croatia, Slovenia, Slovakia, and Serbia. The biological model and feed stuff table is accessible globally through the NorFor Feed Ration Optimizer™ (NFRO), which is an IT tool that allows nutritionists outside the Nordics to apply the NorFor system on farm.

The NorFor system is a model designed to predict digestion of nutrients in the GI-tract by integrating animal characteristics and feed composition to estimate digestion in the rumen and intestines (see Figure 1). The NorFor system also contains submodels for feed intake regulation and dietary physical structure (Volden, 2011).

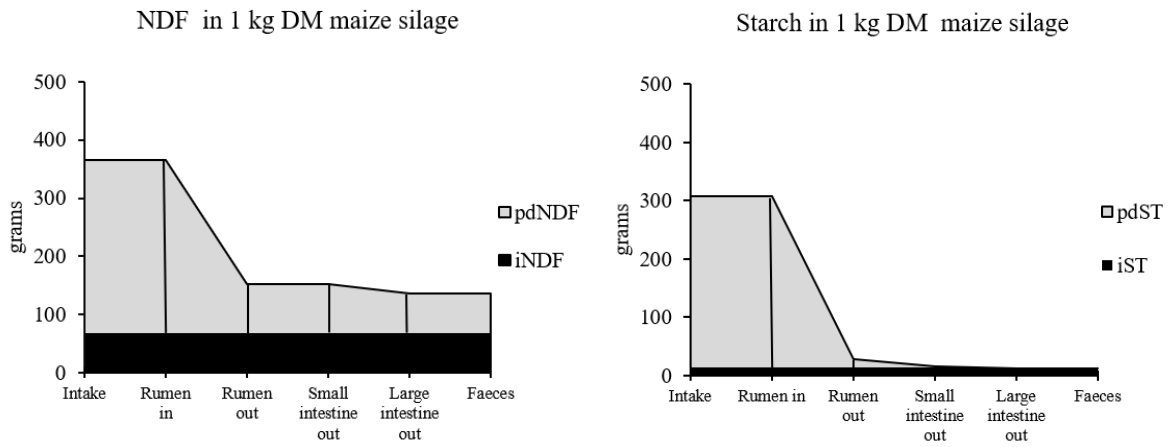


Figure 1 Illustration of digestion of NDF and starch (ST) of their potential digestible (pd) and indigestible (i) fractions in maize silage in different parts of the gastrointestinal tract of cows estimated by the NorFor model.

The NorFor calculation core used by the national advisory software systems and NFRO use a non-linear optimization to suggest diet formulation with the lowest cost while at the same time fulfilling the nutritional requirements for the selected production level.
Overall, NorFor represents a well-established example of long-term Nordic collaboration, combining research, advisory services, and digital tools into a unified system that is widely used both within and beyond its owner organisations.

Cooperation with feed laboratories and feed companies

The NorFor system rely on a close and structured cooperation with both feed companies and feed laboratories. This collaboration ensures that the system remains highly applicable in practical feeding and advisory work.

Feed laboratories play a key role in the NorFor system. Accurate feed evaluation in NorFor relies on detailed chemical analyses, mainly from calibrated prediction methods such as NIR (near-infrared spectroscopy). Feed analysis results are automatically integrated in the NorFor system by the NorFor Feed Analysis System (NorFor FAS). Feed laboratories send the results to NorFor FAS digitally making the analysis results available for specific herds as soon as they have been analysed.

There is also a digital collaboration with feed companies as NorFor provides a software platform where feed companies can add their compound feeds to the NorFor FeedStuff Table (FST). This way compound feeds and their nutritional composition is easily accessible for ration formulation for advisors and farmers using the national advisory software systems or the NorFor Feed Ration Optimizer^{TM}. The compound feeds and feed ingredients that are administered by feed companies are not openly displayed in the NorFor FST online (https://feedstuffs.norfor.info/).

Feed companies can also assess NorFor software in product development, documentation, and advisory support. By applying the same biological model as used in farm advisory services, feed companies can formulate and evaluate feeds in a way that is fully aligned with on-farm ration planning.

Research and development

NorFor works continuously internally and together with researchers to develop new methods and biological features. Some examples are given in the following:

Method for evaluating energy content in concentrates

NorFor developed a model to estimate NEL20 (net energy for lactation at a standardized intake level of 20 kg DM) of compound feeds based on chemical composition and in vitro digestibility, without requiring detailed ingredient information (Álvarez et al., 2022). This provides a practical and standardized way to compare energy values across compound feeds within the NorFor system, despite its otherwise dynamic nature. The work improves transparency of energy content in commercial compound feeds. Overall, the method bridges the gap between mechanistic feed evaluation and routine feed analysis, making energy assessment more operational in practice.

Milk responses to AAT

AAT supply (absorbed amino acids in the small intestine or metabolisable protein) has been evaluated in relation to milk production responses using a meta-analysis of Nordic and international feeding trials (Sommerseth et al., 2026). Results show that increases in AAT supply relative to energy (AAT/NEL) lead to higher ECM and protein yield up to a plateau around 15 g AAT/MJ NEL, with only marginal production gains beyond this level. This indicates that production responses are primarily driven by the balance between ruminal nitrogen availability and energy supply, rather than absolute amino acid supply. Overall, the findings support current NorFor AAT recommendations and suggest that slight reductions in AAT supply could be possible without significantly affecting production, particularly under stricter nitrogen constraints.

Digestion model

In 2021, NorFor introduced several structural changes to the digestion model that resulted in minor adjustments to optimized rations but improved biological realism. The most notable modification is the shift from a curvilinear to a linear ruminal passage rate for forage NDF, leading to slightly higher passage rates, reduced ruminal NDF degradation, and consequently marginal decreases in energy availability and microbial synthesis, while simplifying the model and improving predictions of apparent NDF digestibility. In addition, a unified degradation rate of 60% per hour is applied to the ‘rest carbohydrate fraction’ across both forage and concentrates. The update also reduces endogenous and microbial nitrogen losses in feces, increasing apparent crude protein digestibility and energy values, although slightly decreasing microbial crude protein synthesis and metabolisable protein (AAT). These changes were based on evaluations against a large set of feeding trials (Åkerlind et al., 2019).

Climate

The debate around cows and climate the last 10 years has also had impact on the NorFor system. NorFor can therefore support estimation of the carbon footprint of milk production by providing a biologically consistent and data-driven description of feed intake, nutrient utilisation, and emissions at animal level.

NorFor predicts feed intake and ration composition, which are key inputs for calculating upstream emissions from feed production. By accurately describing what and how much the cows consume, NorFor enables more precise linkage to life cycle assessment (LCA) databases for feed-related emissions. NorFor quantifies nitrogen intake and excretion, which can be used to estimate emissions of nitrous oxide (N_2O) and ammonia (NH_3) from manure management. Since nitrogen efficiency is tightly linked to environmental impact, this provides an important component of whole-farm greenhouse gas assessments.

NorFor includes models for enteric methane emissions, allowing estimation of CH_4 production based on diet composition, intake level, and rumen fermentation characteristics. In recent years, NorFor has focused on improving the prediction of enteric methane emissions. In 2023, models were implemented to quantify the methane-reducing effects of 3-nitrooxypropanol (3-NOP) and nitrate (Karlsson et al., 2024). For both additives, the mitigation effect was modelled as dose dependent. For 3-NOP, the effect is further modulated by diet composition, with a decreasing mitigation response observed at higher levels of roughage NDF per kg of total dry matter intake.

In 2025, an updated methane prediction model for lactating dairy cows was implemented in NorFor. This model improves predictive accuracy by incorporating a broader set of feed-related parameters compared to previous Nordic models. Relative to the earlier NorFor model (Nielsen et al., 2013), the new equation generally predicts lower methane emissions, in line with more recent scientific findings.

Further developments include updated models for growing cattle. New equations for heifers and growing bulls were presented at the Nordic Feed Science Conference in 2026 (Karlsson et al., 2026), representing the latest step in refining methane prediction across animal categories within NorFor.

The NorFor system has the capacity to integrate climate-related parameters into ration evaluation and optimization. The introduction of climate parameters enables farm-specific estimation of greenhouse gas (GHG) emissions by incorporating factors such as forage yield, fertilizer use, carbon sequestration, and methane emissions, expressed as CO_2 equivalents. Additionally, the implementation of a carbon footprint calculator allows quantification of emissions originating from both animal metabolism and feed production, including cultivation, processing, transport, and nitrogen-related emissions. These emissions are aggregated and can be expressed per unit of dry matter intake, per animal, or per unit of production (e.g., energy-corrected milk), enabling comparative assessment of feeding strategies. NorFor provide a comprehensive framework for evaluating the environmental impact of rations. This supports simultaneous optimization of nutrition, productivity, and climate performance.

Future work

Dry matter

Dry matter (DM) concentration is one of the most fundamental feed analyses in ruminant nutrition, as it provides the basis for expressing nutrient concentrations, estimating feed intake, and formulating balanced rations. Because animals regulate intake primarily on a DM basis, inaccuracies in DM determination directly affect predictions of nutrient supply, feed efficiency,

and production responses. The NorFor feed evaluation system defines dry matter of forages as constant weight at 60°C corrected for loss of volatiles (Volden, 2011) to minimize losses of volatile compounds such as organic acids, alcohols, and ammonia, particularly in fermented feeds like silages that occur when drying at 103°C. Kristensen (2024) showed that grass-based forages have a large variation in residual weight loss when first pre-dried at 60°C and thereafter in 103°C, and concluded that correction for residual dry matter might be of importance to improve precision of OMD and chemical methods used in the NorFor feed evaluation system.

Rumen fistulated cows

The in sacco method is challenged in many European countries due to animal welfare reasons. This challenges feed evaluations systems based on the in sacco technique and in vitro methods based on rumen-fluid, such as IVOS (in vitro opløslighed af organisk stof), VOS (vomvätskelöslig organisk substans), Tilley & Terry. INRAe, e.g., has decided to face-out the use of rumen fistulated cows.

References

Álvarez, C., M. Åkerlind, H. Martinussen, E. Brodshaug, D. Clausen, & N. I. Nielsen, 2022. Screening of energy content of compound feeds using the new energy evaluation model, in: Proc.11th Nord. Feed Sci. Conf., Uppsala, Sweden, p. 135-137.

Karlsson, J., C. Alvarez, M. Åkerlind & N. I. Nielsen., 2024. The effect of the methane inhibitors nitrate and 3-NOP on enteric methane in dairy cow, in: Proc.12th Nord. Feed Sci. Conf., Uppsala, Sweden, p. 32-34.

Karlsson, J., F. H. Lau-Jensen, M. Åkerlind & N. I. Nielsen, 2026. Predicting enteric methane from growing cattle, in: Proc.13th Nord. Feed Sci. Conf., Uppsala, Sweden, p. xx-yy. Kristensen, N.B., 2024. Residual weight loss of grass and grass-clover silages dried at 103°C after pre- drying at 60°C., in: Proc.12th Nord. Feed Sci. Conf., Uppsala, Sweden, p. 122-124.

Nielsen, N.I., H. Volden, M. Åkerlind, M. Brask, A.L.F. Hellwing, T. Storlien, J. & Bertilsson, J., 2013. A prediction equation for enteric methane emissions from dairy cows for use in NorFor. Acta Agric. Scand, Sect. A – Anim. Sci., 126-130. https://doi.org/10.1080/09064702.2013.851275

Sommerseth, J.K., I. Schei, M. Åkerlind, A. M. H. Kjeldsen, H. Volden & N. I. Nielsen, 2026. Dietary AAT calculated in NorFor and its effects on responses in ECM- and protein yield in dairy cows. In: Proc.13th Nord. Feed Sci. Conf., Uppsala, Sweden, p. 42-46.

Volden, H. 2011. NorFor - The Nordic Feed Evaluation System. Eur. Assoc. Anim. Prod. Publ. 130. Wageningen Acad. Publ., Wageningen, The Netherlands.

Åkerlind, M. & N.I. Nielsen, 2019. Evaluation of NorFor's prediction of neutral detergent fibre digestibility in dairy cows, in: Proc.10th Nord. Feed Sci. Conf., Uppsala, Sweden, p. 109-116.

The Finnish feed evaluation system serves the livestock sector

M. Rinne, J. Vattulainen & K. Kuoppala
Natural Resources Institute Finland (Luke), Jokioinen, Finland
Correspondence: marketta.rinne@luke.fi

Introduction

The need to compare the productive potential of feeds and to be able to plan nutritionally balanced and economically competitive rations for livestock led to the development of the "modern" animal science during the 19th century. The steps in feed evaluation in Nordic countries until the beginning of the 21st century was reviewed by Weisbjerg *et al*. (2010) at the 1st Nordic Feed Science Conference. At the 8th Nordic Feed Science Conference, the status of the Finnish Feed evaluation system was presented by Rinne *et al*. (2017). The aim of this article is to update the current situation of feed evaluation systems in Finland.

Feed evaluation has a legal status in Finland

Finland is unique in a way that the mandate of Luke to conduct feed evaluation work is included in the national legislation. Feed law (2020) states that the energy and protein values of feed materials and feed mixtures must be based on criteria published by Luke, unless the legislation of European Union states otherwise. The criteria include equations with their default values, the digestibility coefficients published in Feed Tables, and for ruminants, also the values for effective protein degradability in the rumen.

The feed evaluation work is one of Luke's statutory services that is resourced by the Ministry of Agriculture and Forestry to maintain the service and provide guidance to the users in the livestock sector. A Standing Committee for Feed Evaluation supports the activity with members from Luke, University of Helsinki, Finnish Food Authority and Ministry of Agriculture and Forestry of Finland.

The feed evaluation work covers the most important livestock species, i.e., cattle (dairy cows, suckler cows, growing cattle), small ruminants (sheep, goats), pigs (growing and adult pigs), poultry (laying hens, broilers, turkeys) and horses (Table 1). The feed evaluation system is an entity and parts of it can not be combined with some other system. The feed evaluation system consists of:

- Equations to calculate the feed values (energy and protein values)
- Feed values calculated for reference feeds based on their typical composition = Feed Tables
- Nutrient requirements for different livestock species and phases of production

The latest edited version of the Feed Tables and nutrient requirements report was published in 2024 in Finnish (Kuoppala *et al*., 2024a) and in English (Kuoppala *et al*., 2024b). The "Feed Tables and Nutrient requirements" website serves the sector in three languages:

- Finnish: www.luke.fi/rehutaulukot
- Swedish: www.luke.fi/fodertabeller
- English: www.luke.fi/feedtables

Table 1 Feed values for different livestock species in Finland.

	Energy	Protein
Ruminants	Metabolizable energy (ME), MJ/kg dry matter (DM)	Metabolizable protein (MP, previously called AAT) and protein balance in the rumen (PB), g/kg DM
Pigs	Net energy (NE), MJ/kg DM (separately for growing and adult pigs)	Standardized ileal digestible lysine, methionine + cysteine, threonine, tryptophan and valine, g/kg DM for each Digestible crude protein separately for growing and adult pigs, g/kg DM for both
Poultry	Metabolizable energy (ME), MJ/kg dry matter (DM)	Amino acid concentrations, g/kg DM
Horses	Same as for ruminants	Digestible crude protein (dCP), g/kg DM

Finnish feed evaluation and ration formulation system for cattle

Although other Nordic countries have focused on the use NorFor (2026) feed evaluation system for cattle, Finland has continued to use its own system, which is relatively simple and transparent. The Finnish system was reviewed by Rinne *et al*. (2017) and the main points are summarized here:

Relatively simple parameters of feeds required, and thorough development of feed analysis has been undertaken (Huhtanen *et al*., 2006). The system is supported by an official Feed Table reflecting the boreal feed production conditions of Finland.

Simple additive calculations, where associative effects of feeding are covered by empirical correction equation in which level of DM intake, energy value of feeds used and diet CP concentration are considered (Huhtanen *et al*., 2009 with an update in 2026). The advances of the simple approach include less sources of error in calculations and feed analyses. The outcomes have been tested against large dairy cow experimental data to ensure that calculations match the milk production responses.

The equations have been incorporated into the Lypsikki® model (Nousiainen *et al*., 2011, Huhtanen *et al*., 2011a). Lypsikki allows rations to be optimized by e.g. least cost, maximal milk production or minimal environmental load, but at farm level, the most obvious choice is "milk income over feed cost". This requires accurate estimates of feed intake (Huhtanen *et al*., 2007, 2008, 2011b) and milk production responses (Huhtanen & Nousiainen, 2012), which are based on meta-analyses generated using large data sets on diets similar to those used in Finland. The theoretical principles of the approach have been reviewed by Huhtanen & Nousiainen (2014). A ration formulation program CowCompass (in Finnish 'KarjaKompassi', in Swedish 'KoKompassen') launched by ProAgria in 2011 utilizes Lypsikki components.

Update of dairy cow energy requirements in 2026

The energy requirement update for dairy cows has recently been conducted (Huhtanen *et al*., 2026). Several studies have shown that the maintenance energy requirement of dairy cows is higher than previously estimated. Nutrient Requirements of Dairy Cattle in the USA (NASEM,

2021) raised the maintenance requirement to 0.63 MJ ME/kg metabolic live weight, which is 22% higher than the previous Luke energy recommendation. In order to update the Finnish recommendations, a data set of 930 observations was collected from respirometry trials. Based on the meta-analysis, the maintenance energy requirement of cows was significantly higher, but the production energy requirement slightly lower than the previous Luke recommendations. The new maintenance requirement was estimated at 0.63 MJ/kg metabolic live weight (equal to NASEM, 2021) and the production requirement at 5.0 MJ/kg energy-corrected milk (ECM).

The increased maintenance energy requirement is probably due to the increased contribution of metabolically active tissues, intestines and liver, to the live weight as the cows' production level has increased. In addition, previous recommendations for maintenance requirements were based on fasting metabolism experiments.

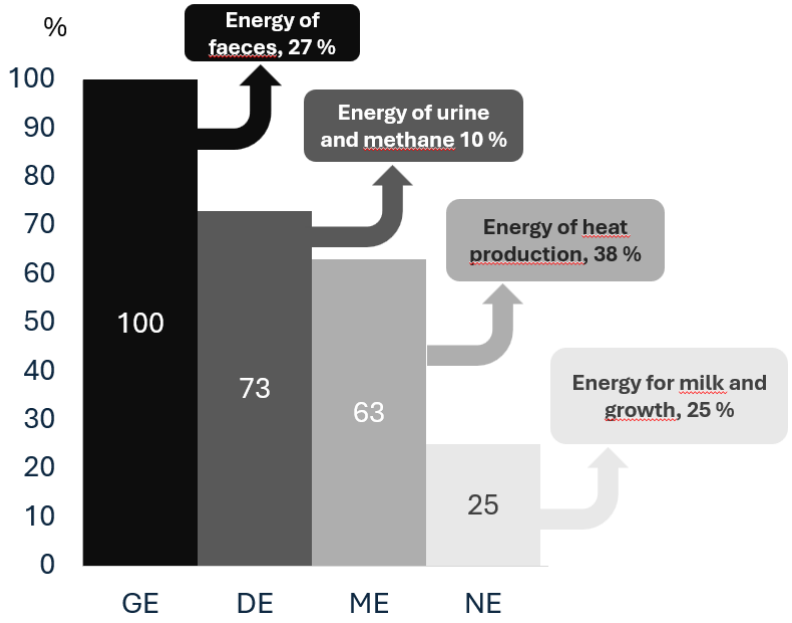


Figure 1 Distrubution of gross energy (GE) and energy losses at different stages according to Huhtanen *et al*. (2026). DE=digestible energy, ME=metabolizable energy, NE=net energy.

Feed evaluation systems for other livestock species

Horses

The energy value for feeds is expressed in megajoules (MJ) of metabolizable energy (ME) and is calculated using the same criteria as presented above for ruminants. In reality, feed digestibility is somewhat lower in horses than in ruminants (Särkijärvi *et al*. 2012), but this difference has been taken into account by increasing the energy requirements accordingly. The digestible crude protein content is obtained by multiplying the crude protein content (CP) of the feed by its digestibility coefficient (cPD). The digestibility coefficients for CP are derived from the ruminant Feed Tables.

Pigs

The feed evaluation system of pigs is French INRAE / AFZ since 2014. The energy value is presented as net energy. Young pigs get less energy from the same feed than older animals, because they do not digest fibre as efficiently so that values are presented separately for young and adult pigs. The protein value is expressed as standardized ileally digested amino acids. The feed values are calculated using EvaPig® program.

The fast changes in the genetic background of the pigs used and feeding programs linked to contract production have resulted in a situation where pig rationing is done by the commercial chains, and the nutrient requirements presented by Luke are indicative.

Poultry

Energy values of feeds are calculated according to WPSA (1986) in line with EU guidelines. The concentrations of amino acids are presented in feed dry matter. Feeding of poultry is conducted according to the contract programs controlled by commercial chains. The genetic lines of the birds used dictate the feeding program used. Thus, the nutrient requirements presented by Luke are indicative.

The Feed Tables

The Finnish Feed Tables have evolved over time and were first published as a dedicated report by Salo *et al*. (1982). The current Feed table contains 280 feeds (ruminants 239, pigs 125, poultry 134; amino acids 72, minerals 201). The chemical composition of a feed material is common to all animal groups, but each animal group has dedicated parameters such as D-value and effective protein degradability in the rumen for ruminants and ileal digestible amino acids for pigs. Maintenance and updating of the feed material list in the Feed Tables is a continuous activity. An excerpt of the Feed Table is presented in Table 2.

Future considerations

The feed evaluation systems are in the very core of livestock production systems. Ration formulation is the most obvious outcome of the feed evaluation systems with significant effects on animal productivity, health and welfare. The rations dictate feed production activities on the farm (forages vs. cereal crops, choices of agronomic management practices, harvesting strategy etc.) as well as needs and costs of purchased feeds. The pricing of commercial feeds is based on feed values, i.e., expected production responses to their use.

The rationing impacts the environmental effects related to feed and manure production. The carbon footprint of feeds and subsequently animal products is of importance as well as efficiency of nutrient utilization, particularly N and P, to limit the environmental impacts of livestock production and maintain societal acceptance of it. The composition, nutritional value and safety (major and minor nutrients including protein, fat, fatty acid profile, minerals, vitamins) of animal-derived foods affect the human health, when consuming milk and meat. Default values for different research and modelling activities, scenarios, excretion calculations, national inventories, legal frameworks etc. are based on data derived from the feed evaluation systems.

The fast development of sensor and information technologies as well artificial intelligence applications may change the concepts of future ration formulation. Majority of Finnish cows is already milked by robots, the number being 62 % of the milk-recorded cows (Hellberg,

2026). Potentially the new ration formulation approaches could be more iterative, i.e., changes caused by manipulation of rations are evaluated automatically, and TMR recipes adjusted based on the "feedback" directly from the cows. However, sound principles of basic feed evaluation are needed as a starting point.

It is obvious that feed evaluation systems are required in the future. At present, the need to update the current feed evaluation systems in Finland is not acute. However, in the long term, the maintenance and development of the systems is needed, and will require significant amount of resources and intellectual capacity.

References

Feed law, 2020. https://www.finlex.fi/eli?uri=http://data.finlex.fi/eli/sd/2020/1263/ajantasa/2025-06-27/fin

Hellberg, T., 2026. Milk recording yields 2025. https://www.proagria.fi/uploads/ProAgria/Tiedostot/Lypsykarjan-tuotosseurannan-tulokset-2025-Teija-Hellberg-ProAgria.pdf

Huhtanen, P. & Nousiainen, J., 2012. Production responses of lactating dairy cows fed silage-based diets to changes in nutrient supply. Livest. Sci. 148, 146–158.

Huhtanen, P. & Nousiainen, J., 2014. Production responses of lactating dairy cows fed silage-based diets to changes in nutrient supply. Recent Advances in Ruminant Nutrition 2013. Eds Garnsworthy, P.J. & Wiseman, J., Nottingham University Press, UK. p. 71-90.

Huhtanen, P., Nousiainen, J. & Rinne, M. 2006. Recent developments in forage evaluation with special reference to practical applications. Agric. Food Sci. 15: 293-323. https://doi.org/10.2137/145960606779216317

Huhtanen, P., Rinne, M. & Nousiainen, J., 2007. Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. Animal 1, 758-770.

Huhtanen, P., Rinne, M. & Nousiainen, J., 2008. Evaluation of concentrate factors affecting silage intake of dairy cows: a development of the relative total diet intake index. Animal 2, 942-935.

Huhtanen, P., Rinne, M. & Nousiainen, J., 2009. A meta-analysis of feed digestion in dairy cows. 2. The effects of feeding level and diet composition on digestibility. J. Dairy Sci. 92, 5031-5042.

Huhtanen, P., Nousiainen, J. & Turtola, E., 2011b. Dairy farm nutrient management model: 2. Evaluation of different strategies to mitigate phosphorus surplus. Agric. Syst. 104, 383 – 391.

Huhtanen, P., Rinne, M., Mäntysaari, P. & Nousiainen, J., 2011a. Integration of the effects of animal and dietary factors on total dry matter intake of dairy cows. Animal 5, 691-702.

Huhtanen, P., Guinguina, A., Kuoppala, K. & Sairanen, A. 2026. Lypsylehmien energiaruokinta suositusten päivitys [Update of the Finnish energy requirements of dairy cows for maintenance and milk production, and correction of metabolizable energy]. Luonnonvara- ja biotalouden tutkimus 21/2026. Natural Resources Institute Finland, Helsinki, Finland. 19 p. https://urn.fi/URN:ISBN:978-952-419-171-5.

Kuoppala, K., Vattulainen, J., Perttilä, S., Saastamoinen, M. & Rinne, M. 2024a. Rehutaulukot ja ruokintasuositukset : Märehtijät, siat, siipikarja, hevoset. Luonnonvara- ja biotalouden tutkimus 17/2024. Luonnonvarakeskus. Helsinki. 98 s. http://urn.fi/URN:ISBN:978-952-380-883-6

Kuoppala, K., Vattulainen, J., Perttilä, S., Saastamoinen, M. & Rinne, M. 2024b. Finnish Feed Tables and Nutrient Requirements : Ruminants, pigs, poultry, horses. Natural Resources and Bioeconomy Studies 70/2024. Natural Resources Institute Finland. Helsinki. 97 p. Available at: http://urn.fi/URN:ISBN:978-952-380-950-5.

NASEM, 2021. Nutrient Requirements of Dairy Cattle, 8th edition. National Academy of Sciences, Washington DC, USA. https://doi.org/10.17226/25806.

NorFor, 2026. www.norfor.info

Nousiainen, J., Tuori, M., Turtola, E. & Huhtanen, P., 2011. Dairy farm nutrient management model. 1. Model description and validation. Agric. Syst. 104, 371-382.

Rinne, M., Kuoppala, K., Vanhatalo, A., Huhtamäki, T., Nousiainen, J. & Huhtanen , P. 2017. Finnish feed evaluation system and Feed Tables. Nordic Feed Science Conference, Uppsala 13-14 June 2017. Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management. Report 296. pp. 97-102. Available at: https://res.slu.se/id/publ/89440

Salo, M.-L., Kiiskinen, T., Tuori, M., 1982. Rehutaulukot ja ruokintanormit: märehtijät - siat - siipikarja - turkiseläimet. 70 p. Helsingin yliopisto, kotieläintieteen laitos, Maatalouden tutkimuskeskus, kotieläinhoidon tutkimuslaitos. https://urn.fi/URN:ISBN:951-45-2807-7

Särkijärvi, S., Sormunen-Cristian, R., Heikkilä, T., Rinne, M. & Saastamoinen, M. 2012. Effect of grass species and cutting time on in vivo digestibility of silage by horses and sheep. Livest. Sci. 144: 230-239. 10.1016/j.livsci.2011.11.019

Weisbjerg, M.R., Rinne, M., Spörndly, R., Ekern, A. & Harstad, O.M., 2010. The history of feed evaluation for ruminants, with special emphasis on the Nordic countries. Proc. 1st Nordic Feed Science Conference, Uppsala, Sweden 23-24 June 2010. Swedish University of Agricultural Sciences, Dept. Animal Nutrition and Management, Report 274, pp. 51-64. Available at: https://res.slu.se/id/publ/34797

WPSA 1986. European Table of Energy Values for Poultry Feedstuffs. First edition. Published by subcommittee Energy of the Working Group nr. 2 Nutrition of the European Feder-ation of Branches of the World's Poultry Science Association. Grafisch bedrijf Ponsen & Looijen, Wageningen, the Netherlands. 24 p.

Table 2 An example from the Finnish Feed Table showing some commonly used feeds (source: www.luke.fi/feedtables).

Feed code and description	In dry matter (DM), g/kg								EPD
	Ash	CP	NDF	iNDF	D-value	ME, MJ	MP	PB	
1001 Barley, 64 - 69 kg/hl	29	115	210	37	821	13.2	96	-29	0.80
1012 Oats, 54 - 58 kg/hl	38	127	290	115	711	12.1	92	-8	0.75
1031 Wheat, 76 - 80 kg/hl	20	133	110	20	843	13.6	96	-12	0.85
1071 Pea, seed	31	230	130	5	849	13.3	116	62	0.80
1072 Faba bean, seed	36	300	160	10	818	12.8	123	125	0.80
2002 Rapeseed meal	80	379	270	125	696	11.4	169	154	0.63
3081 Sugar beet pulp, dried	62	110	450	35	787	12.0	108	-47	0.60
7001 Grass silage, early 1st cut, DM 200-500 g/kg	85	170	520	40	720	11.5	88	39	0.85
7002 Grass silage, average/early 1st cut, DM 200-500 g/kg	80	160	550	70	690	11.0	84	35	0.85
7003 Grass silage, average/late 1st cut, DM 200-500 g/kg	75	145	580	90	660	10.6	80	26	0.85
7004 Grass silage, late 1st cut, DM 200-500 g/kg	75	130	590	110	630	10.1	75	18	0.85
7005 Grass silage, very late 1st cut, DM 200-500 g/kg	70	120	600	140	600	9.6	71	14	0.85
7006 Grass silage, high digestibility 2nd cut, DM 200-500 g/kg	95	170	520	60	680	10.9	84	45	0.85
7007 Grass silage, average digestibility 2nd cut, DM 200-500 g/kg	95	155	530	85	650	10.4	80	36	0.85
7008 Grass silage, low digestibility 2nd cut, DM 200-500 g/kg	90	145	540	115	620	9.9	76	32	0.85
7009 Grass silage, 3rd cut, DM 200-500 g/kg	95	175	510	45	700	11.2	87	46	0.85
7021 Red clover silage, early 1st & 2nd cut, DM 200-500 g/kg	115	230	310	35	700	11.2	101	85	0.80
7022 Red clover silage, average 1st & 2nd cut, DM 200-500 g/kg	110	205	370	90	650	10.4	93	71	0.80
7023 Red clover silage, late 1st & 2nd cut, DM 200-500 g/kg	110	185	430	140	600	9.6	85	62	0.80

CP = crude protein, NDF = neutral detergent fibre, iNDF = indigestible NDF, D-value = digestible organic matter in DM, ME = metabolizable protein, MP = metabolizable protein (amino acids absorbed from the small intestine), PB = protein balance in the rumen, EPD = effective protein degradation in the rumen

A three-stage procedure for determining metabolisable energy of feeds for dairy cattle

K.-H. Südekum on behalf of the Committee for Requirement Standards (AfBN) of the Society of Nutrition Physiology (GfE) in Germany
Institute of Animal Science, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany. Correspondence: ksue@itw.uni-bonn.de

Introduction

The energy value of a feed is calculated in many energy evaluation systems using equations based on the digestible nutrient concentrations. The energy value assigned to each digestible nutrient (or nutrient group) is the digestible (DE) content. In ME equations determined by regression analysis, urinary energy (UE) and CH_4 energy (CH_4-E) are captured by the lower regression coefficients compared to their respective DE values. Despite their high accuracy and reliability, such calculations have limitations, especially concerning their practical applications. Typically, in practice, information regarding the digestibility of individual nutrients is not available, as they must be determined in animal trials. However, organic matter digestibility (OMD), as the central determinant of the energy value of a feed, can be reliably estimated using various *in vitro* methods. The energy digestibility (ED) can be derived from OMD, as these measures differ by a constant value. After determining the energy losses through urine and CH_4, the ME content of the feed can be calculated. The digestible organic matter (DOM) content also serves as a key value for estimating the amount of microbial crude protein (MCP) formed in the rumen (Gresner et al., 2024). This study confirmed that protein supply to ruminants relies mainly on the flow of MCP from the rumen, which primarily depends on energy supply. Moreover, total-tract digestible organic matter (DOM) was at least as precise as ruminally fermented organic matter to predict MCP flow from the rumen. The complete feed evaluation system was published in German in 2023 (GfE, 2023). An English version is in press and will be available later this year.

Brief description of the three-stage procedure for ME determination

The energy evaluation system for dairy cattle in Germany, which was a net energy system for decades, has been entirely newly developed using original data. Energy evaluation of cattle feeds is now based on metabolisable energy (ME). The new procedure presented here for determining the ME content of rations and feedstuffs differs from the previous approach (GfE, 2001), in which the ME was calculated from digestible nutrient concentrations. The key information now is the OMD. As ED differs from OMD by a constant value, the DE content can be determined from the gross energy (GE). Losses in urinary energy (UE) are derived from crude protein (CP) concentration and CH_4 energy (CH_4-E) losses from OMD. Starting with the GE value, the three-stage procedure involves the following calculations:

ED (%) = OMD (%) − 3.3
UE (MJ/kg OM) = 0.0037 · CP (g/kg OM)
CH4-E (MJ/kg OM) = 0.7 + 0.014 · OMD (%)

The equation used to calculate the ME concentration in dry matter (DM) is:
ME (MJ/kg dry matter [DM]) = [(GE (MJ/kg OM) · (OMD (%) − 3.3) / 100 − 0.0037 · CP (g/kg OM) − (0.7 + 0.014 · OMD (%))] · (1 − ash (g/kg DM)/1000).

The ME content of a ration can be derived solely from the determined values of GE, OMD, CP and ash. This ME value represents the standardised energy value (i.e., at a feed intake level

nearby the energy maintenance requirement) determined in cattle or sheep, assuming an N utilisation of 1/3. In the following passage, the three stages are elucidated with some details.

Relationship between organic matter digestibility and energy digestibility

As shown in Figure 1, there is a close relationship between OMD and ED since the crude fat (ether extract) concentration in ruminant diets is generally low and, therefore, the gross energy (GE) values of feed OM vary only slightly. Since the slope of the corresponding regression line does not differ from one, the relationship can be expressed by the mean difference:
OMD (%) − ED (%) = 3.3 (SD = 0.52; n = 102)

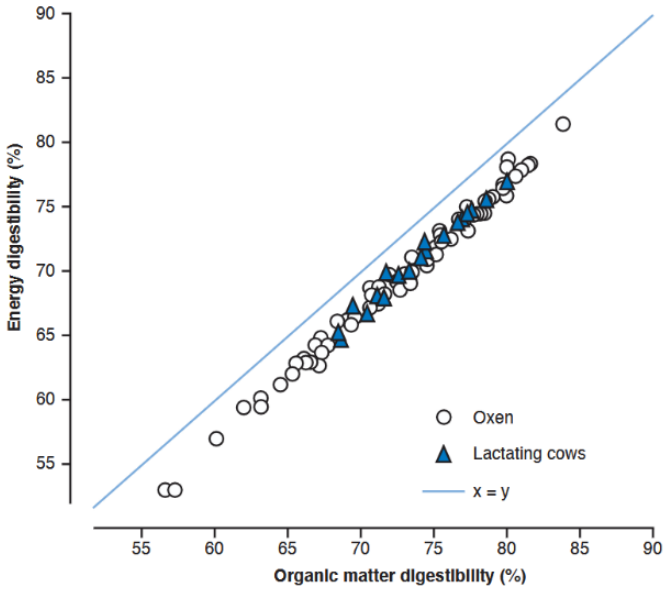


Figure 1 Relationship between the organic matter digestibility (%) and energy digestibility (%) in oxen (n = 102 rations, mean feed intake level 1.1) and lactating cows (n = 20 rations, mean feed intake level 2.4). Feed intake level 1 (FIL1) is defined here as 50 g DM intake per unit of metabolic body size ($kg^{0.75}$). It is evident that the difference between organic matter digestibility and energy digestibility shows very small variation, is constant across the measured range of digestibility, and is not influenced by the feed intake level.

The difference is constant across the measured range of 57–84% OMD. The higher calorific value of faecal organic matter (OM) is primarily due to its microbial substance content and higher lignin content compared to feed OM, which increases with rising OMD. The calorific value of faecal OM is 21.7 kJ/g at 65% OMD and 22.5 kJ/g at 80% OMD, with a mean value of 22.1 kJ/g. The standard deviation (SD) of the mean OMD-ED difference of 0.52 is low, indicating that the ED can be accurately derived from the OMD. The feed intake level does not alter this difference, as shown by the inclusion of 20 additional rations fed to dairy cows at a mean feed intake level (FIL) of 2.4.

Urinary energy losses

Urinary energy losses are significantly influenced by CP intake, ruminal N balance, and milk protein synthesis or body protein accretion. When animals are fed at or near maintenance energy level for the purpose of energy evaluation of feeds, there is no or little protein accretion, resulting in almost all N intake not excreted in faeces being lost via urine. The commonly used reference in the literature linking UE losses to the gross energy of the ration is deemed inappropriate because feed value is related to mass and because, theoretically, UE losses would

increase with higher crude fat concentration. The regression equation derived from data on oxen shows a linear relationship between UE (kJ/kg OM) and the CP concentration of the ration (g/kg OM) with only a slight, non-significant negative y-intercept. Therefore, this relationship can be equally reliably expressed as a ratio, as follows:
UE (kJ) / CP (g) = 5.5 (n = 102; SD = 0.61)

This uniform value of 5.5 kJ UE/g CP must be applied when assessing individual feedstuffs, regardless of the CP concentration. The AfBN has decided that for the purpose of feed energy evaluation, the urinary energy losses of lactating cows should be used rather than of animals at the maintenance metabolism. Nitrogen use efficiency (milk N/N intake) is fixed to be one-third. Accordingly, the UE losses are related to CP as follows:
UE (kJ) / CP (g) = 3.7

Energy losses with methane

Energy losses through CH_4 production were estimated from the 102 rations tested in oxen mentioned above. Of these, 8 rations included starch from unprocessed potatoeswhichwere not considered in the estimate of CH_4 production due to the lack of information on the extent of ruminal starch degradation. For rations containing maize starch concentrations between 75 and 283 (mean 185) g/kg OM, a reduction in CH_4 production was not observed. For the remaining 94 rations, CH_4-E is 1.69 MJ/kg OM (SD = 0.14; SD% = 8.5) and 2.29 MJ/kg DOM (SD = 0.18; SD% = 7.9) (SD%; coefficient of variation = SD/mean in %). The commonly used reference of CH_4-E to GE is less suitable because the majority of CH_4 is produced through carbohydrate fermentation in the rumen, and particularly for rations with varying crude fat, the relationship between CH_4 and OMD can be described as follows:
CH_4-E (MJ/kg OM) = 0.7 + 0.014 · OMD (%) (n = 94; RSD = 0.12)

Accordingly, CH_4 production increases from 1.5 to 1.8 MJ/kg OM as OMD increases from 60 to 80%, or decreases from 2.6 to 2.3 MJ/kg digestible OM. Although OMD has a significant impact on CH_4 production, the coefficient of determination of the above equation is only 28%, indicating that other factors also influence CH_4 production which cannot be outlined here.

The three-stage procedure for determining the ME content has significant advantages over using ME estimation equations based on the digestible nutrient concentrations. Each of the three stages is clearly described and can be experimentally and independently verified. Knowledge of OMD plays a crucial role, as it allows for a reliable and accurate derivation of ED and because energy losses via faeces far exceed those via urine and CH_4. Additionally, CH_4 losses depend on the OMD and can be estimated from it. Urinary energy losses are predominantly caused by the CP content and can thus be determined. This approach opens the possibility of further refining and considering quantifiable influences on each of these stages in the future.

References

GfE, 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt/Main, Germany.

GfE, 2023. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt/Main, Germany.

Gresner, N.,Wild, K., Rodehutscord, M. & Südekum, K.-H., 2024. Prediction of microbial crude protein flow from the rumen of dairy cattle by means of dietary characteristics – a meta-analysis. Arch. Anim. Nutr. 78, 354-380.

Update of NorFor values for beets and beet silages.

E. M. V. Hvas[1], M. Larsen[1], N. I. Nielsen[2], H. Martinussen[2] & M. R. Weisbjerg[1].
[1]*Aarhus University (AU), Department of Animal and Veterinary Sciences, AU Viborg, Research Centre Foulum, Blichers Allé 20, 8830 Tjele, Denmark..*
[2]*SEGES Innovation, Agro Food Park 15, 8200 Aarhus N, Denmark.*
Correspondence: emma.hvas@anivet.au.dk

Introduction

Beet (*Beta vulgaris*) can be fed to dairy cows either as-is or ensiled, with ensiling extending the feeding season and shifting the composition from sugar-rich to ethanol-rich material (Hvas et al., 2025a). The introduction of new beet varieties and the development of modern ensiling methods create a need to update the beet and beet-silage table values in the Nordic Feed Evaluation System (NorFor) to reflect current varieties and practices.

Materials and Methods

Recent Danish research data on beets and beet silages were compiled to update the NorFor Feed Table with values for beets and beets silages. For as-is beets, two dry matter (DM) levels were included (16.5% and 23%). Compositional data were based on 57 samples of dry-cleaned beets (21 samples at 16.5% DM and 36 samples at 23% DM; Hvas et al., unpublished). Data on organic matter digestibility (OMD) were derived from seven washed samples of beets within each DM level (Hartnell et al., 2005), with one additional dry-cleaned sample included for the 23% DM group (Hvas et al., 2025b).

Beet silage data represent three ensiling strategies: the top-layer method, pure beet silage, and the mixing method. Top-layer silages were sampled from three silos on two commercial farms where beets were ensiled on-top of existing maize or grass/clover silage, and samples were collected at one-third and two-thirds of the beet layer, yielding six samples in total. Data on pure beet silage were based on 16 samples, including three replicates of washed beets ensiled in barrels (Hvas et al., 2025a) and 12 replicates ensiled in pallet tanks (Hellwing et al., 2017), and one also ensiled in pallet tank (Olijhoek et al., 2023) but excluded from ash calculations due to unusually high ash content despite washing. For the mixing method, beets were co-ensiled with grass–clover silage in bales at a ration of 37.5:62.5 on a DM basis (beets:grass-clover silage), represented by four replicates (Hvas et al., 2025b).

Relevant analytical parameters used in NorFor, including DM, sugar, and lactate, were extracted when available. For top-layer silages, selected parameters were analysed directly (Hvas et al., 2025b), and simple mean values were calculated across studies. For datasets reporting only L-lactate, total lactate was estimated as twice the reported L-lactate concentration (Johansen et al., 2020).

Results and Discussion

For as-is beets, two types were included that reflected the variation in DM typically found in practice. The high-DM type represented newer beet varieties like those used for sugar production, whereas the low-DM type was more representative of older fodder beet varieties (Evans and Messerschmidt, 2017). Consistent with these varietal differences, high-DM beets had slightly higher sugar concentrations and lower ash concentrations than low-DM beets.

Beets used for pure beet silage were washed before ensiling, whereas the beets used in the remaining silages were only dry-cleaned, contributing to variation in ash content. In general, soil type and the level of cleaning prior to ensiling or feeding as-is have a major impact on ash concentration, and this must therefore be considered when using table values or deciding on chemical analysis.

Table 1 Chemical composition of fresh beets and different types of beet silages used as input for updating NorFor table values. Values represent mean concentrations (g/kg DM unless otherwise stated) based on available samples across studies and on-farm silages.

Item	Beets (16.5 % DM)	Beets (23 % DM)	Ensiled beets (top-layer)	Ensiled beets (barrel/tank)	Ensiled beets (mixed with grass/clover silage)
Dry matter g/kg	165[1]	230[3]	230[5]	206[6]	269[9]
Ash	97.2[1]	55.0[3]	132[5]	40.5[7]	117[9]
Crude protein	53.0[1]	36.0[3]	52.2[5]	58[6]	115[9]
NH_3-N, g N/kg N	-	-	-	53.3[8]	786[9]
NDF	108[1]	90.8[3]	146[5]	146[6]	357[9]
Starch	-	-	-	ND	3.99[9]
Lactate	-	-	45.5[5]	46.9[6]	76.6[9]
Acetate	-	-	56.6[5]	29.7[6]	19.3[9]
Propionate	-	-	1.29[5]	2.04[6]	0.61[9]
Butyrate	-	-	ND	ND	0.16[9]
Alcohols	-	-	94.7[5]	108[6]	112[9]
Sugars	681[1]	771[3]	38.7[5]	123[8]	38.4[9]
Pectins	62.1[1]	53.2[3]	-	-	-
OMD, % of OM	90.7[2]	93.3[4]	-	-	75.5[9]

ND = value below lower limit of detection.
- = Not analysed.
[1]Hvas et al. (Unpublished); n = 21
[2]Hartnell et al. (2005); n = 7
[3]Hvas et al. (Unpublished); n = 36
[4]Hartnell et al. (2005), Hvas et al. (2025b); n = 8
[5]On-farm samples; n = 6
[6]Hvas et al. (2025a), Hellwing et al. (2017), Olijhoek et al. (2023); n = 16
[7]Hvas et al. (2025a), Hellwing et al. (2017); n = 15
[8]Hvas et al. (2025a), Olijhoek et al. (2023); n = 4
[9]Hvas et al. (2025b); n = 4

It is therefore recommended that beet ash content is analysed prior to ensiling. A slight increase in ash concentration during ensiling is expected, as DM losses occur through fermentation, particularly when sugars are fermented by yeasts with substantial CO_2 losses (Borreani et al., 2018). Consequently, when standard table values are used for nutrients, including fermentation products, concentrations should be adjusted to the actual ash content of the beets or silage by applying an organic matter (OM) correction. The adjustment factor, used to scale table values to measured ash, is calculated as: Adjustment factor = (1000 – table value ash content (g/kg DM)) / (1000 – measured ash content (g/kg DM). All nutrients within the OM fraction of beets and silages can then be adjusted to the actual ash content by multiplying by this factor. E.g., adjusting pure beet silage to the ash content of top-layer beet silage: (1000 – 132) / (1000 – 40.5) = 0.90. As full silage analyses, including ethanol quantification, are costly for farmers, this provides a practical compromise using measured ash values to improve NorFor inputs when full analyses are not feasible.

In addition, a considerable part of the DM in ensiled beets remains unexplained. Although mannitol formation (Hvas et al., 2025a; 2025b) and a potential up-concentration of pectin may explain part of this, a fraction of the DM still remains unaccounted for.

Conclusions

The results show substantial variation in nutrient composition and fermentation profiles across beet types and ensiling methods. Ash content and consequently OM proportion vary widely depending on beet variety, cleaning intensity, and ensiling approach, thus adjusted values can be needed. The proposed ash-based correction offers a practical solution for generating more accurate NorFor inputs when full silage analyses are not feasible. However, given the limited dataset, more data are needed to establish robust table values.

References

Borreani, G., Tabacco, E., Schmidt, R.J., Holmes, B.J., Muck, R.E., 2018. Silage review: Factors affecting dry matter and quality losses in silages. J. Dairy Sci. 101, 3952-3979. https://doi.org/10.3168/jds.2017-13837.

Evans, E., Messerschmidt, U., 2017. Review: Sugar beets as a substitute for grain for lactating dairy cattle. J. Anim. Sci. Biotechnol. 8, 25. https://doi.org/10.1186/s40104-017-0154-8.

Hartnell, G. F.; Hvelplund, T.; Weisbjerg, M. R., 2005. Nutrient digestibility in sheep fed diets containing Roundup Ready or conventional fodder beet, sugar beet, and beet pulp. J. Anim. Sci. 83, 400-407.

Hellwing, A. L. F., Messerschmidt, U., Larsen, M., Weisbjerg, M. R. 2017. Effects of feeding sugar beets, ensiled with or without an additive, on the performance of dairy cows. Livest. Sci. 206, 37-44.

Hvas, E. M. V., Hanigan, M. D., Weisbjerg, M. R., Larsen, M., 2025a. Effects of feeding beets and ensiled beets on rumen volatile fatty acid and ethanol metabolism in dairy cows: J. Dairy Sci. 108, 9449-9469. https://doi.org/10.3168/jds.2024-26194.

Hvas, E. M. V., Larsen, M., Andersen, L., Bedenk, U., Weisbjerg, M. R. Unpublished. Nutrient composition in beets grown for cattle feed in Western Europe.

Hvas, E.M.V., Larsen, M., Andersen, L., Bedenk, U., Weisbjerg, M.R. 2025b. Ensiling sugar beets: Sampling, chemical composition, and digestibility of mixed sugar beet silage. Anim. Feed Sci. Tech. 323, 116302. https://doi.org/10.1016/j.anifeedsci.2025.116302.

Johansen, M., Weisbjerg, M.R., Novoa-Garrido, M., Kristensen, N.B., Larsen, M., 2020. Relationship between L-lactate and DL-lactate in different silage types. Proceedings of the 28th General Meeting of the European Grassland Federation, Wageningen Academic Publishers, Helsinki, Finland, 282-284.

Olijhoek, D. W., Lamminen, M., Hellwing, A. L. F., Larsen, M., Weisbjerg, M. R., Knudsen, K. E. B., Lund, P. 2023. Effect of substituting maize silage with fresh or ensiled sugar beets on nutrient digestibility, rumen fermentation and microbial synthesis, and enteric methane emission in dairy cows. Anim. Feed Sci. Tech. 303, 115715. https://doi.org/10.1016/j.anifeedsci.2023.115715.

The effect of processing protein feedstuffs to increase AAT in dairy cow diets in the NorFor system– a meta study focusing on milk production responses

I. Schei[1], J. K. Sommerseth[1], M. Åkerlind[2], A. M. Kjeldsen[3], H. Volden[1,4] & N. I. Nielsen[3]
[1]*TINE SA, R&D-dept, Chr. Magnus Falsens vei 18, 1432 Ås, Norway*
[2]*Växa Sverige, Box 288, 751 05 Uppsala, Sweden*
[3]*SEGES Innovation, Agro Food Park 15, 8200 Aarhus N, Denmark*
[4]*Department of Animal and Aquacultural Science, Faculty of Biosciences, Norwegian University of Life Sciences, P.O.Box 5003, 1432 Ås, Norway*
Correspondence: Ingunn.Schei@tine.no

Introduction

Processing of protein sources, for example through heat treatment or controlled Maillard reactions, aims to reduce ruminal protein degradation while maintaining a high degree of intestinal amino acid digestibility (Reynal and Broderick, 2003). Moreover, in light of increasing focus on precision feeding, reduced protein overfeeding, and stricter environmental sustainability requirements, reduced protein degradability without decreased amino acid availability may improve nitrogen efficiency and reduce N loss. However, processing of protein to reduce the rumen degradable protein (RDP) fraction represents an increased cost. The objective of this study was to conduct a meta-analysis of feeding trials comparing unprocessed and heat- or chemical treated protein feedstuffs to quantify effects on milk production.

Materials and Methods

Data were collected by searching published literature from protein feeding trials where the difference between the rations was due to treatment of the protein source at similar crude protein levels. Seven references included 10 comparisons of unprocessed and processed soybean meal, rapeseed meal and faba beans. Iso-energetic and iso-nitrogenous rations where protein sources were either unprocessed or partially/fully replaced by processed protein sources to reduce ruminal degradability and increase amino acid supply to the small intestine from feed protein were compared. Processed protein sources were expeller and xylose-treated soybean meal, micronized rapeseed meal and heat treated faba beans. Forages were corn silage, grass silage, alfalfa hay or silage, grass clover silage, barley silage or a mixture of these. Cows were from 100 to 200 DIM, with an average of 130 DIM. Data was analyzed with proc mixed in SAS (SAS Institute) with treatment (unprocessed; processed) as the main effect. The feed composition variables NDF, starch, crude fat, PBV (all g/kg DM) and NEL (MJ/kg DM) and their second-order polynomials were included in the model, and backward elimination was used to eliminate non-significant ($P \leq 0.05$) variables. Models were fitted using weighted regression, including study as a random effect to account for between-study heterogeneity, and weights defined as the inverse of the variance in milk yield.

Results and Discussion

Across the dataset (n = 20), cows exhibited a high and variable production level, with an average dry matter intake (DMI) of 24.4 kg/day (range: 18.7–27.1 kg/day). Crude protein concentration was the same within treatments (unprocessed/processed) and averaged 170 g/kg DM among trials, spanning from 140 to 198 g/kg DM, indicating that the experiments covered diets from marginally to clearly adequate protein supply. Mean absorbable amino acids in the small intestine (AAT) supply was 2,335 g/day, corresponding to 96 g AAT/kg DM and 16.0 g

AAT per MJ NEL, reflecting diets designed to meet or exceed metabolizable protein requirements. Positive protein balance in the rumen was indicated by a mean PBV value of 28 g/kg DM, although substantial variation was observed (range: 4–57 g/kg DM).

Table 1 Response in milk production from unprocessed and processed protein sources, and NorFor feed characteristics.

	Unprocessed	Processed	SE	P
ECM, kg/day	35.4	35.2	0.51	0.68
Milk protein yield, g/day	1181	1186	8.85	0.63

Feed characteristics	Unprocessed		Processed	
	Mean	Std Dev	Mean	Std Dev
DMI, kg/day	24.5	2.52	24.2	2.37
NEL, MJ/kg DM	6.68	0.38	6.67	0.39
AAT, g/kg DM	94	9	97	6
PBV, g/kg DM	30	14	25	16
Microbial protein production, g/day	2307	236	2258	217
Rumen undegraded feed protein, g/day	1313	397	1456	325
Dietary AA from feed protein to small the intestine, g/day	766	336	858	260
AAT, g/MJ NEL	15.7	2.8	16.3	2.3
N utilization for milk, %	28.4	3.4	28.6	3.5

The present results show that replacing unprocessed with processed protein sources had no significant effects on ECM and milk protein yield (Table 1). Energy-corrected milk averaged 35.4 and 35.2 kg/day and milk protein yield 1181 and 1186 g/day for unprocessed and processed diets respectively.

Dry matter intake (DMI) was slightly lower for cows fed a processed protein diet compared with an unprocessed protein diet (24.2 vs. 24.5 kg/day. Increased protein protection may slightly reduce intake, possibly due to altered ruminal fermentation patterns or palatability (Olmos Colmenero and Broderick, 2006). Despite this numerical reduction in intake, NorFor calculated higher AAT per kg DM, AAT per MJ NEL, and an increased AAT supply of 93 g/day from feed protein in the processed protein diet compared with the unprocessed diet. Moreover, microbial crude protein synthesis was numerically lower with the processed (2258 vs. 2307 g/day) than the unprocessed diet.

Reynal and Broderick (2003) demonstrated that substituting solvent-extracted soybean meal with expeller soybean meal altered ruminal protein degradability and increased duodenal flow of undegraded protein but also resulted in limited responses in milk yield and milk protein yield. Similarly, Olmos Colmenero and Broderick (2006) reported that increasing rumen undegradable protein (RUP) supply via expeller soybean meal had minimal effects on milk production, while nitrogen efficiency declined when crude protein supply exceeded requirements. The absence of production responses in the current dataset therefore supports the conclusion that increasing dietary RUP does not provide additional benefits when microbial protein synthesis is not limited. The tendency toward numerically lower microbial crude protein synthesis observed with processed protein is also mechanistically plausible. Reducing ruminal protein degradability lowers ammonia availability for microbial growth, which may slightly depress microbial protein synthesis if RDP supply approaches the lower margin of adequacy (Mutsvangwa et al., 2016). The balance between microbial protein and dietary RUP

has also been described by Savari et al. (2018), who showed that shifting protein digestion post-ruminally alters nitrogen partitioning without necessarily improving productive performance.
From an environmental and efficiency perspective, the lack of production response combined with higher AAT supply suggests limited scope for performance gains from replacing unprocessed protein with processed protein under the conditions represented in the dataset. These findings align with Hansen et al. (2021), who reported that improving protein quality rather than quantity can reduce nitrogen losses but does not necessarily increase milk yield in diets already supplying adequate ruminal nitrogen. Nitrogen utilization efficiency for milk production was calculated to 28.4 and 28.6 for unprocessed and processed protein diets respectively, and values below 30 are considered low (Lavery and Ferris, 2021). This may indicate an inefficient utilization of dietary nitrogen for both unprocessed and processed protein diets, and the results suggest that increasing AAT supply beyond the NorFor recommendation of 15 g AAT per MJ NEL is unlikely to improve nitrogen utilization or milk protein synthesis.

Conclusions

The results indicate that processing of protein increased calculated AAT flow, but this increase did not provide any benefit in terms of improved milk production.

References

Chesini, R.G., Takiya, C.S., Dias, M.S.S., Silva, T.B.P., Nunes, A.T., Grigoletto, N.T.S., da Silva, G.G., Vittorazzi Jr., P.C., Rennó, L.N. Rennó, F.P., 2023. Dietary replacement of soybean meal with heat-treated soybean meal or high-protein corn distillers grains on nutrient digestibility and milk composition in mid-lactation cows. J. Dairy Sci. 106, 233–244

Hansen, N.P., Johansen, M., Wiking, L., Larsen, M., Lund, P., Larsen, T., Weisbjerg, M.R., 2021. Fava beans can substitute soybean meal and rapeseed meal as protein source in diets for lactating dairy cows. J. Dairy Sci. 104, 5508-5521.

Lapierre, H., Larsen, M., Sauvant, D., van Amburgh, M. E., & Kristensen, N. B., 2012. Consequences of supply and demand of amino acids on the mammary uptake and metabolism of amino acids in dairy cows. Animal 6, 1623–1632.

Lavery, A., Ferris, C. P., 2021. Proxy measures and novel strategies for estimating nitrogen utilisation efficiency in dairy cattle. Animals 11, 343.

Mutsvangwa, T., Davies, K.L., McKinnon, J.J., Christensen, D.A., 2016. Effects of dietary crude protein and rumen-degradable protein concentrations on milk production in dairy cows J. Dairy Sci. 99, 6298-6310.

Olmos Colmenero, J.J., Broderick, G.A., 2006. Effect of Amount and Ruminal Degradability of Soybean Meal Protein on Performance of Lactating Dairy Cows. J. Dairy Sci. 89, 1635-1643.

Patton, R. A., Hristov, A. N., & Lapierre, H., 2014. Protein feeding and balancing for amino acids in lactating dairy cattle. Veterinary Clinics of North America: Food Animal Practice, 30(3), 599–621.

Ramin, M., Höjer, A., Hetta., M., 2017. The effects of legume seeds on the lactation performance of dairy cows fed grass silage-based diets. Agric. Food Sci. 26, 129-137

Reynal, S.M., Broderick, G.A., 2003 Effects of feeding dairy cows protein supplements of varying ruminal degradability. J. Dairy Sci. 86, 835-843.

Santos, F. A. P., Santos, J. E. P., Theurer, C. B., & Huber, J. T., 1998. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. J. Dairy Sci. 81, 3182–3213.

Savari, M., Khorvash, M. Amanlou, H., Ghorbani, G.R., Ghasemi, E., Mirzaei, M., 2018. Effects of rumen-degradable protein: rumen-undegradable protein ratio and corn processing on production performance, nitrogen efficiency, and feeding behavior of Holstein dairy cows. J. Dairy Sci. 101, 1111-1122.

New windows into rumen metabolism through microbiomes and meta-omics

V. T. E. Aho[1], A. O. Maynez-Perez[1,2], C. M. Kobel[3], A. Schwarm[1] & P. B. Pope[4,5]
[1]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, PO Box 5003, 1432 Ås, Norway.
[2]Facultad de Zootecnia y Ecologia, Universidad Autonoma de Chihuahua, Chihuahua 31453, Chihuahua, Mexico.
[3]Department of Molecular Biology and Genetics, Plant Molecular Biology, Aarhus University, Aarhus, Denmark.
[4]The Centre for Microbiome Research, Queensland University of Technology, 4102 Woolloongabba, Australia.
[5]Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), 1432 Ås, Norway.
Correspondence: velma.tea.essi.aho@nmbu.no

Introduction

Ruminants are entirely dependent on microorganisms to digest their feed. The complex community of archaea, bacteria, fungi and protozoa residing in the rumen is responsible for converting plant polysaccharides into smaller molecules that the host animal can use, such as volatile fatty acids (VFAs), which are considered the main source of caloric energy for ruminants (Bergman, 1990). Furthermore, a majority of the host protein requirement is fulfilled by microbial protein (Dewhurst et al., 2000). The relationship is one of mutual dependence: many of the microorganisms cannot survive outside the specific environmental conditions of the rumen. In fact, the animal and its microbial community can be seen as a single biological unit, a "holobiont" (Kobel et al., 2024). Since the animal and its microbial partners exist in this obligate symbiosis, we cannot fully understand the biological processes taking place in the rumen without understanding the metabolic activities of the microbial community.

Within the past few decades, our ability to measure complex microbial communities has advanced in strides. Much of this progress has been due to the rapid development of DNA sequencing technologies (Escobar-Zepeda et al., 2015). Instead of individual genomes of single microbial species grown in pure culture, we are now able characterize microbial communities directly from environmental or host-associated samples, such as faeces or rumen content. Up to date, most rumen microbiome studies have relied on marker gene sequencing approaches, which only provide information about relative amounts of microbial species (Denman et al., 2018). However, studies that reconstruct collections of microbial genomes, known as metagenome-assembled genomes (MAGs), are becoming increasingly common. MAGs provide a detailed picture of the metabolic capabilities of rumen microbes but can only tell what the organisms are capable of doing, not whether those processes are active. Additional tools enable exploring the activity: metatranscriptomics (sequencing all the RNA), metaproteomics (quantifying all the protein), as well as (meta)metabolomics (quantifying small molecules that are the output of metabolic processes, such as amino acids, fatty acids or sugars). These methods can complement each other: when metagenome data is used as a reference for metatranscriptomic and metaproteomic analyses, it is possible to determine which microbial species or strains are producing the measured RNA or protein. Together, these high-throughput methods for microbial analyses can be described as "meta-omics" and research that combines multiple meta-omics tools as "multi-omics" (Kobel et al., 2024). Here, we present two of our

recent studies (Kobel et al., 2025; Maynez-Perez et al., 2025) that showcase how these tools can be used to open new windows into the microbial metabolic activity in the rumen.

Reconsidering rumen ciliates

Ciliate protozoa, also known as ciliates, are a group of eukaryotic microorganisms that comprise a large proportion of the biomass contained within the rumen, with estimates ranging from 25 to 50% (Firkins et al., 2020). Protozoa participate in key rumen processes such as feed digestion and nitrogen utilization, but also contribute to methane emissions: the hydrogen that is released as a byproduct of their metabolism is taken up by archaea that use it to produce methane (Park, 2024; Xie et al., 2026). Research on rumen ciliates has been hampered by the challenges of growing them in pure culture and the resulting lack of available reference genomes (Li et al., 2022; Park, 2024). Combined with these technical challenges, the fact that ruminants can thrive without their ciliate populations (Williams & Coleman, 1992) has led to ciliates often being overlooked in rumen research. On the other hand, these microorganisms are ubiquitously present in wild and domesticated ruminant species across the world, and in a production setting, animals will inevitably harbour them, since the only way of preventing ciliate colonization is rearing the animal in isolation since birth (Williams & Coleman, 1992).

When we set out to analyze a comprehensive multi-omic dataset of 24 beef cattle steers (n=12 Luing, n=12 Aberdeen Angus cross) from a feedlot trial at Scotland's Rural College (Kobel et al., 2025), we had no particular aim to focus on ciliates. Instead, when our initial explorative data analyses indicated a visual separation of the rumen microbial community into two distinct clusters, we focused on trying to understand the source of this pattern. The two clusters were not related to any documented factor, either methodological (e.g. pen or sampling batch) or biological (e.g. breed), and could be detected in all measured meta-omic layers: metagenomic, metatranscriptomic, metaproteomic and metabolomic data, in both rumen content and post-slaughter rumen wall samples. On closer inspection, we found that many of the differences between clusters were in abundances and activities of ciliates, such as *Polyplastron multivesiculatum* and the genus *Epidinium*. These organisms have long been considered as indicators of two protozoal community types, labelled as type A and B (Eadie, 1962; Williams & Coleman, 1992). Due to reasons that remain unknown, most animals are either type A or type B, containing either *P. multivesiculatum* (type A) or *Epidinium* species and another genus, *Eudiplodinium* (type B), but rarely a mix of these organisms. Our results independently reproduced this originally light microscopy based observation of community structures using omics methods.

While the concept of ciliate community types has existed since the 1960s, modern meta-omics tools enable deeper exploration of microbial activities and the associations of ciliates with other rumen microbial populations. A key finding of our study was that ciliate community differences go hand in hand with equally noticeable differences in abundances and activities of bacteria and archaea, which is why we decided to label our two clusters as "rumen community types" instead of calling them protozoal community types. Furthermore, our results suggested that these community types are reflected in downstream metabolite levels, captured by metabolomics, including those of several amino acids and volatile fatty acids (VFAs): a higher proportion of propionate was seen in type A and higher butyrate in type B animals. The type B community was also associated with higher methane emissions in one out of two cattle breeds included in the trial (Figure 1), which could indicate an interplay between community type and animal genetics. While past studies have shown some degree of heritability in the rumen

microbiome, such analyses have focused on bacteria and archaea (Difford et al., 2018; Martinez-Boggio et al., 2024); the potential relationship of animal genetics and ciliate populations has yet to be explored.

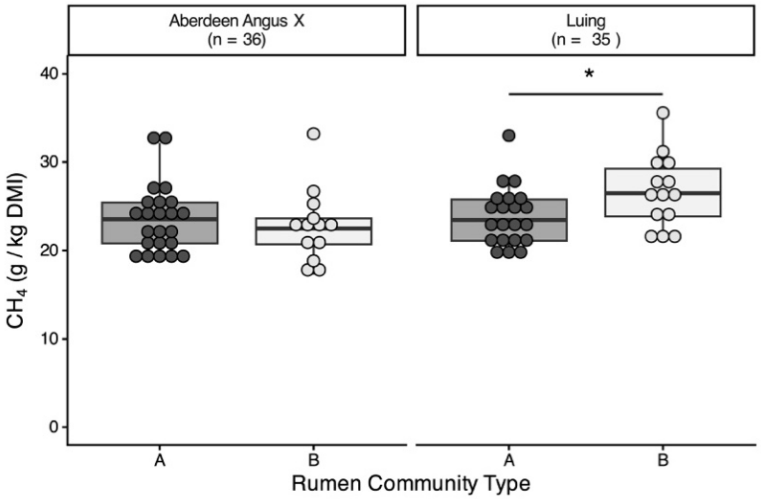


Figure 1 Methane yield (grams per kg dry matter intake, measured in respiration chambers) by breed and rumen community type, indicating a difference in Luing, but not in Aberdeen Angus cross animals. Statistical comparisons between A and B community types performed with Wilcoxon rank-sum tests; * = $P < 0.05$. Previously unpublished figure based on data from Kobel et al. 2025. The data shown is from 71 animals that underwent marker gene based rumen microbiome profiling and methane measurements; the 24 animals which were analysed with the full suite of meta-omic methods are a subset of these 71.

We were only able to study ciliates in detail due to a publication that used culture-independent molecular methods (single-cell sequencing) to generate 52 ciliate genomes (Li et al., 2022), which we utilized as a reference database for our metatranscriptomic and metaproteomic analyses. As technology develops and becomes more affordable, more resources such as this will be published; in fact, a new catalogue of 450 ciliate genomes (Xie et al., 2026) became available during the writing process of this conference manuscript. With improved tools and resources, future research will be able to delve deeper into the interplay of rumen ciliates and other members of the rumen microbial community as well as their relevance to the ruminant host. Considering the community type associated differences we saw in downstream rumen metabolites, it seems justified to suggest that we should pay more attention to ciliates, whether in the context of methane mitigation or that of improving animal productivity.

Microbial perspectives to methane intensity

Different metrics can be used to measure the methane output of an animal, including simple production (grams of methane per day) and ratio-based measures such as methane yield (grams per kilogram of dry matter intake) (Fresco et al., 2023). A measure that connects methane directly to productivity in the context of dairy cows is methane intensity as grams per kilogram of energy-corrected milk (ECM). Two animals that produce the same amount of methane, measured in g/day, differ in methane intensity if one of them has a higher milk production than the other. If the animals consume the same amount of feed, differences in methane intensity reflect differences in energy partitioning. Microbial metabolism is relevant for explaining these differences, since the energy available to the animal depends on microbial metabolism. Specific microbial functions of interest include carbohydrate active enzymes (CAZymes), which are

responsible for the breakdown of dietary plant polysaccharides (Lombard et al., 2014), as well as metabolic pathways that result in VFAs. Producing different VFAs releases or binds different amounts of hydrogen, a key molecule in methane production, and also have different roles in host metabolism and health (Beckett et al., 2021; Mahboubi et al., 2026).

In our recent study focusing on methane intensity, we used a multi-omic approach to analyse rumen samples from 16 Norwegian Red dairy cows housed at the animal facilities of the Norwegian University of Life Sciences (Maynez-Perez et al., 2025). The animals were divided into two groups of equal size so that half of them (n=8) had high and the other half (n=8) low methane intensity, based on methane emissions quantified using a GreenFeed system and ECM calculated from milk yield and composition according to standard energy correction equations. The omics methods consisted of metagenomics and metatranscriptomics: long-read DNA sequencing was used to generate a catalogue of metagenome-assembled genomes, which were used as the reference database to analyse the RNA sequencing data. This combination of methods provided us with information about which bacteria and archaea were present in samples, what functions were encoded in their genomes, and which of those were being actively transcribed.

The high and low methane intensity animals in our study had similar feed intake, digestibility, and methane yield, but the low intensity animals produced more ECM, suggesting a difference in partitioning available energy to milk. Overall microbial communities in the two groups, as measured based on metagenomic abundances, were not significantly different. However, metatranscriptomic analysis revealed differences in the activity of carbohydrate degradation and fermentation pathways: the high methane intensity animals' microbial communities expressed more pectin-degrading CAZymes, while the low methane group had higher activity of fructan-degrading CAZymes as well as enzymes related to butyrate production. Each functional category could be associated with a specific set of bacteria that were actively expressing these enzymes, and we further hypothesized that downstream metabolites from fructan degraders, such as lactate and acetate, might be utilized by butyrate producers through cross-feeding interactions. The pectin degradation in the high methane intensity group is expected to lead to production of methyl esters that can be further converted to methanol and used for methane production. Our metatranscriptomic results implied a trend of higher methane producing activity in the high methane intensity animals, but this was not statistically significant, which is in line with the lack of detected differences in methane output.

All animals in the study were fed an identical diet (grass silage and concentrate with an average ratio of 83:17 on a DM basis), containing both pectins and fructans. However, it appears that the microbial communities of high and low methane intensity animals may have been selective in their activity, preferring one of these carbohydrates over the other. The metatranscriptomic data also suggested differences in butyrate production; unfortunately, our study did not include quantification of VFAs to confirm this. While the study was also limited by a small sample size (n=8 per group) and the lack of detailed digestibility information about specific carbohydrate fractions, the implication that microbial carbohydrate specialization could underlie the differences in methane intensity is nevertheless intriguing and warrants further research.

Conclusions

Meta-omics tools offer a promising way to gain insight into the metabolic activities of rumen microorganisms, far surpassing the microbial abundance information provided by earlier

marker gene based microbiome profiling methods. Our recent work has highlighted the importance of ciliates as shapers of the rumen microbial community and suggested a link between bacterial carbohydrate specialization and differences in methane intensity. However, such results need to be interpreted in the wider context, considering all relevant animal and environmental parameters, and confirmed in more targeted mechanistic studies. Experts in microbial analyses need to come together with researchers specialized in topics such as breeding, nutrition, physiology, and feed science. Microbial discoveries cannot be translated into practical solutions for improved productivity and sustainability without close interdisciplinary collaboration.

References

Beckett, L., Gleason, C. B., Bedford, A., Liebe, D., Yohe, T. T., Hall, M. B., Daniels, K. M., & White, R. R., 2021. Rumen volatile fatty acid molar proportions, rumen epithelial gene expression, and blood metabolite concentration responses to ruminally degradable starch and fiber supplies. J. Dairy Sci., 104(8), 8857–8869.

Bergman, E. N., 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Reviews, 70(2), 567–590.

Denman, S. E., Morgavi, D. P., & McSweeney, C. S., 2018. Review: The application of omics to rumen microbiota function. Animal, 12, s233–s245.

Dewhurst, R. J., Davies, D. R., & Merry, R. J., 2000. Microbial protein supply from the rumen. Anim. Feed Sci. Technol., 85(1), 1–21.

Difford, G. F., Plichta, D. R., Løvendahl, P., Lassen, J., Noel, S. J., Højberg, O., Wright, A.-D. G., Zhu, Z., Kristensen, L., Nielsen, H. B., Guldbrandtsen, B., & Sahana, G., 2018. Host genetics and the rumen microbiome jointly associate with methane emissions in dairy cows. PLoS Genetics, 14(10), e1007580.

Eadie, J. M., 1962. Inter-Relationships between Certain Rumen Ciliate Protozoa. Journal of General Microbiology, 29, 579–588.

Escobar-Zepeda, A., Vera-Ponce de León, A., & Sanchez-Flores, A., 2015. The Road to Metagenomics: From Microbiology to DNA Sequencing Technologies and Bioinformatics. Frontiers in Genetics, 6.

Firkins, J. L., Yu, Z., Park, T., & Plank, J. E., 2020. Extending Burk Dehority's Perspectives on the Role of Ciliate Protozoa in the Rumen. Frontiers in Microbiology, 11.

Fresco, S., Boichard, D., Fritz, S., Lefebvre, R., Barbey, S., Gaborit, M., & Martin, P., 2023. Comparison of methane production, intensity, and yield throughout lactation in Holstein cows. J. Dairy Sci., 106(6), 4147–4157.

Kobel, C. M., Leu, A., Vera-Ponce de León, A., Øyås, O., Lai, W., Altshuler, I., Hagen, L. H., Wollenberg, R. D., Søndergaard, M. T., Bakshani, C. R., Willats, W. G. T., Nicoll, L., McIlroy, S. J., Hvidsten, T. R., Schmidt, O., Greening, C., Tyson, G. W., Roehe, R., Aho, V. T. E., & Pope, P. B., 2025. Protozoal populations drive system-wide variation in the rumen microbiome. Nature Communications, 16(1), 6238.

Kobel, C. M., Merkesvik, J., Burgos, I. M. T., Lai, W., Øyås, O., Pope, P. B., Hvidsten, T. R., & Aho, V. T. E., 2024. Integrating host and microbiome biology using holo-omics. Molecular Omics, 20(7), 438–452.

Li, Z., Wang, X., Zhang, Y., Yu, Z., Zhang, T., Dai, X., Pan, X., Jing, R., Yan, Y., Liu, Y., Gao, S., Li, F., Huang, Y., Tian, J., Yao, J., Xing, X., Shi, T., Ning, J., Yao, B., Huang, H., & Jiang, Y., 2022. Genomic insights into the phylogeny and biomass-degrading enzymes of rumen ciliates. The ISME Journal, 16(12), Article 12.

Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M., & Henrissat, B., 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Research, 42(D1), D490–D495.

Mahboubi, A., Holmström, K., Parchami, M., Uwineza, C., Agnihotri, S., Jomnonkhaow, U., Nadeau, E., & Taherzadeh, M. J., 2026. Volatile fatty acids in ruminants and their role as feed additives: a review. J. Applied Anim. Res., 54(1), 2630934.

Martinez-Boggio, G., Monteiro, H. F., Lima, F. S., Figueiredo, C. C., Bisinotto, R. S., Santos, J. E. P., Mion, B., Schenkel, F. S., Ribeiro, E. S., Weigel, K. A., Rosa, G. J. M., & Peñagaricano, F., 2024. Revealing host genome–microbiome networks underlying feed efficiency in dairy cows. Scientific Reports, 14, 26060.

Maynez-Perez, A. O., Cahyo, H. N., Niu, P., Aho, V. T. E., Pope, P. B., & Schwarm, A., 2025. Intricate microbiome differences observed in lactating cows across methane intensity phenotypes Preprint, bioRxiv. https://doi.org/10.64898/2025.12.11.692445

Park, T., 2024. — Invited Review — Ruminal ciliates as modulators of the rumen microbiome. Animal Bioscience, 37(2), 385–395.

Williams, A. G., & Coleman, G. S., 1992. The Rumen Protozoa. Springer New York.

Xie, F., Jiang, C., Li, Z., Feng, J., Yan, X., Hu, C., He, J., Chai, X., Huang, Z., Xu, Q., Wang, Y., Xiao, Y., Chen, K., Qin, W., Xiao, Y., Zhang, J., Wang, G., Jin, W., Guo, K., Lin, L., Liu, Y., Gao, X., Zheng, L., Shu, X., Wang, R., Wang, M., Si, H., Du, R., Zhu, W., Guan, L. L., Wang, W., Qiu, Q., Mao, S., Xiong, J., & Miao, W., 2026. Rumen ciliates modulate methane emissions in ruminants. Science, 392(6797), eadv4244.

Risk potential of different diets for rumen and hindgut acidosis in dairy cows

Matthias Schilde[1] & Wilfried van Straalen[1]
[1]*Schothorst Feed Research (SFR), Meerkoetenweg 26, 8218NA Lelystad, The Netherlands.*
Correspondence: mschilde@schothorst.nl

Introduction

Hindgut acidosis often occurs in association with rumen acidosis. Low digesta pH and accumulation of volatile fatty acids at the intestinal wall can lead to a transfer of lipopolysaccharides (LPS) into the circulation causing systemic inflammation (Gressley et al., 2011). Research needs to address which dietary factors induce isolated hindgut acidosis.

The study aims at investigating potential effects of varying levels of bypass starch (TBST), rapidly fermentable carbohydrates (RFCH), low aNDFom and bypass NDF (LNDF), and true metabolizable protein (TMP) on rumen and hindgut acidosis, and to derive diet-related risk factors.

Materials and Methods

A subacute ruminal acidosis (SARA) challenge model, as described by Ghozho et al. (2005) (Fig. 1), was applied in a completely randomized block design. In total, 48 Holstein-Friesian cows (82 ± 18.8 (mean ± SD) DIM, 2.4 ± 1.4 lactations) were allocated to 12 blocks based on parity, reticular pH parameters, and fat-protein-corrected milk (FPCM) during a 3-week adaptation period. This was followed by a covariate period to collect cow data for blocking. During the first 31 days, all cows received the same control diet, offered as a partial mixed ration (32% corn silage, 32% grass silage, 4.3% rapeseed straw, 4.4% soybean meal 48% CP, 4.3% formaldehyde-treated soybean meal, 8.5% ground corn, 14.7% ground wheat; DM basis). Within each block, four cows were randomly assigned to one of four experimental diets. In a 3-days build-up phase, diet compositions were gradually adjusted to the targeted composition of the challenge phase. In the subsequent 4-days challenge phase, cows received the diets with the intended final composition. In a subsequent 4-days follow-up, cows returned to the baseline control to assess potential carryover effects of the challenge diets.

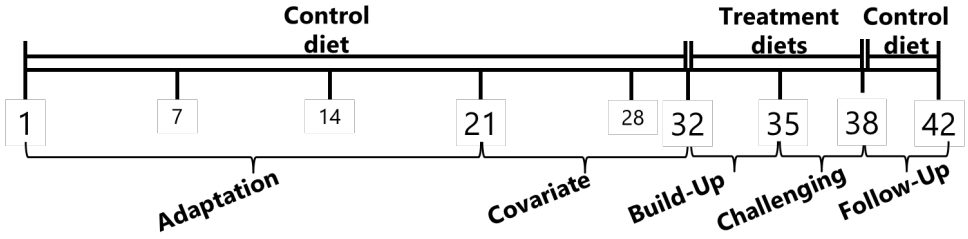


Figure 1 Diet adaptation acc. to the experimental timeline (days) of an acidosis challenge (Gozho et al., 2005).

In treatment 1 (TBST), the content of TBST was increased via ground corn to investigate the isolated effect of high TBST levels on SARA and hindgut acidosis. In treatment 2 (RFCH), concentrations of TBST and RFCH increased using ground corn and wheat to examine the combined effects of high TBST and RFCH levels. In treatment 3 (LNDF), TBST and RFCH contents were increased as in treatment 2, but combined with a reduction in aNDFom and bypass NDF via less rapeseed straw inclusion. In treatment 4 (TMP), the concentration of TMP increased via protected soybean meal, in combination with TBST and RFCH under low aNDFom and bypass NDF supply, to assess the risk of hindgut acidosis.

Daily DM intake and milk yield were recorded individually for each cow. Milk composition was analysed weekly from four consecutive milkings. The milk fatty acid (FA) profile was determined by gas chromatography. Body weight (BW) were automatically recorded after each milking. pH boluses (SmaXtec®) were administered into the reticulum of all cows one week prior to the start of the adaptation phase. Blood samples were collected on days 29, 37, and 38 from coccygeal vein. Concentrations of haptoglobin, serum amyloid A, liver enzyme activity, total bilirubin, LPS-binding protein, and tight junction proteins were analysed. For each cow, faecal pH, faecal consistency (Zaaijer et al., 2001), and faecal particle size distribution (NASCO®) were measured on three consecutive days in each phase. Cow-individual daily data on pH, feed intake, and performance were aggregated into weekly and phase-based means. A mixed model with measurement timepoint as repeated measures was computed in R (v4.5.2), including block and cow as random effects, and treatment group and phase as fixed effects. Measurements from covariate period were included as covariables.

Results and Discussion

Starch concentrations varied between 333 g/kg DM (TBST), 352 g/kg DM (RFCH, TMP), and 362 g/kg DM (LNDF) groups in the challenge phase. The aNDFom concentrations were markedly lower in groups with less rapeseed straw (LNDF, TMP) compared to TBST and RFCH groups (247 vs. 274 g/kg DM). The bypass starch level was higher in the TBST (95 g/kg DM) than in other groups (77 g/kg DM) and clearly exceeded recommendations of 75 g/kg DM. Concentrations of RFCH exceeded recommendations of 180 g/kg DM in all groups except for TBST. The TMP content was 110 g/kg DM in TMP group and greater than in the other three groups (95 g/kg DM) which were fed according to TMP recommendation.

Threshold values for SARA are defined as a daily mean ruminal pH < 6.16 and > 324 min/d with pH < 5.8 (Zebeli et al., 2008). Because reticular pH values are ~0.2 units higher than in the rumen, the threshold values were adjusted to 6.0. SARA thresholds were exceeded in the challenge phase confirming that SARA was successfully induced. The daily mean pH did not differ between groups (Tab. 1). Minimum, maximum, and mean pH were lowest during the challenge phase ($P < 0.001$) and in groups with low aNDFom and bypass NDF (LNDF, TMP). The pH fluctuation, time with pH < 6.0, and AUC with pH <6.0 were greatest in the challenge phase and in groups with lowest levels of aNDFom and bypass NDF fiber and high RFCH levels (LNDF, TMP) ($P < 0.001$). A comparison between the RFCH and LNDF groups shows that, at similar concentrations of RFCH, the lower proportion of aNDFom and bypass NDF in the LNDF group resulted in significantly lower pH values, highlighting the critical importance of adequate fibre supply. In contrast to the RFCH group, increasing the TBST levels did not result in higher ruminal pH values. During the follow-up phase, pH returned to baseline levels.

Faecal pH and score were lowest in challenge phase and in the TBST group compared to the other groups (faecal pH 6.18 vs. 6.47; $P < 0.001$). Although no uniform faecal pH threshold for hindgut acidosis has yet been established, the present results indicate an increased risk of hindgut acidosis if faecal pH values are below 6.2. Faeces was more loose in the TBST group and challenge phase ($P < 0.001$). The faecal particle size distribution, BCS, BW change, milk composition, and FPCM yield did not differ between groups. The DM intake, FPCM, fat and protein yields, and milk fat and protein % decreased in the challenge phase ($P < 0.001$). A fat-to-protein ratio (FPR) of < 1.0 is indicative of SARA. The FPR was lowest (0.99) in the challenge phase and in the RFCH (0.95) and LNDF (0.98) groups. Lower milk urea contents were also observed during the challenge phase and in the LNDF and RFCH groups as

commonly associated with SARA. The lowest concentrations of *trans*-10, *cis*-12 C18:2) were observed in RFCH and LNDF groups (P = 0.04), while *trans*-10 C18:1 concentrations did not differ between treatments. An increase in short- and medium-chain FA, along with a decrease in *iso* C17:0 and *iso* C18:0, was observed in cows with SARA, confirming previous findings (Coleman et al., 2013). No significant changes in serum parameters were detected.

Table 1 Dry matter (DM) intake, milk production and composition, faeces pH and score, and reticular pH of early-lactating cows supplied with diets with high concentrations of true bypass starch (TBST), rapidly-fermentable carbohydrates (RFCH), low aNDFom and bypass NDF (LNDF), and high levels of true metabolizable protein (TMP) in the acidosis challenge phase. During the adaptation phase, all groups received the same diet [1)].

		Milk parameters			**Faeces**		**Rumeno-reticular pH**			
	DM intake	FPCM (kg/d)	Fat-Protein-Ratio	Milk urea (mg/dL)	pH	Score[2)]	Daily mean	Range	Min/d pH<6.0	AUC <6.0
Adaptation[3)]	23.0^{B}	37.9^{B}	1.07^{B}	21.9^{B}	6.62^{B}	3.15^{B}	6.37^{B}	0.89^{A}	102^{A}	13^{A}
Challenge[3)]	21.6^{A}	36.3^{A}	0.99^{A}	16.0^{A}	6.37^{A}	2.82^{A}	6.31^{A}	1.38^{B}	394^{C}	89^{B}
TBST	22.0	36.6	1.04	17.3^{b}	6.18^{a}	2.52^{a}	6.31	1.22^{a}	336	665^{a}
RFCH	21.6	36.2	0.98	16.2^{b}	6.48^{b}	2.90^{bc}	6.30	1.29^{a}	365	77^{a}
LNDF	21.2	36.3	0.95	14.1^{a}	6.46^{b}	2.68^{ab}	6.31	1.51^{b}	420	92^{ab}
TMP	21.5	36.1	1.00	16.3^{b}	6.39^{b}	3.13^{c}	6.30	1.51^{b}	457	121^{b}

[1)] Means with different capital superscripts within a column between experimental periods differ significantly (P<0.05). Means with different lowercase superscripts within a column between groups differ significantly. [2)] Faeces consistency on a scale of 1 to 5 points (Zaaijer et al. 2001). 1 = liquid, 3 = optimal, 5 = very hard. [3)] Rows with name of row in bold depict experimental period means over treatment groups.

Conclusions

High levels of RFCH in combination with starch levels greater than 330 g/kg DM and NDF levels of less than 274 g/kg DM, as fed in the present study, induced a high risk for SARA. High concentrations of TBST, in combination with elevated feed intake and, due to the latter an assumed higher ruminal passage rate, resulted in increased hindgut fermentation representing the primary risk factors for hindgut acidosis. The study outlined that feeding exceeding levels of TBST may lead to isolated hindgut acidosis, even in the absence of SARA. Metabolizable protein was not a risk factor for acidosis. The present short-term challenge possibly did not result in substantial LPS translocation or impairment of intestinal barrier function which could be further investigated in a long-term acidosis challenge.

References

Colman E., Fokkink, W.F., Craninx, M., Newbold, J.R., De Baets, B. & Fievez, V., 2010. Effect of induction of subacute ruminal acidosis on milk fat profile and rumen parameters. J. Dairy Sci. 93, 4759-4773.

Gohzo, G.N., Plaizier, J.C., Krause, D.O., et al., 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. J Dairy Sci., 88, 1399-1403.

Gressley T.F., Hall, M.B. & Armentano, L.E., 2011. Ruminant nutrition symposium: Productivity and health responses to hindgut acidosis. J. Anim. Sci. 89, 1120-1130.

Zebeli, Q., Dijkstra, J., Tafaj, M., Steingass, H., Ametaj, B.N., Drochner, W., 2008. Modeling the Adequacy of Dietary Fiber in Dairy Cows Based on the Responses of Ruminal pH and Milk Fat Production to Composition of the Diet. J Dairy Sci. 91, 2046-2066.

Effects of reduced concentrate feeding on rumen fermentation and methane production in early lactation dairy cows

T. Kokkonen[1], N. Rummukainen[1], S. Salin[1], P. Rissanen[1], M. Kemppainen[1], S.E. Räisänen[2], M. Hovinen[3], A. Halmemies-Beauchet-Filleau[1], M. Lamminen[1] & A. Vanhatalo[1]
[1]Department of Agricultural Sciences, University of Helsinki, Finland. [2]Department of Animal and Veterinary Science (Ruminant Nutrition), Aarhus University, Denmark. [3]Department of Production Animal Medicine, University of Helsinki, Finland. Correspondence: Tuomo.Kokkonen@helsinki.fi

Introduction

A major factor behind production diseases in dairy cows is the imbalance between energy requirements and intake. Often, efforts are made to improve cows' energy intake by increasing concentrate feeding during early lactation, although this may not necessarily improve energy balance because a large portion of the additional energy is directed towards milk production. High concentrate feeding decreases rumen pH (Dijkstra et al., 2012), impairs fibre digestibility in the rumen (Nousiainen et al., 2009), and may slow down the increase of forage intake after calving (Ingvartsen et al., 2001). We aimed to study the effect of two different dietary concentrate proportions on rumen fermentation and methane production in grass silage-based feeding of dairy cows.

Materials and Methods

The experimental design was a randomized block-design, starting from calving, using 30 multiparous Nordic Red cows. Cows were assigned to pairs based on parity, expected calving date, body weight and milk yield during the previous lactation. Planned concentrate proportions in the diet were 37.5% and 50% at the maximum level on concentrate supplementation. Both groups had ad libitum access to the same partial mixed ration (PMR). Concentrate allowances from the milking robot were 2 kg/d and 8 kg/d (as fed), respectively. Rumen fluid samples were collected via oral stomach tubing once a day 1, 3 and 6 wk after calving to study rumen fermentation. Feed intake and milk yield were measured daily, and milk samples for milk constituents were collected during weeks 1, 2, 3, 4, 6 and 8. Methane production was measured using Greenfeed system, based on 20 visits per animal during weeks 5 to 12, i.e. after feed intake was stabilized.

Grass silages used were mainly mixtures of 1st and 2nd cut silages. In addition, 3rd cut grass silage was used in the silage mixture at the end of the experiment. Average D values of 1st and 2nd cut silages were 667 and 660 g/kg DM respectively, whereas the D value of 3rd cut silage was higher (702 g/kg DM). Dietary composition is reported in Table 1.

Table 1 Diet composition based on observed feed intake (weeks 1 to 12)

	Concentrate proportion	
	37.5%	50%
Feed ingredients, % of dry matter		
Grass silage	66.3	55.9
Concentrate mixture	26.5	22.5
Robot feed	7.2	21.6
Chemical composition, % of DM		
Crude protein	17.1	16.4
Neutral detergent fibre	42.3	39.1
Starch	11.4	16.4

Feed and nutrient intakes, milk yield and rumen fermentation were analysed with repeated measures variance analysis using the Mixed procedure of SAS (version 9.4). The model included the fixed effect of treatment, week, treatment x week and the random effect of pair and week x pair. Methane production was analysed with variance analysis using model that included fixed effect of treatment and the random effect of pair. Feed and nutrient intakes were log-transformed prior to statistical analysis to improve normality. Treatment effects were considered statistically significant at $p < 0.05$ and tendencies at $0.05 \leq p < 0.10$.

Results and Discussion

Average concentrate proportions in the diet were 33.7% and 44.1%. Reducing the concentrate in the diet reduced dietary starch concentration and starch intake (Table 2). There was a numerical difference of 0.9 kg/d in total dry matter intake (DMI), but cows on the reduced concentrate proportion had a 2.6 kg/d greater PMR intake. Consequently, there were no differences in crude protein and neutral detergent fibre (NDF) intakes. Reducing dietary concentrate proportion tended to decrease metabolizable energy (ME) intake.

Average milk yield was 10% lower and ECM yield 5.7% lower with reduced concentrate feeding, while feed conversion efficiency and ME balance did not differ between the treatments (Table 2). The current results indicate that higher concentrate allowance in PMR feeding effectively increased ME intake during early weeks of lactation, in agreement with the observations by Kajava et al. (2026). Increased energy intake is primarily diverted to milk production, and hence energy balance was not improved by higher concentrate allowance in this study as was also observed in Kajava et al. (2026).

Table 2 Feed and nutrient intake and milk production (weeks 1 to 12)

	Concentrate proportion			Statistical significance		
	37.5%	50%	SEM	Treatment	Week	Trt x Wk
Total DMI, kg/d	23.6	24.7	0.55	0.187	<0.001	0.100
PMR DMI, kg/d	21.9	19.3	0.54	0.002	<0.001	0.540
Crude protein intake, kg/d	4.04	4.05	0.096	0.970	<0.001	0.498
Starch intake, kg/d	2.67	4.06	0.081	<0.001	<0.001	<0.001
NDF intake, kg/d	9.98	9.64	0.260	0.323	<0.001	0.896
ME intake, MJ/d	273	291	6.3	0.063	<0.001	0.022
Milk, kg/d	37.4	41.5	1.17	0.004	<0.001	0.141
ECM, kg/d	39.5	41.9	1.13	0.068	<0.001	0.040
ECM, kg/DMI, kg	1.76	1.83	0.051	0.338	<0.001	0.166
ME balance, MJ/d	-29.9	-36.8	6.113	0.378	<0.001	0.080

Rumen total volatile fatty acid (VFA) concentration was lower, and pH tended to be higher with reduced concentrate feeding (Table 3), owing to the decrease in intake of rumen-fermentable organic matter. Further, as a typical consequence of an increased forage proportion in the diet and increased pH (Dijkstra et al., 2012), molar proportion of propionate in VFA decreased with reduced concentrate feeding. Proportion of acetate significantly increased only at week 3 (trt x wk interaction P = 0.024). Proportion of butyrate tended to decrease over time with reduced concentrate feeding, while the opposite was observed with higher concentrate allowance (trt x wk interaction P = 0.002). Despite differences in proportion of propionate and pH, no difference was observed in methane production (Table 4). This may be explained by the moderate starch concentration (16.4% in DM) of the diet with higher concentrate

allowance. According to a review by Vanhatalo & Halmemies-Beauchet-Filleau (2020), higher dietary starch concentration (20 to 22% in DM) is needed to mitigate ruminal methanogenesis.

Table 3 Rumen fermentation (weeks 1, 3 and 6)

	Concentrate proportion			Statistical significance		
	37.5%	50%	SEM	Treatment	Week	Trt x Wk
Total VFA, mmol/l	80.7	88.1	1.961	0.007	0.355	0.915
pH	6.68	6.58	0.041	0.067	0.846	0.538
Acetate, mol%	63.7	62.2	0.479	0.017	0.422	0.024
Propionate, mol%	18.1	19.4	0.393	0.014	0.750	0.185
Butyrate, mol%	12.5	12.8	0.259	0.396	0.180	0.002

Table 4 Methane production (weeks 5 to 12)

	Concentrate proportion			Statistical significance
	37.5%	50%	SEM	Treatment
Methane g/d	455	473	15.6	0.378
DMI, kg/d	24.0	25.9	0.76	0.069
ECM, kg/d	41.7	45.1	1.28	0.049
Methane g/kg DM	19.1	18.2	0.65	0.246
Methane g/kg ECM	11.0	10.5	0.37	0.346

Conclusions

Early lactation dairy cows allocated to reduced concentrate feeding from the milking robot could partly compensate decreased concentrate energy allowance by increasing PMR intake. Lower energy intake reduced milk yield and consequently there was no difference in energy balance. The effects on rumen fermentation were not large enough to affect rumen methanogenesis. Dietary concentrate proportion of early lactation dairy cows can be reduced without adverse effect on energy balance and with moderate effects on rumen fermentation. However, reduced milk yield and decreased milk income remain constraining factor.

References

Dijkstra, J., Ellis, J.L., Kebreabb, E., Strathe, A.B., López, S., France, J. & Bannink, A. 2012. Ruminal pH regulation and nutritional consequences of low pH. Anim. Feed Sci. Technol. 172, 22-33.

Ingvartsen, K.L., Aaes, O. & Andersen, J.B., 2001. Effects of pattern of concentrate allocation in the dry period and early lactation on feed intake and lactational performance in dairy cows. Livest. Prod. Sci. 71, 207– 221.

Kajava, S., Sairanen, A. & Rinne, M. 2026. Evaluation of concentrate feeding strategies in an intensive grass silage-based dairy production system. Anim. Feed Sci. Technol. 331, 116595.
Nousiainen, J., Rinne, M. & Huhtanen, P., 2009. A meta-analysis of feed digestion in dairy cows. 1. The effects of forage and concentrate factors on total diet digestibility. J. Dairy Sci. 92, 5019–5030.

Vanhatalo, A. & Halmemies-Beauchet-Filleau, A. 2020. Optimising ruminal function: the role of silage and concentrate in dairy cow nutrition to improve feed efficiency and reduce methane and nitrogen emissions, in: McSweeney, C.S., Mackie, R.I. (Eds.), Improving rumen function. Burleigh Dodds Science Publishing, Cambridge, UK.

Dietary AAT calculated in NorFor and its effects on responses in energy-corrected milk and milk protein yields in dairy cows

J. K. Sommerseth[1], I. Schei[1], M. Åkerlind[2], A. M. H. Kjeldsen[3], H. Volden[4] & N. I. Nielsen[3]
[1]TINE SA, R&D-dept, Chr. Magnus Falsens vei 18, 1432 Ås, Norway
[2]Växa Sverige, Box 30204, 104 25 Stockholm, Sweden
[3]SEGES Innovation, Agro Food Park 15, 8200 Aarhus N, Denmark
[4]Department of Animal and Aquacultural Science, Faculty of Biosciences, Norwegian University of Life Sciences, P.O.Box 5003, 1432 Ås, Norway
Correspondence: jon.kristian.sommerseth@tine.no

Introduction

Increased demand for milk has led dairy farmers to consider strategies to increase milk yield per cow. One proposed measure has been to increase the dietary AAT supply, based on the assumption that this may promote milk yield (Benchaar *et al.*, 2023). However, increased dietary crude protein (CP) supply reduces nitrogen use efficiency in dairy cows and increases nitrogen losses to the environment (Katongole & Yan, 2020; Hristov *et al.*, 2026). To evaluate the effects of increased dietary AAT supply, a NorFor task group has collected data from recent Nordic and international feeding experiments with the aim of evaluating NorFor's current AAT recommendations and assessing whether there is a need for revision.

Materials and Methods

Data: A literature search was conducted using Google Scholar and other scientific databases, applying index terms related to dietary protein supply and production response, including level of CP and/or metabolizable protein (AAT equivalent), milk yield, protein yield, and effects of feed ration. Studies were eligible for inclusion if experimental data were available on animal characteristics and key production variables, including days in milk (DIM), diet composition, dry matter intake (DMI), milk yield, and milk composition. In addition, studies were required to report a measure of variability (SEM or equivalent) for milk and/or protein yield. Furthermore, studies comparing different levels of CP and/or AAT between treatments, involving partial or complete replacement of protein sources, or evaluating responses to different CP levels across stages of lactation, were considered for inclusion. Based on these criteria, the energy-corrected milk (ECM) model included 132 observations from 45 independent studies (random-effect units) derived from 26 papers, whereas the corresponding protein yield model included 120 observations from 40 studies derived from 23 papers due to data availability (Suppl. Materials 1).

Statistic modelling: Models for ECM and milk protein production responses included the variables MJ net energy for lactation per kg DM (NEL), days in milk (DIM), content of NDF, starch (ST), and crude fat (CFat) as g/kg DM, in addition to protein supply variables (AAT/NEL[1], or AAT, CP, and PBV as g/kg DM). AAT and PBV were calculated according to the latest version of the NorFor model (Volden, 2011). All variables were included as second-order polynomials, without interaction effects. Protein supply variables (the selected protein measure and PBV) were forced into the model as second-order terms. Models were fitted using weighted regression with study (as defined above) included as a random effect to

[1] Grams AAT available for milk production/MJ NEL requirement for ECM production

account for between-study heterogeneity, and weights defined as the inverse of the variance in milk yield or protein yield, to account for heterogeneity in precision among experiments. Backward elimination with $P \leq 0.05$ was applied for model reduction. Prediction error was assessed using leave-one-study-out cross validation. Normality of residuals was assumed, acknowledging that this assumption may not be fully met in all cases.

Results and Discussion

The included parameters and their estimates for the selected models for ECM and protein production are shown in Table 1. Both the first and second order term for AAT/NEL and PBV were highly significant, as well as the first order term for dietary constituents. Figure 1 show the predicted responses of ECM and protein yield with varying levels of g AAT/MJ NEL. Responses in both ECM and protein yield exhibited a clear plateau around 16 g AAT/MJ NEL, with only marginal gains above the current recommended minimum level of 15 g AAT/MJ NEL in NorFor (Volden, 2011, Ch. 9). Increasing AAT/NEL from 15.0 to 16.0 g/MJ increases ECM by 0.1 kg and protein yield by 0.01 kg per day. Our findings are consistent with studies showing diminishing production responses once metabolizable protein requirements are met (Benchaar et al., 2023), and with CP-based studies indicating that dietary protein levels around 16-17% of DM are sufficient to maximize milk production (Olmos Colmenero & Broderick, 2006). Within the recommended PBV range (5-40 g/kg DM), predicted ECM responses were small (≤ 0.5 kg), with a maximum around 25 g PBV and little net difference between low and high PBV levels, indicating that PBV had a secondary impact relative to AAT/NEL within the operational range.

Table 1 Included parameters ($P \leq 0.05$) and their estimates for the selected models for responses in ECM and protein production.

Parameters, g/kg DM, unless otherwise stated	Energy-corrected milk		Protein production	
	Estimate	Sign. level[1]	Estimate	Sign. level
Intercept	74.86		2158	
g AAT/MJ NEL	3.618	***	147.7	***
g AAT/MJ NEL (quadratic)	-0.1134	***	-4.424	***
PBV	0.05964	**	4.345	***
PBV (quadratic)	-0.00112	***	-0.07786	***
NEL, MJ/kg DM	-5.592	***	-170	***
NDF	-0.08212	***	-2.954	***
Starch	-0.02683	**	-0.8284	**

[1] * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

The present findings, together with previous literature, suggest that milk production responses to protein supply are primarily driven by interactions between ruminal nitrogen availability and energy supply, whereas post-ruminal amino acid supply plays a secondary role once microbial protein synthesis and energy intake are unlikely to be limiting. This interpretation is consistent with previous work demonstrating that interactions between energy supply and metabolizable protein are key determinants of nitrogen efficiency and production responses in dairy cows (Rius et al., 2010). Even though models based on alternative protein descriptors (grams AAT and CP per kg DM) occasionally yielded slightly better information criteria (AIC/BIC), AAT relative to energy supply (AAT/NEL) was retained, as the results indicate that production responses are primarily driven by the balance between ruminal nitrogen availability and energy supply, rather than by absolute protein supply. Accordingly, providing AAT above the level

required to maximize milk production is unlikely to elicit further production responses and may instead result in increased nitrogen losses rather than increased milk output.

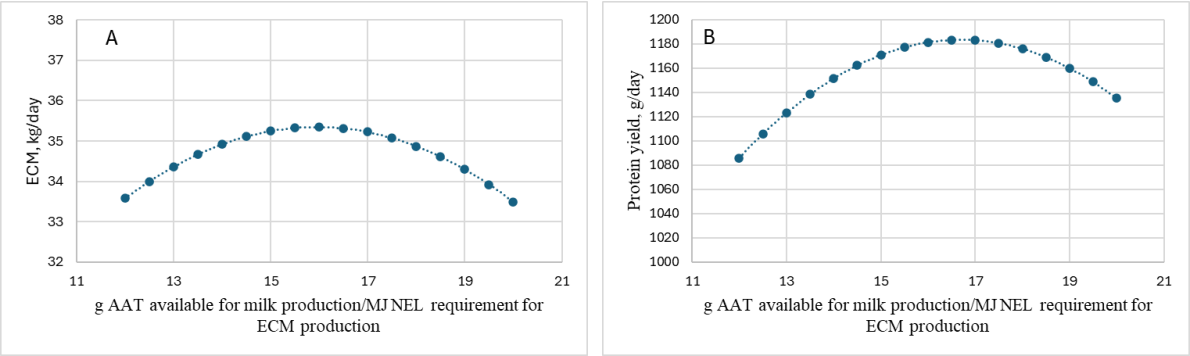


Figure 1 Predicted responses in (A) energy-corrected milk (ECM) and (B) protein yield with increasing AAT/NEL (grams AAT available for milk production/MJ NEL requirement for ECM production) when NEL per kg DM = 6.5 MJ, and PBV, NDF, and starch = 20, 350, and 150 grams per kg DM, respectively.

Conclusions

Given the predicted ECM and protein yield responses identified in this study, there are no scientific reasons for adjusting the AAT/MJ NEL recommendations in the NorFor model. At the same time, the shallow production response around current recommendation levels indicates limited sensitivity of milk yield to small changes in dietary AAT supply.

Supplementary Materials 1: List over studies included in the analysis.

References

Benchaar, C., Hassanat, F., Beauchemin, K.A., Ouellet, D.R., Lapierre, H. & Côrtes, C., 2023. Effect of metabolizable protein supply on milk performance, ruminal fermentation, apparent total-tract digestibility, energy and nitrogen utilization, and enteric methane production of Ayrshire and Holstein cows. *Animals 2023*, 13, 832.

Hristov, A.N., Huthanen, P., van Duinkerken, G. & Pacheco, D., 2026. *Invited review:* Perspectives on nitrogen in dairy cattle nutrition. *Journal of Dairy Science*, 109, 2074-2107.

Katongole, C.B. & Yan, T., 2020. Effect of varying dietary crude protein level on feed intake, nutrient digestibility, milk production, and nitrogen use efficiency by lactating Holstein-Friesian cows. *Animals 2020*, 10, 2439.

Olmos Colmenero, J.J., Broderick, G.A. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. *J. Dairy Sci.*, 89, 1704-1712.

Rius, A.G., McGilliard, M.L., Umberger, C.A., Hanigan, M.D. 2010. Interactions of energy and predicted metabolizable protein in determining nitrogen efficiency in the lactating dairy cow. *J. Dairy Sci.*, 93, 2034-2043.

Volden, H. (Ed.), 2011. NorFor – The Nordic feed evaluation system. EAAP Publication No. 130. Wageningen Academic Publishers, Wageningen, The Netherlands.

Supplementary materials 1

References included in the meta-analysis of ECM and protein yield responses.
References marked with * were not included in the model for protein yield.

*Alstrup, L., Weisbjerg, M.R., Hymøller, L., Larsen, M.K., Lund, P., Nielsen, M.O. 2014. Milk production response to varying protein supply is independent of forage digestibility in dairy cows. *Journal of Dairy Science*, 97: 4412-4422. https://doi.org/10.3168/jds.2013-7585

Barros, T., Quaassdorff, M.A., Aguerre, M.J., Olmos Colmenero, J.J., Bertics, S.J., Crump, P.M., Wattiaux, M.A. 2017. Effects of dietary crude protein concentration on late-lactation dairy cow performance and indicators of nitrogen utilization. *Journal of Dairy Science*, 100: 5434-5448. https://doi.org/10.3168/jds.2016-11917

Benchaar, C., Hassanat, F., Beauchemin, K.A., Ouellet, D.R., Lapierre, H., Côrtes, C. 2023. Effect of metabolizable protein supply in milk performance, ruminal fermentation, apparent total-tract digestibility, energy and nitrogen utilization, and enteric methane production of Ayrshire and Holstein cows. *Animals*, 13: 832. https://doi.org/10.3390/ani13050832

Cabrita, A.R.J., Dewhurst, R.J., Melo, D.S.P., Moorby, J.M., Fonseca, A.J.M. 2011. Effects of dietary protein concentration and balance of absorbable amino acids on productive responses of dairy cows fed corn silage-based diets. *Journal of Dairy Science*, 94: 4647-4656. https://doi.org/10.3168/jds.2012-0497

Chowdhury, M.R., Wilkinson, R.G., Sinclair, L.A. 2023. Feeding lower-protein diets based on red clover and grass or alfalfa and corn silage does not affect milk production but improves nitrogen use efficiency in dairy cows. *Journal of Dairy Science*, 106: 1773-1789. https://doi.org/10.3168/jds.2022-22607

Damborg, V.K., Jensen, S.K., Johansen, M., Ambye-Jensen, M., Weisbjerg, M.R. 2019. Ensiled pulp from biorefining increased milk production in dairy cows compared with grass-clover silage. *Journal of Dairy Science*, 102: 8883-8897. https://doi.org/10.3168/jds.2018-16096

Gidlund, H., Hetta, M., Krizsan, S.J., Lemosquet, S., Huhtanen, P. 2015. Effects of soybean meal or canola meal on milk production and methane emissions in lactating dairy cows fed grass silage-based diets. *Journal of Dairy Science*, 98: 8093-8106. https://doi.org/10.3168/jds.2015-9757

Gidlund, H., Hetta, M., Huhtanen, P. 2017. Milk production and methane emissions from dairy cows fed a low or high proportion of red clover silage and an incremental level of rapeseed expeller. *Livestock Science*, 197: 73-81. https://doi.org/10.1016/j.livsci.2017.01.009

Groff, E.B., Wu, Z. 2005. Milk production and nitrogen excretion of dairy cows fed different amounts of protein and varying proportions of alfalfa and corn silage. *Journal of Dairy Science*, 88: 3619-3632.

*Hymøller, L., Alstrup, L., Larsen, M.K., Lund, P., Weisbjerg, M.R. 2014. High-quality forage can replace concentrate when cows enter the deposition phase without negative consequences for milk production. *Journal of Dairy Science*, 97: 4433-4443. https://doi.org/10.3168/jds.2013-7734

Hynes, D.N., Stergiadis, S., Gordon, A., Yan, T. 2016. Effects of crude protein level in concentrate supplements on animal performance and nitrogen utilization of lactating dairy cows fed fresh-cut perennial grass. *Journal of Dairy Science*, 99: 8111-8120. https://doi.org/10.3168/jds.2016-11110

Jaakkola, S., Saarisalo, E., Heikkila, T. 2009. Formic acid treated whole crop barley and wheat silages in dairy cow diets: effects of crop maturity, proportion in the diet, and level and type of concentrate supplementation. *Agricultural and Food Science*, 18: 234-256

Katongole, C.B., Yan, T. 2020. Effect of varying dietary crude protein level on feed intake, nutrient digestibility, milk production, and nitrogen use efficiency by lactating Holstein-Friesian cows. *Animals,* 10:2439. https://doi.org/10.3390/ani10122439

Kidane, A., Øverland, M., Mydland, L.T., Prestløkken, E. 2018. Interaction between feed use efficiency and level of dietary crude protein on enteric methane emission and apparent nitrogen use efficiency with Norwegian Red dairy cows. *Journal of Animal Science*, 96: 3967-3982. https://doi.org/10.1093/jas/sky256

Law, R.A., Young, F.J., Patterson, D.C., Kilpatrick, D.J., Wylie, A.R.G., Mayne, C.S. 2009. Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. *Journal of Dairy Science*, 92: 1001-1012. https://doi.org/10.3168/jds.2008-1155

Leonardi, C., Stevenson, M., Armentano, L.E. 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. *Journal of Dairy Science*, 86: 4033-4042. https://doi.org/10.3168/jds.S0022-0302(03)74014-4

Letelier, P., Zanton, G.I., Wattiaux, M.A. 2022. Production performance of Holstein cows at 4 stages of lactation fed 4 dietary crude protein concentrations. *Journal of Dairy Science*, 105: 9581-9596. https://doi.org/10.3168/jds.2022-22146

Lobos, N.E., Wattiaux, M.A., Broderick, G.A. 2021. Effect of rumen-protected lysine supplementation of diets based on corn protein fed to lactating dairy cows. *Journal of Dairy Science*, 104: 6620-6632. https://doi.org/10.3168/jds.2020-19835

Olmos Colmenero, J.J., Broderick, G.A. 2006a. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. *Journal of Dairy Science*, 89: 1704-1712.

Olmos Colmenero, J.J., Broderick, G.A. 2006b. Effect of amount and ruminal degradability of soybean meal protein on performance of lactating dairy cows. *Journal of Dairy Science*, 89: 1635-1643. https://doi.org/10.3168/jds.S0022-0302(06)72230-5

Puhakka, L., Jaakkola, S., Simpura, I., Kokkonen, T., Vanhatalo, A. 2016 Effects of replacing rapeseed meal with fava bean at 2 concentrate crude protein levels on feed intake, nutrient digestion, and milk production in cows fed grass silage-based diets. *Journal of Dairy Science*, 99: 7993-8006. https://doi.org/10.3168/jds.2016-10925

Randby, Å.T., Dønnem, I., Prestløkken, E., Martin, A.D., Steen, A., Eknæs, M. 2024. Effect of chop length of grass silage harvested at two maturity stages, and of concentrate protein level, on feed intake and milk production in dairy cows. *Grass and Forage Science*, 79: 211-227. https://doi.org/10.1111/gfs.12647

Rius, A.G., McGilliard, M.L., Umberger, C.A., Hanigan, M.D. 2010. Interactions of energy and predicted metabolizable protein in determining nitrogen efficiency in the lactating dairy cow. *Journal of Dairy Science*, 93: 2034-2043. https://doi.org/10.3168/jds.2008-1777

Wang, C., Liu, J.X., Yuan, Z.P., Wu, Y.M., Zhai, S.W., Ye, H.W. 2007. Effect of level of metabolizable protein on milk production and nitrogen utilization in lactating dairy cows. *Journal of Dairy Science*, 90: 2960-2965. https://doi.org/10.3168/jds.2006-129

*Weisbjerg, M.R., Kristensen, N.B., Hvelplund, T., Lund, P., Løvendahl, P. 2010. Malkekoens produktion ved reduceret kvælstoftildeling. *Husdyrbrug Nr. 22, mai 2010*, Intern rapport, Aarhus Universitet.

Wu, Z., Satter, L.D. 2000. Milk production during the complete lactation of dairy cows fed diets containing different amounts of protein. *Journal of Dairy Science*, 83: 1042-1051. https://doi.org/10.3168/jds.S0022-0302(00)74968-X

Reed (*Phragmites australis*) silage for Icelandic horses- intake and dry matter digestibility

W. Ashworth[1], M. Connysson[2], Ö. Östman[1], & C.E. Müller[3]
[1] *Department of Aquatic Resources, Swedish University of Agricultural Sciences, SE- 750 07 Uppsala, Sweden*
[2]*Department of Animal Biosciences, Swedish University of Agricultural Sciences*
[3]*Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences*
Correspondence: Cecilia.Muller@slu.se

Introduction

The common reed (*Phragmites australis* (Cav.) Steud) is an abundant wetland plant growing in the interface between land and water (Packer et al., 2017) and is both drought and flood resistant (Pagter et al., 2005). As reed stands are dense and most often monospecific (Packer et al., 2017) it has potential to be harvested as a roughage resource for horses and ruminants. Previous studies have reported reed to be rich in fibre and comparatively high in crude protein (CP) concentration, while concentration of water soluble carbohydrates (WSC) and metabolizable energy (ME) value is low (Temel et al., 2023) compared to conventional grass species used for forage production, indicating a particular usefulness of reed as a feed for adult horses in low to moderate exercise. Data on intake and digestibility of reed in horses is however scarce, and therefore two pilot studies on intake and one feeding trial with reed as a roughage for horses have been conducted.

Materials and Methods

In all three studies school horses at Wången National Equine Centre facilities, Alsen, Sweden, were used under ethical permit A15/2023 approved by Umeå ethical board of animal studies, Sweden. The studies were performed in compliance with European Union directives on animal experiments (2010/63/EU; European Union, 2010), and laws (Swedish Constitution, 1988:534) and regulations (Swedish Board of Agriculture Constitution, 2012:26) governing experiments on live animals in Sweden.

In the first pilot study, acceptability of reed silage was tested in 22 horses (18 Icelandic horses and four Gotland ponies). Each horse was fed 1 kg reed silage (320 g DM/kg, 7.3 MJ ME for horses (MEh)/kg DM, 162 g CP/kg DM and 1 g WSC/kg DM) once daily during three consecutive days as an extra feed in addition to the ordinary feed ration which was forage-based and calculated to cover their energy and nutrient requirements (NRC, 2007). The horses had access to the reed silage for 45 minutes during which they were observed for their reed intake, and after the 45 minutes the leftovers were collected and weighed.

In the second pilot study, the same reed silage as in the first study was used in a preference test. Four of the Icelandic horses who consumed all the offered reed silage in the first study were included, and were given simultaneous access during 1 hour to 1 kg of each of the feeds: reed silage, cereal straw (890 g DM, 6.6 MJ MEh/kg DM, 44 g CP/kg DM and 4 g WSC/kg DM), and grass haylage (680 g DM/kg, 9.7 MJ MEh/kg DM, 127 g CP/kg DM, and 74 g WSC/kg DM). The feed preference of the horses was registered to rank the feeds.

In the feeding trial, six Icelandic geldings were selected from a herd of 16 horses based on their acceptance of eating reed silage in a pre-test performed as described for the first pilot study.

Intake and apparent total tract digestibility of DM (ATTDDM) of reed silage (337 g DM/kg, 126 g CP/kg DM, 649 g NDF/kg DM, 2 g WSC/kg DM) and grass haylage consisting of timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* L.) (585 g DM/kg, 123 g CP/kg DM, 635 g NDF/kg DM, 84 g WSC/kg DM) were estimated in a change-over experimental design with two periods of three weeks each, and with three horses per feed in each period. Horses were fed equal amounts of reed silage and grass haylage in DM amount per 100 kg BW and day. A gradual switch between the roughages was performed during one week between the periods. During the final 72 hours of each three-week period, total collection of faeces (using a collection harness) and feed refusals and registrations of daily water and feed intake and body weight (BW) was performed. Samples of feeds, feed refusals, and faeces were collected and analysed for their chemical composition with methods described by Ragnarsson and Lindberg (2008). Data analysis was carried out in R version 4.2.1 (R Core Team, 2022) using mixed models with individual horse as the random factor to account for repeated sampling and with period as a fixed blocking factor. Diet treatment means were accepted as statistically different when $P<0.05$ applying the Kenward-Roger correction for degrees of freedom.

Results and Discussion

In the first pilot study, 6 of 22 horses (27 per cent) consumed all offered reed silage on all three days, while 12 of 22 horses (54 per cent) consumed 0.1 to 0.7 kg reed silage per day, and 4 of 22 horses (18 per cent) refused to eat reed silage completely.

In the second pilot study, all four horses first consumed the grass haylage, thereafter two of them smelled the reed silage without eating it but started to eat cereal straw. The other two horses smelled both reed silage and cereal straw but did not consume any of them.

In the feeding trial, horses consumed on average 0.82 and 1.14 kg DM per 100 kg body weight and day of reed silage and grass haylage, respectively ($P =0.01$). Two horses refused to eat reed silage at different timepoints in the trial, but all horses consumed all the offered grass haylage. Average reed silage leftovers were 0.7 kg DM per horse and day, which corresponded to 20 % of the offered amount. The reed silage leftovers had a lower CP and higher DM and NDF concentration compared to the fed reed silage ($P=0.01$), indicating that horses selected reed parts rich in CP and lower in DM and NDF (Table 1). The ATTDDM was 50 (SD 11.0) and 57 (SD 3.4) per cent for reed silage and grass haylage respectively ($P=0.20$). No data on feeding reed silage to horses has been found in the literature, but studies with reed canary grass (*Phalaris arundinacea* L.) (which is somewhat similar to reed) fed as hay to horses have resulted in voluntary DM intakes of approximately 1 to 1.7 % of BW (Fonnesbeck, 1968; Ordakowski-Burk et al., 2006) and DM digestibilities of 39-46 % Cymbaluk, 1990; Ordakowski-Burk et al., 2006). The ATTDDM of grass haylage was similar to previously reported values for timothy haylage harvested in late plant maturity fed to Icelandic horses (Ragnarsson & Lindberg, 2008).

Horses lost on average 18 kg BW when fed reed silage and 2 kg when fed grass haylage ($P=0.003$). This indicated that both gut fill and energy intake was lower on the reed silage diet and not sufficient for the horses to keep the BW constant, despite the similar ATTDDM between the roughages and despite the selection of the more nutritious parts of the reed silage by the horses. A low intake therefore seems to be the main limiting factor in using reed silage for horses. It is possible that the intake of both DM and energy would have been larger if the horses had been fed reed silage *ad libitum* as they would then have had increased selection

opportunities. Alternative feeding regimes, or combinations with other roughages may be more appropriate for meeting the requirements of energy and nutrients of horses, if a BW loss is not desired.

Table 1 Average (standard error) composition of reed silage and grass haylage as provided and in refusals (no refusals for grass haylage)

Diet	Dry matter, g/ kg	Crude protein, g per kg DM	Neutral detergent fibre, g per kg DM
Reed silage provided	339 (3.4)	126 (0.7)	646 (6.7)
Reed silage refusals	438 (18.1)	88 (7.6)	721 (11.8)
P-value Provided *vs.* refusals	0.01	0.01	0.01
Grass haylage provided	580 (13.9)	124 (3.4)	643 (19.8)
Grass haylage refusals	-	-	-

Conclusion

All horses did not consume reed silage readily. Horses had a lower intake of reed silage compared to grass haylage, and although horses selected the more nutritious parts of the reed silage the ATTDDM was similar for the two roughages. This indicates that a low intake is a more limiting factor for using reed silage as a roughage for horses than a low digestibility.

Funding

These studies were partly funded by FORMAS grant #2021-02425. Reed harvesting expenses were also subsidized by funding from Race For The Baltic, Stockholm, Sweden.

References

Cymbaluk, N.F., 1990. Comparison of forage digestion by cattle and horses. Can. J. Anim. Sci. 70, 601-610.

Fonnesbeck, P.V., 1968. Digestion of soluble and fibrous carbohydrate of forage by horses. J. Anim. Sci. 27, 1336-1344.

National Research Council (NRC), 2007. Nutrient requirements of horses. 6th revised ed. Animal Nutrition Series. The National Academies Press, Washington DC, USA.

Ordakowski-Burk, A.L., Quinn, R.W., Shellem, T.A., & Vough, L.R., 2006. Voluntary intake and digestibility of reed canarygrass and timothy hay fed to horses. J. Anim. Sci. 84, 3104-3109.

Packer, J.G., Meyerson, L.A., Skálová, H., Pyšek, P., & Kueffer, C, 2017. Biological flora of the British Isles: Phragmites australis. J. Ecol. 105, 1123-1162.

Pagter, M., Bragato, C., & Brix, H., 2005. Tolerance and physiological responses of Phragmites australis to water deficit. Aquat. Bot. 81, 285-299.

Ragnarsson, S., & Lindberg, J.E., 2008. Nutritional value of timothy haylage in Icelandic horses. Livest. Sci. 113, 202-208.

Temel, S., Keskin, B., Gümer, Z., & Atalay, A.I., 2023. Determination of yield and quality characteristics of common reed (*Phragmites australis* (Cav.) Trin. Ex Steud) harvested at different growth stages. Turk. J. Field. Crops. 28, 70-78.

Individual and group level production responses to rapeseed meal supply

T. Karhula[1], S. Kajava[2] & A. Sairanen[2]
[1]University of Helsinki (UH), Department of Agricultural Sciences, PL 28 (Koetilantie 5), 00014 Helsingin Yliopisto. [2]Natural Resources Institute Finland (Luke), Halolantie 31 A, 71750 Maaninka
Correspondence: tommi.karhula@helsinki.fi

Introduction

Protein supplementation is well known to have diminishing effect on milk yield (Daniel et al. 2016). Besides adjusting protein supply on herd level also individual protein feeding has been proposed to improve N utilization of dairy cattle (Liu and VandeHaar 2020). However, earlier the reproducibility of individual responses with more than 2 time points has not been tested (Sairanen and Huhtanen 2024). Not considering reproducibility may lead to false estimates of cow's capacity. Economical optimization requires reliable information of responses to nutrient supply. However, in some recent experiments (e.g. Kuoppala et al. 2021) the responses have been smaller than earlier. An experiment was performed to assess the reproducibility of individual production responses in robot milking, and the current level of production response was evaluated through a meta-analysis of rapeseed meal (RSM) supplementation studies published after 1999.

Materials and Methods

18 cows in switch-back experiment of 5 17-day-long periods were used to test reproducibility of three consecutive individual production responses in robot milking. Three protein levels were created by offering concentrates differing in rapeseed meal content at the milking robot. Within a cow, protein level alternated between two levels so that as control was low protein diet (89 g metabolizable protein (MP), according to Finnish feed tables /kg DM) and paired diet was either intermediate or high protein diet (94 or 98 g MP /kg DM), calculated mean requirement being 97 g/kg DM. Proportion of variation explained by cow was tested with proc mixed in SAS Enterprise guide 8.6 with cow and other variables as a random factor. Continuous variables were divided to three classes and included e.g. kg DMI/metabolic bodyweight, energy balance before experiment, parity and DIM. Covariance structure was VC. Cows were classified to three reliability classes by magnitude of random variation observed, e.g. in feed intake or milking robot visit frequency.

In addition to individual response patterns, study assessed the overall milk yield response to RSM. For this literature review, experiments (n=40, 131 treatment means) needed to contain treatments differing in rapeseed protein supply to be included. Publications were searched in PubMed with words "RSM or RSC or Rapeseed expeller or Canola meal AND dairy cow". From Finnish experiments also unpublished data or data published only in thesis were included. Primarily experimental diets were based on grass silage and separate feeding but also experiments using total mixed ratio, maize silage and alfalfa were included. Effect of rapeseed meal was analysed with proc mixed in SAS Enterprise guide 8.6 with model $y = B_0 + B \times x + b_0 + b_1 \times x + C \times x^2 + e$, where B_0 = overall intercept, $B \times x$ and $C \times x^2$ are the fixed effects of rapeseed meal and b_0, $b_1 \times x$ and e are random effects of study (intercept, slope and error). Effect of RSM was estimated with two versions of Lypsikki ration formulation tool (Huhtanen and Nousiainen 2012) by replacing barley with RSM (CP 379 g/kg DM) with estimated intercept of literature model as initial production, liveweight and intake input.

Results and Discussion

Individual differences explained only 17.4 % of the variation in ECM responses, when all reliability classes were included. In line with the small difference between treatment mean responses observed between the low-intermediate and low-high diets (4.05 and 3.62 kg ECM/kg CP) also in the individual data difference in treatment didn't reach significancy. Reliability class, thus the extent of random variation in feed intake and robot visits, went hand in hand with the reproducibility. With best reliability animals only, cow explained already 65 % of the variation in responses. In figure 1 it is possible to see that for some animals the responses were highly repeatable. For large amount of animals observations vary to such extent due to external factors that distinction of individual metabolic efficiency is not possible.

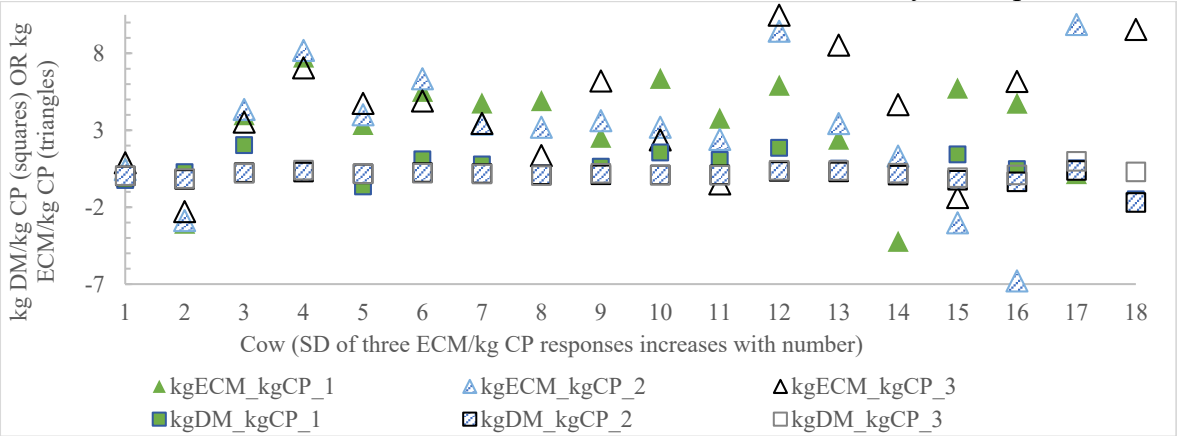


Figure 1 Feed intake (squares) and ECM production (triangles) responses (per kg CP intake) of dairy cows on three consecutive switch back periods 1…3. For animal 18 1st and 2nd responses (-62.0 and -92.1 kg ECM/kg CP) and for animal 17 3rd response (26.6 kg ECM/kg CP) fall out of the figure.

In the literature review rapeseed meal or cake supplementation had significantly ($p<0.001$) quadratic effect both for feed intake and ECM yield (Figure 2). For model predicting ECM yield interaction year×RSM intake had negative slope (-.00998 ± 0.0094) but it was not significant. For model predicting DMI this interaction term was significant ($p<0.05$) but got slightly positive coefficient. Thus, while protein supplementation still

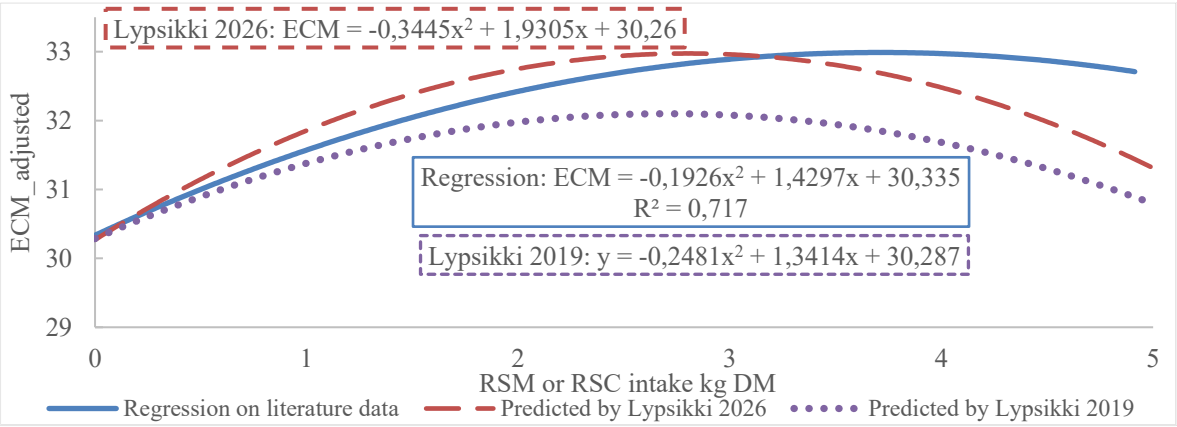


Figure 2 ECM responses to rapeseed meal or rapeseed cake supply in literature dataset of 131 treatment means (line without dots) and predictions by Lypsikki 2019 (line with short dots, currently used for ration formulation in Finland) and 2026 (line with long dots) versions. Experimental observations (ECM_adj) were adjusted for random study effects (intercept and slope).

increases feed intake, ECM response has decreased, even though this development has not been uniform. Variation may be explained by currently unaccounted differences in basal diet's supply of metabolizable protein (Broderick et al. 2010). Newest version of the Lypsikki ration formulation tool (manuscript Huhtanen et al. 2026 in Agricultural and Food Science) appears to predict maximum response more comparably to the literature (literature maximum at 170 g RSM/kg DM) than the 2019 version. This comparison should be taken with caution as some input values (e.g. diet metabolizable protein concentration) needed for the Lypsikki lacked from some of the publications and had to be estimated.

Conclusions

Individual production responses to protein supply are difficult to determine reliably in robot milking due to random variation in milking visits and feed intake. Due to this, strategies to improve N utilization of dairy cattle should predominantly concentrate on group level protein feeding. Group level responses to RSM supply are clearly quadratic and updated version of the Lypsikki ration formulation tool seems to have improved prediction of ECM responses to RSM supply. Further research should concentrate to find reasons for variation in responses.

Acknowledgements

The individual production responses experiment was a part of ViiMa-project (protein-wise milk production), which is funded mainly by the European Agricultural Fund for Rural Development. Analysis of literature data is carried out in SCilf-project (Smart circular livestock farms) which is mainly funded by Finnish Ministry of Agriculture and Forestry.

References

Broderick, G. A., Huhtanen, P., Ahvenjärvi, S., Reynal, S. M. & Shingfield, K. J. 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle—A meta-analysis. Journal of Dairy Science 93: 3216–3230. https://doi.org/https://doi.org/10.3168/jds.2009-2989

Daniel, J. B., Friggens, N. C., Chapoutot, P., Van Laar, H. & Sauvant, D. 2016. Milk yield and milk composition responses to change in predicted net energy and metabolizable protein: a meta-analysis. Animal 10: 1975–1985. https://doi.org/https://doi.org/10.1017/S1751731116001245

Huhtanen, P. & Nousiainen, J. 2012. Production responses of lactating dairy cows fed silage based diets to changes in nutrient supply. Livestock Science 148: 146–158. https://doi.org/https://doi.org/10.1016/j.livsci.2012.05.023

Kuoppala, K., Jaakkola, S., Garry, B., Ahvenjärvi, S. & Rinne, M. 2021. Effects of faba bean, blue lupin and rapeseed meal supplementation on nitrogen digestion and utilization of dairy cows fed grass silage-based diets. Animal 15: 100300. https://doi.org/https://doi.org/10.1016/j.animal.2021.100300

Liu, E. & VandeHaar, M. J. 2020. Low dietary protein resilience is an indicator of the relative protein efficiency of individual dairy cows. Journal of Dairy Science 103: 11401 11412. https://doi.org/https://doi.org/10.3168/jds.2020-18143

Sairanen, A. & Huhtanen, P. 2024. Variation in individual milk production responses to supplementary protein feeding with two types of forages. Livestock Science 280: 105394. https://doi.org/https://doi.org/10.1016/j.livsci.2023.105394

Rumen fermentation pattern of dairy cows fed grass-clover silage or grazing on pasture

D. W. Olijhoek, N. P. Hansen, M. R. Weisbjerg, & M. Larsen
Department of Animal and Veterinary Sciences, AU Viborg, Research Centre Foulum, Aarhus University, DK 8830 Tjele, Denmark.
Correspondence: mogens.larsen@anivet.au.dk

Introduction

Traditional ways of providing grass-clover leys to dairy cows are grazing or conserving as silage. For these, differences in nutrient intakes and rumen degradability are expected and these may influence the rumen environment. This study aimed to examine rumen fermentation patterns of rumen cannulated dairy cows when perennial ryegrass and clover leys were provided as silage or by grazing on pasture.

Materials and Methods

The experiment lasted 11 weeks with 2 weeks of adaptation and 9 experimental weeks (May 6th to July 7th, 2024). In a continuous design, 12 rumen cannulated Danish Holstein dairy cows were allocated to 2 treatments (6 cows/treatment): 1) feeding of a spring growth (first cut) grass-clover silage (white and red clover), and 2) grazing a perennial ryegrass and white clover pasture during day and night. All cows received 5.1 kg DM/d of concentrates. All cows were of second parity and averaged (± standard deviation) 81 ± 22 and 97 ± 8 days in milk (DIM) at the start of the experiment, for the silage and grazing treatments, respectively. Cows were milked twice daily and were weighed after milking. Silage was provided *ad libitum* in automated feed bins for the silage treatment. For the grazing treatment, a rotational grazing system over paddocks was adopted with strip grazing within paddock. Pasture was measured using a platemeter (Grasshopper, True North Technologies, Ireland), with pre-grazing herbage allowance averaging 1,259 kg DM/ha, and pre- and post-grazing sward heights averaging 99 and 68 mm, respectively. Rumen liquid was sampled from the ventral rumen at 6 different timepoints during week 2, 5, and 8, and analysed for pH and rumen VFA. Energy-corrected milk (ECM) yield was calculated according to Sjaunja *et al.* (1991). Pasture dry matter intake (DMI) was calculated based on NorFor energy requirements (Nielsen and Volden, 2011). Data were analysed with a mixed model in SAS (version 9.4, SAS Institute Inc., USA). Body weight, ECM yield and DMI were analysed with a model including fixed effects for treatment (silage or grazing), week (2, 5, and 8), the interaction between treatment and week, and a standardized covariate (mean of 0 and standard deviation of 1), which is an average of body weight, ECM yield, or DMI over the last 5 days before the start of experimental week 1. Rumen parameters were analysed with a mixed model including fixed effects for treatment, week, sampling time (02:30, 06:30, 10:30, 14:30, 18:30, and 22:30 h), DIM at the start of the experiment, and all 2- and 3-way interactions between treatment, week, and time. In both models, week was modelled as repeated measures for cow. Data of glucose concentration in rumen liquid were log transformed before statistical analysis and the results are presented in the table as back-transformed values. *P*-values are considered significant at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

Results and Discussion

Body weight of grazing cows was lower than cows fed silage ($P=0.001$) and declined during the first weeks of the 9-week experiment ($P<0.001$; Table 1). Energy-corrected milk yield did not differ between treatments, but reduced over weeks ($P<0.001$). Total DMI did not differ

between treatments, but increased over the experiment for the silage treatment, while it decreased for the grazing treatment ($P<0.001$). These results show that cows had similar overall ECM yield and DMI while grazing compared to feeding a silage indoors.

Total VFA concentration in rumen liquid was higher and rumen pH consistently lower ($P<0.001$ for both) for the grazing than the silage treatment in the 3 sampling weeks (Table 1; Figure 1) indicating a higher fermentation rate for the grazing than the silage treatment. An interaction was found between treatment and week in the patterns of molar proportions of acetate and propionate ($P<0.001$ for both), with acetate being lower and propionate being higher for grazing cows than cows fed with silage in week 2. The pattern of acetate and propionate molar proportions reversed in weeks 5 and 8. The ratio between acetate and propionate showed an identical pattern for treatments over weeks to the acetate molar proportion. These effects were mainly driven by the grazing treatment, for which the acetate and propionate molar proportions respectively increased and decreased over weeks, while molar proportions of these VFA remained fairly stable over weeks for the silage treatment. This finding indicates that the pasture nutrient composition changed over the experiment, especially compared to week 2 (halfway May). Butyrate molar proportion (Table 1; Figure 1) and glucose concentration (Table 1) were consistently higher ($P<0.001$ for both) for the grazing treatment throughout the sampling weeks, which could relate to a higher sugar content in grazed pasture compared to silage.

Table 1 Performance and rumen fermentation parameters in rumen liquid of dairy cows fed with grass-clover silage and housed indoors or grazing perennial ryegrass and white clover pasture during day and night

				P-values				
Measurement	Silage	Grazing	SEM	Trt	Week	Time	Trt × week	Trt × time
Body weight (kg)	646	606	6.7	0.001	<0.001	-	0.19	-
ECM yield (kg/d)	32.3	32.5	0.70	0.88	<0.001	-	0.20	-
DMI (kg/d)	20.6	20.8	0.48	0.79	0.01	-	<0.001	-
Rumen pH	6.68	6.32	0.03	<0.001	0.75	<0.001	0.04	0.001
Total VFA (mmol/L)	118	127	1.2	<0.001	<0.001	<0.001	0.66	0.07
Acetate (mol/100 mol)	61.9	62.3	0.31	0.47	0.001	<0.001	<0.001	0.001
Propionate (mol/100 mol)	20.2	20.4	0.32	0.59	<0.001	<0.001	<0.001	<0.001
Butyrate (mol/100 mol)	12.6	13.8	0.17	<0.001	0.02	<0.001	0.99	0.05
Acetate:propionate ratio	3.10	3.14	0.059	0.67	<0.001	<0.001	<0.001	<0.001
Glucose (mmol/L)	0.22	0.61	0.022	<0.001	0.001	0.03	0.35	<0.001

Trt = treatment; week = week 2, 5, and 8; time = sampling time 02:30, 06:30, 10:30, 14:30, 18:30, and 22:30 h.
P-values for week × time were significant at $P \leq 0.05$ for all variables.
P-values for treatment × week × time was significant at $P \leq 0.05$ for rumen pH and propionate.

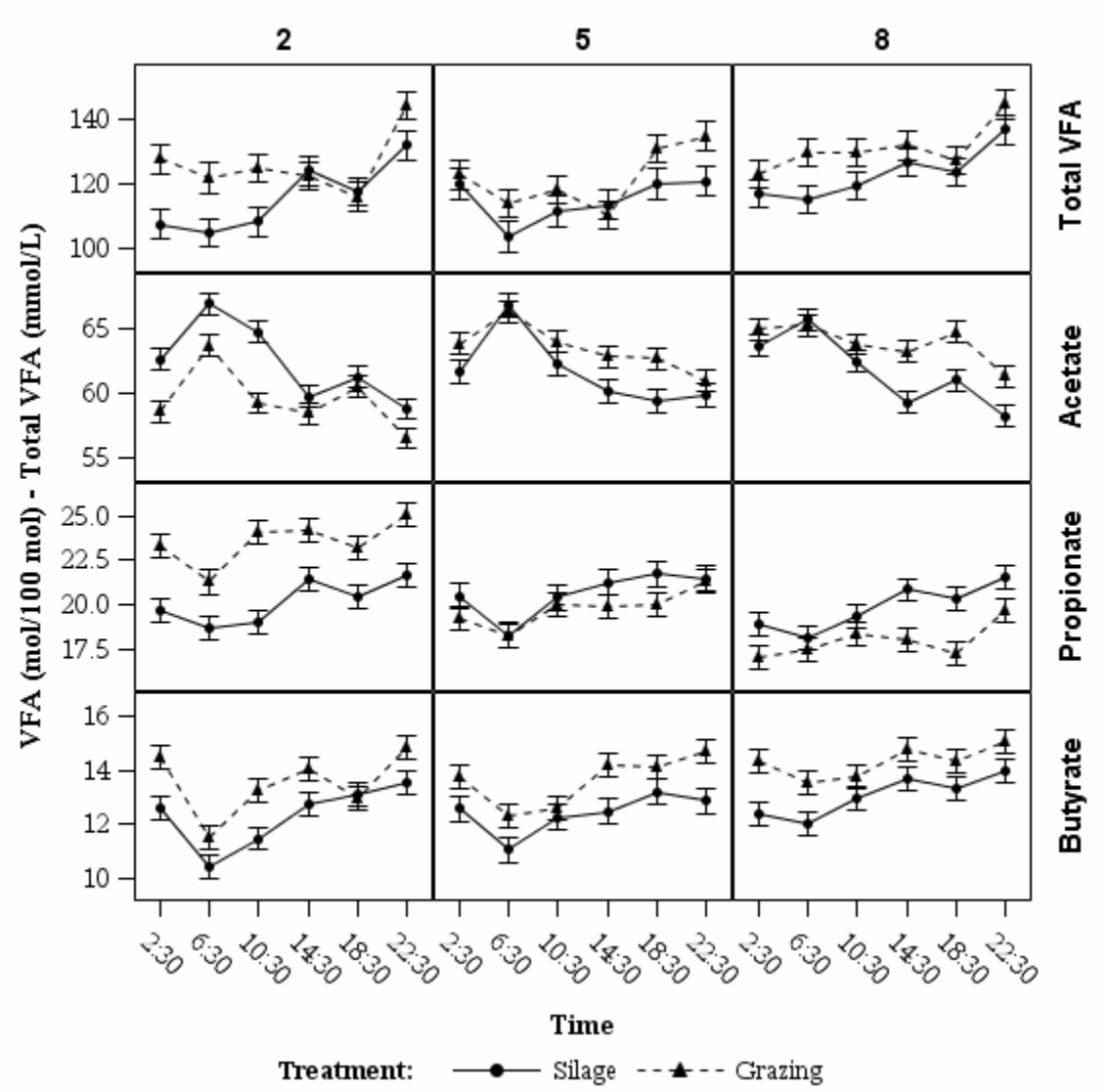


Figure 1 Diurnal total VFA concentration (mmol/L) and individual VFA molar proportions (mol/100 mol) in rumen liquid of dairy cows fed with grass-clover silage and housed indoors or grazing perennial ryegrass and white clover pasture during day and night. Panels represent experimental weeks 2, 5, and 8.

Conclusions

Changes in rumen environments between cows fed grass-clover silage or grazing pasture were driven by the grazing treatment, which may relate to changes in pasture nutrient composition over the experimental weeks.

References

Nielsen, N. I., and Volden, H., 2011. Animal requirements and recommendations, in: NorFor - The Nordic Feed Evaluation System, EAAP publication no. 130. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 85–111.

Sjaunja, L. O., Baevre, L., Junkkarinen, L., Pedersen, J., Setala, J., 1991. A Nordic proposal for an energy corrected milk (ECM) formula, in: EAAP Publication 50: Performance Recording of Animals: State of the Art 1990. Performance Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen, the Netherlands, pp. 156–157.

Effects of silage digestibility and diet concentrate level on performance of finishing bulls

K. Manni[1] & A. Huuskonen[2]
[1]*Natural Resources Institute Finland (Luke), Production Systems, Tietotie 4, 31600 Jokioinen, Finland.*
[2]*Natural Resources Institute Finland (Luke), Production Systems, Halolantie 31 A, 71750 Maaninka, Finland.*
Correspondence: Katariina.Manni@luke.fi

Introduction

In growing cattle, the conventional method of overcoming the deficiencies in energy supply from grass silage (GS) is to supplement the diets with concentrates. The growth responses achieved with concentrate supplementation typically depend on the GS quality, primarily its digestibility (Huuskonen & Huhtanen, 2015). The main objective of the present study was to evaluate the effects of GS digestibility and diet concentrate level on the performance of finishing bulls. In addition, possible GS digestibility × concentrate level interactions were studied.

Materials and Methods

The feeding experiment comprised a total of 90 finishing bulls. During the experiment, 12 bulls were excluded from the study for reasons unrelated to the treatments, and results include 78 bulls. For these bulls, the dams were dairy breed, either Holstein (48 animals) or Nordic Red (30 animals). Sire breeds were Holstein (41 animals), Blonde d'Aquitaine (22 animals) and Nordic Red (15 animals). A 2 × 3 factorial design was used to study the effects of GS digestibility and diet concentrate proportion on animal performance. The two targeted GS digestibilities were medium [670 g digestible organic matter (DOM) in dry matter (DM) (D Value)] and low (620 g DOM in DM). The three concentrate proportions were 0, 250 and 500 g/kg DM. The bulls were fed total mixed rations *ad libitum*. The bulls were housed in an uninsulated barn in pens (10.0 m × 5.0 m) in groups of five bulls in each pen, providing 10 m^2 per bull. The GS used was a mixture of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward. The stands were cut by using a mower conditioner, wilted for 24 h after cutting, harvested using a precision-chop forage harvester, and ensiled in bunker silos with a formic acid-based additive. The concentrate used was rolled spring-sown barley grain.

The statistical model used was $y_{ijklm} = \mu + \delta_k + \alpha_i + \gamma_j + (\alpha\times\gamma)_{ij} + \theta_{ijm} + \beta x_{ijkl} + e_{ijklm,}$ where μ is the intercept and e_{ijklm} is the residual error term associated with l^{th} animal. α_i, γ_j and $(\alpha\times\gamma)_{ij}$ are the effects of i^{th} grass silage digestibility (low, medium) and j^{th} concentrate proportion (0, 250, 500 g/kg DM) and their interaction, respectively, while δ_k is the effect of the breed (k=pure Holstein, pure Nordic Red, Holstein × Blonde d'Aquitaine, Nordic Red × Blonde d'Aquitaine) and θ_{ijm} is the effect of pen. The effect of pen was used as an error term when differences between treatments (silage digestibility, concentrate proportion and their interaction) were compared because treatments were allocated to animals penned together. Initial live weight was used as a covariate (βx_{ijkl}) in the model. Differences between the treatments were tested using orthogonal contrasts: (1) GS digestibility (medium vs. low), (2) linear effect of concentrate proportion, (3) quadratic effect of concentrate proportion, (4) linear interaction between GS digestibility and concentrate proportion, and (5) quadratic interaction between GS digestibility and concentrate proportion.

Results and Discussion

The achieved digestibilities for medium and low digestible silages were 665 and 616 g/kg DM, respectively, and close to pre-planned. The results of the feeding experiment are presented in Table 1. The slaughter age of the bulls was 565 days, on average. No significant silage digestibility × concentrate proportion interactions were observed for any intake parameters. Silage digestibility did not effect DM intake. However, the metabolizable energy (ME) intake of the bulls fed medium digestible silage was 7% higher compared to the bulls fed low digestible silage ($P<0.01$). All intake parameters, DM, ME and CP intakes, increased linearly with increasing concentrate proportion ($P<0.001$).

Carcass gain of the bulls increased 26%, when medium digestible silage was used instead of low digestible silage ($P<0.001$). Carcass gain increased linearly with increasing concentrate proportion ($P<0.001$). A one-gram increase in the D-value of silage increased carcass gain of dairy bulls by 4.1, 3.6 and 2.1 g/d at concentrate levels 0, 25 and 50%, respectively.The most likely explanation for the differences in growth performance is differences in ME intake. Based on the meta-analysis, Huuskonen & Huhtanen (2015) established that ME intake was clearly the most important nutritional variable affecting live weight gain of growing cattle.

For feed conversion parameters two significant ($P<0.05$) linear diet digestibility × concetrate proportion interactions were observed. Increasing concentrate proportion improved both feed (kg DM/kg carcass gain) and CP conversion (kg CP/kg carcass gain) rates of the bulls more on low digestible silage diets compared to medium digestible silage diets. Dry matter, ME and CP conversion rates improved with increasing concentrate proportion and when medium digestible silage was used instead of low digestible silage ($P<0.001$).

No significant ($P<0.05$) silage digestibility × concentrate proportion interactions were observed for any carcass parameters. Carcass weight of the bulls increased with increasing concentrate proportion ($P<0.001$) and when medium digestible silage was used instead of low digestible silage ($P<0.05$). When medium digestible silage was used instead of low digestible silage the dressing proportion and carcass conformation score of the bulls tended ($P<0.10$) to increase 2 and 18%, respectively, and carcass fat score incresed 29% ($P<0.001$). Increasing the concentrate proportion increased dressing proportion ($P<0.001$), carcass conformation score ($P<0.05$) and carcass fat score ($P=0.001$) linearly. Since this study standardized slaughter age instead of slaughter weight, it is probable that slaughter weight affected dressing proportion, carcass conformation and carcass fat score in addition to experimental treatments as these traits generally increase as carcass weight increase.

Conclusions

Energy intake is an important factor for the high performance of beef cattle, particularly for growth rate. Both increasing silage digestibility and including concentrate in the diet are methods to increase energy intake. However, increased concentrate level decreased the growth response of increased D-value of silage.

References

Huuskonen A., Huhtanen, P., 2015. The development of a model to predict BW gain of growing cattle fed grass silage-based diets. Animal 9, 1329-1340.

Acknowledgements

The study was part of the OptiNurmi project, which is co-funded by the European union.

Table 1 Intake, growth performance and carcass traits of the bulls fed total mixed rations with two diferent levels of silage digestibility and three different concentrate levels.

Silage digestibility (S)[1]	Medium			Low			SEM[3]	Orthogonal contrasts[4]				
Concentrate level (C)[2]	Low	Medium	High	Low	Medium	High		S	C_{Lin}	C_{Quad}	$S \times C_{Lin}$	$S \times C_{Quad}$
Number of observations	13	13	13	13	14	12						
Duration of the experiment, d	162	162	162	162	162	162						
Initial live weight, kg	530	528	547	524	528	533	13.3	0.512	0.319	0.627	0.756	0.636
Final live weigh, kg	707	749	792	652	708	759	18.2	0.004	<0.001	0.945	0.536	0.912
Carcass weight, kg	363	395	422	328	366	399	11.4	0.002	<0.001	0.791	0.611	0.946
Slaughter age, d	567	567	565	562	561	568	3.0	0.256	0.514	0.566	0.250	0.385
Intake												
Dry matter (DM), kg/d	12.0	12.6	13.4	11.1	12.5	13.7	0.38	0.523	<0.001	0.938	0.117	0.727
Metabolizable energy (ME), MJ/d	127	141	159	109	134	157	4.1	0.007	<0.001	0.881	0.048	0.798
Crude protein (CP), kg/d	1.46	1.57	1.71	1.36	1.56	1.74	0.045	0.422	<0.001	0.999	0.129	0.741
Carcass gain, g/d	604	806	919	405	630	815	38.4	<0.001	<0.001	0.316	0.209	0.703
Feed conversion												
Kg DM/kg carcass gain	20.7	16.2	14.9	28.6	20.6	17.1	1.26	<0.001	<0.001	0.063	0.022	0.779
MJ ME/kg carcass gain	220	182	177	281	220	197	13.3	<0.001	<0.001	0.104	0.107	0.914
Kg CP/kg carcass gain	2.54	2.02	1.91	3.49	2.57	2.18	0.16	<0.001	<0.001	0.073	0.027	0.802
Carcass characteristics												
Dressing proportion, g/kg	513	527	533	502	516	525	6.5	0.062	0.001	0.542	0.792	0.952
Conformation, EUROP	5.5	5.6	6.2	3.9	5.0	5.8	0.60	0.059	0.034	0.915	0.332	0.663
Fat score, EUROP	5.2	6.3	7.3	3.2	5.3	6.3	0.37	<0.001	<0.001	0.318	0.161	0.453

[1]Medium digestible silage = 665 g digestible organic matter/kg DM. Low digestible silage = 616 g digestible organic matter/kg DM.

[2]Low concentrate level = 0 g barley grain/kg DM, medium concentrate level = 250 g barley grain/kg DM, high concentrate level = 500 g barley grain/kg DM.

[3]Standard error of the mean.

[4]Orthogonal contrasts: (S) medium digestible silage vs. low digestible silage, (C_{Lin}) linear effect of concentrate level, (C_{Quad}) quadratic effect of concentrate level, ($S \times C_{Lin}$) linear interaction between silage digestibility and concentrate level, and ($S \times C_{Quad}$) quadratic interaction between silage digestibility and concentrate level

Effects of decreasing silage particle size by a screw press on rumen passage rate and digestion in dairy cows

B.O. Rustas, M. Managos, & T. Eriksson
Swedish University of Agricultural Sciences (SLU), Department of Applied Animal Science and Welfare, Box 7024, 750 07 Uppsala, Sweden.
Correspondence: bengt-ove.rustas@slu.se

Introduction

Particle size reduction by extrusion, a process where a screw press mix and shear plant material, is utilised in biogas production (Rodriguez et al., 2017). The purpose is to increase access to fibre constituents for bacteria and enzymes for improved fermentation. In an *in vitro* study with rumen fluid we found increased gas production rates, indicating higher rates of digestion, after extrusion of timothy and red clover silages (Elgemark et al., 2020). Particle size reduction of forages may affect intake and Nasrollahi *et al.* (2015) found an increased daily dry matter (DM) intake by 0.5 kg when silage chop length was reduced from 10 to 6 mm. If increased intake is caused by enhanced rumen passage rate it might have negative effects on feed digestibility, counteracting any positive effects of feed processing on digestion rate. The aim of this study was to evaluate how processing by a screw press (extruder) of grass silage harvested at different maturities affect digestion and digestive kinetics in dairy cows.

Materials and Methods

A grass-dominated (70% timothy) ley crop was harvested as round bale silage at first cut on June 13 (Harvest 1) and 10 days later (Harvest 2) at Lövsta research centre, Uppsala, Sweden. At feed out, bales were mixed and chopped in a feed mixer and half of each bale was further processed in a double screw press (Bio-Extruder MSZ-B15e, LEHMANN Maschinenbau GmbH). Particle size distribution of silages was determined with a set of sieves (Penn State Particle Separator, Nasco, Fort Atkinson, WI, USA) with four levels.

Four rumen fistulated cows (Swedish Red, 143±38 DIM, 35±8 kg ECM) were enrolled in a Latin square design experiment with four periods of 21 days and 4 treatments: silage from harvest 1 or 2 fed chopped or chopped and extruded, in a 2 × 2 factorial arrangement. Silage was fed separately *ad libitum* together with 2 kg soybean meal, 6 kg compound feed (Komplett Norm 180, Lantmännen, Sweden) and 120 g of a mineral mix.

Rumen evacuations were performed at 1300 h on d 12 and d 19 in each period. During emptying, every tenth handful of rumen content was collected in a separate container, thoroughly mixed, sampled and frozen at –20°. Spot samples of faeces were collected twice a day on d 15 to 19 and frozen at -20 ºC. Rumen and faecal samples were later pooled within cow and period after thawing, mixed, sampled and a freeze dried.

All samples were dried at 60ºC, milled on a hammer mill (1 mm) and analysed for ash, NDF (Chai and Udén, 1988; with undiluted detergent solution, α-amylase, and sodium sulfite), N (Kjeldahl) and mineral content (ICP-AES). Dry matter was determined at 60ºC for silages and 103ºC for other samples. *In vitro* organic matter digestibility (IVOMD) was determined after 96 h incubation with rumen fluid (Lindgren, 1979). Indigestible NDF (iNDF) was determined in silages, concentrates, rumen contents and faeces after 288 h incubation in sacco in two nonlactating fistulated dairy cows with samples milled through a 1.5 mm screen on a knife mill (Åkerlind et al., 2011).

Rumen kinetics expressed as rate in % h were calculated according to Robinson et al. (1987) with intake (k_i) = 100*intake (kg/24h)/rumen pool (kg), with passage (k_p) = 100* faecal output (kg/24 h)/rumen pool (kg) and with digestion (k_d) = k_i - k_p. Total tract digestibility was calculated using iNDF as internal marker.

Statistical analysis was performed on means from each period by the mixed procedure of SAS (SAS System for Windows, release 9.4, SAS Institute Inc., Cary, NC, USA). The model included period, cow, harvest, silage processing and the interaction between harvest and processing, cow were considered random and the other factors fixed effects.

Results and Discussion

The NDF content was similar across harvests (Table 1) which resulted in treatments not confounding NDF content and digestibility which is often the case when comparing grass crops of different maturities.

Table 1 Composition and particle size distribution of silages

	Harvest 1		Harvest 2	
	Chopped	Extruded	Chopped	Extruded
DM, g/kg	458	477	505	524
Ash, g/kg DM	80	80	71	69
CP, g/kg DM	125	124	106	105
NDF, g/kg DM	548	549	551	552
IVOMD, g/kg OM	799	801	713	722
PSD[1], % 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

[1]PSD=particle size distribution on sieves of different size.

Intake increased after extrusion but was not affected by maturity (Table 2). Dry matter intake increased by 1.5 kg per day after particle size reduction which is more than the 0.5 kg Nasrollahi *et al.* (2015) found in a meta analysis. Even though average particle size was not determined in this study the sieving results indicate a larger difference in particle length between treatments than the 10 vs 6 mm reported by Nasrollahi *et al.* (2015).

Table 2 Intake of dry matter (DMI) and NDF (NDFI) of silages and diets and digestion of organic matter (OMD) and NDF (NDFD) of diets fed to cows in the experiment

	Harvest 1		Harvest 2			P-value		
	Chopped	Extruded	Chopped	Extruded	SED	Harvest	Process	H x P
DMI, silage, kg	18.0	19.2	17.2	19.3	0.33	0.21	<0.001	0.079
DMI, tot, kg	25.0	26.0	24.2	26.2	0.35	0.28	0.001	0.087
NDFI, kg	11.5	12.1	11.1	12.3	0.17	0.50	<0.001	0.09
OMD, %	72.5	72.7	66.4	68.5	1.37	0.002	0.28	0.36
NDFD, %	68.8	69.1	58.6	61.3	1.97	0.001	0.33	0.40

The greater intake of extruded silages did not seem to be caused by faster passage through the cows as the passage rate of iNDF did not differed between chopped and extruded silages (Table 3). This is supported by Ali et al. (2024) who did not find differences between chopped and extruded silage in total tract mean retention time, based on pulse dosing of chromium mordanted fiber in this study. As rumen pool size of NDF did not differ due to processing it was probably the increased digestion rates that caused the difference in intake. Faster digestion cleared digestible NDF quicker for extruded silage compared to chopped silage and facilitated increased intake.

Conclusion

By passing grass silage through a screw press digestion rate, but not digestibility or passage rate, of NDF increased.

Table 3 Rumen pool size for dry matter (DM), neutral detergent fibre (NDF), indigestible NDF (iNDF) and potentially digestible NDF (dNDF), and kinetic parameters describing rate of passage (k_p) and digestion (k_d).

	Early harvest		Late harvest			P-value		
	Chopped	Extruded	Chopped	Extruded	SED	Harvest	Process	H x P
Rumen pool size, kg								
Digesta	110.3	107.6	113.9	113.0	4.80	0.23	0.62	0.80
DM	13.6	13.2	14.0	15.0	0.58	0.034	0.50	0.14
NDF	8.13	7.88	8.84	9.48	0.38	0.005	0.50	0.15
iNDF	3.03	2.92	4.07	4.64	0.28	<0.001	0.29	0.13
dNDF	5.10	4.96	4.77	4.84	0.21	0.17	0.82	0.50
Kinetics, % per hour								
dNDF, k_d	6.56	7.06	5.73	6.56	0.34	0.030	0.031	0.51
iNDF, k_p	2.6	2.8	2.8	2.6	0.25	0.73	0.85	0.30

References

Ali, S., Rustas, B.O. & Kronqvist, C., 2024. Effects of silage particle size reduction on feed total tract retention time and magnesium absorption in dairy cows. Proc.12th Nord. Feed Sci. Conf., Uppsala, Sweden, 85–87.

Elgemark, E., Eriksson, T. & Rustas, B.O., 2020. Effect of mechanical processing of ley crop silages on *in vitro* gas production and neutral detergent fibre digestion. Grassl. Sci. Eur., 25, 632-634.

Nasrollahi, S.M., Imani, M. & Zebeli, Q., 2015. A meta-analysis and meta-regression of the impact of forage particle size, level, source and preservation method on feed intake, nutrient digestibility, and performance in dairy cows. J. Dairy Sci. 98, 8926–8939.

Robinson, P.J., Tamminga, S. & van Vuuren, A.M., 1987. Influence of declining level of feed intake and varying proportion of starch in the concentrate on rumen ingesta quantity, composition and kinetics of ingesta turnover in dairy cows. Livest. Prod. Sci. 17, 37–62.

Rodriguez, C., Alaswad, A., Benyounis, K.Y. & Olabi, A.G., 2017. Pretreatment techniques used in biogas production from grass. Renew. Sustain. Energy Rev., 68, 1193–1204.

Effect of harvest time of ensiled reed on intake and digestibility in dry dairy cows

W. Ashworth[1], Ö. Östman[1] & B.O. Rustas[2]
[1] Swedish University of Agricultural Sciences, Department of Aquatic Resources, Box 7018 750 07, Uppsala, Sweden. [2] Swedish University of Agricultural Sciences (SLU), Department of Applied Animal Science and Welfare, Box 7024, 750 07 Uppsala, Sweden.
Correspondence: bengt-ove.rustas@slu.se

Introduction

Common reed (*Phragmites australis*) is a fibrous plant found growing in wetlands and coastal habitats across the globe (Packer et al., 2017), with the potential to be used as a roughage for livestock. It is a fibrous feed with a relatively high crude protein content, low digestibility, and low energy value (Silliman et al., 2014). The high fibre content constrains intake and therefore may limit energy supply even further (Allen, 1996). This combination of characteristics makes reed a potentially suitable feed for dry dairy cows, having low energy and moderate protein requirements. Despite its potential, few studies have conducted feeding trials with reed as a roughage source. This study aims to evaluate the effect of harvest date on intake and digestibility of reed silage in dry dairy cows.

Materials and Methods

The experiment was conducted at the Swedish Livestock Research Centre, SLU and was approved by the Uppsala Ethics Committee for Animal Research, Uppsala, Sweden (diary number 5.8.18-01637/2022) and by the Board for research animals at SLU (d.n. SLU ua 2023.4.1-2092).

Reed was harvested in the Baltic Sea at a coastal bay in Östhammar municipality, east of Uppsala (60.19511, 18.440858), between 6-12 July 2023 (Early harvest) and at similar bays in Vaxholm municipality, close to Stockholm (59.422431, 18.289729), between 9-15 August 2023 (Late harvest). The crop was cut 20 cm above water surface with a flail chopper mounted on a flat-bottomed boat driven by an aircraft propeller. An inoculant (*Lactobacillus Plantarum, Lactobacillus Paracasei, Lactobacillus Brevis*; Xtrasil bio ultra, Konsil Scandinavia AB, Tvååker, Sweden) was added in the chopper. On land, bales of 50 kg were produced from a high-density hay baler and wrapped in ten layers of 25µm thick stretch film (Silograss, Armando Alvarez Group/ASPLA, Spain) using a mini-bale wrapper (FW500/4K, Wolagri, Remanzacco, Italy). Wrapped bales were stacked on pallets, transported to the experimental station, and stored until the trial started. During the experiment, bales from each harvest were unwrapped twice a week and the reed silage was then mixed and chopped in a horizontal, double screw TMR mixer (SMW 17, Delaval, Tumba, Sweden). To improve crude protein content urea was added during mixing, at an inclusion rate of 0.9 and 1.1 % of dry matter for Early and Late harvest, respectively. Plastic containers were filled with 20 kg of mixed reed and stored in a fridge at 4°C.

Eight dry cows (Swedish Red, 707 ± 21 kg) in 200 (± 12) days of pregnancy were used in a change-over trial with two 3-week periods, 2 weeks for adaptation and the last for sampling. Cows were randomly allocated to one of two treatment sequences and were either fed reed from the first harvest in period one and then from the second harvest in period two or vice versa. Cows were housed in a free stall barn with feed bins for automatic intake recording

(CRFI, Biocontrol A/S, Rakkestad, Norway). Each animal used a single bin throughout the experiment. Early or late harvest reed silage was offered at 12.00 each day at a level ensuring at least 10% orts. A mineral mix (100 g, Mixa Optimal, Lantmännen, Sweden) was distributed on top of the silage. Intake registration and sampling of feed, orts and faeces (spot sampling twice a day) were carried out during 5 days in the collection periods. Samples were frozen at -20°C and later pooled within experimental period. Pooled samples of silage and orts were dried at 60ºC and faeces were freeze dried. All dried samples were milled on a hammer mill with a 1-mm screen. Dry matter was determined at 103°C. Analysis of ash, crude protein (CP), neutral detergent fibre (aNDFom), acid insoluble ash (AIA), 96-h in vitro organic matter digestibility (IVOMD) and fermentation acids were performed with routine methods (Eriksson and Rustas, 2014). Apparent digestibility was calculated using AIA as internal marker.

Statistical analysis was performed on period averages with a general linear model in the software Minitab (Minitab LLC, version 18). The model included period, treatment and animal. A p-value less than 0.05 was considered significant in comparisons.

Results and Discussion

Fermentation quality (Table 1) was similar to that of reed silage used in a previous experiment (Rustas et al., 2024) supporting the ensilability of reed.

Table 1 Composition of reed silages fed to dry dairy cows

		Early harvest	Late harvest
Dry matter	%	36	41
Ash	g/kg DM	66	60
Crude protein (CP)	g/kg DM	119	91
NDF[1]	g/kg DM	640	693
IVOMD[2]	%	71	60
Lactic acid	g/kg DM	60	44
Acetic acid	g/kg DM	9	8
Propionic acid	g/kg DM	1	<0.4
Butyric acid	g/kg DM	<0.4	<0.4
Ethanol	g/kg DM	12	9
Ca	g/kg DM	2.0	2.0
P	g/kg DM	1.6	1.2
K	g/kg DM	15.4	9.7
Mg	g/kg DM	1.8	1.7
Na	g/kg DM	2.8	3.2
S	g/kg DM	4.1	4.0

[1]Neutral detergent fibre. [2]In vitro organic matter digestibility.

Dry matter intake declined when feeding reed from the late harvest (Table 2), probably due to increased fibre content and reduced fibre digestibility (Allen, 1996).

Organic matter digestibility (OMD) of the early harvest was a bit lower than the 55% reported for dairy heifers fed ensiled reed harvested from mid-July to the beginning of August (Rustas et al., 2024). According to Swedish feed tables (Spörndly, 2003) OMD for reed harvested in

the summer is 46% which is well in line with present findings for harvest in July, although the table values are based on determinations with wethers on maintenance level.

Calculated energy intake according to Norfor (Norfor, 1.0.80; Foderopti, Växa Sverige), based on OMD in the experiment, was 87 MJ ME/d for early harvest and 54 MJ ME/d for late harvest. Swedish recommendations (Spörndly, 2003) for energy supply to a 700 kg dry cow in 7th month of pregnancy is 78 MJ ME/day and in 8th month 84 MJ. Feeding only the reed silage harvested in July would therefore be sufficient to support dry cows' energy demand with more than 4 weeks to calving.

It should be noted that as the two harvests took place in separate locations both temporal and spatial effects might have influenced the results.

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

	Early harvest	Late harvest	SED[1]	*P*-value
Intake				
Dry matter, kg/d	12.8	10.8	0.49	0.007
Dry matter, % of live weight	1.7	1.5	0.05	0.010
NDF[2], kg/d	8.2	7.5	0.32	0.080
NDF, % of live weight	1.1	1.0	0.05	0.136
Digestibility, %				
Dry matter	47	33	2.5	0.001
Organic matter	48	35	2.5	0.002
Crude protein	56	42	2.5	0.001
NDF	52	41	2.4	0.006

[1]Standard error of difference. [2]Neutral detergent fibre.

Conclusion

Digestibility of the reed harvested in July was poor and it was even poorer in the reed harvested in August. The results suggest that ensiled reed harvested in July may fulfil energy requirements of dry dairy cows.

References

Allen, M.S., 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74, 3063-3075.

Rustas, B.O., Thelin, H., and Kiessling, A. 2024. Effect of particle size reduction by extrusion on intake and digestion of reed silage in dairy heifers. Proc.11th Nord. Feed Sci. Conf., Swedish Univ. Agric. Sci., Uppsala, Sweden, pp. 51–53.

Spörndly, R., 2003. Fodertabeller för idisslare (Feed table for ruminants). Rapport 257. Dept. Anim. Nutr. Mgmt., Swed. Univ. Agric. Sci., Uppsala, Sweden.

Silliman, B.R., Mozdzer, T., Angelini, C., Brundage, J.E., Esselink, P., Bakker, J.P., Gedan, K.B., van de Koppel, J. and Baldwin, A.H. 2014. Livestock as a potential biological control agent for an invasive wetland plant. Peer J. 2, p. 567.

Packer, J.G., Meyerson, L.A., Skálová, H., Pysek, P. and Kueffer, C. 2017. Biological Flora of the British Isles: Phragmites australis. Journal of Ecology, 105 (4), pp. 1123-1162.

Excretion calculations of horses in Finland

J. Vattulainen, H. Högel & M. Saastamoinen
Natural Resource Institute Finland (Luke), Tietotie 4, 31600 Jokioinen, Finland
Correspondence: jenni.vattulainen@luke.fi

Introduction

The Natural Resources Institute Finland (Luke) conducts annual national excretion calculations that quantify dry matter (DM), organic matter (OM), and nutrient excretion from livestock, including horses. These calculations support national emission inventories, evaluations of nutrient loading to air and waters, manure management planning, and circular economy strategies. Excretion is computed using a nutrient balance approach, i.e, nutrient excretion = nutrient intake – nutrient retention in the animal or its products. The nutrients quantified include nitrogen (N), phosphorus (P), and potassium (K, not presented here), alongside DM and OM. Because horses represent a highly heterogeneous population in Finland, varying in breed, size, use, age, and activity level, categories based on size and work intensities are used in the calculations. This compensates for the lack of actual feeding data and wide variation in management practices (Uotila et al., 2012). Calculation method of horses was renewed during 2020-2023 (Saastamoinen et al., 2024).

Materials and Methods

Horses were categorized by size (adult horses 550 kg; ponies/small horses 350 kg; small ponies 225 kg) and activity level (maintenance, light, moderate, heavy work) according to the calculation purposes. Categories were in line with Finnish feeding recommendations and nutrient requirements for horses (Kuoppala et al., 2024). In the absence of exact feed intake and feed composition data, intake of DM, OM and nutrients were estimated from the metabolizable energy requirement (ME_{req}) based on Finnish nutrient requirements, and the ME content of the diet based on Finnish feed tables (Kuoppala et al., 2024). ME_{req} were calculated per kilogram of live weight for each individual horse category. Feed intake was then derived by dividing ME_{req} by the diet's ME density. The diets used in the calculations were forage-dominated, reflecting Finnish feeding practices (Saastamoinen & Hellämäki, 2012), with roughage-to-concentrate ratios of 100:0 for maintenance, 80:20 for light work, 70:30 for moderate work and 60:40 for heavy work. Average roughage composition was based on the publication of Saastamoinen & Hellämäki (2012). Oats were used as the representative concentrate. Nutrient intakes (DM, OM, CP, N, P, K) were calculated from diet composition and estimated feed intake. Digestibility coefficients for OM, DM, and crude protein (CP) were derived from published equations (Jensen et al., 2014; Ragnarsson & Lindberg, 2008; Palmgren Karlsson et al., 2000) and Finnish feed table values (Kuoppala et al., 2024). Faecal output was calculated from undigested DM and OM, assuming 21% DM content in faeces (Särkijärvi & Saastamoinen, 2019). Retention of nutrients was not taken into account in these calculations.

Results and Discussion

Main results from the calculations representing year 2025 are presented in Table 1. In the national level, majority on N, P and faeces were excreted by adult horses. Adult horses included in the heavy work category had the highest excretion, but since their proportion of all horses was small, they only contributed around 16% of the total excretion (Figure 1).

Table 1 Annual excretion of nitrogen, phosphorus and faeces (kg/a) per animal in different activity levels and size categories.

Excretion, kg/a/animal		Adult horse	Pony and small horse	Small pony
Number of horses	Maintenance	8400	530	2500
	Light work	12800	2700	0
	Moderate work	25000	3500	670
	Heavy work	6900	110	370
Nitrogen	Maintenance	35.5	23.4	15.8
	Light work	38.9	25.5	17.1
	Moderate work	46.3	30.2	20.2
	Heavy work	55.2	35.9	23.8
	Weighted average	44.0	27.9	17.4
Phosphorus	Maintenance	5.59	3.55	2.28
	Light work	6.52	4.15	2.67
	Moderate work	8.04	5.12	3.29
	Heavy work	9.89	6.29	4.05
	Weighted average	7.53	4.63	2.66
Amount of faeces	Maintenance	6419	4085	2626
	Light work	6182	3934	2529
	Moderate work	6946	4420	2841
	Heavy work	7781	4951	3183
	Weighted average	6787	4211	2724

The change in the calculation method decreased overall the nitrogen excretion in all horse categories, when compared to the previous results. The average amount of N excreted per year per animal in Finland is four kg less than for example in Sweden (Carlen et al., 2026).

There is a wide variety of feeding practices, forage quality, management, and work intensity among stables and horses in Finland. Since there is no accurate data of the actual feeding practices or feeds used, there is a lot of uncertainty in the calculations. The methodology presented herein, estimates nutrient excretion at the Finnish national level using categorized average values, with the best possible data available. Forage based diets are favoured horse feeding in Finland. Mineral supplementations and commercial concentrate feeds were not included in the calculations, since the group of possible feeds is variable and more data would be needed. However, many horses receive some other supplementary feed besides hay and oats in their everyday life. Also, the quality of roughage might vary drastically between different stable systems.

Conclusions

Adult horses, especially in the moderate work intensity group, contribute the most to total national nutrient loads due to their population size and higher absolute nutrient turnover. Improving the precision of excretion estimates would require more data on actual feeding practices, feed intake, roughage analyses, and live weights of horses, of which feed intake and feed composition, particularly roughage quality, should be prioritized, as they directly determine nutrient input and excretion.

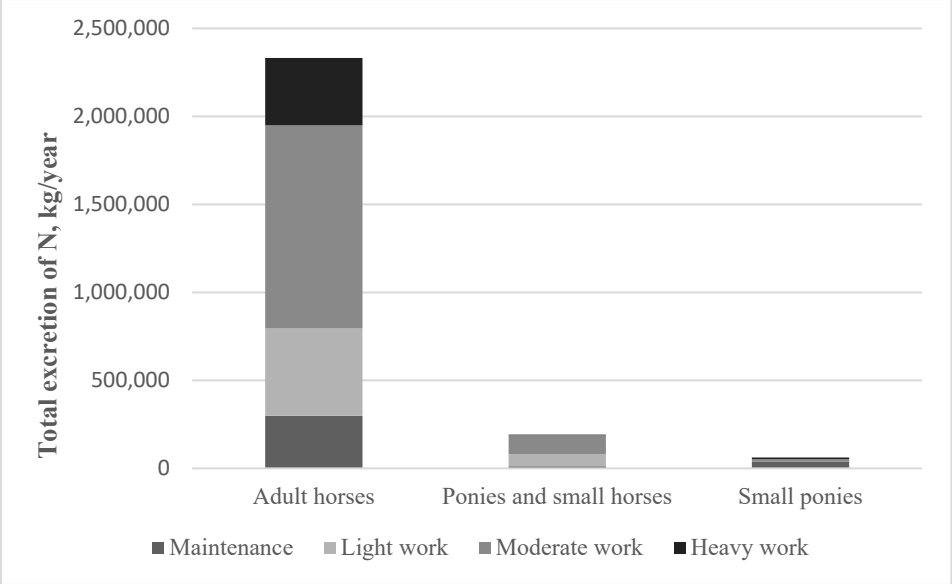


Figure 1 Total annual nitrogen excretion in different work and size categories of horses.

References

Carlén, E., Kanth, M., Bengtsson, J., Szudy, M., Eklund, V., Kellner, M., Hytteborn, J., Mawdsley, I. & Lundblad, M. 2025. National Inventory Document Sweden 2025: Greenhouse Gas Emission Inventories 1990–2023. Swedish Environmental Protection Agency.

Jensen, R.B., Austbø, D., Bach Knudsen, K.E. & Tauson, A.-H. 2014. The effect of dietary carbohydrate composition on apparent total tract digestibility, feed mean retention time, nitrogen and water balance in horses. Animal 8: 1788–1796.

Kuoppala, K., Vattulainen, J., Perttilä, S., Saastamoinen, M. & Rinne, M. 2024. Finnish Feed Tables and Nutrient Requirements : Ruminants, pigs, poultry, horses. Natural Resources and Bioeconomy Studies 70/2024. Natural Resources Institute Finland. Helsinki. 97 p.

Palmgren Karlsson, C., Lindberg, J.E. & Rundgren, M. 2000. Associative effects on total tract digestibility in horses fed different ratios of grass hay and whole oats. Livest. Prod. Sci. 65: 143–153

Ragnarsson, S. & Lindberg, J.E. 2008. Nutritional value of timothy haylage in Icelandic horses. Livest. Sci. 113: 202–208.

Saastamoinen, M. & Hellämäki, M. 2012. Forage analyses as a base of feeding of horses. In: Saastamoinen, M., Fradinho M.J., Santos, A.S., Miraglia, N. (eds.). Forages and grazing in horse nutrition. EAAP publication no. 132. pp. 305–314.

Saastamoinen, M., Särkijärvi, S., Högel, H., Vattulainen, J. & Nousiainen, J. 2024. Excretion calculation of horses in Finland. Natural Resources and Bioeconomy Studies 67/2024. Natural Resources Institute Finland. Helsinki. 30 p.

Uotila, R., Thuneberg, T. & Saastamoinen, M. The usage of forage analyses in optimizing horse nutrition in Finland. In: Forages and grazing in horse nutrition. Saastamoinen, M., Fradinho M.J., Santos, A.S., Miraglia, N. (eds.). EAAP publication no. 132. pp. 331- 334

Enteric methane emission in dairy cows on early vs late summer pasture and effect of 3-nitrooxypropanol

E. Økland[1], T. Undheim[1], A. Kidane[1], A. Schwarm[1], A. Perez[1] & E. Prestløkken[1]
[1]Norwegian University of Life Sciences (NMBU), Department of Animal and Aquacultural Sciences, Oluf Thesens vei 6, 1433 Aas, Norway
Correspondence: erlend.podhorny.okland@nmbu.no

Introduction

Quantification of enteric methane (CH_4) is important to estimate emissions from ruminant production. Grazing is an important part of cattle production, and quantifying enteric CH_4 emissions from dairy cows on pasture is important, as emissions vary between grazing systems and indoor feeding (Kar-Klootwijk et al., 2026). Additionally, enteric CH_4 may vary throughout the grazing season. 3-nitrooxypropanol (3-NOP) is a promising additive for reducing enteric CH_4 emissions. However, there is a knowledge gap on the effect of 3-NOP in grazing systems. This study investigates the effects of the grazing season with and without 3-NOP supplementation as a CH_4 inhibitor. Preliminary results are presented.

Materials and Methods

Two grazing experiments were conducted on early and late summer pasture, in August 2024 and May 2025 at the Norwegian University of Life Sciences (NMBU), with 30 Norwegian Red dairy cows in each experiment. Based on milk yield, body weight and days in milk, the cows were allocated into a control group (mineral premix only) and a treatment group given 3-NOP (Bovaer[10], DSM-Firmenich Nutrition Products Ltd) at 1.2 g per cow per day, in a mineral premix. Each trial lasted for 21 days, starting with 6 days covariate period where all cows were fed the same diet, and 15 days of measurement with 15 cows in each of the two groups. The cows were grazing together in the same paddock and milked twice daily (0600 and 1600 h local time), and in the measurement period, 3-NOP was administered to the treatment cows during milking blended with a mineral premix. All cows received 75 grams of premix per milking, without 3-NOP for the control group. During milking, the cows received 6 kg of concentrate per day in early season, and 3 kg per day in late season. Enteric CH_4 was measured using two GreenFeed (GF) units (C-Lock Inc., Rapid City, SD). One GF unit was located close to the milking barn where breath samples were analysed from each cow after morning and evening milking. The second GF unit was located on pasture and visited by the cows on a voluntary basis. In the GFs, the cows were offered 1.5 kg of concentrate per day combined between the two GFs, if they visited a total of 5 times per day. Herbage samples for chemical analysis were taken throughout the trial period in both seasons. Samples were taken before the cows were moved to a new pasture, and after the cows were moved off pasture, giving five samples pre- and post-grazing, both in early and late season.

In the measurement period, CH_4 data was aggregated per day, with the measured values weighted depending on the duration in time since 3-NOP was administered. In short, the day was divided into five time periods and to include data from a specific day in the analysis, a cow was required to have at least one measurement after 3-NOP administration after milking and one measurement on pasture that day. The method and justification of the method are described further in an abstract for a poster at this conference (Økland et al., 2026).

In addition to daily CH_4 emissions, energy corrected milk yield (ECM) and methane intensity (gram CH_4 / kg ECM) were estimated per cow and day. Covariates for CH_4, ECM and methane

intensity were calculated by averaging the measurements of CH_4, ECM and methane intensity from the covariate period, respectively.

The aggregated CH_4 data, ECM and methane intensity was analysed with linear mixed-effects model using the lme function in the nlme package in R (version 4.5.2). The model used Season (Early and Late), Group (Control and Treatment), measurement day and their two- and three-way interactions as fixed effects. Cow in Season was used as random effect. The model also used Parity, DIM at the start of experiment, concentrate intake and the respective covariate centred within Season as covariates. Additionally, first-order autoregressive correlation (AR(1)) was used for repeated observations for cow, and observation-level weights were specified using varFixed, giving greater weight to observations with measurements which covered a larger number of time bins. Significance was defined as $P < 0.05$.

Results and Discussion

Based on Figure 1, enteric CH_4 appered to be greater in late season compared to early season. In both early and late summer season, enteric CH_4 appeared to be reduced in the treatment (3-NOP) group right after 3-NOP administration. At the same time, the control group appeared to have increased CH_4 right after both milkings, although to different extent. This could be due to the cows receiving concentrate which might have increased the microbial activity and CH_4 production in the rumen.

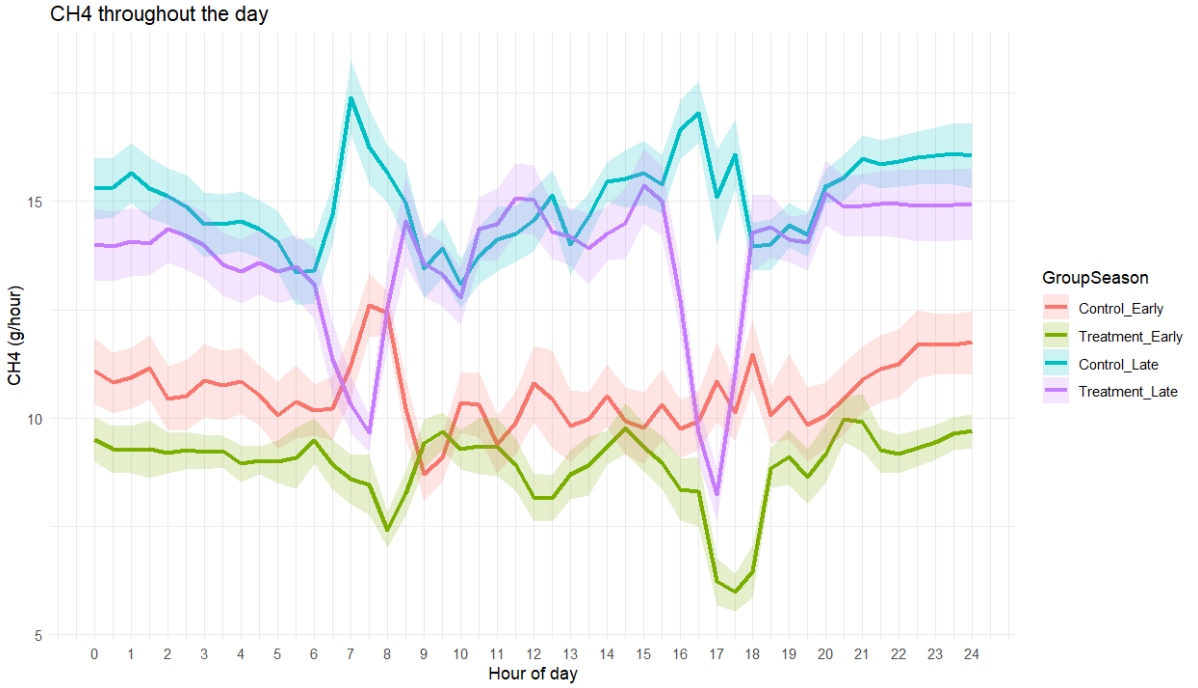


Figure 2 Enteric methane (g/h) throughout 24 hours in early and late summer season, with 3-NOP administered at 0600 and 1600

Compared to early season, CH_4 emissions per day was greater ($P < 0.05$) in late season, by over 120 grams per day (Table 1). This corresponds to more than 50% increase in daily CH_4 from early to late season. With the method applied, compared to the control group, the treatment group produced 14% less CH_4 in early season and 10% less CH_4 in late season. The numerical difference in relative reduction between early and late season is probably due to lower daily

CH_4 in early control (EC) compared to late control (LC), as the absolute reduction differed by only a few grams between early and late season.

Energy corrected milk yield was greater ($P < 0.05$) in early season and numerically greater in late season for cows given 3-NOP (Table 1). In early season, ECM increased by almost 3 kg per day in cows fed 3-NOP, corresponding to 10% increase from the control group. This might be explained by increased energy utilization, but as feed intake has not been estimated yet, conclusions cannot be made regarding this.

Table 1 Absolute and relative contrasts between Control and Treatment group in Early and Late season and between Control groups of Early and Late season

	Contrast[1]	Absolute difference (g/d)[2]	Relative difference %[2]	SE	P-value[3]
CH_4 g/d	LC - EC	124.4	51.8	21.5	***
	ET - EC	-33.6	-14.0	11.1	*
	LT - LC	-36.8	-10.1	11.3	***
ECM kg/d	LC - EC	-6.22	-21.1	2.11	*
	ET - EC	2.97	10.1	1.06	*
	LT - LC	0.91	3.9	1.06	NS
CH_4 g/kg ECM	LC - EC	8.12	96.3	0.98	***
	ET - EC	-1.55	-18.4	0.50	*
	LT - LC	-2.12	-12.8	0.51	***

[1]EC, Early Control; ET, Early Treatment (3-NOP); LC, Late Control; LT, Late Treatment (3-NOP)
[2]Negative number means a reduction while positive number means an increase
[3]P-values for absolute reduction
***$P < 0.001$, *$0.01 < P < 0.05$, NS Not significant

For the control groups, CH_4 intensity (CH_4 g/kg ECM) increased ($P < 0.05$) by over 95% from early to late season (Table 1), which is explained by greater CH_4 and lower ECM production in late compared to early season. In cows fed 3-NOP, CH_4 intensity decreased ($P < 0.05$) by 18% compared to the control group in early season and by about 13% in late season (Table 1). The higher relative reduction in early season compared to late season might be due to lower NDF content in the grass in early season, which resulted in lower daily CH_4 and higher ECM in early season compared to late season. However, this cannot be confirmed before NDF analyses are finished.

Conclusions

Enteric CH_4 emissions were lower in the early than in the late grazing season, at unknown feed intake. The absolute reduction in CH_4 by 3-NOP did not differ between the early and late season, whereas the relative reduction was greater in the early season.

References

Kar-Klootwijk, C. C. W., Koning, L., Holshof, G., Klop, A., & Bannink, A. 2026. Enteric methane emission of dairy cows in 3 grass-based systems: Unrestricted grazing, zero-grazing, and grass silage. *J. Dairy Sci.* https://doi.org/https://doi.org/10.3168/jds.2025-27697

Økland, E, A. Kidane, A. Schwarm & E. Prestløkken, 2026. Methods to estimate daily enteric methane from cows given pulse doses of short-lived methane inhibitors using the GreenFeed system. In: Proc.13th Nord. Feed Sci. Conf., Uppsala, Sweden, p. 89-91.

CO_2 prediction in lactating dairy cows using an international dataset, intended for use with sniffer in on-farm settings

M.H. Kjeldsen[1], M. Johansen[1], M.R. Weisbjerg[1], A.L.F. Hellwing[1], A. Bannink[2], S. Colombini[3], L. Crompton[4], J. Dijkstra[5], M. Eugène[6], A. Guinguina[7,8], A.N. Hristov[9], P. Huhtanen[8], A. Jonker[10], M. Kreuzer[11], B. Kuhla[12], C. Martin[6], P.J. Moate[13], P. Niu[14], N. Peiren[15], C. K. Reynolds[4], S.R.O. Williams[13], and P. Lund[1]

[1]Aarhus University, Denmark. [2]Wageningen Livestock Research, Wageningen University and Research, the Netherlands. [3]University of Milan, Italy. [4] University of Reading, United Kingdom. [5]Animal Nutrition Group, Wageningen University and Research, the Netherlands. [6] INRAE, France. [7]Swedish University of Agricultural Sciences, Sweden. [8]Luke, Finland. [9]The Pennsylvania State University, USA. [10] Bioeconomy Science Institute, New Zealand. [11]ETH Zurich, Switzerland. [12]FBN, Germany. [13]Agriculture Victoria Research, Australia. [14]Norwegian University of Life Sciences, Norway. [15]Flanders Research Institute for Agriculture, Fisheries and Food, Belgium.

Correspondence: Maria.kjeldsen@anivet.au.dk

Introduction

Three CO_2 prediction models have been published by Kjeldsen et al. (2024), intended for converting concentrations of CO_2 and CH_4, obtained by sniffer equipment, to CH_4 production. Large-scale measurement of CH_4 emission from dairy cows is important for generating reliable and accurate CH_4 breeding values. The conversion of sniffer generated gas concentrations to gas production based on the ratio of $[CH_4]$:$[CO_2]$ and predicted CO_2 production, is expected to be more reliable than using CH_4 gas concentrations alone. Given the use of variables not routinely available on farms in Kjeldsen et al. (2024) models, the aim is to develop CO_2 prediction models based on routinely recorded farm data.

Materials and Methods

The dataset used (n = 2,244 individual cow observations) is the same as that used in Kjeldsen et al. (2024). Data pre-processing and model evaluation (performed with "opmetrics" function from the R package mod-MetricsR by Giagnoni (2023)) followed the same steps as described in Kjeldsen et al. (2024), and we refer to that study for summary statistics and details about the test datasets (respiration chamber data (RC), GreenFeed data (GF), and GreenFeed data when using 3-NOP, nitrate, and their combination (GF+)). Foreseen limitations in data availability on commercial farms necessitated restricting the candidate predictor variables in the current model re-evaluation to energy-corrected milk (ECM), days in milk (DIM), parity, breed, and the concentrations of milk crude fat (milk CF), milk crude protein (milk CP), and milk lactose. Breed and parity were categorical variables, the rest were numerical variables. However, milk CF and milk CP were highly correlated (r = 0.54), so milk CP was excluded.

Results and Discussion

Two models were developed in the current study: model 4 and model 5. Model 1, 2, and 3 can be found in Kjeldsen et al. (2024). Model 4 and model 5 were generated because milk CF was a significant main effect, but the difference in conditional R^2 values was negligible (0.7361 and 0.7363 for model 4 and model 5, respectively). The models are as follows:

Model 4: CO_2 (g/d) = b_0 + (b_1 × ECM) + (b_2 × DIM) + (b_3 × Milk CF) + breed + parity + ($b_{ECM, DIM}$ × ECM × DIM) + ($b_{ECM, breed}$ × ECM) + ($b_{DIM, breed}$ × DIM) + ($b_{parity, breed}$) + ($b_{milkCF, parity}$ × Milk CF)

Model 5: CO_2 (g/d) = b_0 + (b_1 × ECM) + (b_2 × DIM) + breed + parity + ($b_{ECM, DIM}$ × ECM × DIM) + ($b_{ECM, breed}$ × ECM) + ($b_{DIM, breed}$ × DIM) + ($b_{breed, parity}$)

Table 1 Coefficients and model parameter estimates of the 2 models to predict CO_2 (g/d) from lactating dairy cows.

Item	Model 4	Model 5
Intercept (b_0)	12,783	12,629
ECM (b_1, kg/d)	-39.9	-41.4
DIM (b_2, d)	-14.9	-15.3
Milk CF (b_3, g/kg)	-3.08	
Breed		
Ayrshire	0	0
Holstein	-2,910	-2,653
Jersey	-5,825	-5,410
Others/crossbreeds	-3,944	-3,911
Parity		
First	0	0
Second	497	339
Third and higher	1,597	686
ECM × DIM	0.471	0.485
ECM × Ayrshire	0	0
ECM × Holstein	83.7	79.6
ECM × Jersey	99.1	89.0
ECM × Others/crossbreeds	79.3	73.4
DIM × Ayrshire	0	0
DIM × Holstein	6.07	5.61
DIM × Jersey	7.35	6.51
DIM × Other/crossbreeds	10.0	10.4
First parity × Ayrshire	0	0
Second parity × Ayrshire	0	0
Third parity × Ayrshire	0	0
First parity × Holstein	0	0
Second parity × Holstein	659	671
Third parity × Holstein	566	676
First parity × Jersey	0	0
Second parity × Jersey	353	262
Third parity × Jersey	946	729
First parity × Other/crossbreeds	0	0
Second parity × Other/crossbreeds	437	494
Third parity × Other/crossbreeds	162	231
Milk CF × first parity	0	
Milk CF × second parity	-3.60	
Milk CF × third parity	-19.9	

Model coefficients are presented in Table 1. Examples of the use of model 1, 2, and 3 can be found in Kjeldsen et al. (2024). Shortly, when tested on the RC test dataset, model 4 had a higher dispersion expressed as % of MSE (18.8% and 18.3% for model 4 and 5, respectively). In contrast, when tested on the two GF datasets (GF or GF+), model 5 had the highest dispersion as % of MSE. A detailed discussion of the model evaluation is not included here, instead we refer to inspection of Table 2 and 3 (where the models were tested on the training dataset itself, see explanation in Kjeldsen et al. (2024)). As with the previous three models in Kjeldsen et al. (2024), the model performance was better for both models when tested on the two GF test datasets (Table 2), where mean bias (calculated as observed – predicted, MB) was lower for both models (MB across models and with/without additives was -153 g/d), compared to when tested on the RC data (MB across models was 1,789 g/d). Therefore, the models were also tested on a modified dataset, where observed CO_2 production was corrected by MB from model evaluation on the test datasets (see details in Kjeldsen et al. (2024)). When evaluated on the modified dataset, nearly all error was attributable to dispersion error, with negligible mean- or slope bias (SB). Evaluation of the models using the training dataset (Table 3) showed that

excluding milk CF has only a limited impact on model predictive performance. Likewise, excluding dietary crude fat (going from model 3 in Kjeldsen et al. (2024) to model 4 in the current study) has limited impact on predictive performance. Both model 4 and 5 have a low SB (see the residuals in Figure 1), indicating good predictive performance regardless of the CO_2 production level. However, model 4 and model 5 have a higher MB and a lower CCC value compared to model 3 in Kjeldsen et al. (2024) (within test dataset), which together indicate reduced agreement and increased bias compared with model 3 in Kjeldsen et al. (2024).

Conclusions

The two models were developed to replace model 3 (the "reduced on-farm" model) in Kjeldsen et al. (2024), when access to predictor variables is limited. Both models have high predictive performances, with low slope bias similar to the "reduced on-farm" model. However, both models exhibited higher mean bias and lower CCC compared with the "reduced on-farm" model.

References

Giagnoni, G. 2023. modRMSE. https://github.com/giuliogiagnoni/modRMSE/#modrmse.

Kjeldsen, M. H., M. Johansen, M. R. Weisbjerg, A. L. F. Hellwing, A. Bannink, S. Colombini, L. Crompton, J. Dijkstra, M. Eugène, A. Guinguina, A. N. Hristov, P. Huhtanen, A. Jonker, M. Kreuzer, B. Kuhla, C. Martin, P. J. Moate, P. Niu, N. Peiren, C. Reynolds, S. R. O. Williams, P. Lund. 2024. Predicting CO_2 production of lactating dairy cows from animal, dietary, and production traits using an international dataset. J. Dairy Sci. 107:6771-6784. https://doi.org/10.3168/jds.2023-24414.

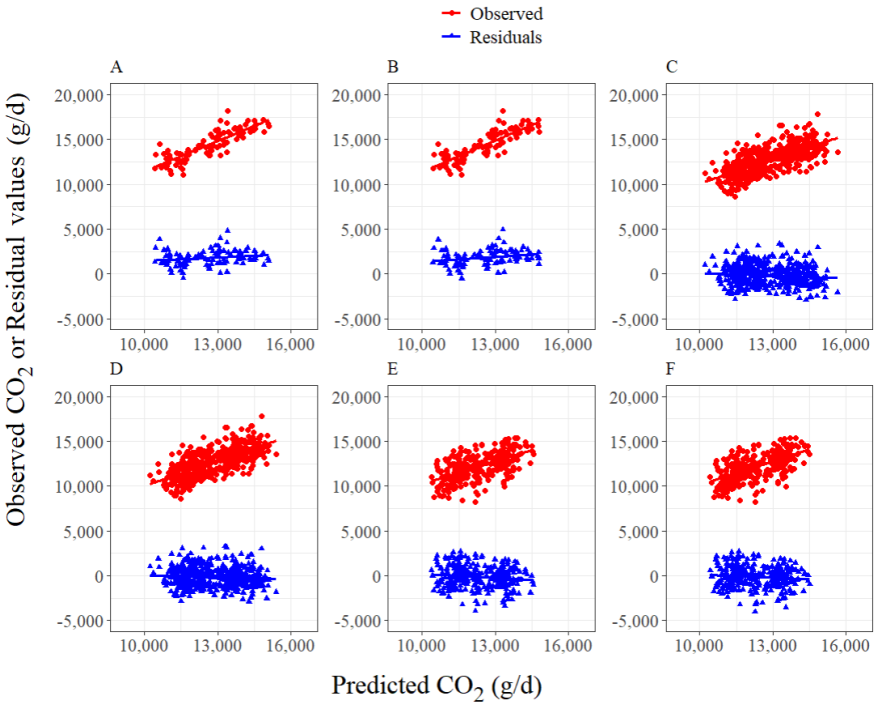


Figure 1 Observed (red) CO_2 production (g/d) in the test dataset, and residual values (blue) of CO_2 production of the 2 models plotted against predicted values of CO_2 production for model 4 and model 5 in : (A) and (B), respectively, at respiration chamber data (n = 103); (C) and (D), respectively, at GreenFeed data (n = 478); and (E) and (F), respectively, at GF data, only including diets containing 3-NOP, nitrate or both 3-NOP and nitrate (n = 295).

Table 2 Model evaluation of the 2 models to predict CO_2 production (g/d) from lactating dairy cows[1].

Item	Test dataset	RMSE	RMSE, % mean	MAE	CCC	RSR	MB	SB	MB, % MSE	SB, % MSE	Dispersion, % MSE
Model performance[2]											
Model 4	RC	1,976	13.8	1,784	0.48	1.1	1,774	0.126	80.6	0.646	18.8
	GF	1,129	8.94	914	0.66	0.74	-185	-0.097	2.69	1.01	96.3
	GF+	1,208	9.93	957	0.54	0.82	-133	-0.148	1.22	1.50	97.2
Model 5	RC	2,009	14.0	1,814	0.49	1.2	1,804	0.173	80.6	1.07	18.3
	GF	1,114	8.82	898	0.66	0.73	-153	-0.066	1.88	0.453	97.7
	GF+	1,201	9.88	949	0.54	0.82	-140	-0.119	1.35	0.937	97.7
Model performance[3]											
Model 4	Modified	1,119	8.87	890	0.66	0.73	-0.211	-0.075	0.000	0.590	99.4
Model 5	Modified	1,111	8.83	882	0.66	0.72	0.164	-0.044	0.000	0.198	99.8

[1]Model performance was evaluated based on either of the following: i) Observations from 3 test datasets (both from Aarhus University, Denmark) obtained from respiration chambers (RC, n = 103) or GreenFeed units (GF, n = 478) without additives, GF only including diets containing nitrate, 3-NOP, or both (GF+, n = 295) or ii) a modified dataset, with RC, GF, and GF+ test datasets merged together (n = 876). The CO_2 production (g/d) in the modified dataset was calculated by subtracting mean bias (if measurements were obtained by RC) or adding mean bias (if measurements were obtained by GF and GF+) from evaluation of the specific model on RC, GF, and GF+ test datasets. RMSE = root mean square error, MAE = mean absolute error, CCC = concordance correlation coefficient, RSR = ratio of RMSE to standard deviation of measured data, MB = mean bias, SB = slope bias, and MSE = mean square error. [2]Test dataset based on observed CO_2 production. [3]Test dataset corrected for mean bias of CO_2 production.

Table 3 Model evaluation of the 2 models to predict CO_2 production (g/d) from lactating dairy cows based on the training dataset (n = 2,244) (explanation of abbreviations in Table 2).

Item	Test dataset	RMSE	RMSE, % mean	MAE	CCC	RSR	MB	SB	MB, % MSE	SB, % MSE	Dispersion, % MSE
Model 4	Training dataset	1,546	12.5	1,230	0.59	0.76	159	0.0144	1.06	0.0146	98.9
Model 5	Training dataset	1,562	12.6	1,243	0.58	0.77	198	0.0008	1.60	0.00005	98.4

Cow efficiency and diet effects on milk production and methane emissions

M. Vadroňová[1], P. Fant[1], K. Kozicka[1], P. Huhtanen[2] & M. Ramin[1]
[1]*Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Umeå, Sweden*
[2]*Production Systems, Natural Resources Institute Finland (LUKE), 31600 Jokioinen, Finland*
Correspondence: mariana.vadronova@slu.se

Introduction

Feed efficiency (FE) describes how effectively animals convert feed into outputs such as milk or meat, while accounting for maintenance and losses (Rauw et al., 2025). As feed is the largest cost in dairy production, improving its conversion into milk is important both economically and environmentally. Consequently, enhancing FE is a key goal for sustainable dairy systems, influencing feed costs, greenhouse gas emissions, and resource use efficiency (Huhtanen et al., 2025). Residual feed intake (RFI) is a commonly used measure of feed efficiency and is widely applied in research and breeding programs (Connor, 2015). However, residual heat production (RHP), which focuses on energy losses rather than intake, has been proposed as an alternative indicator (Huhtanen et al., 2021; Huhtanen et al., 2025). Variation in heat production explains a considerable part of the differences in FE between animals, reflecting differences in maintenance and metabolism (Guinguina et al., 2020). Dietary factors such as the forage-to-concentrate (F:C) ratio influence dry matter intake and milk production (Olijhoek et al., 2018). However, it is still uncertain whether efficiency phenotypes remain consistent across different diets, which is important for applying FE metrics in varied production systems.

This study aimed to assess FE in dairy cows using RHP as an indicator, and to examine how efficiency phenotype and dietary F:C ratio affects feed intake, milk yield, and methane (CH_4) emissions based on gas exchange measurements from the GreenFeed system (GF, C-lock Inc., Rapid City, SD). It was hypothesized that FE phenotype varies with diet and is not stable across dietary conditions.

Material and Methods

A total of 44 lactating Nordic Red dairy cows were screened for two weeks using two GF units to identify the most and least efficient animals based on RHP calculations by Huhtanen et al. (2025). From these, 32 cows (604 ± 71.4 kg BW, 86 ± 33.7 DIM, and 33.6 ± 6.6 kg/d milk yield) were selected and classified as efficient (n = 16) or inefficient (n = 16) according to their RHP, where lower RHP meant higher efficiency. The cows were then enrolled in a switch-back design with two dietary treatments and three experimental periods. Cows were grouped by efficiency and then paired within each group based on parity, RHP, milk yield, and days in milk. Within each pair, one cow was randomly assigned to either a low-forage diet (55:45 F:C; F55) or a high-forage diet (70:30 F:C; F70). The forage consisted of grass silage from first-cut perennial leys dominated by timothy (*Phleum pratense*) with approximately 5% red clover (*Trifolium pratense*). The concentrate used was Fiber 165B Komplett (Malmö, Sweden). Diet composition and chemical properties are presented in Table 1. Individual feed intake was continuously monitored using Roughage Intake Control feeders (Hokofarm Group B.V., Marknesse, the Netherlands), while milk yield was recorded at each milking with gravimetric meters (SAC, S.A. Christensen and Co. Ltd., Kolding, Denmark). For analysis, only data from the last 10 days of each experimental period were included.

Data were analyzed using SAS (v9.4; MIXED). The model included fixed effects of efficiency group, diet, diet × efficiency, period, and sequence (for most parameters it was not significant), with block and cow as nested random effects. Mean values are presented as least square mean obtained by the LSMEANS statement in SAS. The differences were presented significant if $P \leq 0.05$.

Results and Discussion

Dry matter intake (DMI), milk yield and CH_4 production is presented in Table 2. There was no significant interaction ($P > 0.01$) between diet and efficiency phenotype for all variables, only a tendency ($P = 0.09$) for energy corrected milk (ECM). Cows fed the F55 diet had higher ($P < 0.01$) DMI than cows fed the F70 diet, irrespective of their efficiency. Similarly, Olijhoek et al. (2018) reported a higher DMI when cows were fed high-concentrate diet and noted no difference for efficiency phenotype based on their RFI. In our study, both milk yield and ECM were affected by efficiency phenotype as by the diet (both $P < 0.05$), where efficient cows and the F55 diet resulted in higher yields. This is, however, partly in contrast with Olijhoek et al. (2018), where ECM yield did not differ between efficient and inefficient cows, but authors reported higher milk and ECM yield for the high-concentrate diet. In our study, both CH_4 yield (g/kg DMI) and intensity (g/kg ECM) were affected by efficiency and diet ($P < 0.05$). Inefficient cows showed higher CH_4 intensity than the efficient ones, and the F70 diet increased CH_4 intensity compared with the F55 diet. Although Olijhoek et al. (2018) reported higher CH_4 production for high-forage diets, they did not report any differences in CH_4 intensity between the RFI groups.

Increased DMI, milk yield (Ormston et al., 2025), as well as the decreased CH_4 intensity are typical for high-concentrate diets (Olijhoek et al., 2022). Notably, in this study, cows classified as efficient based on RHP remained consistently efficient across contrasting diets, with no evidence of re-ranking. Even though RFI is commonly used to assess feed efficiency, rankings can vary across diets (Higgins et al., 2024). Since RHP reflects residual metabolic energy losses linked to FE (Huhtanen et al., 2025), our results suggest that it may capture a more stable efficiency phenotype than RFI, potentially offering advantages for identifying efficiency across different dietary conditions.

Table 1 Dietary composition and chemical properties of diets.

Item	**Diet[1]**	
	F55	**F70**
Dietary composition, % of DM		
Grass silage	54.7	69.5
Komplett Fiber 165, 2024	44.7	29.8
Mineral mix (Mixa intensitive MG, 2023)	0.59	0.28
Chemical composition, % of DM		
Ash	7.63	7.91
CP	16.4	16.5
Starch	13.3	9.04
Ether extract	4.06	3.82

[1]The experimental diets were 55:45 forage-to-concentrate ratio (F55) and 70:30 forage-to-concentrate ratio (F70).

Table 2 Total dry matter intake (DMI), milk yield, and methane production from efficient and inefficient cows fed high- and low-forage diets.

Item	Efficient		Inefficient		SEM	*P* value		
	F55	F70	F55	F70		Eff	Diet	Eff × Diet
Total DM intake, kg/d	23.2	21.0	22.0	19.9	0.78	0.316	< 0.01	0.941
Yield, kg/d								
Milk	33.3	31.0	25.7	24.4	1.30	< 0.01	< 0.01	0.14
ECM[1]	38.4	35.6	32.3	30.9	1.51	0.02	< 0.01	0.09
Methane, g/d	371	371	418	415	23.9	0.20	0.73	0.68
Methane yield, g/kg DMI	16.0	17.7	19.1	20.9	0.87	0.018	< 0.01	0.65
Methane intensity, g/kg ECM	9.7	10.5	13.2	13.5	0.59	< 0.01	0.013	0.28

The experimental diets were 55:45 forage-to-concentrate ratio (F55) and 70:30 forage-to-concentrate ratio (F70). [1]ECM = energy corrected milk (3.14 MJ/kg)

Conclusion

Feed efficiency was affected by both diet and efficiency phenotype. Cows fed the low-forage diet showed higher DMI and milk yield, and lower CH_4 emissions. Efficient cows consistently outperformed inefficient cows across diets. Contrary to the hypothesis, no re-ranking occurred, indicating that RHP reflects a stable efficiency trait across the dietary conditions of this study. This suggests that RHP may be a robust indicator for improving productivity and reducing CH_4 intensity in dairy systems.

References

Connor, E.E., 2015. Improving feed efficiency in dairy production: challenges and possibilities. Animal, 9, 395–408.

Guinguina, A., Yan, T., Lund, P. & Huhtanen, P., 2020. Between-cow variation in maintenance energy requirement and efficiency of metabolizable energy utilization in dairy cows. J. Dairy Sci., 103, 156–167.

Higgins, M.G., Kenny, D.A., Fitzsimons, C., et al., 2019. The effect of breed and diet type on the hepatic transcriptome in beef cattle divergent for feed efficiency. BMC Genomics, 20, 525.

Huhtanen, P. & Bayat, A.R., 2025. Potential of novel feed efficiency traits for dairy cows based on respiration gas exchanges measured by respiration chambers or GreenFeed. J. Dairy Sci. 12340–1235.

Huhtanen, P., Bayat, A.R. & Lund, P., 2021. Residual carbon dioxide production as an index of feed efficiency in dairy cows. J. Dairy Sci., 104, 5332–5344.

Olijhoek, D.W., Løvendahl, P... & Lund, P.2018. Methane production, rumen fermentation, and diet digestibility of Holstein and Jersey dairy cows divergent in residual feed intake and fed two forage-to-concentrate ratios. J. Dairy Sci., 101, 9926–9940.

Olijhoek, D.W., Hellwing, A.L.F., & Børsting, C.F., 2022. Feeding up to 91% concentrate to Holstein and Jersey dairy cows: effects on methane emission, rumen fermentation, digestibility, production, and feeding behavior. J. Dairy Sci., 105, 9523–9541.

Ormston, S., Yan, T., Chen, X., Gordon, A.W., Theodoridou, K., Huws, S. & Stergiadis, S., 2025. Impact of dietary forage proportion and crossbreeding on feed efficiency and methane emissions in lactating dairy cows. Anim. Nutr. 20, 419–429.

Rauw, W.M., Baumgard, L.H. & Dekkers, J.C.M., 2025. Feed efficiency and metabolic flexibility in livestock. Animal, 19, 101376.

Evaluating equations predicting enteric methane emissions from lactating dairy cows fed grass silage-based diets

A. R. Bayat[1], T. Kokkonen[2], A. Sairanen[3] & S. Hietala[4]
[1]*Natural Resources Institute Finland (Luke), Animal Production, 31600 Jokioinen, Finland.*
[2]*University of Helsinki, Department of Agricultural Sciences, 00014 Helsinki, Finland.*
[3] *Natural Resources Institute Finland (Luke), Animal Production, 71750 Maaninka, Finland.*
[4]*Natural Resources Institute Finland (Luke), Bioeconomy and Environment, 90570 Oulu, Finland.*
Correspondence: alireza.bayat@luke.fi

Introduction

Environmental impacts of livestock production, especially of cattle-based production, are well acknowledged (Gerber *et al.,* 2013). Regarding climate change impact, one of the largest greenhouse gas (GHG) emission sources is enteric fermentation related to feed digestion of ruminants. While different mitigation measures, e.g., diet optimisation and genetics, are utilised to reduce enteric methane (CH_4) production, it is similarly important to evaluate the accuracy of the CH_4 prediction methods. These prediction methods are commonly utilised in, e.g., Life Cycle Assessment (LCA) or national inventory of GHG, to evaluate the climate impact of livestock products and the sector. The objective of this study was to evaluate the performance of different dairy cow CH_4 prediction methods and to form a method recommendation for Finnish cattle LCA.

Materials and Methods

Sixteen equations for estimating CH_4 production from the literature, selected based on their statistical criteria especially their low prediction error, were evaluated with a dataset (n=482) collected from 18 experiments conducted with Nordic Red lactating dairy cows (n=480) or Holstein (n=22) fed grass silage-based diets during 2011-2025 in Luke, Jokioinen (n=447, 16 experiments), Luke, Maaninka (n=25, one experiment) and University of Helsinki (n=10, one experiment) using respiration chambers (n=303), GreenFeed (n=146) and ruminal SF_6 tracer technique (n=33). The database was characterized with body weight (BW, 660±68.1 kg), dry matter intake (DMI, 23.9±3.5 kg/d), gross energy intake (GEI, 436±60.6 MJ/d), milk yield (34.5±7.3), diet composition (OM=925±6.1, CP=163±11.3, NDF=388±27.9, EE=35.6±3.9, forage ratio= 538±68 and OM digestibility 708±28.1 g/kg DM) and CH_4 production (506±71.1 g/d). Relationships between observed and predicted values using the equations were evaluated using a Mixed regression model (SAS 9.4) including random effect of experiment. Outliers in the database were identified using distribution of conditional residuals analyzed by proc Mixed when experiment, diet and method of CH_4 measurements were considered as random effects in the model. The values (n=6) outside of the range of 2 × SD were removed as outliers. Dietary treatments influencing CH_4 emissions significantly such as lipids or 3-NOP were removed from the database. Intercept, slope, R^2, root mean square prediction error (RMSPE, root of the sum of the squared residual errors divided by the number of observations) and %RMSPE (RMSPE/mean) were used to compare the models. Lin's concordance correlation coefficient (CCC) between observed and predicted CH_4 values was calculated according to Lin (1989). Pearson correlation coefficient and a bias correction factor are included in CCC which provide measures of precision and accuracy, respectively. The following equations were used for the comparison:

1. Hristov *et al.* (2013): CH_4 (MJ/d) = 1.54 + 0.0392 × GEI + 0.00791 × NDF - 0.0653 × EE + 0.00586 × BW
2. IPCC (2006): CH_4 (MJ/d) = GEI × 0.065
3. Ramin and Huhtanen (2013-I): CH_4 (L/d) = 62 + 25.0 × DMI
4. Ramin and Huhtanen (2013-II): CH_4 (L/d) = 20 + 35.8 × DMI - 0.50 × DMI^2
5. Ramin and Huhtanen (2012-I): CH_4 (L/d) = 51.5 × $DMI^{0.792}$
6. Ramin and Huhtanen (2012-II) : CH_4 (L/d) = 976 × [(1 - $e^{(0.0407 \times DMI)}$]
7. Niu *et al.* (2018-I): CH_4 (g/d) = 124 + 13.3 × DMI
8. Niu *et al.* (2018-II): CH_4 (g/d) = 76 + 13.5 × DMI - 0.955 × EE + 0.224 × NDF
9. Ellis *et al.* (2007-I): CH_4 (MJ/d) = 3.14 + 2.11 × NDFI
10. Ellis *et al.* (2007-II): CH_4 (MJ/d) = -1.02 + 0.681 × Forage ratio
11. Moraes *et al.* (2014): CH_4 (MJ/d) = 0.225 + 0.0422 × GEI + 0.0125 × NDF - 0.0329 × EE
12. Yan *et al.* (2009): CH_4 (L/d) = 14.7 + 35.1 × DMI
13. Lypsikki-I: CH_4 (L/d) = 31.9 × $DMI^{0.85}$
14. Lypsikki-II: CH_4 (L/d) = 25.4 + 9.0 × OMD) × $DMI^{0.86}$
15. Lypsikki-III: CH_4 (L/d) = (29.3 + 1.09 × BW) × $DMI^{(0.84 - 0.78 \times EE)}$
16. Lypsikki-IV: CH_4 (L/d) = =31.2 × $DMI^{(0.89 - 0.66 \times EE - 0.04 \times Concetrate\ ratio)}$

When CH_4 was given as L/d or MJ/d, the conversion factors of 0.714 and 0.05565, respectively were used to convert the unit to g/d.

Results and Discussion

Hristov (2013) followed by Niu (2018-II) equations had the lowest RMSPE% (8.46 and 8.50% of mean; Table 1). However, they had noticeable mean biases (57.1 and 92.9 g/d) and low CCC (0.26 and 0.45), respectively. IPCC(2006) equation indicated the lowest mean bias (2.4 g/d) followed by Ramin and Huhtanen (2013-I) and Lypsikki-II, Lypsikki-III and Lypsikki-I (Huhtanen *et al.* unpublished; 12.0, 25.2, 31.7 and 38.6 g/d, respectively). Niu_(2018-I), Hristov (2013) and Moraes (2014) equations had the lowest slope bias for the regression line when fitted against observed data (slope close to 1) but they had high mean biases (69-110 g/d) and low CCC (0.21-0.37). Ramin and Huhtanen (2013-I) had the best CCC followd by IPCC (2006), Lypsikki-II, Lypsikki-III and Lypsikki-I (0.70, 0.69, 0.67, 0.63 and 0.59, respectively). Overall, aforementioned equations outperformed other equations while they were different in terms of different fit criteria.

Conclusions

Even though there were differences between the tested equations in predicting CH_4 emissions of lactating dairy cows, based on the statistics criteria evaluated, there was no obvious single equation outperforming others to be introduced for LCA analysis. The simplest equation (i.e., IPCC 2006) had the lowest mean bias while other equations including Hristov (2013) and Niu (2018-II) had the smallest errors among other equations. Ramin and Huhtanen (2013-I), IPCC (2006) and Lypsikki equations (I, II and III) had the highest CCC values indicating the best precision and accuracy. Other criteria, such as data availability would determine which of the satisfactory equations should be used for estimating enteric CH_4 emissions from lactating dairy cows.

Table 1 Statistical comparison of equations used to predict CH_4 production of dairy cows

	Intercept	Slope	R^2	RMSPE	RMSPE%	Mean bias	CCC
IPCC (2006)	191	0.63	0.49	44.62	8.77	2.4	0.69
Ellis *et al.* (2007-I)	143	0.90	0.46	44.27	8.69	103	0.23
Ellis *et al.* (2007-II)	148	1.13	0.52	44.03	8.67	189	0.09
Yan *et al.* (2009)	190	0.53	0.50	44.55	8.75	-96.5	0.37
Ramin and Huhtanen (2012-I)	105	0.90	0.52	44.32	8.69	45	0.58
Ramin and Huhtanen (2012-I)	-28	1.25	0.52	44.38	8.70	67.7	0.44
Ramin and Huhtanen (2013-I)	143	0.76	0.52	44.33	8.69	12	0.70
Ramin and Huhtanen (2013-II)	-141	1.56	0.50	44.76	8.79	81.2	0.35
Hristov *et al.* (2013)	94	1.00	0.50	43.05	8.46	92.9	0.26
Moraes et al. (2014)	104	1.01	0.49	43.80	8.60	110	0.21
Niu *et al.* (2018-I)	72	0.99	0.50	44.55	8.75	69.2	0.37
Niu *et al.* (2018-II)	38	1.05	0.52	43.38	8.50	57.1	0.45
Lypsikki-I	140	0.78	0.50	44.55	8.75	38.6	0.59
Lypsikki-II	151	0.74	0.52	44.89	8.74	25.2	0.67
Lypsikki-III	152	0.75	0.51	43.50	8.55	31.7	0.63
Lypsikki-IV	128	0.83	0.51	43.99	8.65	51.9	0.51

References

Ellis, J.L., Kebreab, E., Odongo, N.E., McBride, B.W., Okine, E.K., & France, J., (2007). Prediction of methane production from dairy and beef cattle. J. Dairy Sci. 90, 3456–66.

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A. & Tempio, G., (2013). Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. FAO, Rome.

Hristov, A. N., Oh, J., Firkins, J. L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H. P., et al. (2013). Special topics - Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci., 91, 5045–5069.

IPCC (2006). Guidelines for National Greenhouse Gas Inventories. IGES. Intergovernmental Panel on Climate Change, Kanagawa, Japan.

Lin, L. I., 1989. A concordance correlation-coefficient to evaluate reproducibility. Biometrics 45, 255–268.

Moraes, L.E., Strathe, A.B., Fadel, J.G., Casper, D.P., & Kebreab, E., (2014). Prediction of enteric methane emissions from cattle. Glob. Change Biol. 20, 2140–2148.

Niu, M., Kebreab, E., Hristov, A.N., Oh, J., Arndt, C., Bannink, A., Bayat, A.R.,..... & Yu, Z., (2018). Prediction of enteric methane production, yield, and intensity in dairy cattle using an intercontinental database. Glob. Change Biol. 24, 3368–3389.

Ramin, M., & Huhtanen, P., (2012). Evaluation of the Nordic dairy cow model Karoline in predicting methane production. Acta Agric. Scand. Section A - Anim. Sci. 62, 254–258.

Ramin, M., & Huhtanen, P., (2013). Development of equations for predicting methane emissions from ruminants. J. Dairy Sci. 96, 2476–2493.

Yan, T., Porter, M.G., & Mayne, C.S., (2009). Prediction of methane emission from beef cattle using data measured in indirect open-circuit respiration calorimeters. Animal 3, 1455–1462.

Predicting enteric methane from growing cattle

J. Karlsson[1], F. H. Lau-Jensen[2], M. Åkerlind[1] & N. I. Nielsen[2]
[1]Växa, Box 288, 751 05 Uppsala, Sweden. [2]Livestock Innovation, SEGES, 8200 Aarhus N, Denmark. Correspondence: Johanna.Karlsson@vxa.se

Introduction

Some dairies and slaughterhouses have introduced incentive schemes that provide additional payments to milk and beef producers who calculate and achieve low climate impact of the production on farm. In parallel, countries that have signed the Paris Agreement are required to report their national greenhouse gas inventories to the United Nations every second year. These calculations rely on equations for predicting enteric methane from cattle, making it essential that such equations are both based on available data and provide accurate estimates. In recent years, a substantial amount of new research on methane production in growing cattle has become available. Therefore, updated models to predict enteric methane production in growing heifers and bulls based on dietary information have been developed, for implementation within the NorFor model.

Materials and Methods

A literature search with "methane OR enteric OR CH4 OR GreenFeed OR "respiratory chamber*" OR chamber*" in title was carried out in the "Web of Science" database. Articles with growing heifers and bulls (Bos taurus) fed indoors or in feedlots, written in English, were selected. Treatment means were excluded if diet contained methane reducing additive such as 3-NOP, nitrate or tannins.

The dataset used for development and evaluation contained 101 treatment means from 32 studies including heifers (n = 73) and bulls (n=34), with publication year ranging between 2003 and 2025. Descriptive statistics of the data is presented in Table 1.

Table 1 Descriptive statistics of dataset for heifers (73 treatment means) and bulls (34 treatment means)

	Heifers				Bulls			
Variable	mean	std	min	max	mean	std	min	max
Body weight (kg)	488	131.2	220	732	308	105.7	78.6	621
Live weight gain(g/d)	1004	404.2	100	2070	1314	289.8	810	2100
Age (days)	404	155.1	198	718	262	79.2	63	427
Roughage intake (kg DM/d)	6.00	2.705	0.73	12.0	4.23	1.827	0.67	8.03
Concentrate intake (kg DM/d)	2.42	2.826	0.00	10.8	2.61	1.956	0.52	8.93
Crude fat (g/kg DM)	32.7	11.30	16.1	75.1	34.5	8.606	22.6	59.2
NDF (g/kg DM)	388	98.6	164	675	368	82.58	218	506
Starch (g/kg DM)	205	162.1	0.5	564	212	97.03	38.2	408

A linear mixed model was fitted to predict methane production (CH4, g/d) using the mixedlm function from the statsmodels package in Python. The model included a set of independent variables as fixed effects, while study was included as a random effect to account for study-level variation. All models included an intercept term. Forward selection was applied.

Statistical significance for variables was declared at $P \leq 0.05$. Models were evaluated on Bayesian information criterion (BIC), root mean square error (RMSE), and concordance correlation coefficient (CCC).

Results and Discussion

Some of the models developed for predicting the production of enteric CH_4 in growing heifers and bulls are presented in Table 2.

Table 2 Evaluation of models predicting enteric CH4 production in growing heifers and bulls. Numbers in grey indicate non-significant (p-value >0.05) variables in model for selected dataset

Dataset	Total	Heifers	Bulls	Total	Heifers	Bulls	Total	Heifers	Bulls
Model	BIC			RMSE			CCC		
DMI	750	539	254	38.3	36.8	35.8	0.635	0.472	0.753
DMI + CFat	753	540	257	38.0	35.8	35.8	0.644	0.514	0.752
DMI + conc_share	717	514	255	31.7	30.0	34.7	0.767	0.667	0.765
DMI + NDF	708	522	241	30.4	31.7	28.0	0.789	0.652	0.851
DMI + NDFr	701	514	244	29.4	30.0	29.5	0.804	0.680	0.831
DMI - ST	712	515	240	30.9	30.2	27.7	0.783	0.697	0.854
DMI - STc	703	504	245	29.7	28.0	29.9	0.794	0.712	0.830
DMI - CFat - STc	706	505	249	29.5	27.4	29.9	0.800	0.730	0.830
DMI + NDF - STc	694	506	242	27.7	27.6	26.9	0.826	0.733	0.862
DMI + NDFr - STc	698	507	246	28.3	27.8	28.6	0.818	0.724	0.843
DMIr + DMIc	719	511	258	32.1	29.4	36.1	0.764	0.685	0.743
DMIr + DMIc + NDF	705	511	256	29.2	28.7	33.5	0.810	0.715	0.779
DMIr + DMIc + NDF - STc	701	511	248	28.0	27.8	28.0	0.823	0.731	0.850
DMI - CFat - STc + NDF	700	508	244	27.9	27.3	26.6	0.825	0.741	0.866

Dry matter intake (DMI, kg DM/d), roughage intake (DMIr, kg DM/d), concentrate intake (DMIc, kg DM/d), crude fat (CFat, g/kg DM), concentrate share (conc_share, % of DM), neutral detergent fibre (NDF, g/kg DM), NDF from roughage (NDFr, g/kg DM), starch (ST, g/kg DM), starch from concentrate (STc, g/kg DM), starch from roughage (STr, g/kg DM)

Dry matter intake is the major explanator for predicting enteric methane emissions in most ruminants, primarily by increasing the supply of fermentable organic matter in the rumen (Nielsen et al., 2013; Ramin & Huhtanen, 2013). Here DMI alone explained 24% of variance in the heifer dataset and 59% of variance in the bull dataset. Several of the developed models had non-significant variables with the bull dataset, which could be explained by a smaller dataset but also by the fact that the bull dataset had lower variation for many variables compared to the heifer dataset.

The two models with similar and high performance (low BIC, low RMSE and high CCC) and having all variables being significant in all three datasets were:

1. $CH4\ (g/d) = 77.7 + 17.3 \times DMI - 0.649 \times CFat - 0.168 \times STc$

2. CH4 (g/d) = - 28.4 + 18.4 × DMI + 0.159 × NDFr

A third model including more parameters and having relatively high performance for the total dataset were:

3. CH4 (g/d) = 33.5 + DMI × 17.5 – CFat × 0.578 – STc × 0.133 + NDF × 0.088

here presented with coefficients from the total dataset including both heifers and bulls, with dry matter intake (DMI; kg DM/d), crude fat (CFat; g/kg DM), starch from concentrate (STc; g/kg DM), NDF from roughage (NDFr; g/kg DM), starch (ST; g/kg DM), and NDF (g/kg DM).

Dietary fat and the carbohydrates starch and NDF along with concentrate proportion have previously shown to influence enteric methane production (Arndt et al., 2022; Zhang et al., 2024). Fat reduces methane by inhibiting protozoa and methanogens, and providing alternative hydrogen sinks via biohydrogenation of unsaturated fatty acids. Increasing dietary starch or concentrate proportion has generally been associated with reduced enteric methane emissions, particularly when expressed per unit of intake or production, with meta-analyses demonstrating a negative relationship between starch content and methane output. This effect is primarily mediated by a shift in rumen fermentation toward propionate formation at the expense of hydrogen availability for methanogenesis. NDF on the other hand, enhance acetate formation during ruminal fermentation, which generates hydrogen that is subsequently utilized by methanogens to produce methane.

Conclusions

Several models for predicting the enteric CH4 production in growing heifers and bulls were developed and evaluated. The best performing models included DMI and NDF from roughage or crude fat and starch from concentrate, where DMI and NDF increases methane and crude fat and starch from concentrate decreases methane

Supplementary Materials: List over studies included in the analysis

References

Arndt, C., Hristov, A.N., Price, W.J. & Yu, Z., 2022. Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5 °C target by 2030 but not 2050. Proc. Natl. Acad. Sci. U.S.A. 20, e2111294119. https://doi.org/10.1073/pnas.2111294119

Nielsen, N.I., Volden, H., Åkerlind, M., Brask, M., Hellwing, A.L.F., Storlien, T. & Bertilsson, J., 2013. A prediction equation for enteric methane emissions from dairy cows for use in NorFor. Acta Agric. Scand., Section A— Anim. Sci. 63, 126-130. https://doi.org/10.1080/09064702.2013.851275

Ramin, M. & Huhtanen, P., 2013. Development of equations for predicting methane emissions from ruminants. J. Dairy Sci. 96, 2476-2493. https://doi.org/10.3168/jds.2012-6095

Zhang, C., Jiang, X., Wu, S., Zhang, J., Wang, Y., Li, Z. & Yao, J. 2024. Dietary fat and carbohydrate-balancing the lactation performance and methane emissions in the dairy cow industry: A meta-analysis. Anim. Nutr. 17, 347-357. https://doi.org/10.1016/j.aninu.2024.02.004

Supplementary Materials: List over studies included in the analysis of Karlsson et al. on Predicting enteric methane from growing cattle in Proceeding of the 13th Nordic Feed Science Conference, 2026.

Aboagye I.A., Oba, M., Koenig, K.M., Zhao, G.Y. & Beauchemin K.A., 2019. Use of gallic acid and hydrolyzable tannins to reduce methane emission and nitrogen excretion in beef cattle fed a diet containing alfalfa silage. J. Anim. Sci. 97, 2230-2244. https://doi.org/10.1093/jas/skz101

Beauchemin K.A. & McGinn S.M., 2005. Methane emissions from feedlot cattle fed barley or corn diets. J. Anim. Sci. 83, 653-661. https://doi.org/10.2527/2005.833653x

Beauchemin K.A. & McGinn S.M., 2006. Methane emissions from beef cattle: Effects of fumaric acid, essential oil and canola oil. J. Anim. Sci. 84,1489-1496. https://doi.org/10.2527/2006.8461489x

Beauchemin K.A. & McGinn S.M., 2006. Enteric methane emissions from growing beef cattle as affected by diet and level of intake. Can. J. Anim. Sci. 86, 317-441.

Beauchemin K.A., McGinn S.M. & Petit H.V., 2007 Methane abatement strategies for cattle: Lipid supplementation of diets. Can. J. Anim. Sci. 87, 431-440.

Beauchemin, K.A., McGinn, S.M., Martinez, T.F. & McAllister, T.A., 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. J. Anim. Sci. 85, 1990-1996. https://doi.org/10.2527/jas.2006-686

Beauchemin, K.A., Tamayao P., Rosser C., Terry S.A. & Gruninger, R., 2022. Understanding variability and repeatability of enteric methane production in feedlot cattle. Frontiers in Anim. Sci. 3, 1029094. https://doi.org/10.3389/fanim.2022.1029094

Burgers, E.E.A., Bannink, A., Walker, N., Zihlmann, R. & Van Gastelen, S., 2025. Effects of 3-nitrooxypropanol on methane emission, growth and feed intake in growing calves from 5 months of age. J. Anim. Sci. 103, skaf379. https://doi.org/10.1093/jas/skaf379

Cowley F.C., Kinley R.D. Mackenzie S.L., Fortes M.R.S. Palmieri C.Simanungkalit G. Almeida A.K. & Roque B.R., 2024. Bioactive metabolites of Asparagopsis stabilized in canola oil completely suppress methane emissions in beef cattle fed a feedlot diet. J. Anim. Sci. 102, skae109. https://doi.org/10.1093/jas/skae109

Crowley S.B., Purfield D.C., Conroy S.B., Kelly D.N., Evans R.D., Ryan C.V. & Berry D.P., 2024. Associations between a range of enteric methane emission traits and performance traits in indoor-fed growing cattle. J. Anim. Sci. 102, skae346. https://doi.org/10.1093/jas/skae346

De Mulder T., Peiren N., Vandaele L., Ruttink T., De Campenere S., van de Wiele T. & Goossens K., 2018. Impact of breed on the rumen microbial community composition and methane emission of Holstein Friesian and Belgian Blue heifers. Livest. Sci. 207, 38-44. https://doi.org/10.1016/j.livsci.2017.11.009

Doreau M., Arbre M., Popova M., Rochette Y. & Martin C., 2017. Linseed plus nitrate in the diet for fattening bulls: effects on methane emission, animal health and residues in offal. Animal 12, 501-507. https://doi.org/10.1017/S1751731117002014

Hammond K.J., Humphries D.J., Crompton L.A., Kirton P. & Reynolds C.K., 2015. Effects of forage source and linseed supplementation on methane emissions from growing dairy cattle of differing body weights. J. Dairy. Sci. 98, 8066-8077. https://doi.org/10.3168/jds.2015-9669

Hellwing A.L.F., Lund P. Mogensen L. & Vestergaard M., 2018. Growth, feed intake, methane emissions and carbon footprint from Holstein bull calves fed four different rations. Livestock Sci. 214, 51-61. https://doi.org/10.1016/j.livsci.2018.05.003

Hellwing A.L.F., Sorensen M.T., Weisbjerg M.R. Vestergaard M & Kund P., 2013. Can rapeseed lower methane emission from heifers? Acta Agric. scand. Section A Anim. Sci. 62, 259-262. https://doi.org/10.1080/09064702.2013.788203

Keller M. Kreuzer M., Reidy B. Scheurer A., Liesegang A. & Giller K., 2022. Methane emission, nitrogen and energy utilisation of beef cattle when replacing or omitting soybean meal in a forage-based diet. Anim. Feed Sc. Tech. 290, 115362. https://doi.org/10.1016/j.anifeedsci.2022.115362

Keller M., Scheurer A., Reidy B., Liesegang A., Amelchanka S.L., Kreuzer M. & Giller K., 2023. Nitrogen and energy losses and methane emissions from beef cattle fed diets with gradual replacement of maize silage and concentrate with grass silage and corn-cob mix. Animal 17, 100722. https://doi.org/10.1016/j.animal.2023.100722

Kirwan S.F., Tamassia L.F.M., Walker N.D., Karagiannis A., Kindermann M. & Waters S.M., 2023. Effects of dietary supplementation with 3-nitroxypropanol on enteric methane production, rumen fermentation and performance in young growing beef cattle offered a 50:50 forage:concentrate diet. J. Anim. Sci. 102, skad399. https://doi.org/10.1093/jas/skad399

Lee C., Araujo R.C., Koenig K.M. & Beauchemin K.A., 2015. Effects of encapsulated nitrate on enteric methane production and nitrogen and energy utilization in beef heifers. J. Anim. Sci. 93, 2391-2404. https://doi.org/10.2527/jas.2014-8845

Managos M., 2025 Feed intake, growth rate, and greenhouse gas emissions from pregnant dairy heifers fe either grass -clover or a mix of grass-clover silage and whole crop wheat silage Doctoral thesis, Acta Universitatis Agriculturae Sueciae 2025:70. Swedish University och Agricultural Sciences https://doi.org/10.54612/a.1929uke7ci

Oretomiloye F., Coates T., Hayes M. & Terry S.A., 2025. Effect of whole seaweed and seaweed extract on rumen fermentation, nutrient digestibility, and methane emissions of beef heifers fed a high-forage diet. Can. J. Anim. Sci. 105, 1-9. https://doi.org/10.1139/cjas-2024-0129

Parnian-Khajehdizaj F., Noel S.J., Johansen M., Weisbjerg M.R., Hellwing A.L.F. Hojberg O., Hall M.B. & Lund P., 2023. Methane emission, nutrient digestibility, and rumen microbiota in Holstein heifers fed 14 different grass or clover silages as the sole feed. J. Dairy. Sci. 106, 4072-4091. https://doi.org/10.3168/jds.2022-22638

Renand G., Vinet A., Decruyenaere V., Maupetit D. & Dozias, D., 2019. Methane and Carbon Dioxide Emission of Beef Heifers in Relation with Growth and Feed Efficiency. Animals 9, 1136. doi:10.3390/ani9121136

Riesgraf K.A., Akins M.S., Laporta J. & Weigel K.A., 2024a. Long-term growth, feed efficiency, enteric methane emission, and blood metabolite responses to in utero hyperthermia in Holstein heifers. J. Dairy. Sci. 107, 6358-6370. https://doi.org/10.3168/jds.2023-24417

Riesgraf K.A., Weigel K.A., Akins M.S. & Van Os J.M.C., 2024b. Long-Term Effects of Pre-Weaning Individual or Pair Housing of Dairy Heifer Calves on Subsequent Growth and Feed Efficiency. Animals 14, 716. https://doi.org/10.3390/ani14050716

Romero-Perez A., Okine E.K., McGinn S.M., Guan L.L., Oba M., Duval S.M., Kindermann M. & Beauchemin K.A., 2014. The potential of 3-nitrooxypropanol to lower enteric methane emissions from beef cattle J. Anim. Sci. 93, 4682-4693 https://doi.org/10.2527/jas.2014-7573

Romero-Perez K.A., Okine E.K., McGinn S.M., Guan L.L., Oba M., Duval S.M. & Kindermann M., 2015. Sustained reduction in methane production from long-term addition of 3-NOP to a beef cattle diet. J. Anim. Sci. 93,1780-1791. https://doi.org/10.2527/jas.2014-8726

Santos-Silva, J., Alves S.P., Francisco A., Portugal A.P., Dentinho A.T., Almeida J., Regedor da Silva J.D., Fialho L., Cachucho L., Jerónimo E., Barradas A., Rodrigues A., Rodrigues N., Teixeira R.F.M., Domingos T. & Bessa R.J.B., 2023. Forage based diet as an alternative to a high concentrate diet for finishing young bulls - Effects on growth performance, greenhouse gas emissions and meat quality. Meat Science 198, 109098. https://doi.org/10.1016/j.meatsci.2023.109098

Schönhusen U., Zitnan R., Kuhla S., Jentsch W. Derno M. & Voigt J., 2010. Effects of protozoa on methane production in rumen and hindgut of calves around time of weaning. Archives of Anim Nutr 57, 279-295. https://doi.org/10.1080/00039420310001594423

Smith P.E., Waters S.M., Kenny D.A., Kirwan S.F. Conroy S. & Kelly A.K, 2021. Effect of divergence in residual methane emission on feed intake and efficiency. J. Anim. Sci. 99, skab275. https://doi.org/10.1093/jas/skab275

Staerfl S.M., Zeitz J.O. Kreuzer M. & Soliva C.R., 2012.Methane conversion rate of bulls fattened on grass or maize silage as compared with the IPCC default values, and the long-term methane mitigation efficiency of adding acacia tannin, garlic, maca and lupine. Agriculture, Ecosystem & Environment 148, 111-120. https://doi.org/10.1016/j.agee.2011.11.003

Vyas D., Alazzeh A., McGinn S.M., McAllister T.A., Harsyad O.M., Holo H. & Beauchemin, K.A., 2016. Enteric methane emissions in response to ruminal inoculation of Propionibacterium strains in beef cattle fed a mixed diet. Anim. Prod. Sci. 56, 1035-1040. https://doi.org/10.1071/AN14801

Williams M.S., O'Hara E., Coates T., McAllister T.A., Gruninger R.J. & Terry S.A., 2025. The effect of a sugarcane polyphenol extract on intake, apparent total tract digestibility, rumen fermentation, pH, methane emissions, and the rumen microbiome of beef heifers fed a high forage diet. Can. J. Anim. Sci. 105, 1-9 https://doi.org/10.1139/cjas-2024-0124

Zhang X.M., Smith M.L., Gruninger R.J., Kung L., Vyas D., McGinn S.M., Kindermann, M., Wang M., Tan Z.L. & Beauchemin K.A., 2021. Combined effects of 3-nitrooxypropanol and canola oil supplementation on methane emissions, rumen fermentation and biohydrogenation, and total tract digestibility in beef cattle. J. Anim. Sci. 99, skab081. https://doi.org/10.1093/jas/skab081

Comparison of enteric methane emission from rumen cannulated and intact dairy cows

D. W. Olijhoek, N. P. Hansen, M. Maigaard, M. R. Weisbjerg, P. Lund & M. Larsen
Department of Animal and Veterinary Sciences, AU Viborg, Research Centre Foulum, Aarhus University, DK 8830 Tjele, Denmark.
Correspondence: mogens.larsen@anivet.au.dk

Introduction

Enteric methane (CH_4) emission from cattle can be measured with techniques that measure from breath only, such as GreenFeed (GF) and sulphur hexafluoride tracer gas. When using rumen cannulated cows, there is a risk of underestimating emissions due to escape of gases through the cannula. Breath gas measurements of rumen cannulated cows are therefore not recommended (Hristov *et al*., 2025). This study aimed to compare CH_4 and carbon dioxide (CO_2) emission from rumen cannulated and intact Danish Holstein dairy cows measured using GF and when housed and fed under the same conditions in an indoor or grazing system.

Materials and Methods

The experiment was of continuous design and lasted 11 weeks with 2 weeks of adaptation and 9 weeks of experiment (May 6th to July 7th, 2024). Treatments were: 1) indoor housing with feeding of a spring growth (first cut) grass-clover silage and separate feeding of concentrates, and 2) grazing during day and night with additional feeding of concentrates. Twelve rumen cannulated (6 cows per treatment) and 11 intact lactating dairy cows (6 for the indoor and 5 for the grazing treatment) were used. All cows were of second parity and averaged (± standard deviation) 89 ± 18.1 and 116 ± 46.3 days in milk (DIM) at the start of the experiment, for cannulated and intact cows, respectively. These cows were part of a larger study and the effects of grazing based on all cows will be published elsewhere.

Cows were milked twice daily (05:00 and 16:00 h) in a milking parlour. Rumen cannulas were purchased from Bar Diamond Inc. (USA). Methane emission was measured with the GF system (C-Lock Inc., USA) using 2 units indoors and 2 units placed in huts on pasture. A concentrate pellet was provided to all cows as bait in the GF with an allowance of up to 2.4 kg dry matter/d (DM). The same pellet was provided twice daily in the milking parlour at a fixed amount (in total 2.7 kg DM/d). Indoor cows were loose-housed and had free access to grass-clover silage in automated bins. Grazing cows grazed a perennial ryegrass and white clover pasture in a rotational system over paddocks and with strip grazing within paddock. A fresh strip of pasture was provided after each milking. Pre- and post-grazing herbage mass per paddock was measured and strip size determined before grazing commenced using a platemeter (Grasshopper, True North Technologies, Ireland). The pre-grazing herbage allowance was on average 1,259 kg DM/ha, and the pre- and post-grazing sward heights were on average 99 and 68 mm, respectively. Total dry matter intake (DMI) was the sum of DMI of concentrate and grass-clover silage or pasture. The DMI of pasture was calculated based on NorFor energy requirements (Nielsen and Volden, 2011). Energy-corrected milk yield (3.14 MJ/kg) was calculated according to Sjaunja *et al.* (1991). Variables were averaged per week before statistical analysis. Only gas measurements of cows with more than 10 GF visits per week were included. Data of the last 5 d of the adaptation period were averaged per cow, standardized within cow type with a mean of 0 and standard deviation of 1, and considered as covariate in the analysis. Data were analyzed in SAS (version 9.4, SAS Institute Inc., USA). The mixed model included fixed effects for type of cow (cannulated or intact), treatment (indoor or

grazing), week (1 to 9), DIM at experimental onset, covariate for the respective dependent variable, and all 2- and 3-way interactions between type of cow, treatment, and week. Week was modelled as repeated measures for cow. Least squares means were adjusted with the Tukey-Kramer test. *P*-values are considered significant at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

Results and Discussion

Over the 9-week experiment and when only considering cow-weeks with more than 10 visits per cow, intact cows visited the GF in total 1,617 and 586 times for the indoor and grazing treatment, respectively, while cannulated cows visited 1,582 and 813 times, respectively. Figure 1 shows similar diurnal patterns between type of cow on the same treatment.

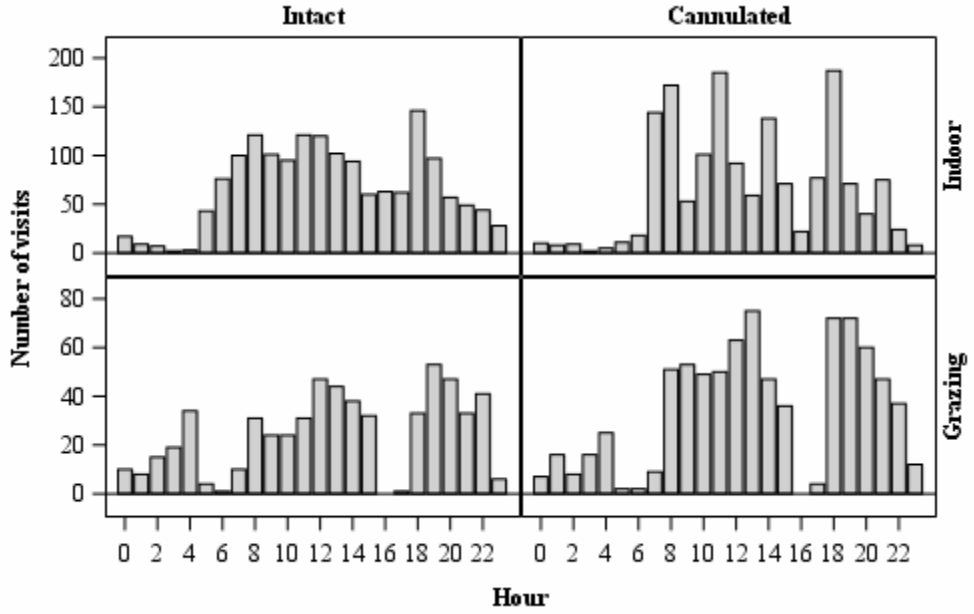


Figure 1 Diurnal summed visits to GreenFeed over week 1 to 9 by type of cow and housing system.

No significant differences were observed between cannulated and intact cows for BW, ECM yield, total DMI, concentrate DMI, and visits to GF (Table 1). These findings indicate that cannulated cows behaved similarly to intact cows when fed with grass-clover silage indoors or when grazing on pasture. However, CH_4 emission (all expressions) tended to be lower for cannulated than intact cows and the CH_4:CO_2 ratio was significantly lower (P=0.01), while daily CO_2 emission was unaffected by type of cow. This suggests unrepresentative escape of gases from the rumen cannula and dilution of CO_2 escaping from the cannula with CO_2 originating from respiration. Therefore, total emission of CH_4 from breath of rumen cannulated cows is not similar to intact cows and values are likely underestimated.

Most variables differed between treatments, except ECM yield and total DMI, which were unaffected by type of cow, treatment, and their interaction. Concentrate intake from GF was lower when cows were grazing than cows remaining indoors (P<0.001), which relates to the number of weekly visits to GF. The number of visits per cow were lower for grazing intact and cannulated cows than for both types of cows when indoors (P=0.02). Emission of CH_4 (all expressions) and CO_2, and the CH_4:CO_2 ratio were lower when cows were grazing compared to feeding of a grass-clover silage indoors (all $P \leq 0.05$).

Table 1 Least squares mean for rumen cannulated and intact dairy cows fed and housed indoors with grass-clover silage or grazing perennial ryegrass and white clover pasture during day and night

	Indoor		Grazing			*P*-values		
Measurement	Intact	Cannulated	Intact	Cannulated	SEM	Type	Trt	Type × trt
Body weight (kg)	653	649	615	607	9.94	0.57	0.001	0.83
ECM yield (kg/d)	32.2	32.7	32.7	33.0	0.68	0.54	0.60	0.92
Total DMI (kg/d)	20.8	21.0	21.4	21.0	0.53	0.83	0.58	0.56
Concentrate DMI from GreenFeed (kg/d)	2.71	2.68	1.56	1.89	0.11	0.20	<0.001	0.11
Average visits to GreenFeed (n/week)	29.5[a]	28.7[a]	15.5[b]	19.4[b]	1.03	0.18	<0.001	0.02
CH_4 (g/d)	476	425	319	295	21.2	0.09	<0.001	0.52
CH_4 (g/kg ECM)	14.6	12.9	9.76	9.60	0.53	0.09	<0.001	0.14
CH_4 (g/kg BW)	0.73	0.66	0.52	0.48	0.03	0.07	<0.001	0.51
CO_2 (g/d)	14,199	14,173	13,055	12,800	438	0.74	0.01	0.78
CH_4:CO_2 ratio	0.034	0.029	0.025	0.023	0.001	0.01	<0.001	0.28

Trt = treatment (indoor, grazing); type = type of cow (intact, cannulated).
Different superscripts for type × trt differ significantly at $P \leq 0.05$.
P-values for week were significant at $P < 0.001$ for all variables.
P-values for treatment × week were significant at $P \leq 0.05$ for body weight, total DMI, concentrate intake from GreenFeed (GF), visits to GF, CO_2, CH_4:CO_2; and a tendency for ECM yield.
P-value for week × type was a tendency for ECM yield.
P-values for treatment × week × type were significant at $P \leq 0.05$ for concentrate intake from GF and a tendency for body weight.

Conclusions

In conclusion, CH_4 emission measured with GF from the breath of rumen cannulated cows seem lower (tendency) than that of intact cows when animals were similar in performance and feeding. The ranking of treatments was similar for intact and cannulated cows. Therefore, CH_4 emission of rumen cannulated cows are likely underestimated when measured in breath. Main differences were observed between systems with gas emissions being lower when cows were grazing than when cows were fed indoors with grass-clover silage.

References

Hristov, A.N, Bannink, A., Battelli, M., Belanche, A., Sanz, M.C.C., Fernandez-Turren, G., Garcia, F., Jonker, A., Kenny, D.A., Lind, V., Meale, S.J., Zilio, D.M., Muñoz, C., Pacheco, D., Peiren, N., Ramin, M., Rapetti, L., Schwarm, A., Stergiadis, S., Theodoridou, K., Ungerfeld, E.M., van Gastelen, S., Yáñez-Ruiz, D.R., Waters, S.M., Lund, P., 2025. Feed additives for methane mitigation: Recommendations for testing enteric methane-mitigating feed additives in ruminant studies, J. Dairy Sci. 108, 322–355.

Sjaunja, L.O., Baevre, L., Junkkarinen, L., Pedersen, J., Setala, J., 1991. A Nordic proposal for an energy corrected milk (ECM) formula, in: EAAP Publication 50: Performance Recording of Animals: State of the Art 1990. Performance Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen, the Netherlands, pp. 156–157.

Methods to estimate daily enteric methane from cows given pulse doses of short-lived methane inhibitors using the GreenFeed system

E. Økland[1], A. Kidane[1], A. Schwarm[1], E. Prestløkken[1]
[1]Norwegian University of Life Sciences (NMBU), Department of Animal and Aquacultural Sciences, Oluf Thesens vei 6, 1433 Aas, Norway
Correspondence: erlend.podhorny.okland@nmbu.no

Introduction

It is common to quantify enteric methane (CH_4) emissions and the mitigation effects of different strategies in ruminants. Enteric CH_4 can be measured in several ways, one of which is voluntary measurement using the GreenFeed (GF) System (C-Lock Inc., Rapid City, SD). However, the results may be affected by the experimental setup. When 3-nitrooxypropanol (3-NOP) was fed in pulse doses, Costigan et al. (2024) reported a transient effect of the additive on CH_4 emissions. If this effect is not accounted for, daily CH_4 emissions and the mitigation potential of inhibitors might be over- or underestimated. This work explores the possibility of using various aggregation methods to estimate enteric CH_4 emissions when short-lived CH_4 inhibitors are evaluated using the spot-sampling GF system.

Materials and Methods

Experimental data from two grazing trials with Norwegian Red dairy cows conducted at the Norwegian University of Life Sciences (NMBU) in August 2024 and May 2025 were used for the analyses. In each experiment, 15 cows were allocated to a control group, and 15 cows were given 3-NOP (Bovaer[10], DSM-Firmenich Nutrition Products Ltd). The first 6 days was a covariate period without 3-NOP followed by 15 days of trial period, where the treatment group received 1.2 grams of 3-NOP per cow per day, divided between two milkings (0600 and 1600 h local time). During milking, the cows received 6 kg of concentrate per day in early season and 3 kg per day in late season. Enteric CH_4 was measured using two GF units. One GF unit was placed close to the milking barn and visited by each cow after milking, while another unit was located on pasture and visited voluntarily. Combined between the GF units, the cows could receive a total of 1.5 kg of concentrate per day, if they visited 5 times per day.

The data was visualized to investigate how long 3-NOP appeared to be effective. Based on this, the duration of the effect was set to 2.5 h after each administration, corresponding to a total of 5 h per day. The day was divided into 5 time of day bins (0000-0600 h, 0600-0830 h, 0830-1600 h, 1600-1830 h, 1830-0000 h). Each bin covered either a period without reduced CH_4 emissions (bin 1, 3 and 5) or a period with reduced CH_4 emissions.

Five methods of aggregating CH_4 data were compared. (1) Pure mean (PuM); simple daily average per cow. (2) Barn mean (BaM); daily average from the GF placed in the milking barn. (3) Pasture mean (PaM); daily average from the GF on pasture. (4) Weighted mean (WeM); measurements were weighted based on when during the day they were taken. For a day to be included per cow, the cow needed at least one measurement in bins 1, 3 and 5 combined (unaffected CH_4) and one in bins 2 and 4 combined (reduced CH_4). The average CH_4 for bins 1, 3 and 5 was weighted by 19/24 h, while the average CH_4 for bins 2 and 4 was weighted by 5/24 h. The two weighted averages were then summed. The fifth method was (5) Area Under the Curve (AUC); using interpolation, data per week were used to make graphs over 24 hours per cow, with one datapoint every 0.2 hours. Area was determined for each graph and divided by 24 to give average CH_4 emissions over 24 hours.

Data from each aggregation method was analysed separately with linear mixed-effect models using the lme function in the nlme package in R (version 4.5.2). The models used Group (Control, Treatment), Season (Early, Late), Time variable (Day in Season or week in Season (for AUC)) and their two- and three-way interactions as fixed effects. Cow in Season was used as random effect, and Parity, DIM at experimental start, concentrate intake and average CH_4 emissions from the covariate period were used as covariates. First order autoregressive correlation (AR(1)) was used to model correlation between repeated observations for a cow, for all methods except AUC. For WeM, observation-level weights were specified using varFixed, giving more weight to observations based on a greater number of time bins. Significance was defined as $P < 0.05$.

Results and Discussion

For the treatment group, the CH_4 emissions dropped right after administrating 3-NOP until 1 to 1.5 h after administration, before rising again within 1 to 1.5 h after nadir (Figure 1). There was a higher concentration of measurements during these hours compared with the rest of the day. If all measurements are taken while 3-NOP is effective, the estimated reduction in CH_4 emissions will be higher compared to if all the measurements are taken while 3-NOP has low or no effect. Thus, as the short-lived 3-NOP was given in two pulse doses, the inhibition results will highly depend on measurement pattern relative to administration.

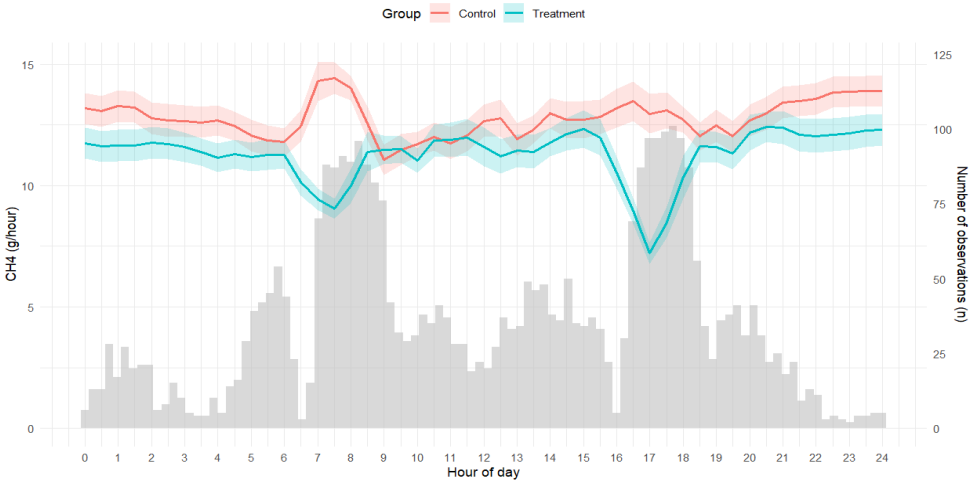


Figure 1 Enteric CH_4 in g/hour and number of observations across 24 hours, with 3-NOP administered at 0600 and 1600

The estimated difference between the control- and treatment group for the different aggregation methods is presented in Table 1. All methods except PaM produced a significant difference between the two treatments. The relative difference varied from less than 6 % in PaM to almost 30 % in BaM, representing the periods with low and high effect of 3-NOP, respectively. The relative difference estimated by PuM, is somewhere between BaM and PuM. However, as there were more observations while 3-NOP had high effect, PuM might overestimate the effect of 3-NOP as the method enlarge the difference between the treatments. In contrast, fewer observations while 3-NOP has high effect would possibly underestimate reduction in CH_4 emissions as the difference between control and treatment would be reduced. This variation shows that the uneven spread in measurements needs to be accounted for.

For WeM, differences in the number of measurements within each bin are less important, provided that the measurements are representative of that bin and that the bin widths correctly reflect whether CH_4 emissions are inhibited or not. In our study, the WeM method should therefore account for the uneven distribution of measurements and provide a more accurate estimate of the treatment effect than PuM, BaM and PaM.

We found that AUC is an alternative to WeM that can account for uneven distribution of measurements, as it does not assign greater weight to periods with more frequent measurements, but instead provides greater accuracy during those periods compared with periods with fewer measurements. However, a prerequisite is that the measurements are sufficient in number (readings) and distribution to give fairly accurate and representative graphs for the cows during the day or measurement period. This could be obtained by either having more measurements per cow per day, or using longer aggregation intervals, like weekly or fortnightly periods. An indication that WeM and AUC might be better methods to account for uneven emissions and frequency of measurements compared to the methods based on averages, is that WeM and AUC estimate fairly similar contrasts between the control- and treatment group, while the estimated contrasts from PuM, BaM and PaM vary more in between each other.

Table 1 Least Square means (SE) for CH_4 (g/day) for each group and aggregation method, and absolute and relative contrast between Control group and Treatment group for each aggregation method

Aggregation Method[1]	Group[2]		Contrast		P-value	
	Control	3-NOP	Absolute difference g/d[3]	Relative difference %[4]	Treatment[5]	Contrast[6]
PuM	308 (5.11)	252 (5.11)	55.9 (7.16)	18.15	<0.001	<0.001
BaM	318 (6.76)	224 (6.82)	94.1 (9.62)	29.59	<0.001	<0.001
PaM	294 (7.08)	277 (6.61)	17.1 (8.99)	5.82	0.018	0.064
WeM	302 (5.52)	267 (5.54)	35.2 (7.72)	11.66	<0.001	<0.001
AUC	305 (5.42)	267 (5.42)	38.3 (7.61)	12.56	<0.001	<0.001

[1]PuM, Pure Mean; BaM, Barn Mean; PaM, Pasture Mean; WeM, Weighted Mean; AUC, Area Under the Curve
[2]LSmean (SE) for Control and Treatment group
[3]Contrast (SE) based on Tukey between Control and Treatment group
[4]Reduction in CH_4 with 3-NOP relative to Control
[5]P-value of the effect of treatment on the model
[6]P-value for the contrast between Control and Treatment group

Conclusions

The aggregation method affects the result when estimating the reduction potential of CH_4 inhibitors with short-lived mitigation effects, such as 3-NOP, especially when using spot-sampling CH_4 measurement techniques. Under these circumstances, weighted means and area under the curve may be more suitable methods than average-based methods, as they better account for uneven emissions and variation in measurement frequency when estimating daily CH_4 emissions in cows.

References

Costigan, H., Shalloo, L., Egan, M., Kennedy, M., Dwan, C., Walsh, S., Hennessy, D., Walker, N., Zihlmann, R., & Lahart, B. 2024. The effect of twice daily 3-nitroxypropanol supplementation on enteric methane emissions in grazing dairy cows. *J. Dairy Sci. 107*(11), 9197–9208. https://doi.org/https://doi.org/10.3168/jds.2024-24772

Evaluation of high-purity tribromoethanol (TBE) and rumen fluid from efficient or inefficient cows on *in vitro* methane production and fermentation pattern

A. Alipour[1,2], M. Vadroňová[1] & M. Ramin[1]
[1]*Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Umeå, Sweden*
[2]*Department of Animal Science, Faculty of Agriculture, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*
Correspondence: mohammad.ramin@slu.se

Introduction

Enteric methane (CH_4) emission from ruminants is a major sustainability challenge in ruminant systems and reflects a loss of dietary energy for the host (Morgavi et al., 2023). Compounds that inhibit methyl-coenzyme M reductase (MCR), the terminal enzyme of methanogenesis, represent a targeted mitigation strategy (Duin et al., 2016). However, the response to antimethanogenic additives may depend on rumen microbial background, as cows differing in efficiency or CH_4 phenotype show differences in fermentation and rumen microbial characteristics (Ahvenjärvi et al., 2024; Stepanchenko et al., 2025). Therefore, this study evaluated the effects of high-purity tribromoethanol (TBE), a CH_4 inhibitor (Fekri et al., 2026) and rumen fluid from efficient or inefficient cows on gas production, CH_4 kinetics, and fermentation parameters in a 2 × 2 *in vitro* factorial design.

Materials and Methods

In a 2 × 2 factorial design, a 70:30 forage to concentrate ratio substrate (dried and milled; DM, 903 g/kg; OM, 925 g/kg DM; CP, 163 g/kg DM; EE, 37.4 g/kg DM; starch, 320 g/kg DM) was incubated (1 g/bottle) in an automated *in vitro* gas production system described by Ramin and Huhtanen (2012). Bottles were filled with buffered rumen fluid from efficient or inefficient cows (from an ongoing experiment from the farm at Röbäcksdalen, Umeå), with or without TBE (provided by Agteria AB, Stockholm, Sweden), resulting in four treatments: E (efficient rumen fluid without additive), EA (efficient rumen fluid with additive), I (inefficient rumen fluid without additive), and IA (inefficient rumen fluid with additive). Each treatment consisted of six replicate bottles in each of two experimental runs.

Total gas production was recorded automatically. Headspace gas samples (200 µL) were collected at 2, 4, 8, 24, and 48 h and analyzed for CH_4 concentration using gas chromatography. Cumulative total gas and CH_4 data were used to estimate *in vivo* gas and CH_4 production assuming a mean rumen retention time of 50 h (Ramin & Huhtanen, 2012). At the end of the incubation period (48 h), the incubation was terminated, pH was measured and liquid samples were collected and volatile fatty acid (VFA) analysis by gas chromatography.

Data was analyzed using the PROC MIXED procedure in SAS. Efficiency status, additive, run, and their interactions were fitted as fixed effects. Bottles were included as the random error term. Significance was declared at $P < 0.05$. Residuals were evaluated for normality and homogeneity of variance.

Results and Discussion

Total gas and CH_4 production parameters are shown in Table 1. The addition of TBE reduced estimated *in vivo* total gas production ($P = 0.006$). Gas production rate (Gas K_d) was affected by rumen fluid source ($P < 0.01$). Estimated *in vivo* CH_4 production was affected by rumen fluid source and additive ($P < 0.01$; $P < 0.01$, respectively). There was also a significant rumen fluid × additive interaction for CH_4 production ($P = 0.017$). Methane production rate (CH_4 K_d) was also affected by rumen fluid source, additive, and their interaction ($P = 0.023$; $P < 0.01$; $P = 0.036$, respectively). As indicated in Table 2, total VFA concentration was not affected by the main effects or their interaction. The molar proportion of acetate was decreased by the additive ($P < 0.01$). The molar proportion of propionate was affected by rumen fluid source, additive, and their interaction ($P = 0.005$; $P < 0.01$; $P = 0.032$, respectively: Table 2). The molar proportion of butyrate was affected by rumen fluid source and additive ($P = 0.048$ and $P < 0.01$, respectively: Table 2). As reported by Shao et al. (2026), MCR-based additives reduce CH_4 by inhibiting the terminal step of methanogenesis, which markedly lowers CH_4 formation. The simultaneous increase in propionate is also consistent with previous work showing that, when methanogenesis is suppressed, reducing equivalents are redirected from CH_4 toward alternative hydrogen sinks such as propionate (Xuan et al., 2024; Fekri et al., 2026). The rumen fluid effect suggests that the efficient rumen fluid was inherently less methanogenic and more propionogenic, which is in line with reports that the rumen microbiome of efficient cows is associated with more lactate utilizing and propionate forming pathways, whereas inefficient cows tend to exhibit a more methanogenic fermentation pattern (Mizrahi & Jami, 2018). The significant interaction of rumen fluid × additive on CH_4 and propionate production suggests that the CH_4 inhibitor efficacy depended on rumen microbial background (Mizrahi & Jami, 2018).

Table 1 Least squares mean of total gas and CH_4 production parameters for each experimental group.

Item	Experimental groups				SEM	P-Value		
	E	EA	I	IA		RF	A	RF×A
Total Gas production, ml/g DM	159	150	167	149	4.5	0.456	0.006	0.286
Gas K_d, h^{-1}	0.150	0.152	0.127	0.134	0.0033	< 0.01	0.179	0.507
Total CH_4 production, ml/g DM	30.0	0.842	34.2	2.35	0.484	< 0.01	< 0.01	0.017
CH_4 K_d, h^{-1}	0.060	0.015	0.061	0.028	0.0026	0.023	< 0.01	0.036

The experimental groups contain: basal diet + rumen fluid collected from efficient cows (E); basal diet + rumen fluid collected from efficient cows (EA) + Additive; basal diet + rumen fluid collected from inefficient cows (I); basal diet + rumen fluid collected from inefficient cows + Additive (IA).

Table 2 Least squares mean of fermentation parameters for each experimental group.

Item	Experimental groups				SEM	P-Value		
	E	EA	I	IA		RF	A	RF×A
Total VFA, mmol/g DM	5.45	4.85	5.60	5.22	0.249	0.363	0.123	0.678
Acetate, mmol/mol total VFA	570	446	587	441	5.05	0.293	< 0.01	0.100
Propionate, mmol/mol total VFA	284	370	252	361	3.51	0.005	< 0.01	0.032
Butyrate, mmol/mol total VFA	95.9	134	103	145	3.35	0.048	< 0.01	0.628

The experimental groups contain: basal diet + rumen fluid collected from efficient cows (E); basal diet + rumen fluid collected from efficient cows (EA) + Additive; basal diet + rumen fluid collected from inefficient cows (I); basal diet + rumen fluid collected from inefficient cows + Additive (IA).

Conclusions

The results of the present study indicate that TBE was effective in reducing both the amount and rate of CH_4 production, while the inherent characteristics of the rumen microbial background also influenced CH_4 production and fermentation efficiency *in vitro*. Most importantly, additive efficacy depends on rumen fluid efficiency. Thus, an efficient rumen fluid may enhance CH_4 mitigation strategies while supporting more efficient fermentation.

References

Ahvenjärvi, S., Bayat, A.-R., Toivanen, M., Mäntysaari, P., & Tapio, I. (2024). The effects of residual energy intake on nutrient use, methane emissions and microbial composition in dairy cows. *Sci. Rep*, *14*(1), 613.

Duin, E. C., Wagner, T., Shima, S., Prakash, D., Cronin, B., Yáñez-Ruiz, D. R., Duval, S., Rümbeli, R., Stemmler, R. T., & Thauer, R. K. (2016). Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. *Proc. Natl. Acad. Sci*, *113*(22), 6172–6177.

Fekri, A., Menni, G., Fant, P., & Ramin, M. (2026). Effects of high-purity tribromoethanol (TBE) on methane production and ruminal fermentation across diets with different nutrient compositions using an in vitro gas production system. *J. Agric.Food Res*, 102928.

Mizrahi, I., & Jami, E. (2018). Review: the compositional variation of the rumen microbiome and its effect on host performance and methane emission. *Anim*, 12(S2): S220–S232.

Morgavi, D., Cantalapiedra-Hijar, G., Eugène, M., Martin, C., Noziere, P., Popova, M., Ortigues-Marty, I., Muñoz-Tamayo, R., & Ungerfeld, E. (2023). Reducing enteric methane emissions improves energy metabolism in livestock: is the tenet right? *Anim*, *17*, 100830.

Ramin, M., & Huhtanen, P. (2012). Development of an in vitro method for determination of methane production kinetics using a fully automated in vitro gas system—A modelling approach. *Anim. Feed Sci. Technol*, *174*(3-4), 190–200.

Shao, Z., Lu, Z., Wu, L., Liu, W., Abdullah, M., Hassan, F., Chen, X., Cao, R., & He, B. (2026). Methyl Coenzyme M Reductase as a Target for Inhibition of Methanogenesis in Ruminants: Challenges and Opportunities. *Front. Microbiol*, *16*, 1704809.

Stepanchenko, N., Wasson, D., Indugu, N., Cueva, S., Martins, L., Narayan, K., Pitta, D., & Hristov, A. (2025). Effects of methane inhibitors on ruminal fermentation and microbial composition in vitro using inoculum from phenotypically high-and low-enteric-methane-emitting cows. *J. Dairy Sci*, *108*(10), 10746–10765.

Xuan, T., Zheng, T., Li, T., Wu, B., Li, T., Bao, W., & Qin, W. (2024). The Effects of different doses of 3-NOP on ruminal fermentation parameters, methane production, and the microbiota of lambs In Vitro. *Fermentation*, *10*(9), 440.

Evaporation of ethanol from the feed bunk when including sugar beet silage

E. M. V. Hvas[1], J. R. Thyssen[1] & M. Larsen[1]
[1]*Aarhus University (AU), Department of Animal and Veterinary Sciences, AU Viborg, Research Centre Foulum, Blichers Allé 20, 8830 Tjele, Denmark.*
Correspondence: emma.hvas@anivet.au.dk

Introduction

Beets (*Beta vulgaris*) can be ensiled to extend their use in dairy cow rations. However, beet silage typically contains high concentrations of ethanol (Hvas et al., 2025). Because ethanol has a $\log_{10}$ Henry's law constant of -3.67 compared with -5.12 for acetate (Hafner et al., 2013), it is highly volatile and may evaporate from the feed mix at the feed bunk. Such evaporation represents both an energy loss and a potential environmental concern, as volatile organic compounds (VOC), including ethanol, act as ozone precursors (Wilkinson and Muck, 2019). The degree of evaporation from the feed bunk may also be influenced by cows' rooting and sorting behaviour, which changes the surface area exposed to air. The aim of the present study was therefore to examine the evaporation of VOC including ethanol, acetate, and lactate and the loss of dry matter (DM) from a TMR containing beet silage, with and without simulated rooting behaviour.

Materials and Methods

The experiment was conducted in February 2025, when outdoor temperatures ranged from -8 to 5 °C, in a unheated barn environment. The barn doors were kept shut to prevent wind interference. Two feed mixtures were prepared: a control total mixed ration (CON) and a second mixture in which 19.9% of the CON ration (DM basis) was replaced with beet silage (ETH). The CON TMR consisted, on a DM basis, of maize silage (22.1%), grass/clover silage (18.5%), rapeseed cake (10.4%), rapeseed meal (10.4%), dried beet pulp (6.7%), spring barley (4.2%), and vitamins and minerals (1.2%). Each mixture was placed on a cleaned concrete floor in six stacks of 26.2 kg DM, corresponding to the daily ration of a high-yielding Danish Holstein cow. Three stacks per mixture were subjected to simulated rooting behaviour by manually flipping each corner of the stack toward the center with a dedicated shovel and then levelling it back to its original size; the remaining three stacks were left unrooted. Each stack followed a unique randomised sampling scheme using a 3×3 grid to determine sampling positions over time. Samples were collected at 0, 0.5, 1, 1.5, 2.5, 3.5, 5, 8.5 and 12 h, with rooting performed every 10 min until 1.5 h, every 15 min until 5 h, and every 30 min until 12 h. When sampling and rooting time coincided, samples were collected before rooting. At each sampling, approximately 500 g of surface material was removed, subdivided, and frozen for DM determination and for ethanol, VFA and L-lactate analysis. Water extracts of the TMR and silage samples were prepared for chemical analysis (Hvas et al., 2025).

Statistical analysis was performed in R 4.3.2 (R Core Team, 2021). Because of the large inherent difference in ethanol concentration between CON and ETH, the two feed mixtures were analysed separately. Differences between rooted and unrooted stacks were evaluated using linear mixed-effects models fitted (nlme function, nlme package), followed by a one-way ANOVA. Data was log-transformed for analysis and are reported as back-transformed values.

Results and Discussion

Ethanol was the only compound that showed measurable changes in concentration across the two TMRs (Table 1 and Table 2). In ETH, rooting reduced the mean ethanol concentration ($P = 0.046$). However, there was no time effect and no effect of rooting depending on the time after feed-out, indicating that the difference between rooted and unrooted stacks occurred early and that both treatments followed a similar decline thereafter. In CON, ethanol evaporation dynamics differed between rooted and unrooted stacks depending on the time after feed-out ($P = 0.021$).

Table 1 Average volatile organic compounds (VOC) and dry matter (DM) values12-hour for feed stacks with beet silage (ETH). Values are mean concentrations (g/kg DM for VOC and % for DM) across sampling times. The slope describes the change per sampling interval. RCON denotes the mixed feed stack.

		Treatment			*P*-values		
Item	Parameter	ETH	RETH	SEM	Rooting (R)	Time (T)	R × T
Ethanol	Mean concentration	20.3	18.5	0.035	0.046	0.322	0.296
	Slope	-0.117	-0.285	0.167			
Acetate	Mean concentration	31.0	29.9	0.054	0.535	0.879	0.980
	Slope	0.286	0.296	0.402			
L-lactate	Mean concentration	42.2	41.8	0.280	0.880	0.156	0.186
	Slope	-0.226	0.164	0.290			
DM	Mean concentration	38.8	39.6	0.011	0.126	0.172	0.171
	Slope	0.133	-0.019	0.109			

Across the 12-hour period, predicted ethanol losses ranged from 6.6% in ETH to 16% in RETH, and 7.5% in CON to 14.8% for RCON, relative to initial concentrations of 21.4 g/kg DM for the ethanol-rich TMR and 6.87 g/kg DM for the control TMR. Thus, although the percentage losses with and without rooting were similar in the ethanol-rich TMR and in the control TMR, the absolute losses were considerably smaller in CON and RCON due to the lower initial concentrations.

The ethanol losses observed in the current study appear to have only a minor impact on the overall energy value of the feed. According to the prediction model, ethanol losses over 12 hours were 1.4 g/kg DM (ETH), 3.4 g/kg DM (RETH), 0.5 g/kg DM (CON), and 1.0 g/kg DM (RCON). The highest loss of 3.4 g/kg DM corresponds to 0.1 MJ and given that 3.14 MJ is required to produce 1 kg ECM (Volden, 2011), this represents only a very small fraction of the total dietary energy supply.

In contrast to ethanol, concentrations of acetate, L-lactate, and DM remained stable throughout the sampling period. This pattern aligns with the substantially lower vapor pressures of acetate and lactate relative to ethanol. Furthermore, de Evan et al. (2025) observed that ethanol in ethanol-rich beet silages is almost completely lost during drying across freeze drying and oven drying with temperatures from 50 to 100°C, whereas acetate and lactate exhibit more temperature-dependent and incomplete evaporation. These findings support the greater stability of acetate and L-lactate observed in the present study.

Ethanol loss was greatest immediately after feed-out, reflecting the expected rapid volatilisation when silage is first exposed to air. As anticipated, rooting accelerated ethanol evaporation relative to unrooted stacks, particularly in feed with higher initial ethanol content. The experiment was conducted during one of the colder months of the year and in the absence of wind. Under typical farm conditions, higher temperatures and wind speeds would be expected to increase evaporation rates, as temperature, wind velocity, and feed porosity are strong drivers of volatilisation processes (Hafner et al., 2010). Thus, the losses observed here likely represent conservative estimates relative to on-farm conditions.

Table 2 Average volatile organic compounds (VOC) and dry matter (DM) over 12-hours in control feed stacks (CON). Values are mean concentrations (g/kg DM for VOC and % for DM) across sampling times. The slope describes the change per sampling interval. RCON denotes the mixed feed stack.

		Treatment			*P*-values		
Item	Parameter	CON	RCON	SEM	Rooting (R)	Time (T)	R × T
Ethanol	Mean concentration	6.54	6.64	0.005	0.432	0.022	0.021
	Slope	-0.043	-0.085	0.018			
Acetate	Mean concentration	11.3	11.4	0.004	0.304	0.873	0.866
	Slope	-0.020	-0.023	0.020			
L-lactate	Mean concentration	40.7	40.9	0.008	0.667	0.070	0.072
	Slope	0.059	-0.039	0.053			
DM	Mean concentration	40.3	40.3	0.002	0.126	0.172	0.171
	Slope	0.036	0.050	0.025			

Conclusions

Ethanol was the only VOC that declined over time, with rooting accelerating losses in ethanol-rich TMR. However, absolute losses were small, and the associated energy loss was negligible. Concentrations of acetate, L-lactate, and DM remained stable. Overall, ethanol evaporation from TMR appears limited and of minor nutritional relevance under the studied conditions.

References

de Evan, T., Hvas, E. M. V., Larsen, M., Weisbjerg, M. R. 2025. Effect of drying method for dry matter determination on losses of fermentation compounds in mixed beet silages. Anim. Feed Sci. Technol. 330, 116565. https://doi.org/10.1016/j.anifeedsci.2025.116565.

Hafner, S. D., Howard, C., Muck, R. E., Franco, R. B., Montes, F., Green, P. G., Mitloehner, F., Trabue, S. L., Rotz, C. A. 2013. Review – Emission of volatile organic compounds from silage: Compounds, sources, and implications. Atmos. Environ. 77, 827-839. https://doi.org/10.1016/j.atmosenv.2013.04.076.

Hvas, E. M. V., Hanigan, M. D., Weisbjerg, M. R., Larsen, M., 2025. Effects of feeding beets and ensiled beets on rumen volatile fatty acid and ethanol metabolism in dairy cows: J. Dairy Sci. 108, 9449-9469. https://doi.org/10.3168/jds.2024-26194.

Volden, H. 2011. NorFor - The Nordic feed evaluation system. EAAP publication no. 130. Wageningen Academic Publishers.

Tracking atmospheric methane over Swedish dairy farms using multi-year TROPOMI observations

K. Kozicka[1], J. Oliveira[2], J. Karlsson[3] & M. Ramin[1]
[1]*Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, 901 83, Umeå, Sweden*
[2]*Swedish University of Agricultural Sciences, Department of Crop Production Ecology, 901 83 Umeå, Sweden*
[3]*Växa Sverige, 756 51 Uppsala, Sweden*
Correspondence: katarzyna.kozicka@slu.se; mohammad.ramin@slu.se

Introduction

Methane (CH_4) is one of the most important greenhouse gases, contributing significantly to global warming due to its high radiative forcing and relatively short atmospheric lifetime (Jacob et al., 2016). Agricultural activities, particularly livestock production, represent a major anthropogenic source of CH_4 emissions, with dairy farming playing a key role in countries such as Sweden (Swedish Environmental Protection Agency, 2025). Satellite observations provide a unique opportunity to monitor atmospheric CH_4 concentrations at regional to global scales. Instruments such as the TROPOspheric Monitoring Instrument (TROPOMI) onboard Sentinel-5P offer daily global coverage and enable the analysis of spatial and temporal variability in column-averaged dry-air mole fractions of atmospheric CH_4 (XCH_4) (Hasekamp, et al., 2021; Jacob et al., 2016). However, detecting moderate CH_4 emissions from agricultural sources remains challenging, particularly in high-latitude regions. Therefore, the aim of this study was to evaluate the capability of TROPOMI to capture XCH_4 variability at both national and farm scales in Sweden, and to assess the potential for detecting emissions from dairy farms under high-latitude conditions.

Material and Methods

The study was conducted over Sweden, with a focus on twelve selected dairy farms distributed across different regions of the country. The farms vary in herd size and management conditions, representing typical Swedish dairy production systems. Atmospheric CH_4 observations were obtained from the TROPOMI instrument onboard Sentinel-5P. The analysis covered the period from 2019 to 2025. Standard quality filtering (e.g. cloud screening and quality assurance thresholds) was applied to ensure reliable retrievals of XCH_4. Monthly mean XCH_4 values were calculated using pixel-weighted averaging of all valid observations. Analyses were performed at two spatial scales: (i) national scale, using all valid pixels over Sweden, and (ii) local scale, using circular 10 km buffers around each farm. Long-term temporal trends in XCH_4 concentrations were evaluated using linear regression. Due to strong seasonal limitations in satellite observations at high latitudes, particularly during winter, further analyses were restricted to the May–September period, which provides the most consistent data coverage. At the national scale, spatial patterns and long-term trends in XCH_4 concentrations were assessed. At the local scale, mean XCH_4 values and temporal trends were compared between farms and against the national average.

Results and Discussion

TROPOMI observations revealed a clear north–south pattern in XCH_4 concentrations across Sweden, with lower values in the north and higher concentrations in southern regions (Figure

1a). In addition, a consistent increase in XCH_4 was observed over the study period, with an average national trend of approximately 11 ppb yr^{-1} (Table 1), consistent with reported global atmospheric CH_4 growth rates (Lan et al., 2025). Spatial differences between 2019 and 2025 indicate a widespread increase in XCH_4 concentrations across the country (Figure 1b).
At the farm scale, XCH_4 concentrations within 10 km buffers were similar across sites and close to the national mean (Table 1). Temporal trends at all sites closely followed the national increase, indicating that local variability is largely driven by regional and hemispheric background concentrations. This highlights the difficulty of isolating farm-level signals in satellite-derived XCH_4 observations. This limitation is consistent with previous studies showing that XCH_4 observations integrate signals over large spatial areas and are strongly influenced by atmospheric transport and background variability (Jacob et al., 2016; Varon et al., 2018). As a result, emissions from individual farms are diluted within the broader atmospheric signal and remain difficult to detect. Overall, the results demonstrate that while TROPOMI effectively captures regional XCH_4 patterns and long-term trends, its ability to resolve moderate agricultural emissions at the farm scale is limited under high-latitude conditions.

Conclusion

TROPOMI observations provide robust information on regional XCH_4 variability and long-term trends over Sweden, capturing both spatial patterns and the overall increase in atmospheric XCH_4 concentrations. However, at the scale of individual dairy farms, XCH_4 variability is largely dominated by regional background concentrations, which limits the detectability of local emission sources. These findings highlight the challenges of using satellite-derived column measurements to identify moderate agricultural emissions in high-latitude regions, where atmospheric mixing and spatial averaging reduce the visibility of local signals.

Table 3 Farm-level and national mean XCH_4 (column-averaged dry-air mole fractions of atmospheric CH_4) concentrations for the May–September period, derived from TROPOMI observations. Farm-level values represent averages calculated within 10 km buffers surrounding each dairy farm. Annual trends were estimated using linear regression over the available observation period for each site.

Farm/ National	Multi-year mean XCH_4 [ppb], 2019–2025	Mean XCH_4 [ppb], 2019	Mean XCH_4 [ppb], 2025	Annual trend [ppb yr^{-1}]
F01	1854 ±0.25	1812	1873	11.2
F02	1861 ±0.35	1811	1883	11.0
F03	1862 ±0.39	1811	1882	11.7
F04	1867 ±0.49	1811	1878	10.9
F05	1862 ±0.42	1812	1885	12.1
F06	1865 ±0.27	1817	1888	12.0
F07	1865 ±0.25	1818	1889	12.1
F08	1858 ±0.38	1811	1880	11.1
F09[a]	1863 ±0.58	–	1874	9.1*
F10[b]	1882 ±0.85	–	1896	10.9
F11	1876 ±0.44	1815	1904	13.1
F12[b]	1872 ±0.33	–	1893	9.1
Sweden	**1867 ±0.01**	**1815**	**1892**	**12.0**

* Trend not statistically significant ($p \geq 0.05$).[a] Data available only for 2020–2022 and 2024–2025 due to limited TROPOMI data availability. [b] Data available only for 2020–2025 due to limited TROPOMI data availability.

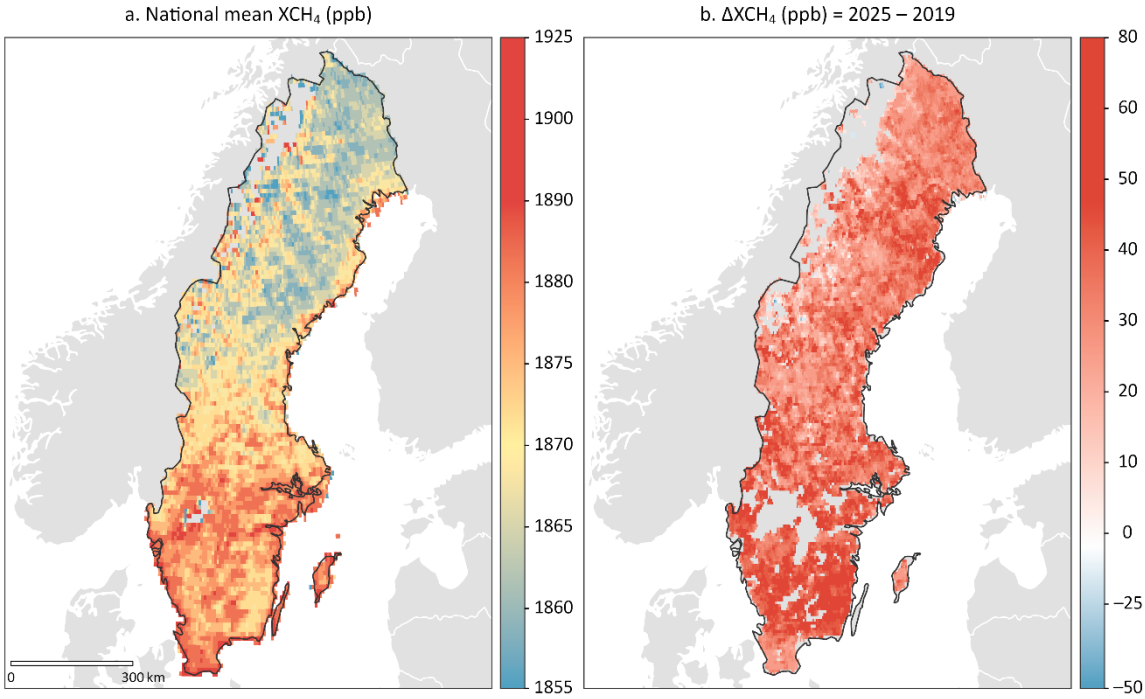


Figure 3. Spatial distribution of atmospheric XCH_4 (column-averaged dry-air mole fractions of atmospheric CH_4) over Sweden derived from TROPOMI: a. Pixel-weighted annual mean XCH_4 concentration (ppb) for the full study period 2019–2025; b. Difference in annual mean XCH_4 (ppb) between 2025 and 2019.

References

Hasekamp, O., Lorente, A., Hu, H., Butz, A., aan de Brugh, J., Landgraf, J., 2021. Algorithm Theoretical Baseline Document for Sentinel-5 Precursor methane retrieval (No. SRON-S5P-LEV2-RP-001). KNMI, De Bilt, The Netherlands.

Jacob, D.J., Turner, A.J., Maasakkers, J.D., Sheng, J., Sun, K., Liu, X., Chance, K., Aben, I., McKeever, J., Frankenberg, C., 2016. Satellite observations of atmospheric methane and their value for quantifying methane emissions. Atmos. Chem. Phys. 16, 14371–14396. https://doi.org/10.5194/acp-16-14371-2016

Lan, X., Thoning, K., Dlugokencky, E., NOAA Global Monitoring Laboratory, 2025. Trends in globally-averaged CH4, N2O, and SF6. https://doi.org/10.15138/P8XG-AA10

Swedish Environmental Protection Agency, 2025. Sweden National Inventory Report 2025. Swedish Environmental Protection Agency, Stockholm, Sweden.

Varon, D.J., Jacob, D.J., McKeever, J., Jervis, D., Durak, B.O.A., Xia, Y., Huang, Y., 2018. Quantifying methane point sources from fine-scale satellite observations of atmospheric methane plumes. Atmos. Meas. Tech. 11, 5673–5686. https://doi.org/10.5194/amt-11-5673-2018

Research on sustainable feed for livestock in Norway: a knowledge summary

I. Dønnem[1], T. Persson[2], H. Steinshamn[2], A.K. Uhlen[1], E. Prestløkken[1], A.K. Bakken[2], E. Byers[2], M. Höglind[2], L.T. Mydland[1], Å. Ergon[1], V. Kvakkestad[2] & H.H. Simonhjell[2]
[1]Faculty of Biosciences, Norwegian University of Life Sciences, PO Box 5003, 1432 Ås, Norway. [2]Division of Food Production and Society, Norwegian Institute of Bioeconomy Research (NIBIO), PO Box 115, 1431 Ås, Norway.

Introduction

Livestock production is a central component of Norwegian agriculture, contributing to food security and the provision of ecosystem services. At the same time, feed production and utilization is associated with greenhouse gas emissions, nutrient losses, and land-use pressures. Sustainable livestock production is also challenged by feed import dependency. In response to these challenges, the Norwegian Government launched a social mission on sustainable feed in 2023, with the overall objective that all livestock feed should come from sustainable sources and contribute to reduced climate impacts across food systems.

The Research Council of Norway commissioned in 2024 a knowledge summary of Norwegian research on sustainability of livestock feed which has been conducted from 2017 and onwards. The objective of the knowledge summary was to provide an accessible and systematic overview of the research results, and to identify knowledge gaps to inform future research and innovation priorities.

In this review, sustainable feed and feed system is understood as feed to livestock that is produced and utilized in ways that are environmentally sound, economically viable, and socially responsible. This includes minimizing negative environmental impacts across feed production and use, ensuring good animal health and welfare, and supporting viable local communities involved in feed production and use. Sustainable feed is further understood as feed that enables livestock production systems to align with national agricultural policies and international commitments while maintaining long-term productivity and resilience.

Materials and Methods

The knowledge synthesis was conducted as a scoping review, following the PRISMA guidelines (Tricco et al., 2018). A structured review protocol (Steinshamn et al., 2025) was developed and published as the first step in the review process.

Systematic literature searches were performed in major scientific databases, including Web of Science and Scopus, and grey literature. Search strings combined terms related to livestock species, feed and feeding, sustainability, and Norway. The literature emerging from the searches included peer-reviewed scientific articles, conference papers, research reports, book chapters, and MSc and PhD theses.

Inclusion criteria required studies to (i) be conducted in Norway or in Norway in combination with other Nordic countries, (ii) be published between 2017 and April 2025, (iii) be written in Norwegian or English, and (iv) address the production of, or response to, feed , feed ingredient feed system of feed rations intended for livestock.

Studies focusing exclusively on aquaculture, pets, or non-agricultural animals were excluded, as were reviews based on data from non-Nordic contexts. After screening and eligibility assessment, a total of 381 publications met the inclusion criteria.

Metadata and thematic information were extracted using AI-assisted tools (ChatGPT Plus), followed by manual verification and quality control by members of the project group. Publications were categorized according to publication type, livestock species, feed type, sustainability dimension, methodological approach, disciplinary affiliation, and institutional authorship. Studies were further classified as effect studies, mapping studies, or sustainability studies depending on their research methods.

A stakeholder workshop with representatives from industry, research institutions, advisory services, and public authorities was conducted to contribute identifying perceived knowledge gaps and innovation barriers.

Results and Discussion

The 381 identified publications included 249 peer-reviewed scientific articles, 49 research reports, 52 MSc theses, 20 PhD dissertations and 11 book chapters. Research activity remained relatively stable throughout the period, with no clear trends in volume or thematic focus. Articles were published across 119 different scientific journals. About half of the publications involved collaboration between two or more institutions, and 70% of the publications had authors exclusively from Norwegian research institutions. Citation analyses showed that a substantial share of articles achieved moderate international visibility.

Environmental sustainability was addressed in 56% of publications, particularly climate-related issues such as greenhouse gas emissions and carbon footprint. Economic sustainability was addressed in 26% of the publications, often in combination with environmental analyses, especially through cost benefit studies. Social sustainability including farm resilience, labour conditions, social acceptability, and cultural values was addressed in only 3% of the publications.

A notable finding was that few studies operationalized or defined sustainability. Fully integrated sustainability studies combining environmental, economic, and social dimensions were rare.

The most extensively studied feed categories were forage (excl. pasture), pasture, and concentrate feeds, reflecting their central role in Norwegian livestock production.

Forages (103 publications): Research addressed forage cultivation, conservation, feeding practices, and effects on feed quality, animal performance, and greenhouse gas emissions. Research emphasized crop management, harvest timing, silage additives, forage species diversity, and nutrient utilization. Only a few studies explicitly framed forage management within a holistic sustainability assessment.

Pasture and grazing (86 publications): Research focused on grazing systems, including both lowland pastures and tundra-based reindeer husbandry. The studies predominantly examined vegetation and ecosystem dynamics, biodiversity, and climate interactions. Sustainability was frequently discussed, but explicit indicators or integrated assessments were rare.

Concentrate feeds (93 publications): Research addressed concentrate feed ingredients and feeding strategies, with strong emphasis on replacing imported soybean meal with Norwegian protein sources such as rapeseed, field peas, and faba beans. Processing technologies (e.g.

pelleting, fractionation, phytase use), feed efficiency, and mitigation of greenhouse gas emissions were central themes. Life cycle assessments to evaluate system-wide impacts were used in a limited number of studies.

Novel Feed Resources (48 publications): Research on novel feed resources was limited in scope and scale. Yeast-based proteins and macroalgae (particularly brown and red seaweeds) were the most studied alternatives, with promising results for nutrient value and, in some cases, methane reduction. Insect-based feeds and grass juice protein were represented by only a small number of studies, often at laboratory or pilot scale.

Non-conventional feed resources (28 publications): Research including the use of food waste, agricultural by-products, and animal by-products, was sparse. Where such studies exist, they mainly addressed nutritional value and economic feasibility, with few comprehensive sustainability assessments.

Conclusions

This knowledge summary showed that Norwegian research on sustainable feed is extensive but unevenly distributed across sustainability dimensions and research topics. While environmental impacts especially climate-related issues are well studied, social sustainability and integrated system performance are still largely underexplored. A key finding is the lack of a unified and operational definition of sustainability in feed and livestock production.

The report identified two main perspectives on sustainability: a system-oriented perspective focusing on the resilience and present and future performance of feed production systems, and a product-oriented perspective focusing on the attributes of specific feed products.

Significant knowledge gaps were identified, including:

- Integration of environmental, economic, and social dimensions
- Insufficient data on upscaling, economic viability, and safety of novel feed resources
- System-level analyses linking soil, plants, animals, and food systems
- Limited research on circular resource use (including food waste, agricultural and animal by-products)
- Insufficient understanding of nutrient resource efficiency (N and P)
- Lack of climate adaptation research

Going forward, it will be important to continue research on traditional feed resources, while increasing attention to alternative protein sources and circular resource use. At the same time, interdisciplinary and system-oriented research will be needed to understand interactions within real production systems.

Overall, while the existing research provides a strong starting point, filling these gaps will be key to developing more sustainable and resilient feed systems in Norway.

References

Steinshamn, H., Dønnem, I., & Persson, T. 2025. Protokoll for en kartleggingsoversikt (scoping review) om norsk forskning på bærekraft av produksjon av fôr til husdyr. OSF, April 2. https://doi.org/10.17605/OSF.IO/A6MH3

Tricco, A.C., Lillie, E., Zarin, W., O'Brien, K.K., Colquhoun, H., Levac, D., Moher, D., et al., 2018. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. Ann Intern Med. 169(7):467-473. https://doi.org/10.7326/m18-0850

Effect of different CH_4 mitigation strategies on milk vitamins B_2 and B_{12} concentrations

M. H. Kjeldsen[1], M. Thorsteinsson[1], S. Fedosov[2] C. F. Børsting[1], M. R. Weisbjerg[1], S. K. Jensen[1], P. Lund[1] & S. Lashkari[1].
[1]*Department of Animal and Veterinary Sciences, AU Viborg–Research Centre Foulum, Aarhus University, 8830 Tjele, Denmark*
[2] *Department of Molecular Biology and Genetics, Aarhus University, Universitetsbyen 81, 8000, Aarhus C, Denmark*
Correspondence: Maria.kjeldsen@anivet.au.dk

Introduction

Vitamin B12 (cobalamin) and vitamin B2 (riboflavin) are essential vitamins for synthesis of red blood cells and energy metabolism in mammals. Animals cannot synthesize these vitamins, but the vitamins are produced by microorganisms in order to sustain the microorganisms' own growth and production of among others propionate, CH_4, and methionine (Akins, 2012). Therefore, human vitamin B12 supply depends largely on dietary animal products, particularly from ruminants, due to the ruminal microbial fermentation. Archaea are the key contributors to B12 synthesis in the rumen (Franco-Lopez et al., 2020). Yet, the population and activity of archaea are expected to be altered when CH_4 formation is changed by dietary changes. The consequences of changes in diets for dairy cows for milk vitamin B2 and B12 concentrations were investigated in the current study.

Materials and Methods

Milk samples were collected from 3 different experiments (exp): 1) a 4 × 4 Latin square design with 4 dairy cows fed a control diet (CON) or a diet with concentrations of di-bromomethane (DBM) at 7 (LOW), 14 (MEDI), or 21 mg/kg DM (HIGH), 2) a 4 × 4 Latin square design with 4 dairy cows fed a grass silage or maize silage based diet combined with a saturated or unsaturated fat source, and 3) a cross-over design with 2 periods and 8 cows fed either a high (F63) or low forage (F35) diet (63% or 35% forage of DM, respectively). All exp were performed at Aarhus University, with Holstein dairy cows. One morning milk sample per cow was collected in each exp after at least 12 days of adaptation to the specific diet. All milk samples were analysed for vitamin B12, whereas vitamin B2 was only analysed in exp 2 and 3. Vitamin B12 was measured by an in-house ELISA method, where biotin-labelled B12 competed with the extracted B12 for the B12-specific capturing protein intrinsic factor. Biotin molecules (associated with the capturing protein on the surface of the ELISA well) were detected with the help of streptavidin, labelled by horseradish peroxidase. Vitamin B2 was analysed by LUFA-ITL GmbH, AGROLAB Group, Kiel, Germany (CEN, 2014). A Proc Mixed procedure for statistical analyses was performed in SAS (SAS Institute Inc., Cary, NC, USA) for each exp with dose of DBM (exp 1), forage type, fat source, period, and the interaction between forage and fat (exp 2), and treatment and period (exp 3) included as fixed effects. Cow was included as a random effect in all 3 models. Due to the dose response set-up in exp 1, the observations were tested for a polynomial contrast.

Results and Discussion

Milk vitamin B12 concentrations were not affected by the dietary changes in exp 1 and exp 3, and the milk concentration of vitamin B2 was not affected by the forage level in exp 3 (Table 3). In addition, there were no linear, quadratic, or cubic responses to doses of DBM on milk vitamin B12 concentration in exp 1 (Table 1). Di-bromomethane is a potent feed additive to

reduce CH_4 production, and in exp 1 the CH_4 yield (g/kg DM intake) was 15% lower for the HIGH diet compared to the CON diet (Thorsteinsson et al., unpublished). The archaea were therefore expected to be inhibited, and the absence of an effect on the milk concentration of vitamin B12 in exp 1 was surprising.

Table 1 Concentrations of vitamin B12 (µg/100 ml) in milk from cows in experiment 1, where cows received dibromomethane added to their diet in concentrations (mg/kg DM) as follows: a control diet (CON, 0), LOW (7), MEDI (14), and HIGH (21) (n = 4)

	CON	LOW	MEDI	HIGH	SEM	P-values			
						Diet	Linear	Quadratic	Cubic
Vitamin B12	0.74	0.79	0.85	0.76	0.08	0.77	0.73	0.38	0.67

Table 2 Concentrations of vitamin B2 (mg/100 ml) and B12 (µg/100 ml) in milk from cows in experiment 2, where cows were fed four combinations of grass silage (GRA) or maize silage (MAI) together with a saturated (SAT) or unsaturated fat source (rapeseed, RAP) (n = 4)

	GRA-SAT	GRA-RAP	MAI-SAT	MAI-RAP	SEM	P-values		
						Forage	Fat	Forage × fat
Vitamin B2	0.21	0.21	0.18	0.20	0.01	0.08	0.54	0.32
Vitamin B12	0.86	0.70	0.76	0.62	0.14	0.06	0.01	0.96

Table 3 Concentrations of vitamin B2 (mg/100 ml) and B12 (µg/100 ml) in milk from cows in experiment 3, where cows were fed either a low forage diet (F35, 35% of DM) or a high forage diet (F63, 63% of DM) (n = 16)

	F35	F63	SEM	P-value
Vitamin B2	0.18	0.19	0.01	0.42
Vitamin B12	0.70	0.70	0.04	0.95

In exp 3 the forage level, and thereby the concentrate and starch level, differed between the F35 (239 g starch/kg DM) and F63 diets (150 g starch/kg DM). Starch does not have a direct inhibition on the archaea, but fermentation of starch re-directs H2 from methanogenesis into propionate synthesis. Yield of CH_4 was 26% lower for cows fed F35 compared to cows fed F63 in exp 3 (Kjeldsen et al., 2024). Schwab et al. (2006) found a tendency ($P < 0.10$) toward a negative correlation between starch intake (kg/d) and daily apparent ruminal synthesis of vitamin B12, whereas the correlation with vitamin B2 was positive in their study ($P < 0.05$). Seck et al. (2017) also reported higher daily apparent ruminal synthesis of vitamin B2 in cows fed a low-forage diet ($P < 0.001$), but no effect on ruminal synthesis of vitamin B12. However, in general the ruminal apparent synthesis should be interpreted with caution since feed intake is not accounted for, and feed intake, and thereby also passage rate, will most likely affect the microbes and their synthesis of vitamins in the rumen. In exp 2 there was no interaction between forage type and fat source with respect to milk vitamin B2 and B12 concentrations. In contrast to exp 3, which showed no effect of forage-level, forage-type tended to affect B2 and B12 concentrations in exp 2, with lower concentrations found in the maize-based diets ($P = 0.08$ and $P = 0.06$, respectively), compared with grass-based diets. The average starch content was 190 g/kg DM and 279 g/kg DM for the grass- and maize based diets, respectively, and CH_4 yield was reduced by 18% in the maize based diets in exp 2 (Lashkari et al., unpublished). Furthermore, dietary supplementation of rapeseed fat reduced the milk concentration of

vitamin B12 (Table 2, P = 0.01), but vitamin B2 concentrations were unaffected. The reduced concentration of vitamin B12 aligns with the expectation, since both fibrolytic bacteria and methanogens are negatively affected by fat, as especially the unsaturated fatty acids are toxic to fibrolytic bacteria and methanogens, unless they are rapidly bio-hydrogenated in the rumen (Patra, 2013).

Conclusions

No adverse effect of DBM (exp 1) and forage level (exp 2) on milk concentration of vitamin B2 and vitamin B12 were observed. However, a higher proportion of maize silage, and thereby a higher proportion of starch from maize silage, tended to reduce concentration of both vitamin B2 and vitamin B12 in milk (exp 3). Furthermore, unsaturated fat negatively affected the concentration of vitamin B12 in milk, but not on vitamin B2 (exp 3).

References

Akins, M.S., 2012. Effects of cobalt supplementation and vitamin B12 injections on lactation performance and metabolism of Holstein dairy cows. PhD Thesis. University of Wisconsin-Madison.

CEN, 2014. Foodstuffs. Determination of vitamin B2 by high performance liquid chromatography. EN 14152:2014. Brussels: European Committee for Standardization.

Franco-Lopez, J., Duplessis, M., Bui, A., Reymond, C., Poisson, W., Blais, L., Chong, J., Gervais, R., Rico, D.E., Cue, R.I., Girard C.L. & Ronholm, J., 2020. Correlations between the composition of the bovine microbiota and vitamin B12 abundance. mSystems, e00107-20. https://doi.org/10.1128/msystems.00107-20.

Kjeldsen, M.H., de Evan Rozada, T., Noel, S.J., Schönherz, A., Hellwing, A.L.F., Lund, P. & Weisbjerg, M.R., 2024. Phenotypic traits related to methane emissions from Holstein dairy cows challenged by low or high forage proportion. J. Dairy Sci. 107, 10787-10810. https://doi.org/10.3168/jds.2024-24848.

Patra, A.K., 2013. The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis. Livest. Sci. 155, 244-254. https://doi.org/10.1016/j.livsci.2013.05.023.

Schwab, E.C., Schwab, C.G., Shaver, R.D., Girard, C.L., Putnam, D.E. & Whitehouse, N.L., 2006. Dietary forage and nonfiber carbohydrate contents influence B-vitamin intake, duodenal flow, and apparent ruminal synthesis in lactating dairy cows. J. Dairy Sci. 89, 174-187. https://doi.org/10.3168/jds.S0022-0302(06)72082-3.

Seck, M., Voelker Linton, J.A., Allen, M.S., Castagnino, D.S., Chouinard, P.Y. & Girard, C.L., 2017. Apparent ruminal synthesis of B vitamins in lactating dairy cows fed diets with different forage-to-concentrate ratios. J. Dairy Sci. 100, 1914-1922. https://doi.org/10.3168/jds.2016-12111.

Harvested forages for horses from a feeding perspective – a systematic literature review

S. Ringmark[1], M. Connysson[1], K. Arvidsson Segerkvist[2], C.E. Müller[2]
[1]*Department of Animal Biosciences, Swedish University of Agricultural Sciences, Sweden*
[2]*Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Sweden*
Correspondence: Cecilia.Muller@slu.se

Introduction

Horses are evolutionary adapted to a grass-based diet in their feed intake behaviour and how they digest their feed, meaning that forage is the most important feedstuff for the horse. In Sweden, about 1/3 of all ley on arable land is used as feed for horses (Cederberg & Henriksson, 2020). Due to the important role of forages in equine diets and the comparably large proportion of cultivated ley that is used for horses, the aim of this project was to perform a systematic literature review within the area of forage for horses from a feeding perspective. The objectives were to identify the currently available published peer-reviewed literature on feeding forages to horses, critically evaluate the quality of the literature, summarize the content to present the existing current knowledge, and identify knowledge gaps of importance for a sustainable feeding of forages to horses, with special reference to Northern European conditions.

Materials and Methods

A search strategy for literature was developed, and a literature search was performed in databases containing scientific publications which resulted in 11316 hits. After removal of duplicates, papers outside the study scope, and papers not passing the inclusion criteria, 312 publications remained which were critically reviewed and summarized, and knowledge gaps identified. Inclusion criteria comprised full descriptions of experimental and statistical design, forage types, methodology if digestibility was studied, forage analytical methods, type and number of horses, that at least one treatment was present where horses were fed forage only, that the study should be possible to repeat (except for case reports), and that the paper was written in English, Swedish, Danish or Norwegian language.

Results and Discussion

The 312 studies that were included ranged from years 1903 to March 2024, and were mainly about forage intake, forage digestion/digestibility/nutritive value, pre-feeding treatments of forages for horses (such as e.g. soaking in water), and forage hygienic quality in relation to health issues in horses (Figure 1). Both voluntary intake and dry matter or organic matter digestibility was found to be largely influenced by plant maturity at harvest from summarizing the included literature on intake and performing meta-analysis on dry- and organic matter digestibilities, but it was not the only explanatory factor for any of the variables. Other factors such as plant species, physical form of the forage, and feeding level also had an influence, as had physiological status of the horse. To be able to fully make use of different forage types for different horse categories, more knowledge is needed about e.g. interactions between plant species/ varieties/maturity at harvest and equine intake and digestibility of them. The possibility to use forage-only diets for all horses should be further explored, and particularly for broodmares and foals there is a scarcity of knowledge about forage types that may suit this horse category.

Several studies aiming at comparing different forage conservation methods for e.g. intake or digestibility in horses were confounded in their experimental design as the forages that were

compared were not from the same origin. The results could therefore be due to different plant species or -maturity as well as the conservation method, restricting the possibility to draw any clear conclusions on the impact of various forage conservation methods on e.g. intake or digestibility.

Forage hygienic quality and its influence on horse health was mainly covered in case reports rather than in comparative studies. One exception to this were studies on the amount of respirable particles in different forages, how it could be managed and its association to respiratory health in horses. In these studies, silage and haylage were reported to in general have lower levels of respirable particles compared to hay, and to result in healthier airways in horses. Other topics on forage hygienic quality mainly covered in case reports were botulism, presence of moulds and mycotoxins including endophytic fungi, various contaminants and methods for assessment of forage hygienic quality. It was noted that research on endophyte-infected grasses and possible implications of using such forages for horses was performed primarily in the USA, and studies in European conditions were lacking almost completely.

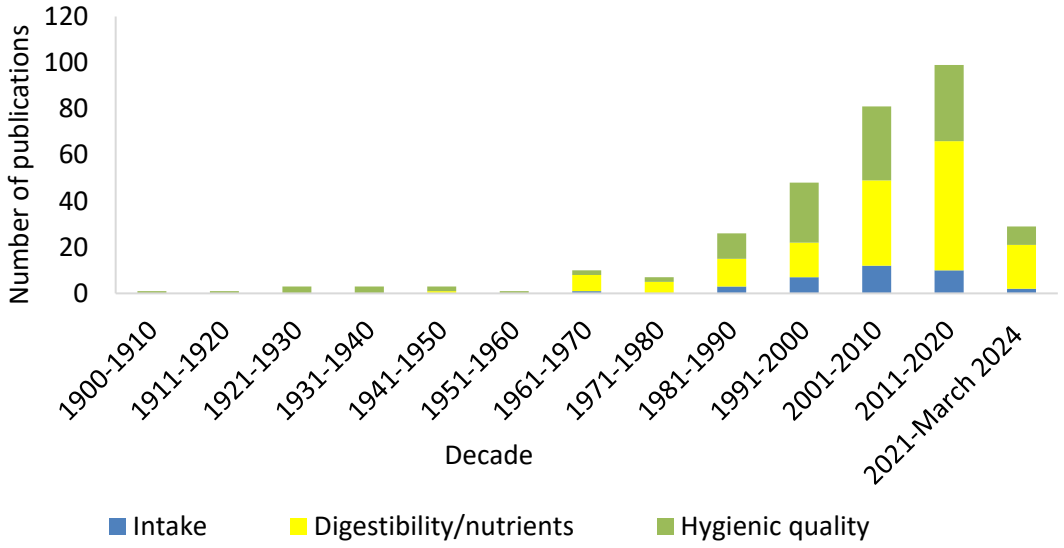


Figure 1 Number of publications passing the inclusion criteria, distributed over the decades from year 1900 to March 2024 and divided over the main themes forage intake, forage digestion, digestibility and/or nutritive value, and forage hygienic quality.

Conclusion

In conclusion, considering the importance of forages in the diet for a hindgut fermenter such as the horse, further research within the area is needed to develop strategies for a sustainable forage feeding of horses in Northern Europe. Several research areas are of high priority, such as if/how forages can be designed to fit the requirements of both feed intake time and energy and nutrient requirements in different horse categories, and health impacts on horses from endophyte-infected grasses, among others.

References

Cederberg C., Henriksson M. 2020. Gräsmarkernas användning i jordbruket. Rapport från Chalmers Tekniska Högskola, Göteborg, Sweden.

Ringmark, S., Connysson, M., Arvidsson Segerkvist, K., Jansson, A., Müller, C.E. 2023. Forages for horses from a feeding perspective- a collection of knowledge from 1903 to 2022. 2nd ed. Report 308, Department of Animal nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Germination of *Echinochloa crus-galli* seeds could be reduced by ensiling

M. Rinne[1], I. Leino[1,2], M. Franco[1], P. Ruuttunen[1] & T. Hyvönen[1]
[1]*Natural Resources Institute Finland (Luke), Jokioinen, Finland*
[2]*University of Helsinki, Finland*
Correspondence: marketta.rinne@luke.fi

Introduction

The Boreal climate has limited the presence of many harmful weeds, but climate change and increasing import of seed mixtures e.g. for biodiversity purposes has boosted the spreading of several alien species into Finland. Barnyard grass (*Echinochloa crus-galli*) is one of the most harmful of such species due to strong competitive ability, high reproductive capacity and widespread resistance to herbicides. It can infest several crops including grass leys and cereals. Controlling of harmful plant species requires efficient control and holistic combination of different means. The aim of this study was to evaluate the viability of barnyard grass seeds in ensiled feeds. Barnyard grass has been used as feed, so that it does not pose risks to animals consuming it. If the seeds loose their ability to germinate during the ensiling phase of feeds, crops contaminated with barnyard grass could be used as feeds without a risk of further spreading it.

Materials and Methods

Two ensiling experiments were conducted during summer 2025 to evaluate the viability of barnyard grass seeds. In Exp.1, pure red clover (*Trifolium pratense*) stand was cut, chopped and artificially dried to a DM content of 208 g/kg. The red clover was ensiled without any additive or using a lactic acid bacteria inoculant (Sila-Bac© 1188, Pioneer Hi-Bred International, Johnston, IA, USA) with 4 replicates per treatment. Exp. 2 used crimped barley (*Hordeum vulgare*) grains obtained from a commercial dairy farmer. The experimental treatments included moisture level (achieved by adding tap water) and use of a formic acid based additive (FA; AIV 2 Plus Na, Eastman, Oulu, Finland) in a 2 × 2 factorial design, and 3 replicates per treatment were prepared. Both feeds were ensiled in laboratory scale into one-litre glass jars.

The barnyard grass seeds were collected from South-Western Finland during summer 2024. The seeds were sealed into polyester bags (50 seeds per a bag with dimensions of 4 × 5 cm) and inserted into the middle of the jars while filling them with the feeds. The fermentation period lasted for 3 months, after which the feeds were sampled for analysis of the fermentation quality using standard laboratory methods of Luke Jokioinen laboratory (for details of methods see Franco et al., 2022).

At jar opening, the seed bags were removed, and seed viability was tested using the Jacobsen germination test, where seeds were placed on top of papers above a temperature-controlled (+24 °C) water bath. Strips of paper reached the water to supply moisture to seeds, which were covered by glass domes with a hole on top for ventilation. Artificial light was provided for 14 hours per day. The germinated seeds were calculated after 7 and 14 days of incubation. Control seed batches were germinated simultaneously.

The statistical analysis was conducted using SAS statistical package using one-way Anova for Exp. 1 and a model with the main effects (moisture level and additive) as well as their interaction as fixed effects (Exp. 2), and replicates as random effects in the statistical model.

Results and Discussion

The raw material composition for both experiments is presented in Table 1. The DM concentration of the red clover was low for a grass silage, and the moisture content was low for ensiled grains. The fermentation quality of both red clover silages was moderate based on the somewhat elevated pH and ammonia N proportion values (Table 2). Lactic acid bacteria inoculation significantly increased the conversion of water-soluble carbohydrates into lactic acid ($P<0.001$). The moisture content of crimped grains increased from 254 to 326 g/kg due to the water addition, which significantly increased the fermentation indicated by lower pH, and higher ammonia N and lactic acid concentrations ($P<0.001$; Table 3). Addition of FA decreased pH, and ethanol and lactic acid concentrations ($P<0.01$). The interaction effect was also significant for most fermentation parameters.

The control seed germination rate was 66 and 54 % in Exps 1 and 2, respectively. None of the seeds incubated in red clover silage germinated. In Exp. 2, two seeds in one replicate of low moisture FA treated grains germinated, while all other samples resulted in zero germination of barnyard grass seeds. The treatment resulting in viable seeds (germination rate 1 %) was the one with most limited fermentation, with a total fermentation acid production of only 1.4 g/kg DM.

Table 1 Composition of the red clover (Exp. 1) and barley grain (Exp. 2) raw materials.

	Red clover	Crimped barley grains
Dry matter (DM), g/kg	208	768
In DM, g/kg		
Ash	107	27
Crude protein	165	106
Starch	-	669
Water soluble carbohydrates	78	-
Neutral detergent fibre	371	198

Table 2 Fermentation quality of the red clover silage (Exp. 1) without additive (Control) or inoculated with lactic acid bacteria (LAB).

	Control	LAB	SEM	P-value
Dry matter (DM), g/kg	202	195	3.0	0.142
pH	4.43	4.41	0.033	0.645
Ammonium N, g/kg N	86	78	3.2	0.126
In DM, g/kg				
Water soluble carbohydrates	31	5	2.7	0.006
Ethanol	10	10	0.4	0.261
Lactic acid	88	122	5.6	0.005
Acetic acid	27	31	1.1	0.053
Propionic acid	0.1	0.1	0.02	0.199
Butyric acid	0.5	0.4	0.29	0.765
Fermentation losses, g/kg DM	6.0	5.7	0.74	0.795
Barnyard seed germination, %	0	0	-	-

Table 3 Fermentation quality of crimped ensiled barley grains (Exp. 2) affected by moisture level (M) and treatment without additive (Control) or using a formic acid based additive (FA).

Moisture	High		Low		SEM	P-value		
Additive	Control	FA	Control	FA		M	FA	M×FA
Moisture, g/kg	329	323	256	250	1.8	<0.001	0.006	0.963
pH	4.57	4.64	6.12	4.84	0.053	<0.001	<0.001	<0.001
Ammonium N, g/kg N	27.8	15.7	7.3	6.9	0.54	<0.001	<0.001	<0.001
In dry matter, g/kg								
Ethanol	6.0	1.8	6.2	0.1	1.14	0.445	0.001	0.377
Lactic acid	13.9	1.7	1.5	0.1	0.76	<0.001	<0.001	<0.001
Acetic acid	3.4	2.4	1.5	1.1	0.21	<0.001	0.008	0.085
Propionic acid	0.09	0.10	0.07	0.08	0.010	0.046	0.498	0.917
Butyric acid	1.63	0.03	0.01	0.01	0.100	<0.001	<0.001	<0.001
Fermentation losses, g/kg DM	22.1	6.1	10.0	0.0	6.83	0.169	0.064	0.630
Barnyard seed germination, %	0	0	0	1	-	-	-	-

Conclusions

The results of this study are promising as they indicate that fermentation of barnyard grass seeds during ensiling eliminates the viability of them, when the extent of fermentation is high or moderate. Such conditions can be expected in forage preservation with typical extent of wilting. However, when fermentation is restricted by limiting moisture potentially accompanied with a formic acid additive, the risk of viable seeds increases. Such conditions can result in relatively dry crimped grain ensiling, or possibly with extensively wilted haylage. Additional risks in feed use include spreading of feed biomass including barnyard grass seeds while transporting the biomass from the fields to the silos.

Further research would be needed to elucidate the growth span of barnyard grass relative to feed harvesting strategy, i.e., the maturation of barnyard grass seeds in relation to feed crop harvesting strategy. Additional information of wider range of ensiling conditions affecting weed seed viability would also be useful.

References

Franco, M., Tapio, I., Pirttiniemi, J., Stefański, T., Jalava, T., Huuskonen, A. & Rinne, M., 2022. Fermentation quality and bacterial ecology of grass silage modulated by additive treatments, extent of compaction and soil contamination. Fermentation 8, 156. https://doi.org/10.3390/fermentation8040156

Acknowledgements

The funding provided by MTK (Farmers' Union) foundation, MTK and Eastman is gratefully acknowledged. The ensiling experiments were conducted in cooperation with OptiNurmi and OptiVilja projects co-funded by the European Union.

In vitro total gas and methane production of emerging forage species for ruminants

N. Ayanfe[1], B. Micke[2], D. Parsons[2] & P. Fant[1]
[1]*Swedish University of Agricultural Sciences (SLU), Department of Applied Animal Science and Welfare, Skogsmarksgränd, 901 83 Umeå, Sweden.*
[2]*Swedish University of Agricultural Sciences (SLU), Department of Crop Production Ecology, Skogsmarksgränd, 901 83 Umeå, Sweden.*
Correspondence: nisola.ayanfe@slu.se

Introduction

Leys play an important role in ruminant production as a basis for forage supply while also providing various ecosystem services such as carbon sequestration, nitrogen fixation, and biodiversity. Moreover, biodiverse leys enhance the resilience and sustainability of forage production. Beyond agronomic benefits, some forage species may also influence enteric methane (CH_4) emissions, partly through secondary metabolites (e.g., tannins, phenols, flavonoids, alkaloids, essential oils, and saponins) present in legumes and forbs (Ku-Vera *et al.,* 2020). However, information about the CH_4 mitigation potential of native and emerging Swedish forage species remains limited. Therefore, this study aimed to determine the *in vitro* CH_4 and gas production of emerging forage species relevant to biodiverse ley systems.

Materials and Methods

Ten novel and emerging Swedish forage species were selected, including eight forbs and two legumes. The forbs were yarrow (*Achillea millefolium* L.), native common chicory (*Cichorium intybus* L.), chicory cultivar Spadona (*Cichorium intybus* L. cv. Spadona), golden marguerite (*Cota tinctoria* (L.) J. Gay), oxeye daisy (*Leucanthemum vulgare* Lam.), hoary plantain (*Plantago media* L.), ribwort plantain (*Plantago lanceolata* L.), and salad burnet (*Sanguisorba minor* Scop.). The legumes were bird vetch (*Vicia cracca* L.) and bush vetch (*Vicia sepium* L.). Timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) were included as commonly used reference species. Third-cut forage samples were collected from a field at the Röbäcksdalen research station (63.81°N, 20.24°E) and second-cut forage samples from outdoor demonstration pots in September and October 2025, respectively. The field experiment included *A. millefolium*, *C. intybus*, *C. intybus* cv. Spadona, *L. vulgare*, *P. lanceolata*, *P. pratense*, *V. cracca*, and *V. sepium*. The pot experiment consisted of *C. tinctoria*, *P. media*, *S. minor*, and *T. pratense*. All samples were dried, milled to 1 mm, and sub-samples were taken for chemical analysis. A 1.0 g sample was incubated in triplicate for 48 h over 3 experimental runs. Each run also contained 6 blank bottles and 2 bottles with an internal standard. Rumen fluid was collected from 2 fistulated Nordic Red dairy cows fed a total mixed ration of grass silage and concentrate (60:40; dry matter [DM] basis). Samples were mixed with 60 mL of buffered rumen fluid in 250 mL glass bottles (pre-flushed with CO_2) and incubated in a water bath at 39 °C. Total gas production was measured following Cone et al. (1996) using a fully automated gas production system (Gas Endeavour, Bioprocess control, Lund, Sweden). Gas samples were collected from each bottle at 24 and 48 h of incubation and analysed for CH_4 concentration by gas chromatography. Final pH was recorded after 48 h of incubation. Data were analysed using PROC GLIMMIX in SAS, with species as a fixed effect and experimental run as a random effect. Pot and field data were analysed separately, and pairwise comparisons between means were performed using the LSD test. Statistical significance was considered at $P \leq 0.05$, and tendency at $0.05 < P < 0.10$.

Results and Discussion

The chemical composition of the forage species is reported in Table 1. As expected, legumes, whether grown in pots or field, had numerically higher crude protein concentration (235–283 g/kg DM) than the grass (117 g/kg DM) and field-grown forbs (105–175 g/kg DM). Neutral detergent fibre concentration varied widely among field-grown forbs (282–469 g/kg DM) but ranged from 274 to 322 g/kg DM in legumes across both growing conditions. Ash concentration showed great variability among field-grown forbs (83.8–186 g/kg DM) while legumes had a narrower range (82.7–98.4 g/kg DM) regardless of growing conditions.

Table 1 Chemical composition of forage species harvested from the field or pots and evaluated *in vitro*

	Source	Dry matter, g/kg	g/kg dry matter		
			Ash	Crude protein	aNDFom[1]
Grass					
Phleum pratense	Field	214	95.0	117	502
Legumes					
Trifolium pratense	Pot	244	92.7	235	274
Vicia cracca	Field	186	98.4	283	322
Vicia sepium	Field	162	82.7	279	289
Forbs					
Cota tinctoria	Pot	259	94.4	ND[2]	ND
Plantago media	Pot	185	159	ND	ND
Sanguisorba minor	Pot	262	83.8	ND	ND
Achillea millefolium	Field	135	162	175	330
Cichorium intybus (native)	Field	128	128	138	369
Cichorium intybus cv. Spadona	Field	117	136	134	383
Leucanthemum vulgare	Field	82	186	156	282
Plantago lanceolata	Field	134	121	105	469

[1]aNDFom = Neutral detergent fibre free of residual ash; [2]ND = Not determined.

In vitro total gas and CH_4 production of forage species grown in pots or the field are shown in Table 2. Among pot-grown species, CH_4 production (mL/g DM) at 24 h of incubation was higher for *P. media* and *T. pratense* than for *C. tinctoria* and *S. minor* ($P = 0.003$), with *T. pratense* maintaining the highest CH_4 at 48 h ($P = 0.002$). However, total gas production did not differ among species, which suggests alterations in fermentation pathways rather than reduced fermentation. In line with this, *T. pratense* had the highest CH_4/Gas ratio among pot-grown species. In contrast, *S. minor* had the lowest CH_4/Gas ratio, consistent with its high content of tannins and extractable phenols (135 and 171 g/kg DM, respectively; Kapp-Bitter *et al.,* 2021) compared to the other forbs and *T. pratense*, which have low to moderate tannins. For field-grown species, total gas production tended to vary among forage species at 24 h ($P = 0.089$) and 48 h ($P = 0.052$). Methane production (mL/g DM) at 24 h of incubation was lower for *P. lanceolata* compared to other species, but differences diminished by 48 h, resulting in a similar CH_4/Gas ratio among forbs and *P. pratense*. Lower CH_4 production observed for *P. lanceolata* during the early stages of fermentation, along with numerically lower total gas production, may partly be attributed to reduced fermentation. For *P. pratense*, the low CH_4/Gas ratio is likely due to high water-soluble carbohydrates (not analysed), which promote propionate production during fermentation (as indicated by the lower final incubation pH), thereby reducing methanogenesis (Meale *et al.,* 2012). It is noteworthy that some numerical differences among field-grown species may suggest biologically meaningful variations. However, these results should be interpreted with caution, as not all observed differences were statistically significant.

Conclusions

S. minor showed the lowest CH_4-to-total gas ratio among pot-grown species, while *P. lanceolata* had the lowest CH_4 production per unit of DM among field-grown species during the early fermentation phase. These findings suggest species-related variation in *in vitro* CH_4 production. Nevertheless, confirming their CH_4-mitigating potential will require further research under comparable growing conditions.

Table 2 *In vitro* total gas and methane production, and pH of forage species harvested from the field or pots

Source		pH[1]	Total gas, mL/g DM[2]		CH_4, mL/g DM		CH_4/total gas (48 h)
			24 h	48 h	24 h	48 h	
Pot	Legumes						
	Trifolium pratense	6.57^{a}	196	206	28.8^{a}	42.5^{a}	0.207^{a}
	Forbs						
	Cota tinctoria	6.50^{b}	195	201	24.6^{b}	33.9^{c}	0.171^{b}
	Plantago media	6.56^{a}	205	216	28.5^{a}	36.3^{b}	0.171^{b}
	Sanguisorba minor	6.45^{b}	187	202	22.0^{b}	31.9^{c}	0.160^{c}
	SEM[3]	0.026	5.0	4.7	0.76	1.69	0.0085
	Species	0.012	0.105	0.115	0.003	0.002	0.002
Field	Grass						
	Phleum pratense	6.32^{e}	195	216	25.0^{bc}	36.5^{ab}	0.169^{c}
	Legumes						
	Vicia cracca	6.63^{a}	176	186	25.9^{b}	37.9^{ab}	0.204^{ab}
	Vicia sepium	6.58^{bc}	178	186	29.0^{a}	39.7^{a}	0.218^{a}
	Forbs						
	Achillea millefolium	6.60^{b}	170	175	23.2^{c}	33.4^{bc}	0.191^{bc}
	Cichorium intybus (native)	6.55^{d}	184	190	24.5^{bc}	33.3^{bc}	0.176^{c}
	Cichorium intybus cv. Spadona	6.54^{d}	182	186	25.1^{bc}	33.3^{bc}	0.179^{bc}
	Leucanthemum vulgare	6.57^{c}	179	182	25.0^{bc}	33.0^{bc}	0.181^{bc}
	Plantago lanceolata	6.54^{d}	154	161	20.8^{d}	28.4^{c}	0.177^{c}
	SEM[3]	0.020	8.0	9.5	0.82	1.94	0.0116
	Species	<0.001	0.089	0.052	<0.001	0.007	0.013

[1]pH = measured at 48 h *in vitro* incubation; [2]DM = Dry matter; [3]SEM = Standard error of the mean; [a-e]Means in columns with different superscript letters differ significantly ($P \leq 0.05$).

References

Cone, J.W., van Gelder, A.H., Visscher, G.J. & Oudshoorn, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Technol. 61, 113-128.

Kapp-Bitter, A.N., Dickhoefer, U., Kreuzer, M. & Leiber, F., 2021. Mature herbs as supplements to ruminant diets: effects on in vitro ruminal fermentation and ammonia production. Ani. Prod. Sci. 61, 470-479. https://doi.org/10.1071/AN20323

Ku-Vera, J.C., Jiménez-Ocampo, R., Valencia-Salazar, S.S., Montoya-Flores, M.D., Molina-Botero, I.C., Arango, J., Gómez-Bravo, C.A., Aguilar-Pérez, C.F. & Solorio-Sánchez, F.J., 2020. Role of secondary plant metabolites on enteric methane mitigation in ruminants. Front. Vet. Sci. 7, 584. https://doi.org/10.3389/fvets.2020.00584

Meale, S.J., Chaves, A.V., Baah, J. & McAllister, T.A., 2012. Methane production of different forages in in vitro ruminal fermentation. Asian-Australas. J. Anim. Sci. 25, 86-91. http://dx.doi.org/10.5713/ajas.2011.11249

Predicting microbial community in sugar beet silage based on fermentations patterns

E. M. V. Hvas[1], M. Larsen[1] & M. R. Weisbjerg[1]
[1]Aarhus University (AU), Department of Animal and Veterinary Sciences, AU Viborg, Research Centre Foulum, Blichers Allé 20, 8830 Tjele, Denmark.
Correspondence: emma.hvas@anivet.au.dk

Introduction

Beets (*Beta vulgaris*) can be used as feed for dairy cows, and their value has increased with the development of new high dry matter (DM) varieties containing more sugar and less potassium. Pure beet silages show substantial DM losses but negligible energy losses, due to sugar fermentation to ethanol and CO_2, as well as effluent losses caused by their high water content. Yeasts and heterofermentative lactic acid bacteria (LAB) are the main contributors to ethanol and CO_2 production during fermentation (Borreani et al., 2018). Co-ensiling beets with higher DM feeds, such as grass/clover silage, reduces effluent loss but alters the fermentation profile compared with pure beet silage (Hvas et al., 2024), likely through the introduction of microbial communities or alternative substrates. Microorganisms produce characteristic fermentation profiles, thus, their activity may be inferred from the concentrations of fermentation end products. The aim of this study was therefore to use multiple linear regression to predict the main silage-associated microbes, and their fermentation pathways, based on final concentrations of fermentation products and total fermentation weight loss, in order to evaluate differences in fermentation pathways in pure and mixed beet silages.

Materials and Methods

Beets harvested in 2021 were ensiled in November 2021 (Set 1) and January 2022 (Set 2) using vacuum-packed silage bags (0.63 ± 0.028 kg fresh matter). Beets were dry cleaned, crushed (maximum diameter 62 mm), and mixed with the respective mixer feeds. Set 1 included nine mixed silages and one pure beet silage (Table 1) and Set 2 included three mixed silages and one pure beet silage (Table 2). For each silage, 10 - 20 bags were used to measure gaseous weight loss by repeated weighing on days 1, 4, 8, 15, 22, 30, 54, 92, 113 and 181. Further, 9 bags were frozen at -20°C after 30, 92 and 181 days (n = 3 per time point) and used for chemical analyses. Chemical analyses of water extracts included ethanol, mannitol, VFA, and L-lactate (Hvas et al., 2024).

Statistical analyses were performed in R 4.1.2 (R Core Team, 2021). Fermentation pathway contributions were predicted using multiple linear regression ("lmList" function, "lme4" package). For each silage, the proportion of total fermentation products attributed to ethanol, mannitol, VFA, lactate, and CO_2 (estimated from measured weight loss) was calculated and used as response variables. Predictor variable was the relative contribution of fermentation pathways attributes to homofermentative LAB, heterofermentative LAB, enterobacteria, clostridia, and yeast. Concentrations of ethanol, mannitol, VFA and L-lactate were corrected for the initial concentration in mixer feeds. Total lactate was calculated as two times the concentration of L-lactate (Johansen et al., 2020). Differences among mixer feeds were tested using linear models with a one-way ANOVA, using fermentation pathway as the response variable and mixer feed as the fixed effect.

Results and Discussion

Across all fermentation pathways, differences were observed among silages in Set 1 (Table 1). The same pattern was seen in Set 2, except for the clostridial pathway, which did not differ among silages (Table 2). In both sets, the yeast pathway represented the largest proportion of total fermentation activity.

Table 1 Proportion of fermentation pathways in pure and mixed beet silage in Set 1.

	Fermentation pathway				
Mixer feed	LAB HO[1]	LAB HE[2]	Enterobacteria[3]	Clostridia[4]	Yeast[5]
Pure beets	0.007^{bc}	0.090^{d}	0.059^{bc}	0.005^{b}	0.838^{a}
Maize silage	<0.001^{c}	<0.001^{f}	0.140^{abc}	0.035^{ab}	0.824^{a}
Grass/clover silage	<0.001^{c}	0.021^{ef}	0.045^{c}	0.044^{ab}	0.889^{a}
Grass seed straw	<0.001^{c}	0.268^{a}	0.116^{abc}	0.020^{ab}	0.595^{c}
Beet pulp, dried	<0.001^{c}	0.132^{bc}	0.157^{ab}	0.015^{ab}	0.695^{bc}
DDGS[6]	0.004^{c}	0.042^{e}	0.131^{abc}	0.045^{ab}	0.778^{ab}
Wheat bran	0.001^{c}	0.117^{cd}	0.186^{a}	0.060^{a}	0.636^{c}
Rapeseed meal	0.020^{a}	0.124^{c}	0.185^{a}	0.037^{ab}	0.634^{c}
Sunflower meal	0.015^{ab}	0.164^{b}	0.173^{a}	0.038^{ab}	0.610^{c}
Soybean hulls	0.004^{c}	0.148^{bc}	0.181^{a}	0.030^{ab}	0.637^{c}
SEM	0.002	0.007	0.023	0.010	0.027
P-value	<0.01	<0.01	<0.01	0.02	<0.01

[a-f]Different superscripts for mixer feed differ significantly ($P \leq 0.05$)
[1] Homofermentative lactic acid bacteria: 1 glucose → 2 lactate
[2] Heterofermentative lactic acid bacteria: 1 glucose → 1 lactate + 1 ethanol + 1 CO_2 and Fructose → 1 lactate + 1 acetate + 2 mannitol + 1 CO_2
[3] Enterobacteria: 2 glucose → 2 lactate + 1 acetate + 1 ethanol + 2 CO_2
[4] Clostridia: 2 lactate → 1 butyrate + 2 CO_2 + 2 H_2
[5] Yeast: 1 glucose → 2 ethanol + 2 CO_2
[6] DDGS: Wheat dried distillers' grain solubles.

In Set 1, pure beets and beets co-ensiled with maize silage or grass/clover silage had higher proportions of yeast fermentation compared with silages co-ensiled with grass seed straw, dried beet pulp, wheat bran, rapeseed meal, sunflower meal, or soybean hulls. In Set 2, beets co-ensiled with grass/clover silage or maize gluten feed showed the highest yeast contribution, whereas pure beets displayed the greatest proportion of the heterofermentative LAB pathway.

Based on their high sugar content, pure beet silages would generally be expected to show strong yeast-dominated fermentation. However, the differences observed between Set 1 and Set 2 indicate that fermentation patterns in pure beet silages can vary considerably between batches, even under controlled conditions, and that the link between sugar availability and yeast activity is not straightforward. After 181 days, pure beet silage in Set 2 contained 123 g mannitol/kg DM, compared with 75.6 g/kg DM in Set 1. Ethanol concentrations likewise differed (287 vs. 345 g/kg DM). For comparison, Hvas et al. (2025) reported ethanol and mannitol levels of 182 and 87.9 g/kg DM in larger-scale barrel silages, whereas Weisbjerg et al. (2018) found ethanol levels of 125–134 g/kg DM and mannitol levels of 5.6–66.0 g/kg DM in silages ensiled for 26–

146 days without additives. Together, these findings show that fermentation profiles in pure beet silage can differ markedly between silages and that yeast activity depends not only on sugar content.

Table 2 Proportion of fermentation pathways in pure and mixed beet silage in Set 2.

	Fermentation pathway				
Mixer feed	LAB HO[1]	LAB HE[2]	Enterobacteria[3]	Clostridia[4]	Yeast[5]
Pure beets	0.000[b]	0.199[a]	0.134[b]	0.034	0.633[b]
Grass/clover silage	0.005[b]	0.022[b]	0.071[b]	0.037	0.866[a]
Beet pulp, fresh	0.000[b]	0.069[b]	0.525[a]	0.026	0.380[c]
Maize gluten feed	0.019[a]	0.019[b]	0.147[b]	0.027	0.788[a]
SEM	0.003	0.022	0.040	0.016	0.039
P-value	<0.01	<0.01	<0.01	0.95	<0.01

[a-c]Different superscripts for mixer feed differ significantly ($P \leq 0.05$)
[1] Homofermentative lactic acid bacteria: 1 glucose → 2 lactate
[2] Heterofermentative lactic acid bacteria: 1 glucose → 1 lactate + 1 ethanol + 1 CO_2 and Fructose → 1 lactate + 1 acetate + 2 mannitol + 1 CO_2
[3] Enterobacteria: 2 glucose → 2 lactate + 1 acetate + 1 ethanol + 2 CO_2
[4] Clostridia: 2 lactate → 1 butyrate + 2 CO_2 + 2 H_2
[5] Yeast: 1 glucose → 2 ethanol + 2 CO_2

Conclusions

Fermentation pathways in both pure and mixed beet silages varied markedly when predicted from final fermentation products. Pure beet silages showed considerable batch variation, which also affects the fermentation profile of mixed silages. This may infer a need for inoculation to obtain a high ethanol content in beet silage.

References

Borreani, G., Tabacco, E., Schmidt, R.J., Holmes, B.J., Muck, R.E., 2018. Silage review: Factors affecting dry matter and quality losses in silages. J. Dairy Sci. 101, 3952-3979. https://doi.org/10.3168/jds.2017-13837.

Hvas, E. M. V., Hanigan, M. D., Weisbjerg, M. R., Larsen, M., 2025. Effects of feeding beets and ensiled beets on rumen volatile fatty acid and ethanol metabolism in dairy cows: J. Dairy Sci. 108, 9449-9469. https://doi.org/10.3168/jds.2024-26194.

Hvas, E.M.V., Larsen, M., Andersen, L., Bedenk, U., Weisbjerg, M.R. 2024. Ensiling sugar beets: Effect of mixer feed used for co-ensiling on fermentation products and losses when ensiled in vacuum bags. Anim. Feed Sci. Tech. 318, 116101. https://doi.org/10.1016/j.anifeedsci.2024.116101.

Johansen, M., Weisbjerg, M.R., Novoa-Garrido, M., Kristensen, N.B., Larsen, M., 2020. Relationship between L-lactate and DL-lactate in different silage types. Proceedings of the 28th General Meeting of the European Grassland Federation, Wageningen Academic Publishers, Helsinki, Finland, 282-284.

Weisbjerg, M.R., Bedenk, U., Hellwing, A.L.F., Larsen, M., Hilscher, E., 2018. Wet ensiling of sugar beets with or without ensiling additive. XVIII International Silage Conference, University of Bonn, Bonn, Germany, 552-553.

Possibility of using foam in forage conservation

M. Knicky & P. Melin
RISE-Research Institutes of Sweden, Department of Agriculture and Environmental Engineering, BOX 7033, 750 07 Uppsala, Sweden.
Correspondence: martin.knicky@ri.se

Introduction

A successful ensiling process is associated with low losses, as lactic acid bacteria efficiently convert substrates into fermentation products, while other undesirable microorganisms are less efficient and therefore cause greater fermentation losses (McDonald et al., 1991). Studies have shown that silage losses in bunker silos can be high, reaching up to 26% of dry matter (DM) (Spörndly and Nylund, 2016). These high losses are largely due to handling during filling and feed-out (Muck and Holmes, 2000). Today, plastic films are used to cover silage and thereby maintain anaerobic conditions. The conventional method of covering the silo with plastic films is labour-intensive and involves potential safety risks during covering and uncovering. In addition, recycling and disposal of the film after use is laborious and problematic from an environmental perspective. In Germany, the Technologie- und Förderzentrum institute (TFZ) has developed a spray-on foam composed of natural oils, fibres, alginate, and natural rubber. Once applied, the foam hardens into an elastic surface that adheres to the silage mass and can be removed mixed with the feed. This study aimed to evaluate the potential and limitations of the foam method for bunker-silo sealing and feed-out protection.

Materials and Methods

The foam formula used in the test was obtained from Technologie- und Förderzentrum institute (TFZ, Straubing, Germany) and consisted of two main fractions that are mixed together immediately upon application. Each fraction consists of several raw materials that are mixed separately. The foam consisted of rapeseed oil, sodium alginate, cellulose fiber, calcium sulfite in one fraction; and natural rubber, water, sodium benzoate, sorbic acid, glycerol in second fraction. The foam is intended to be spray-applied to the surface of the silo. Unfortunately, a lack of the necessary equipment meant that the foam in this experiment was applied using a pouring method. As a conservation biomass grass–legume herbage from second (50% of legumes, mainly red clover) and third cut (57 % of legumes, mainly white clover) was used, both wilted to 35% DM content and chopped to 3 cm length. No silage additives were applied. Two types of experiments were performed. The first, the storage trial (third cut forage), where three foam thicknesses (9, 17, 21 mm) were compared with a control where tightnes was achieved with fermentation lock on the lid to prevent air entry. For this purpose, 1.7 L laboratory silos were used, and forage was packet at density of 167 kg DM/m³. In the second, the feed-out simulation trial (second cut forage), two PVC tubes (11 cm × 1.8 m) were filled with wilted forage at density of 168 kg DM/m³. All silos were stored at temperatures ranging from 3 to 21 °C for 180 days. After the storage, silages were analyses for chemical and microbial composition. Microbial analyses were performed by Eurofins by certified methods. Tube silos were cut by 15 cm weekly for a period of five weeks to simulate slow feed-out process and kept in room temperature about 20 °C. One silo tube was foam-covered after each removal at the thickness of 6 mm and the other remained uncovered. Weight losses, temperature, mold, yeast and chemical composition were monitored in removed silage fractions. Statistical analyses were performed using the GLM procedure in the SAS computer package (SAS Institute Inc., 1990). Analysis of variance in a completely randomized design

was used to evaluate the effect of foam thickness on silage quality. Differences between effects were calculated by t-test and expressed as least significant difference (LSD, P <0.05).

Results and Discussion

The chemical composition of forages from both cuts gave fermentation coefficient 37 and 39 which indicated similar predisposition for successful ensiling process (Weissbach et al., 1974). However, forage from third cut used in storage trial contained more legumes resulting in low water-soluble carbohydrate (WSC) content (6.2 g/kg DM) and a high buffering capacity (8.8 g lactic acid/100 g DM). Both forages were highly contaminated by aerobic microflora (5.6 and 5.5 log cfu/g sample for yeasts and moulds respectively).

Table 1 Chemical and microbial composition of silage in the foam-thickness test in the storage trial (n=3). Control = lid with fermentation lock; Foam 1 = 0.9 cm; Foam 2 = 1.7 cm; Foam 3 = 2.1 cm.

Analyses	Unit	Treatment				LSD*	P-value
		Control	Foam 1	Foam 2	Foam 3		
DM	%	34.7	34.9	35.1	34.6		
pH		5.2	5.3	5.2	5.2	0.18	0.2
NH_3-N	% total N	8.0^{c}	8.4^{b}	8.9^{a}	9.1^{a}	0.32	0.001
Lacic acid	% DM	1.9^{b}	2.2^{b}	2.4^{b}	3.8^{a}	1.07	0.02
Acetic acid	% DM	0.4^{a}	0.2^{b}	0.3^{b}	0.3^{b}	0.06	0.004
Propion. acid	% DM	0.6^{b}	0.5^{a}	0.6^{b}	0.7^{b}	0.10	0.02
Butyric acid	% DM	<0.05	<0.05	<0.05	<0.05	0.11	0.3
Ethanol	% DM	0.8^{a}	0.4^{c}	0.5^{b}	0.6^{b}	0.08	0.001
2.3-butandiol	% DM	0.6	0.6	0.5	0.6	0.16	0.3
Yeast	log cfu/g	1.7^{a}	4.2^{c}	4.2^{c}	3.9^{b}	0.22	0.001
Moulds	log cfu/g	1.7^{b}	4.3^{a}	2.7^{b}	1.7^{b}	1.36	0.01
Weight losses	% DM	4.7^{b}	16.4^{a}	15.4^{a}	15.3^{a}	6.87	0.001

Means in the same column with different uppercase letters differed (P <0.05). * Least signifiant difference.

Results from storage trail are presented in Table 1. Overall fermentation quality of silages was poor across all treatments, mainly due to low WSC content and high buffer capacity of the forage. All silages showed high pH and low lactic acid production, indicating dominance of heterofermentative lactic acid bacteria.

Table 2 Silage quality during feed-out simulation test (n=1).

Treatment	Exposure time	DM (%)	pH	Moulds	Yeasts
				log cfu/g	
Foam	1st week	34.5	4.9	<1.7	5.8
Control		35.8	5.0	<1.7	5.8
Foam	2nd week	34.6	5.0	<1.7	6.3
Control		36.2	5.1	<1.7	6.2
Foam	3rd week	34.4	5.0	3.5	6.2
Control		35.1	5.2	5.4	7.0
Foam	4th week	34.5	5.0	4.2	5.9
Control		32.5	8.1	8.0	8.0
Foam	5th week	34.5	5.0	5.2	7.0
Control		34.4	5.6	6.0	7.2

Mould growth declined with increasing foam thickness, and at ~17 mm mould was almost fully excluded. This result differs from TFZ recommendations (5–8 mm), potentially due to poorer application quality from manual pouring. However, yeast populations remained high in all foam treatments compared to control. Silage losses were greater in foam-covered silos compared with the control. Some losses in foam treatments were likely caused by evaporation

of water from the foam, which contains ~20% moisture. Results from feed-out simulation trial showed that foam covering during feed-out reduced aerobic spoilage substantially (Table 2). The foam-covered tube exhibited lower temperature increases, lower aerobic microbial growth and markedly reduced weight losses (Figure 1) compared with the uncovered control. Differences in temperature were exhibited from third week of exposure. These results show promise for foam as a feed-out barrier, especially during prolonged removal intervals.

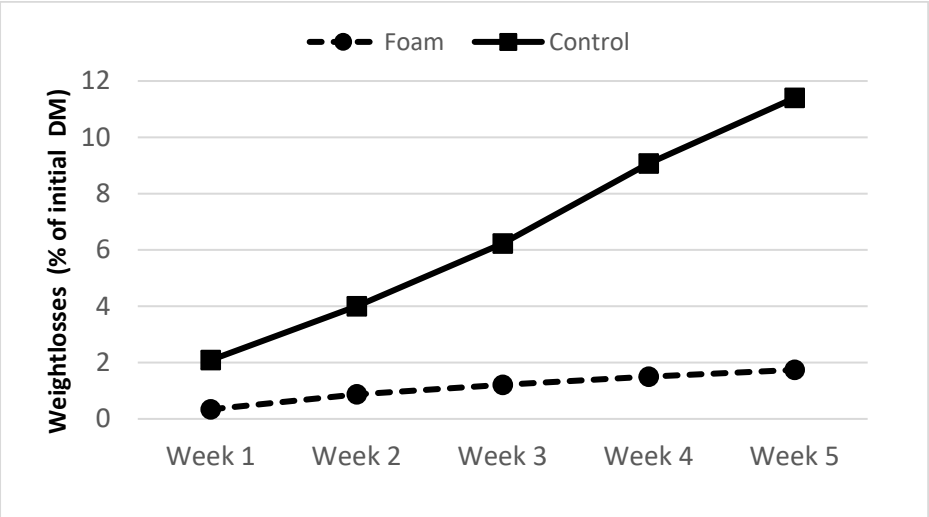


Figure 1 Accumulated weight losses of dry matter during feed-out test (n=1).

Conclusions

Foam as a silage cover shows potential, particularly for protecting exposed surfaces of silo during feed-out where it reduced aerobic losses. However, without a proper equipment foam did not achieve fermentation quality comparable to air-tight control. Proper application appears critical, especially regarding uniform thickness.

References

McDonald, P., Henderson, A.R. and Heron. S.J.E. (1991). The Biochemistry of Silage. Chalcombe Publications, 13 Highwoods Drive, Marlow Bottom, Marlow, Bucks, UK.

Muck, R.E and Holmes, B.J. (2000). Preventing silage storage losses. Biological Systems Engineering Department, University of Wisconsin, Madison & Agricultural Engineer, USDA, Agricultural Research Service, US Dairy Forage Research Center. Madison, Wisconsin.

Spörndly, R. and Nylund, R. 2016. Temperature development and dry matter losses of grass silage in bunker silos. In Udén et al (editors), Proceedings of the 7th Nordic feed science conference, Uppsala, Sweden.

Weissbach, F., Schmid, L. & Hein, E. 1974. Method of anticipation of the run of fermentation in silage making based on chemical composition of green fodder. Proc. 12th Inter. Grassland Congress, Moscow, 3, 663-673.

Ensiling characteristics of intermediate wheatgrass (IWG), Kernza ®: effects of harvest timing and silage additives.

K. Lätt[1], F. Dahlström[1], D.-A. Danielsson[1], V. Picasso[2], O. Hallin[3] and K. Arvidsson Segerkvist[1]

[1] Swedish University of Agricultural Sciences (SLU), Department of Applied Animal Science and Welfare, Skara
[2]SLU, Department of Crop Production Ecology, Uppsala
[3]Hushållningssällskapet Sjuhärad, Länghem
Correspondence: kajsa.latt@slu.se

Introduction

In Sweden and northern Europe, climatic conditions require that forage is harvested during summer and preserved as silage for winter feeding, making high-quality silage production essential for year-round livestock nutrition. Intermediate wheatgrass (*Thinopyrum intermedium*, IWG), marketed as Kernza®, is a recently introduced perennial dual-purpose wheatgrass in Sweden that can be harvested for both grain and forage. Under a changing climate, its long, deep root system confers both drought tolerance and resilience to heavy rainfall, making it a relevant complementary forage option. Recent forage evaluations show that Kernza can meet nutritional targets, while cutting frequency and timing determine dry matter (DM), water-soluble carbohydrates (WSC) and fibre at harvest, highlighting the need to evaluate fermentation quality in Swedish forage systems reliant on stored winter feed (Favre et al., 2019; Culman et al., 2023). Despite growing interest in IWG, the interaction between harvest timing and silage additives on fermentation, DM losses and aerobic stability (ASTA) remains unclear, limiting recommendations and adoption in northern Europe. The aim of this study is to evaluate the effects of harvest timing in the first cut and the use of silage additives on fermentation characteristics, DM-losses and aerobic stability of ensiled IWG.

Materials and Methods

The ensiling study of Kernza® was conducted at Rådde experimental farm in Länghem, Sweden. Fresh matter was harvested from the primary growth on 24th May and 4th June 2024 (early and late harvest) with a fertilization rate of 105 kg N/ha. After mowing, the forage was wilted to 25–30% DM, chopped and prepared for ensiling. The forage was ensiled in 0.3- or 1.5-liter glass jars. Five treatments were applied: three bacterial inoculants (homofermentative LAB_{Ho} heterofermentative LAB_{He} and combined LAB_{HoHe}), a chemical salt-based additive CHEM, and a control CON. The 0.3-liter jars were opened after three days to measure pH, while the 1.5-liter jars were opened after 111 and 114 days for early and late harvest, respectively. Aerobic stability was assessed using the method of Honig (1986). Analyses included dry matter (60 °C and 103 °C), ash (550 °C), pH, and fermentation products (lactic, acetic, propionic and butyric acid, ethanol and ammonium nitrogen).

All statistical analyses were performed in R (R Core Team, 2025). Data were analysed using a model including the effects of harvest timing, additive treatment and their interaction. Pairwise comparisons were performed using Tukey's adjustment, and differences were considered significant at $p < 0.05$.

Results and Discussion

Overall, IWG silage showed good fermentation across all treatments, with low levels of butyric acid detected. Harvest time affected several fermentation parameters and was generally more influential than additive treatment (Table 1). Changes in pH from day three to opening and after ASTA reflected fermentation rate and aerobic stability. Treatment differences appeared more pronounced at the early harvest than at the late harvest, and the additive treatments followed a similar pattern throughout the fermentation process, in line with their expected effects. For the fermentation products, IWG silage showed concentrations comparable to those of conventional grass-clover silage (Auerbach and Nadeau, 2020). DM losses tended to be higher at the early harvest than at the late harvest, with significant differences between additive treatments. All additive treatments, including inoculants and the chemical additive, numerically improved aerobic stability at the early harvest, while the chemical additive resulted in the highest aerobic stability overall.

Conclusions

IWG forage showed good ensiling properties and resulted in high-quality silage. Additives contributed to fermentation quality and improved aerobic stability, depending on the type of inoculant or chemical used.

References

Auerbach, H., & Nadeau, E. (2020). Effects of additive type on fermentation and aerobic stability and its interaction with air exposure on silage nutritive value. Agronomy, 10, 1229.

Culman, S., Pinto, P., Pugliese, J., Crews, T., DeHaan, L., Jungers, J., Larsen, J., Ryan, M., Schipanski, M., Sulc, M., Wayman, S., Wiedenhoeft, M., Stoltenberg, D., Picasso, V., 2023. Forage harvest management impacts "Kernza" intermediate wheatgrass productivity across North America. Agronomy Journal 115, 2424–2438. https://doi.org/10.1002/agj2.21402

Favre, J.R., Castiblanco, T.M., Combs, D.K., Wattiaux, M.A., Picasso, V.D., 2019. Forage nutritive value and predicted fiber digestibility of Kernza intermediate wheatgrass in monoculture and in mixture with red clover during the first production year. Animal Feed Science and Technology 258, 114298. https://doi.org/10.1016/j.anifeedsci.2019.114298

Honig, H., 1986.Evaluation of aerobic stability. In Proceedings of the EUROBAC Conference, Uppsala, Sweden,12–16 August 1986.

R Core Team (2025). _R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Table 1 Ensiling characteristics, fermentation profile and aerobic stability of Kernza® harvested at two harvest time from the primary growth during 2024, g kg^{-1} dry matter unless stated otherwise.

Parameter	Early					Late					P-value		
	CON	LAB_{Ho}	LAB_{HoHe}	LAB_{He}	CHEM	CON	LAB_{Ho}	LAB_{HoHe}	LAB_{He}	CHEM	Harvest time	Additive	H×A
DM (%)	25.5^{bc}	25.7^{cd}	25.1^{ab}	24.7^{a}	25.7^{cd}	25.4^{bc}	25.6^{cd}	26.3^{e}	25.5^{bc}	26.0^{de}	<0.001	<0.001	<0.001
DM loss (%)	5.0^{bc}	4.1^{ab}	5.4^{c}	6.6^{d}	4.6^{abc}	4.5^{abc}	4.2^{ab}	4.3^{ab}	4.5^{abc}	3.6^{a}	<0.001	<0.001	<0.001
Ash (%)	7.29^{b}	7.18^{b}	7.31^{b}	7.73^{c}	7.39^{bc}	5.94^{a}	5.91^{a}	5.92^{a}	5.98^{a}	5.83^{a}	<0.001	0.008	0.049
pH day 3	5.34^{a}	4.12^{a}	4.06^{a}	4.37^{a}	5.54^{a}	5.12^{a}	4.06^{a}	4.09^{a}	4.14^{a}	4.73^{a}	0.206	0.001	0.693
pH at opening	4.01^{cd}	3.89^{ab}	3.97^{c}	4.12^{e}	4.04^{d}	3.90^{ab}	3.86^{a}	3.88^{ab}	3.91^{b}	4.04^{d}	<0.001	<0.001	<0.001
WSC	0.24^{a}	1.67^{d}	0.13^{a}	0.12^{a}	0.86^{b}	0.28^{a}	0.21^{a}	0.16^{a}	0.26^{a}	1.35^{c}	0.001	<0.001	<0.001
Lactic acid	83.62^{b}	84.90^{b}	86.94^{b}	72.40^{b}	80.20^{b}	65.04^{b}	67.72^{b}	65.13^{b}	65.08^{b}	40.55^{a}	<0.001	0.007	0.022
Acetic acid	9.37^{bc}	4.45^{a}	6.82^{ab}	9.67^{bc}	10.84^{c}	10.74^{c}	8.50^{bc}	10.23^{bc}	10.85^{c}	10.03^{bc}	<0.001	<0.001	0.018
Propionic acid	1.87^{c}	0.70^{a}	1.41^{b}	1.41^{b}	1.42^{b}	0.68^{a}	0.69^{a}	0.67^{a}	0.69^{a}	0.65^{a}	<0.001	<0.001	<0.001
Butyric acid	0.703^{a}	0.702^{a}	0.705^{a}	0.707^{a}	0.937^{a}	0.916^{a}	0.689^{a}	0.667^{a}	0.692^{a}	0.654^{a}	0.678	0.607	0.243
Ethanol	9.37^{d}	3.74^{abc}	16.68^{e}	24.05^{f}	5.19^{bc}	3.65^{abc}	3.21^{ab}	6.67^{cd}	9.00^{d}	1.54^{a}	<0.001	<0.001	<0.001
NH3-N	0.207^{d}	0.121^{a}	0.175^{c}	0.209^{d}	0.206^{d}	0.126^{ab}	0.120^{a}	0.122^{a}	0.123^{a}	0.152^{bc}	<0.001	<0.001	<0.001
pH after ASTA	7.20^{d}	6.17^{bcd}	4.47^{ab}	4.37^{ab}	4.00^{a}	7.07^{cd}	7.30^{d}	5.20^{abc}	5.93^{abcd}	4.27^{ab}	0.010	<0.001	0.258
Aerobic stability (h)	191^{a}	240^{abc}	282^{abc}	316^{bc}	336^{c}	212^{ab}	203^{a}	230^{abc}	209^{ab}	325^{c}	0.013	<0.001	0.080

CON= No additive treatment, LAB_{Ho} = additive with homofermentative lactic acids bacteria, LAB_{HoHe} = additive with a combination of homofermentative and heterofermentative lactic acids bacteria, LAB_{He} = additive with heterofermentative lactic acids bacteria, CHEM = salt-based additive. ASTA= aerobic stability. LSmeans with superscripts, unlike letters means $p < 0.05$.

Assessing the energy and protein value of timothy hay harvested at different maturities for horses – A pilot study

R.B. Jensen[1] & C.E. Müller[2]
[1]*Aarhus University (AU), Department of Animal and Veterinary Sciences, Blichers Allé 20, 8830 Tjele*
[2]*Swedish University of Agricultural Sciences (SLU), Department of Applied Animal Science and Welfare, Box 7024, 75007 Uppsala, Sweden.*
Correspondence: rbj@anivet.au.dk

Introduction

Several feed evaluation systems are used for horses (NRC, 2007; GfE, 2014, 2025; CVB, 2023; INRA, 2015), complicating direct comparisons of energy and protein values and creating challenges across the equine sector due to limited harmonisation. Forage, the main component of equine diets, is often harvested at later developmental stages than for ruminants, yet shows considerable variation in nutritional value, necessitating reliable analyses. Near-infrared spectroscopy (NIRS) is widely applied by commercial laboratories to estimate forage nutritive value; however, variability in NIRS results and derived energy and protein estimates has been reported (Harris et al., 2018; Le Cocq et al., 2022). Therefore, this pilot study compared NIRS-based estimates of energy and protein values in forage harvested at different developmental stages with values derived from chemical analyses and aimed to provide guidance for future feed evaluation in the Nordic countries regarding the energy and protein value of feeds.

Materials and Methods

Timothy grass (*Phleum pratense L.*) harvested every sixth day from three experimental plots at the Norwegian University of Life Sciences (NMBU), Ås, Norway, between 22 May and 3 July 2021 was used in this pilot study. This resulted in a total of 24 samples representing eight distinct developmental growth stages. The experimental plots were fertilized with 30 kg N/ha in autumn 2020 and 100 kg N/ha in spring 2021. Grass was cut manually using electric scissors at 17:00 h. Plant biomass from each plot and harvest was oven-dried at 45 °C for 48 h to preserve the samples in a form representative of hay production. The dried samples were subsequently milled to pass a 1-mm screen and stored until further analyses. Samples were analysed for crude protein (CP) using standard chemical methods (Thorringer and Jensen, 2021). In addition, all samples (approximately 100 g) were analysed by four commercial laboratories using NIRS to estimate energy and protein values. It was exactly the same samples that were sent to all laboratories. Agreement between NIRS-based estimates of energy value from the laboratories and between NIRS-derived CP and chemical analyses of CP was evaluated using Bland–Altman plots. Differences between paired measurements were plotted against the mean of the two values, and the 95% confidence interval represented the expected range within which 95% of the differences would lie (Bland & Altman, 1999). Energy and protein values were estimated according to the German feed evaluation system (GfE, 2014; 2025), as described below.

Estimating energy value of feeds

The metabolizable energy (ME) content was calculated based on the following equation:

1) ME (MJ/kg DM) = Gross Energy (GE, MJ/kg DM) × Energy Digestibility (ED, %) – Urinary Energy (UE, MJ/kg DM) – Methane Energy (CH_4-E, MJ/kg DM)

Where GE, ED, UE and CH_4-E are calculated as:

2) GE (MJ/kg DM) = 23.6 × CP (g/kg DM) + 39.8 × crude fat (CF, g/kg DM) + 17.3 × starch (ST, g/kg DM) + 16.0 × SU (sugar, g/kg DM) + 18.9 × organic residue (OR, g/kg DM)
    a. OR (g/kg DM) = Organic matter (OM) – CP – CF – ST – SU
3) ED (%) = OM digestibility (OMD_{Horse}) (%) – 3.4
    a. OMD_{Horse} = -K + 1.15 × $OMD_{Ruminant}$
    b. K-values for different feeds are presented in GfE (2025) and varies between 11.5 to 22.0. A K-value of 17.5 is used which also corresponds to the values used in the Dutch system when converting $OMD_{Ruminant}$ to OMD_{Horse}
4) UE (MJ/kg DM) = 0.33 + 0.0043 × CP (g/kg DM)
5) CH_4-E (MJ/kg DM) = 0.00083 × OR (g/kg DM)

Estimating protein value of feeds

The protein value of feeds is expressed as pre-caecal digestible crude protein (pcdCP) in the German system (GfE, 2014), based on analysis of CP and neutral detergent insoluble CP (NDICP) and subsequent estimation of the neutral detergent soluble CP (NDSCP) fraction.

6) NDSCP (g/kg DM) = CP – NDICP
7) $pcdCP_{Analysed}$ (g/kg DM) = 0.9 × NDSCP

Bockisch et al. (2023) suggested to used the following equation when the NDICP is not known:

8) $pcdCP_{Estimated}$ (g/kg DM) = -32.36 + 0.860 × CP (g/kg DM)

Results and Discussion

Energy and protein values were reported differently among laboratories, as expected. All laboratories reported CP, and pcdCP was estimated using equation 8. Mean values for pcdCP and ME are shown in Figure 1. Bland–Altman analysis showed that the bias ± SD for pcdCP was 8.4 ± 6.7, −1.6 ± 5.5, 1.4 ± 8.7, and 3.2 ± 7.5 g/kg DM when chemical analyses were compared with NIRS results from laboratories 1–4, respectively. The good agreement between chemical analyses and NIRS for CP has also been reported previously (Harris et al., 2018), indicating that pcdCP can be estimated and compared across laboratories. However, further validation and *in vivo* studies on feeds used for horses in the Nordic countries, particularly forages, are needed to ensure biological relevance. One laboratory reported ME_{Horse}, and two laboratories reported $OMD_{Ruminant}$, enabling calculation of ME_{Horse} according to equations 1–5. The last laboratorie did not report OMD. Bland–Altman analysis showed biases ± SD for ME of 0.20 ± 0.28 (NIRS1 vs. NIRS2), −0.06 ± 0.38 (NIRS1 vs. NIRS3), and −0.26 ± 0.38 (NIRS2 vs. NIRS3). The results from this pilot study indicate that it is possible for laboratories to report ME_{Horse} and pcdCP in a comparable way when calculations are based on equations from GfE (2014) for protein and GfE (2025) for energy. These recommendations support ongoing work on updating and standardising Nordic feeding recommendations for horses (Jensen and Müller, 2026).

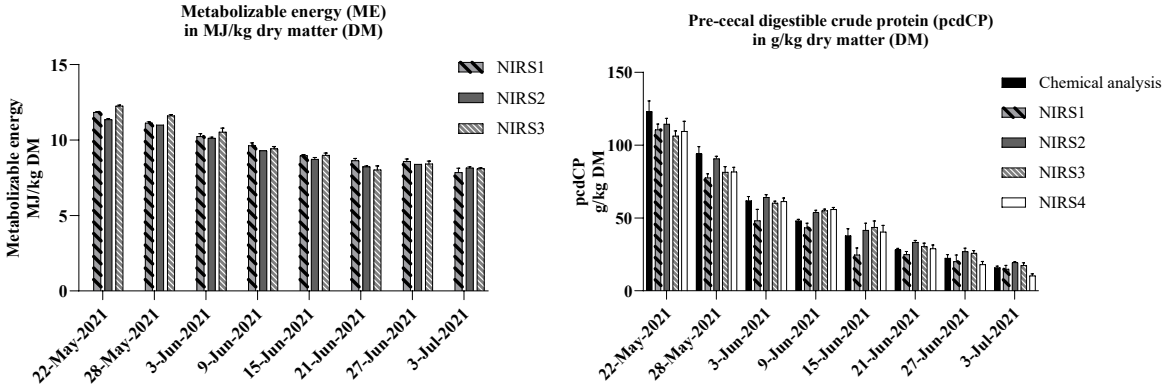


Figure 1 Metabolisable energy (ME) and pre-caecal digestibility of crude protein (pcdCP) of timothy grass harvested at eight different developmental stages. Both ME and pcdCP were estimated using near-infrared spectroscopy from four commercial laboratories (NIRS1-4), while pcdCP was additionally determined by chemical analysis for comparison.

Conclusions

The results demonstrate that ME and pcdCP of forage can be reported consistently according to the German feed evaluation system. However, further work should focus on validation using feeds and feeding conditions commonly utilized in the Nordic countries. Procedures should be further standardised and adopted by laboratories to ensure transparency in how results are derived and to improve comparability across laboratories.

References

Bockisch, F., Taubert, J., Coenen, M., and Vervuert, I. 2023. Protein Evaluation of feedstuffs for horses. Animals, 13, 2624.

CVB, 2023. Chemical composition and nutritional values of feedstuffs. Stichting CVB, The Netherlands.

GfE, 2014. Empfehlungen zur energie- und nährstoffversorgung von pferden. DLG-VErlag Gmbh, Frankfurt am Main, Germany.

GfE, 2025. The three-step procedure for determinig the metabolisable energy of feeds for horses. In: Proceedings of the society of nutrition physiology, Göttingen, Germany.

Harris P.A., Nelson, S., Carslake, H.B., Argo, C.M., Wolf, R., Fabri, F.B., Brolsma, K.M., van Oostrum, M.J., Ellis, A.D. 2018. Comparison of NIRS and wet chemistry methods for the nutritional analysis of haylages for horses. J. Equine Vet. Sci. 71, 13-20

INRA, 2015. Equine nutrition. Wageningen Academic Publishers, Wageningen, The Netherlands

Jensen, R.B and Müller, C.E. 2026. Energy and protein evaluation of horse feeds in the Nordic countries. Proceedings of the 13th Nordic Feed Science Conf., Uppsala, Sweden.

Le Cocq, K., Harris, P., Bell, N., Burden, F.A., Lee, M.R.F., Davies, D.R. 2022. Comparisons of commercially available NIRS-based analyte predictions of haylage quality for equid nutrition. Anim. Feed Sci. Technol. 283, 115-158.

NRC, 2007. The nutrient requirements of horses. 6th edition. National Academy Press, Washington DC.

Thorringer, N.W. and Jensen, R.B. 2021. Methodical considerations when estimating nutrient digestibility in horses using the mobile bag technique. Animal, 15, 100050.

Beet types high in dry matter have higher sugar proportions of organic matter, diluting other nutrients

E. M. V. Hvas[1], M. Larsen[1], L. Andersen[2], U. Bedenk[3], M. R. Weisbjerg[1].
[1]Aarhus University (AU), Department of Animal and Veterinary Sciences, AU Viborg, Research Centre Foulum, Blichers Alle 20, 8830 Tjele, Denmark.
[2]KWS SCANDINAVIA A/S, Lysholt Allé 10, 7100 Vejle, Denmark
[3]KWS SAAT SE & Co. KGaA, Grimsehlstr. 31, 37574 Einbeck, Germany.
Correspondence: martin.weisbjerg@anivet.au.dk

Introduction

Beets (*Beta vulgaris*) are cultivated both for feed and for industrial sugar production. A high yield of digestible biomass per area combined with a long growth season that reduces nitrogen leakage from soil makes beets one of the most interesting annual crops in some temperate regions. Traditionally, fodder beets with lower dry matter (DM) concentration were used as feed for cattle and other production animals, while sugar beets with higher DM were used for sugar production. Feeding fresh beets is still practiced, but ensiled beets have become the most common way to feed beets to dairy cows in Denmark, resulting in feed with high ethanol concentrations due to the high sugar content before ensiling (Hvas et al., 2025). Ensiling has increased interest in high DM beets to reduce effluents (Hvas et al., 2024), and this has brought the DM concentration of fodder beets closer to that of sugar beets. However, the impact of beet DM concentration on nutrient composition in DM is not well described. Soil contamination, and thereby ash concentration, can vary considerably in beets and may obscure the effect of DM concentration on nutrient composition when expressed per kg DM rather than per kg organic matter (OM). The aim of the present study was to examine the impact of beet DM concentration on nutrient composition in beet OM and DM.

Materials and Methods

A total of 89 beet samples were collected over three growing seasons (2021–2023) from six European countries (Denmark, Sweden, Germany, Netherlands, Belgium, France) and categorised as high or medium DM types (≥190 or <190 g DM/kg fresh matter (FM), respectively). Nine high and three medium DM varieties were sampled. All samples were collected following a defined sampling protocol. Beets were collected from piles 2–4 weeks after harvest (mid-October to early December). Each sample was transferred into three mesh bags of 20 kg each and transported directly after sampling to the processing facility at the KWS station in Klein Wanzleben, Germany. Samples were processed immediately or stored outside (5–10°C) for no longer than three days before processing. Beets were either dry-cleaned or washed before subsamples from approx. 60 kg were obtained using an eight-bladed saw. The resulting brei was homogenised, shock frozen at –40°C, and stored at –20°C. Chemical analyses were performed either on frozen brei or freeze-dried samples and included ash (550°C), sugars (Larsson and Bengtsson, 1983), crude protein (CP, Dumas), NDF (Fibertec), minerals (ICP), and chloride (Potentiometric titration with silvernitrate, LaCroix et al., 1970).

All statistical analyses were performed in R 4.1.2 (R Core Team, 2021). Data were analysed using a linear model (lm function, lme4 package; Bates et al., 2015) with one-way ANOVA.

Results and Discussion

Dry-cleaned beets (N=57) contained 230 and 165 g DM/kg FM, 218 and 149 g OM/kg FM, and 55 and 97 g ash/kg DM, while washed beets (N=32) contained 214 and 156 g DM/kg FM, 209 and 148 g OM/kg FM, and 26 and 48 g ash/kg DM for high and medium DM types, respectively. Due to the large variation in ash, especially between washed and dry-cleaned beets, chemical composition in Table 1 is expressed per kg OM to eliminate the effect of soil contamination.

Table 1 Composition of nutrients in proportion of organic matter (g/kg OM) in beets with high and medium dry matter concentration

	High	Medium	*P*-values
N	52	37	
Total sugar	816	754	<0.01
Crude Protein	38	59	<0.01
NDF	96	120	<0.01
Cloride	0.1	0.4	<0.01
Sodium	0.9	2.9	<0.01
Potassium	9.9	22.2	<0.01
Magnesium	1.5	1.6	0.14

Sugar concentration in OM was 62 g/kg higher in high DM than in medium DM beets (Table 1). At the same time, other nutrients had lower concentrations (CP -21, NDF -24, chloride -0.4, sodium -2.1, potassium -12.3, magnesium -0.1 g/kg OM). Thus, increased DM concentration in high DM beets is mainly due to increased sugar, and the higher sugar content dilutes other nutrients in OM.

The increase in sugar (62 g/kg OM) was similar to the increase in OM (66 g/kg FM, weighted averages (215 and 149, respectively) of above reported OM concentration in FM in dry-cleaned and washed beets) from medium to high DM beets, indicating that increased DM is largely driven by increased sugar. Average dilution of non-sugar organic compounds compared to sugar was 0.69 in high compared to medium DM beets. If OM composition changed only due to dilution with sugar, all other nutrients should decrease proportionally. However, as shown in Figure 1, this is not the case. In high DM beets, CP reduced more than NDF, and for minerals chloride, sodium, and potassium reduced much more than magnesium. This inconsistent dilution pattern may be explained by root morphology. Medium DM beets have a larger portion of the root above ground with a higher proportion of hypocotyl tissue, while high DM beets develop deeper underground roots composed mainly of storage tissue. The hypocotyl region contains more minerals and protein, explaining the lower CP content in high DM beets (Enchev and Bozhanska, 2022). Consequently, high DM beets allocate more biomass to parenchyma cells with large vacuoles for sucrose storage, reducing the relative concentration of other nutrients.

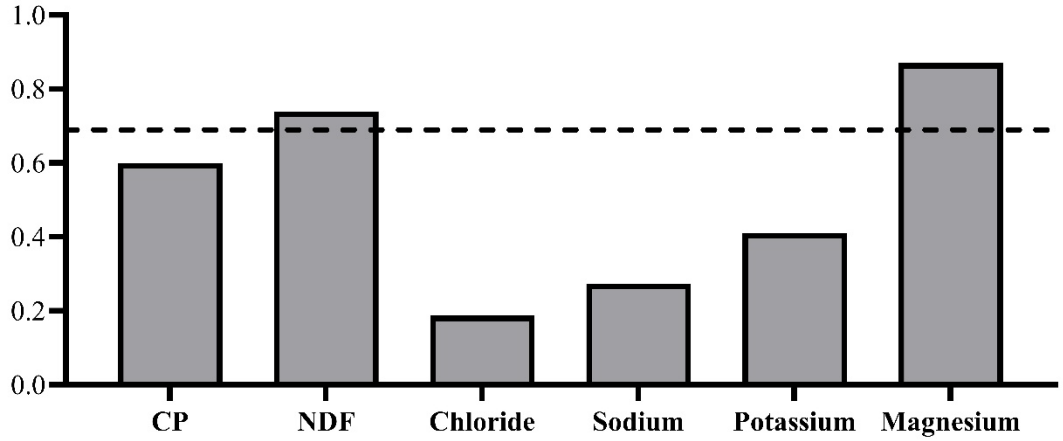


Figure 1 Relative dilution of some organic and inorganic nutrients with increasing sugar content, expressed as the average ratio of nutrient-to-sugar in high DM beets compared to medium DM beets calculated as (nutrient/total sugar)$_{\text{high DM beets}}$/(nutrient/total sugar)$_{\text{medium DM beets}}$. Stipulated line (0.69) is the average ratio of non-sugar organic nutrients.

Conclusions

Beets with high DM compared to medium DM have a higher sugar proportion of OM, thereby diluting the proportion of other nutrients. However, this is not a simple dilution, as nutrients other than sugar do not all decrease by the same factor.

References

Enchev, S., Bozhanska, T., 2022. Chemical composition of sugar beet, fodder beet and table beet depending on the harvest period. Bulg. J. Agric. Sci. 28, 1034-1039.

Hvas, E. M. V., Hanigan, M. D., Weisbjerg, M. R., Larsen, M., 2025. Effects of feeding beets and ensiled beets on rumen volatile fatty acid and ethanol metabolism in dairy cows: J. Dairy Sci. 108, 9449-9469. https://doi.org/10.3168/jds.2024-26194.

Hvas, E.M.V., Larsen, M., Andersen, L., Bedenk, U., Weisbjerg, M.R. 2024. Ensiling sugar beets: Effect of mixer feed used for co-ensiling on fermentation products and losses when ensiled in vacuum bags. Anim. Feed Sci. Tech. 318. 116101. https://doi.org/10.1016/j.anifeedsci.2024.116101.

LaCroix, R.L., Keeney, D.R., Walsh, L.M. 1970. Potentiometric titration of cloride in plant tissue extracts using the chloride ion electrode. Soil Science and Plant Analysis 1, 1-6.

Larsson, K., Bengtsson, S., 1983. Determination of readily available carbohydrates in plant material. Methods report No. 22, National Laboratory of Agricultural Chemistry, Uppsala, 514 SE.

Determination of water concentration in cattle feed by Karl Fischer titration

N. B. Kristensen
Department of Animal and Veterinary Sciences
Aarhus University, Blichers Allé 20, DK-8830 Tjele, Denmark
Correspondence: nielsbastian@anivet.au.dk

Introduction

The NorFor feed evaluation system defines dry matter (DM) of silage from recovered mass after drying samples to constant weight at 60°C followed by correction for loss of volatiles (Volden, 2011). It was previously reported that residual weight loss of grass silages, dried at 103°C, following initial drying at 60°C (WL60-103), was a trait with large variability and correlated to key feed evaluation traits like OMD (Kristensen, 2024). The Karl Fischer (KF) titration method has previously been suggested as a reference for NIR calibrations to be used for predicting water concentrations in silage (Abrams et al., 1988). The present study was undertaken to compare water concentrations obtained from KF titration of cattle feed with NorFor DM and to use KF titration for determination of residual water concentrations in samples dried at 60°C.

Materials and Methods

Silage samples were collected as face samples from open silage clamps or obtained as dried and milled samples from SEGES Innovation P/S (Aarhus N, Denmark). Dry samples were milled on a 0.5 mm screen (Peppink 200CM, Peppink Mills b.v., Olst, The Netherlands), scanned by FT-NIR (Bruker MPA, Bruker, Ettlingen, Germany), and residual weight loss (drying at 103°C) was predicted from inhouse prediction models (SEGES Innovation P/S).

A dedicated titrator (TitroLine® 7500 KF, SI Analytics, Xylem Inc., Washington, DC, USA) was used for KF titrations. The two-component titration method was used based on Hydranal Solvent Medium (methanol, imidazole and sulphur dioxide) and Hydranal Titrant 5 (Honeywell / Fluka). Solvent extractions were performed using dry methanol and dry formamide (1:1, Honeywell / Fluka) except for initial tests comparing extractions in dry methanol with methanol:formamide extraction.

Approximately 4 g (exact weight recorded) of silage samples and approx. 5 g of dry milled samples were weighed into 100-mL oven dried screw cap bottles. Into the bottles were added 50 mL of dry methanol and 50 mL of dry formamide. The bottles were closed and mixed vigorously, then allowed to sit for approx. 20 min and mixed again. Bottles were incubated for 20 h at room temperature. A solvent blank was included in each batch. Before titration, approx. 10 mL of solvent was transferred to a 16 mm plastic tube (also blank extraction) and centrifuged at 3.000 × g for 10 min at room temperature.

Samples were injected into the titration vessel with a two-component syringe (#309110; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and a 21G × 80 mm hypodermic needle (Sterican®, B. Braun Melsungen AG, Melsungen, Germany). All incubations were measured gravimetrically using a 4-digit lab scale. The sample was aspirated with a small air gab in the needle, the needle was wiped clean, and capped. The syringe with solvent, needle, and cap was weighed. After weighing the sample was immediately injected into the titration vessel, and finally the syringe with needle, and cap was weighed. Injected amount was calculated by difference.

Before analysing a sample batch, the titrant was adjusted by injecting a water standard with a known water concentration close to 1.000 mg/g (Honeywell / Fluka). Then the solvent blank was titrated to determine the water background of the solvent mix.

Data was processed with two iterations. The first iteration was used to correct the solvent volume in the incubation bottle i.e. the total amount of solvent in the bottle was set to the amount of methanol, formamide, and water in the sample. Water in the sample (mg water/g sample) was calculated as: water equivalent of titrant (mg/mL) × solvent in the incubation bottle (g) × [titrant consumed in titration (mL) / injected amount (g)] / sample amount (g). From the first iteration the amount of water in the sample was added to the total solvent in the extraction bottle and the calculation repeated. The difference between the two iterations had no effect on dry samples; however, when titrating silages with low DM concentrations and water-based solutions the correction increased the calculated water concentration of the sample.

Data was analysed using PROC UNIVARIATE and PROC MEANS in SAS (SAS Institute Inc., Cary, NC, USA). Water concentrations from KF titrations are presented as % water in the sample ± standard error of the mean.

Results and Discussion

Initially water extraction by methanol and 1:1 mixture of methanol and formamide was compared in dried and milled samples with and without storage after milling. The observed water concentration ranged from 0.9 to 4.2 % with a mean of 1.9 ± 0.2 % and the difference between the extraction methods was 0.19 ± 0.04 % units ($P < 0.001$). The largest water concentrations were observed in samples extracted by a mixture of methanol and formamide.

Formamide is often used to improve extraction of water in complex matrices and has been used previously for analysis of forage-based feed samples (Thiex and Van Erem, 1999). The increased water extraction observed in the present study in combination with previously published methods was the reason for using a methanol formamide mixture for all following extractions.

A small sample set of three grass silages was dried using three different methods: freeze drying, drying at 60°C and drying at 103°C. NIR predicted WL60-103 was 9.4 ± 0.6, 9.1 ± 2.3, and 5.3 ± 2.1 % for samples dried by freeze drying, 60°C, and 103°C, respectively. After NIR scanning the samples were stored at lab bench conditions for several days and then water concentration of the samples was determined by KF titration. The water concentration determined by KF titration was lower (difference 5.3 ± 0.6 % units; $P < 0.001$) compared with NIR predicted WL60-103 and found to be 3.6 ± 0.3, 2.3 ± 0.2, and 2.1 ± 0.2 % water, respectively. Data indicates that WL60-103 over predicts water concentration, that most of the weight loss is supposed to be loss of volatiles or water from condensation reactions.

A set of 8 grass silages were transported about 1 h by car in closed containers immediately after NIR scanning at SEGES Innovation P/S. These samples were weighed into extraction bottles immediately after arrival at the lab and the KF water concentration was on average 1.08 ± 0.07 %. KF titration of dried and milled samples showed that the vast majority of the WL60-103 weight loss is not water. Lactic acid polymerization by condensation with loss of water probably explain a large part of the weight loss when drying a 103°C (Garlotta, 2001).

To evaluate the use of KF titration for determination of water in feed samples before drying, a small sample set, containing 1 sample of corn silage, 3 samples of compound feed, and 16 samples of grass silage / haylage was analyzed by KF titration parallel to drying at 60°C (forages) or 103° (compound feed). The DM of forage was corrected using the simple NorFor correction (Volden, 2011). Figure 1 shows the relative KF non-water fraction / NorFor DM.

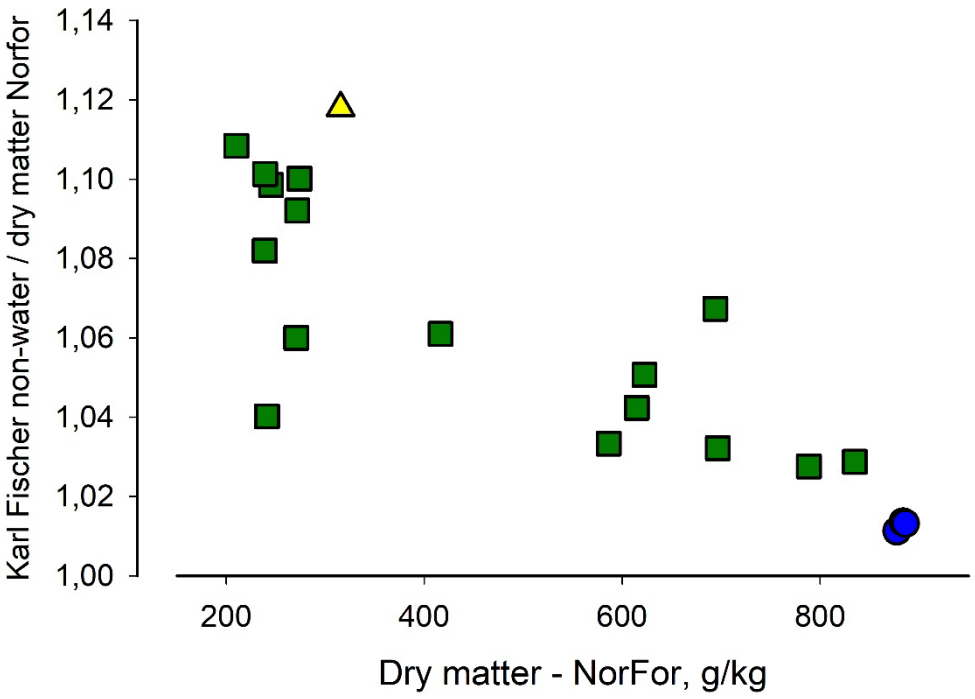


Figure 1 Non-water fraction (based on Karl Fischer titration) relative to dry matter determined according to NorFor (forage dried at 60°C with simple correction for volatile loss and compound feed dried at 103°C). In forage the non-water loss with drying is variable and not accounted for by simple NorFor correction. One data point per silage is shown. Data represents 1 sample of corn silage (triangle), 3 samples of compound feed (circles), and 16 samples of grass silage / haylage (squares).

It was not possible to account for the loss of volatiles in forages by simple correction of drying loss and the variability between samples were apparently increasing with decreasing DM. It appears that the NorFor feed evaluation system underestimates the non-water intake which will affect the calculated energy efficiency.

KF titration is not an operational tool for routine analyses of forage samples. To implement the KF method in NorFor it is necessary to implement use of double NIR scanning i.e. scanning of samples as received (AsIs scanning) in combination with scanning of dry milled samples for prediction of nutrient composition. This approach would need redefinition of dry matter in NorFor and reparameterization of the model.

Conclusions

Drying loss of forage samples at 103°C after initial drying at 60°C is only to a small extent related loss of free water. A water concentration of approx. 1 % was observed in samples processed immediately after drying at 60°C. Samples took up water from air at storage. Considerable variation between NorFor corrected DM and non-water fraction of silage were observed with largest variation for silages with low DM concentrations. Drying loss of volatiles not accounted for by DM correction will lead to erroneously low energy values of especially low DM silages.

References

Abrams, S. M., J. S. Shenk, and H. W. Harpster. 1988. Potential of near infrared reflectance spectroscopy for analysis of silage composition. J. Dairy Sci. 71,1955-1959. https://10.3168/jds.S0022-0302(88)79766-0

Garlotta, D. 2001. A literature review of poly(lactic acid). J. Polym. Environ. 9, 63-84. https://doi.org/10.1023/A:1020200822435

Kristensen, N. B. 2024. Residual weight loss of grass and grass-clover silages dried at 103°C after pre-drying at 60°C Pages 122-124 in Proceedings of the 12th Nordic Feed Science Conference. SLU Uppsala, Sweden. https://doi.org/10.54612/a.4h6nuvh43i

Thiex, N. and T. Van Erem. 1999. Comparisons of Karl Fischer method with oven methods for determination of water in forages and animal feeds. J. AOAC Int. 82,799-808. https://doi.org/10.1093/jaoac/82.4.799

Volden, H. 2011. NorFor - The Nordic Feed Evaluation System. Eur. Assoc. Anim. Prod. Publ. 130. Wageningen Acad. Publ., Wageningen, The Netherlands.

Horse preference for silage press cake from green biorefinery compared to lucerne - preliminary studies

C.E. Müller[1*], E. Brodin[2], F. Karlsson Lindgren[2], & J. Yngvesson[1]
[1]Swedish University of Agricultural Sciences (SLU), Dep. of Applied Animal Science and Welfare, SE-75007 Uppsala, Sweden, [2]Axevalla Horse Centre 1, SE-53292 Axvall, Sweden
Correspondence: Cecilia.Muller@slu.se

Introduction

Green biorefinery of freshly harvested ley crops or silage include pressing the forage to obtain juice that can be further processed to a protein-rich feed for primarily pigs, poultry and fish. The remaining fibre pulp, also known as press cake, has been reported to have a higher concentration of fibre and insoluble protein and a lower concentration of N and P compared to the original silage (Damborg et al., 2018). This can make it a feed of interest for adult horses in low to moderate exercise, where requirements of energy and protein are comparably low. Feeding a roughage that does not oversupply N or P to the horse can contribute to decrease plant nutrient leakage from horse management, which is desirable from environmental and sustainability perspectives (Saastamoinen *et al*., 2020, 2021). To evaluate silage press cake as a feed for horses, the first step was to test if horses would accept to eat it as it is well known that horses may show feed refusal of novel feeds (van den Berg *et al*., 2016), and if the feed is not voluntarily consumed it is of little value. Feed acceptance can be studied in several ways, of which preference tests can provide more information compared to simple acceptance tests. In this study, horse preference for fresh and pelleted silage press cake was therefore compared to lucerne (*Medicago sativa* L.) chaff and lucerne pellets. Lucerne was chosen as the comparative feed as it is rather well-studied and is generally regarded as a feed of high preference in horses.

Materials and Methods

Two separate experiments were conducted where horse preference for fresh silage press cake compared to lucerne chaff was studied in Experiment I (February 2024), and pelleted silage press cake compared to pelleted lucerne was studied in Experiment II (March 2025). The horses in the study were all adult Icelandic horses, Standardbred trotters and warmblood riding horses used for equitation education purposes at Axevalla Horse Centre, Axvall, Sweden. There were 23 horses in Experiment I (eight Icelandic, eight warmblood and seven standardbred horses) and 22 horses in Experiment II (six Icelandic, eight warmblood and eight Standardbred horses). No ethical permit was required for the study.

In Experiment I, horse preference was studied for fresh silage press cake and dried lucerne chaff (Rumiluz poultry SB, Desialis, France). The silage press cake originated from biorefined (screw-pressed silage with separation into silage juice and the remaining press cake) bunker silo silage from the primary harvest of a ley consisting of 45 % timothy (*Phleum pratense* L.), 25 % meadow fescue (*Festuca pratensis* Huds.), 10 % perennial ryegrass (*Lolium perenne* L.), 15 % red clover (*Trifolium pratense* L.) and 5 % white clover (*Trifolium repens* L.). In Exp. II, horse preference was studied for pelleted silage press cake and pelleted lucerne (Hippo Lusern, Svenska Foder AB, Lidköping). The pelleted silage press cake came from biorefined round bale silage from the primary harvest of a ley consisting of 91.5 % timothy and meadow fescue, 5.2 % unspecified weeds, and 3.3 % red and white clover.

In both experiments, the horses were given simultaneous access to the two experimental feeds (0.5 kg dry matter (DM) per horse for each of the feeds) for 30 minutes per day during three

consecutive days. For the pelleted feeds, 500 ml water was added just prior to feeding (to reduce the risk of oesophageal obstruction in the horses at fast consumption). The feeds were sampled each test day and analysed for chemical composition as described by Müller and Udén (2007). The feeds under study were fed as an extra feed and were not included in the ordinary feed ration, which was calculated to cover the individual energy and nutrient requirements of the horses. The ordinary feed ration consisted of 1.3-2.2 kg DM haylage per 100 kg body weight depending on the breed/horse type and exercise level. All horses also received 50-125 g mineral feed per day and had free access to drinking water and a saltlick. In addition, Standardbreds were fed 0.5-3 kg concentrate feeds, 0.3 kg molassed sugar beet pulp and 20 g table salt per horse and day, and Icelandic horses had access to cereal straw.

Preference was observed as the first feed the horses chose to eat from ("First choice", defined as the first feed the horse approached and was observed to ingest), and the consumed amount of each feed (in kg) was registered. Within each of the experiments, "First choice" was analysed by Chi^2 tests, feed consumption by a one-way ANOVA mixed model procedure with individual horse as random factor to account for repeated measurements, and the composition of the experimental feeds by a one-way ANOVA and general linear models, all in SAS version 9.4 for Windows (SAS Institute, Cary, USA), where $P<0.05$ were interpreted as showing statistical difference.

Results and Discussion

The results showed that all experimental feeds differed from each other in all analysed variables within each experiment (Table 1). In Experiment I, the horses choose fresh silage press cake as frequently as lucerne chaff as their first choice (Figure 1). The horses consumed on average 40 per cent of the offered amount of fresh silage press cake and 64 per cent of the offered amount of lucerne chaff ($P<0.001$).

In Experiment II, horses choose pelleted lucerne more often than pelleted silage press cake (Figure 1). On average, horses consumed 6 per cent of the offered amount of pelleted silage press cake, and 94 % of the offered amount of pelleted lucerne ($P<0.001$).

Table 1 Chemical composition (per kg dry matter if not otherwise mentioned) in experimental feeds used in Experiment I and II. Mean values and standard error in brackets, n=3

Variable	Experiment I		Experiment II		P-value
	Fresh silage press cake	Lucerne chaff	Pelleted silage press cake	Pelleted lucerne	
Dry matter g/kg	445^{a} (0.3)	802^{b} (0.2)	840^{d} (0.1)	835^{c} (0.0)	<0.001
Metabolizable energy for horses, MJ	12.4^{c} (0.10)	7.3^{a} (0.04)	10.5^{b} (0.07)	7.5^{a} (0.33)	<0.001
Digestible crude protein, g	86^{c} (1.4)	68^{b} (2.3)	40^{a} (2.3)	136^{d} (1.4)	<0.001
Neutral detergent fibre, g	504^{c} (4.9)	489^{b} (3.5)	653^{d} (5.3)	432^{a} (5.4)	<0.001
Water soluble carbohydrates, g	71^{c} (1.6)	105^{d} (2.0)	10^{a} (0.2)	26^{b} (0.9)	<0.001

^{abc} Different superscript letters denote difference at the reported P-value.

The results showed that the horses preferred lucerne over silage press cake in pelleted form, and that the horses consumed less silage press cake than lucerne in both forms. Reasons may include differences in chemical composition or nutrient concentrations (DeBoer *et al*., 2017), but also differences in smell from the feeds (van den Berg *et al*., 2016). All ensiled feeds will smell different from dried feeds, and it has been reported that silage compared to hay may give both higher (Müller och Udén, 2007) and lower (Moore-Colyer och Longland, 2000)

consumption in horses. The pelleted silage press cake was dusty (very fine feed particles) despite the addition of water prior to feeding, which may have contributed to the low preference for it in the horses.

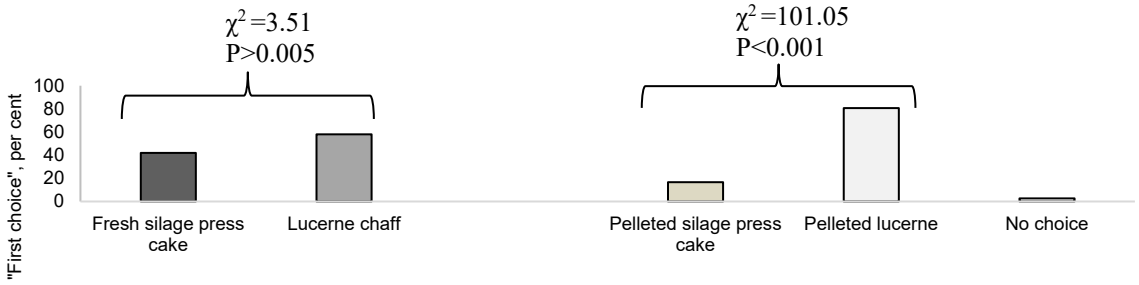


Figure 1 "First choice" (in per cent of possible times) of the horses in Experiment I where fresh silage press cake was compared to lucerne chaff, and in Experiment II where pelleted silage press cake was compared to pelleted lucerne.

Conclusion

The horses preferred pelleted lucerne over pelleted silage press cake. Fresh silage press cake was chosen as frequently as lucerne chaff, but the consumed amount of fresh silage press cake was lower compared to lucerne chaff. This may limit the inclusion of silage press cake in equine diets, but further research could include using it as a diet enrichment for horses where a limited feed intake is required. Digestibility and nutritive values for horses also needs to be studied.

Financing

Green Valleys 2.0 Interreg Öresund-Kattegatt-Skagerak, partly financed through the European Union, project number 20358654 financed this study.

References

Damborg V.K., Stødkilde L., Jensen S.K. & Weisbjerg M.R. 2018. Protein value and degradation characteristics of pulp fibre fractions from screw pressed grass, clover, and lucerne. Anim Feed Sci Technol. 244, 93–103.

DeBoer M.L., Sheaffer C.C., Grev A.M., Catalano D.N., Wells M.S., Hathaway M.R. & Martinson K.L. 2017. Yield, nutritive value, and preference of annual warm-season grasses grazed by horses. Agron J 109, 2136–2148.

Moore-Colyer M.J.S. & Longland A.C. 2000. Intakes and *in vivo* apparent digestibilities of four types of conserved grass by ponies. Anim Sci 71, 527–534.

Müller C.E. & Udén P. 2007. Preference of horses for grass conserved as hay, haylage or silage. Anim Feed Sci Technol 132, 66–78.

Saastamoinen M., Särkijärvi S. & Valtonen E. 2020. The effect of diet composition on the digestibility and fecal excretion of phosphorus in horses: A potential risk of P leaching? Animals 10, 140.

Saastamoinen M., Särkijärvi S. & Suomala H. 2021. Protein source and intake effects on diet digestibility and N excretion in horses - A risk of environmental N load of horses. Animals 11, 3568.

van den Berg M., Giagos V., Lee C., Brown W.Y. & Hinc G.N. 2016. Acceptance of novel food by horses: The influence of food cues and nutrient composition. Appl Anim Behav Sci 183, 59–67.

Interaction between breed and feeding

M. Kargo[1,2], C. Rosman[2] & S. Agenäs[3]
[1] Center for Kvantitativ Genetik og Genomforskning (QGG), Aarhus University, C. F. Møllers Allé 3, 8000 Aarhus*, Denmark*
[2] VikingGenetics, Ebeltoftvej 16 8960 Randers SØ., Denmark
[3] *Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, P. O. Box 7024, 750 07 Uppsala, Sweden.*
Correspondence: mokar@vikinggenetics.com

Introduction

Red dairy cattle have for more than one century been very important for dairy production in the Nordic countries. In Norway the breed is Norwegian Red cattle (NRF) in Finland it is the Finish Ayrshire (FAY), in Sweden Swedish Red Cattle (SRB), and in Denmark it is Danish Red (RDM). The last three mentioned populations have, for nearly 20 years, had a commonly owned breeding program VikingRed (VR) where selection of breeding animals is done across populations followed by use of the selected AI-bulls across the three countries. All the red breeds have for more than 40 years selected breeding animals according to a total merit index including both production, health, functional and conformation traits summing up to highest overall profitability (Kargo et al., 2014) – the so-called Nordic profile. However, even though the Red breeds have followed the Nordic profile to the largest degree, there has been a decline in number of red cows over the last decades. This may be due to lack of understanding of the value of the invisible traits included in the Nordic profile, but it may also be because red cows need to be fed differently from Holstein. The last point is the focus of this paper.

Reduced numbers of red dairy cow - despite "Same bottom line – differently composed"

Overall, the number of dairy cows has decreased over the last decade, but the reduction within the red cows in Denmark, Sweden and Finland have been larger than within the other dairy breeds. In Norway the reduction has been smaller. The overall size of the Nordic red population is today approximately 290.000 cows in milk control.

Table 1 Number of red cows in Denmark, Sweden, Finland, and Norway in 2025.

Denmark	Sweden	Finland	Norway	All
21.349	48.814	56.281	162.389	288,833

The number of red cows in Denmark, Sweden and Finland is less than half of what it was 15 years ago. The reason why this reduction is seen is not known for sure. One reason may be that the production level for VR is lower than for Holstein (HF), and dairy farmers focus very much on production level instead of overall profitability. Probably because yield level for each herd can be seen in all National reports from yield organizations, while overall profitability isn't published at herd level. The same holds for health traits and functional traits e.g. fertility and calving traits belonging to the so-called invisible traits. These invisible traits are, however, important for the herd profitability.

The importance of the invisible traits was highlighted in the report "Economical comparison of Ayrshire and Holstein breeds in Finland" (Keisolmi, 2026). In this report the performance of VR cows was compared to HF cows within 296 Finnish mixed herd with both VR and HF

cows based on data from 2021 until 2024. It was required that: 1) The herd size was above 50 cows, 2) The herd had at least 20 % of either breed, and 3) The proportion of the two main breeds had not changed more than 20 % in the herd between 2017 and 2023. In these 296 selected herds differences between the performance of VR and HF cows were calculated. These within-herd differences were then averaged across herds. By doing the comparisons within herd, the results were by default corrected for different herd management levels. In Table 2 a short summary of main results is given.

Table 2 Short summary of breed differences between VR and HF in Finland (Keisolmi, 2026), differences market with F when favorable for VR and with U when unfavorable.

Trait	Holstein	VikingRed	Difference (VR-HF)
ECM (kg)	10,570	9,737	- 833 **U**
Cow mortality (%)	8.7	5.3	- 3.4 **F**
Mastitis freq. (%)	33.6	30.1	- 3.5 **F**
Claw diseases freq. (%)	43.0	27.8	- 15.2 **F**
Marginal contribution, Euro per cow year*	2941 (low) 3472 (high)	3024 (low) 3524 (high)	+ 84 **F (**low) + 52 **F** (high)

***** Calculation of Marginal contribution carried out by Simherd with a milk price of 48 EURO cents per liter (low) and 53 EURO cents per liter (high)

The conclusion is that the overall profitability from VR and HF is equal - with higher income from milk production in HF, but lower costs in VR due to less feed requirement, less health problems and lower mortality. This means that the strengths of VR lie particularly in efficiency and cost savings, while Holstein's advantages are driven by higher milk yield. These results agree with earlier results from Sweden (Stålhammar, 2014) and findings in Denmark in 2021 (unpublished). The assumption in all these comparisons is that the feed efficiency is at same level within the breeds, and that the cows are fed according to the breed requirements. This may not be true in all situations.

Challenges with feeding Nordic Red dairy cattle

From practical dairy production it is experienced that VR (FAY, SRB, and RDM) heifers react differently on the same feeding compared to HF heifers, and the VR heifers obtain higher BCS. This was investigated in a trial at AU-Campus Viborg (Johansen et al., 2024). These results showed that both HF, VR and Crossbred F1 heifers all grow well on standard diet, but when introducing more straw into the roughage ratio - to the level where the energy concentration was reduced by 15% in the total mixed ratio - the breed groups reacted differently. The VR heifers had the same growth rate on the more roughage-based feeding compared to the standard diet, the F1 heifers had nearly the same growth rate, while the HF heifers had significantly lower growth rate when feeding was more roughage based.

Same heifers as in the Johansen et al. (2024) experiment were kept as lactating cows at the AU research farm, and both HF, F1- and VR cows were offered the same standard feeding as other cows at the AU herd not in trail with the following dietary composition in g/kg dry matter: maize silage 345, grass-clover silage 1. cut 83, grass-clover silage 2. cut 91, rolled barley 98, rapeseed cake 157, rapeseed meal 103 and dried sugar beet pulp 112.

Still unpublished results show that these cows had significantly different yield in second parity depending on heifers feeding. Furthermore, we saw a clear breed times heifer feeding interaction as can be seen in Table 3.

Table 3 Daily yield of VR, F1 crosses and HF cows in different lactations stages in second lactation fed differently as heifers. STD: Heifer feeding according to standard diet, LOW: Heifer feeding with 15% lower energy concentration as STD (Hein and Kargo in preparation for JDS).

	HF		F1		VR	
DIM	STD	LOW	STD	LOW	STD	LOW
5-60	41.2 (1.5)^{ab}	39.2 (2.1)^{ab}	37.7 (1.7)^{ac}	42.5 (1.7)^{b}	33.3 (1.9)^{c}	40.5 (2.2)^{ab}
61-120	40.5 (1.1)^{a}	38.8 (2.1)^{ab}	39.5 (1.4)^{a}	41.8 (1.4)^{a}	34.1 (1.6)^{b}	40.2 (1.6)^{a}
121 -160	38.2 (1.0)^{a}	39.3 (2.1)^{a}	36.1 (1.3)^{ab}	38.4 (1.7)^{a}	32.3 (1.5)^{b}	37.7 (1.5)^{a}

The results show that VR second parity cows react differently to heifer feeding compared to HF cows. VR cows have a higher yield in second parity when fed a lower energy concentration as heifers, while there is no difference for the HF cows.

This poses the challenge that such interactions between breed and energy concentration in the cow feed also can be found. In fact, testimonies from practical experiences point in that direction. Feeding advisor Bruno Due, at the Danish farmer owned advisory company SAGRO, have tried to reduce the energy concentration in a RDM herd with 600 cows, which resulted in slightly increased ECM yield and lower feeding costs. In facts this herd after the feeding correction obtained the lowest feeding cost per produced kg ECM among a group of 8 dairy herds with same yield level – approximately 11.500 kg of ECM per cow year. This suggests that many VR cows are being fed incorrectly.

Towards a new concept on feeding the middle-sized cow

The above-mentioned challenges may be one of the key issues for the decline in number of red cows. There have therefore been meetings between VikingGenetics, Vaxa and SLU with the aim of creating a common Nordic effort on breed time feeding interactions. As a starting point a concept note will be written concentrating on this important issue and suggesting common efforts to be made. By doing this we hope to create an interest in a broader research collaboration within the area.

References

Johansen K., Vestergaard, M., Spleth, P., Hein L., Nielsen H.M. & Kargo M., 2024. Growth and feed efficiency of Nordic Red Dairy Cattle, Holstein, and their F1 crossbreeds when limiting feed energy concentration in prepubertal heifers. J. Dairy Sci. 107:7973-7982.

Kargo, M., L. Hjortø, M. Toivonen, J. A. Eriksson, G. P. Aamand, and J. Pedersen. 2014. Economic basis for the Nordic Total Merit Index. J. Dairy Sci. 97(12):7879–7888

Keisolmi, R., 2026. Economical comparison of Ayrshire and Holstein breeds in Finland. VikingGenetics report, 37 pages.

Stålhammer H. 2014. SRB och Holstein ger samma lönsamhet. http://media.srb-foreningen.se/L%C3%B6nsamhetsber%C3%A4kning-2013.pdf

In situ and in vitro degradation of concentrates for dairy-beef calves

M. R. Weisbjerg[1], N. P. Hansen[1], H. Martinussen[2], K. S. Jensen[2], C. B. Geven[2], N. B. Kristensen[1] & M. Vestergaard[1,2]
[1]Aarhus University (AU), Department of Animal and Veterinary Sciences, AU Viborg, Research Centre Foulum, Blichers Alle 20, 8830 Tjele, Denmark.
[2]SEGES Innovation P/S, Agro Food Park 15, 8200 Aarhus N, Denmark.
Correspondence: martin.weisbjerg@anivet.au.dk

Introduction

Production and fattening of dairy-beef calves in Denmark rely on concentrate-rich diets, with focus on increasing live weight gain while reducing feed costs and climate impact. Therefore, potential new feedstuffs are continuously being evaluated. One such feed is palm kernel cake, not traditionally included in fattening diets in Denmark. Due to its content of mannose polymers, palm kernel cake is a special feed that has caused major challenges in developing enzymatic in vitro digestibility methods to assess in vivo digestibility (Weisbjerg & Hvelplund, 1993), and doubts have been raised whether palm kernel cake is evaluated correctly. The aim of this study was to describe protein and neutral detergent fiber (NDF) degradation and digestibility characteristics of feedstuffs with potential for dairy-beef calf fattening, and to compare palm kernel cake with other feeds.

Materials and Methods

Concentrate feedstuffs were sampled from Danish feed companies in 2024-2025. Feeds comprised (Table 1, number of samples in brackets) fava beans (1), fava bean hulls (1), rapeseed cake (2), sunflower meal (1) and cake (2), palm kernel cake (3), malt sprouts (1) and potato protein (1). Crude protein (CP) in feeds was analysed using the Dumas principle, and CP in residues after incubation quantitatively using the Kjeldahl method. NDF was analysed in Fibertech using amylase and reported ash-free. In vitro enzymatic digestibility of organic matter (EDOM) was analysed according to Álvarez et al. (2020) at a commercial lab (Eurofins Agro Testing Denmark A/S, Vejen). Rumen NDF degradation profile (0, 2, 4, 8, 16, 24, 48, 96, 168 h incubation, 38 µm pore size), indigestible NDF (iNDF, 288 h incubation, 12 µm pore size), rumen dry matter (DM) and CP degradation (0, 2, 4, 8, 16, 24, 48, 96 h incubation, 38 µm pore size), and mobile bag digestibility (12 µm pore size, 16 h rumen pre-incubation, pepsin HCl treatment in lab, duodenum–faeces passage) were performed in situ according to Norfor standards (Åkerlind et al., 2011). Rumen incubations were performed in 3 dry rumen-fistulated cows fed a standard ration; mobile bags were rumen pre-incubated in the same cows and introduced into the duodenal cannula of lactating rumen-, duodenal- and ileal-fistulated cows for duodenum-to-faeces passage. Degradation parameters were estimated from exponential models, for NDF including lag time and for CP without lag time. Degradability of DM at 48 h and effective degradation of CP were corrected for particle losses according to Hvelplund & Weisbjerg (2000).

Results and Discussion

Neutral detergent fibre concentration and degradation characteristics are presented in Table 1. The examined feeds were highly diverse in both NDF concentration and degradation. Fava bean hulls were high in NDF concentration, high in potential NDF digestibility measured as degradation asymptote and as 1000 - iNDF, but low in degradation rate, resulting in rather low

effective NDF degradability. Comparing palm kernel cakes with other oilseed by-products (rapeseed and sunflower cake and sunflower meal) samples showed that palm kernel cakes were higher in NDF, effective NDF degradability and lag time, but lower in iNDF and degradation rate. The lag time observed for palm kernel cakes was extremely long, 4.4 to 11.8 h.

Table 1 Neutral detergent fiber concentration and degradation characteristics, 48 h DM degradation and in vitro enzymatic OM digestibility

	NDF (g/kg DM)	iNDF (g/kg NDF)	dNDF[1] (g/kg NDF)	Kd[2] (%/h)	Lag time (h)	ENDFD[3] (g/kg NDF)	48 h DMD[4] (g/kg DM)	EDOM (g/kg OM)
Fava beans	227	16	896	7.62	1.5	690	970	970
Fava bean hulls	591	72	1000	1.72	8.7	389	726	726
Rapeseed cake 1	252	528	417	13.02	0.0	361	827	827
Rapeseed cake 2	325	478	478	10.01	1.3	388	813	813
Sunflower meal	335	432	544	9.07	0.0	445	833	833
Sunflower cake 1	371	526	370	9.55	0.0	306	759	759
Sunflower cake 2	331	596	433	15.22	0.0	382	754	754
Palm kernel cake 1	727	214	768	5.70	10.6	460	704	704
Palm kernel cake 2	731	212	780	4.75	4.4	503	721	721
Palm kernel cake 3	686	207	793	6.45	11.8	478	705	705
Malt sprouts	498	187	781	5.72	0.0	579	787	787
Potato protein	79	162	773	27.48	0.0	720	966	966

[1] Asymptote; [2] Fractional rate of NDF degradation; [3] Effective NDF degradation at 2%/h fractional passage rate; [4] 48 h rumen DM degradability corrected for particle losses

Concentration of CP, CP degradation characteristics and mobile bag digestibility are presented in Table 2. The feeds varied considerably in CP concentration, from (g/kg DM) 131 in fava bean hulls to 835 in potato protein. Total tract digestibility (g/kg CP) measured using the mobile bag method was very high for sunflower meal and cakes (954-957), and high for fava beans (921), rapeseed cakes (902-905) and potato protein (887), moderate to low for malt sprouts (842), low for palm kernel cakes (760-784), and very low for fava bean hulls (660). Effective rumen CP degradabilities (g/kg CP) were also very low for palm kernel cakes (162-246), therefore small intestinal digestibility (g/kg CP, calculated) of rumen escape CP was reasonably comparable with the other feeds, like fava beans (i.e., 681-742), in line with rapeseed cakes (681-712), lower than sunflower meal and -cakes (818-855) and potato protein (836), but higher than fava bean hulls (203), malt sprouts (524) and fava beans (638). In vitro enzymatic digestibility (g/kg OM, EDOM, Table 1) was low for palm kernel cakes (704-721), lower than fava bean hulls (726), sunflower meal and -cakes (754–833), malt sprouts (787), rapeseed cakes (813-827), potato protein (966) and fava beans (970). However, regressing (data not shown) EDOM on 48 h rumen DM degradability corrected for particle losses as a proxy for in vivo digestibility showed that EDOM values for palm kernel cakes related to 48 h DM degradation similar to most other feeds and close to y=x. The only clear exceptions were potato protein and fava bean hulls, where EDOM values were considerably higher than 48 h DM degradation.

Conclusions

Potential concentrate feeds for dairy-beef calves vary greatly in composition, digestibility and degradability. Palm kernel cake is special and showed a very long lag time for NDF degradation. Comparisons of EDOM and 48 h in situ DM degradation indicated that EDOM correctly evaluates palm kernel cake digestibility. However, the unusual digestion behaviour with extremely long lag times for NDF degradation calls for in vivo studies to more accurately evaluate the feed value of palm kernel cake.

Table 2 CP concentration and degradation characteristics

	CP (g/kg DM)	a[1] (g/kg CP)	b[2] (g/kg CP)	Kd[3] (%/h)	EPD[4] (g/kg N)	CPD[5] (g/kg CP)	Calculated escape CPD[6] (g/kg CP)
Fava beans	287	597	397	12.68	782	921	638
Fava bean hulls	131	390	509	7.80	582	660	203
Rapeseed cake 1	308	465	466	14.62	707	905	681
Rapeseed cake 2	362	199	758	9.30	660	902	712
Sunflower meal	398	294	681	14.50	753	954	818
Sunflower cake 1	317	603	359	16.01	738	957	843
Sunflower cake 2	317	550	410	12.97	706	956	855
Palm kernel cake 1	171	212	788	1.73	289	779	699
Palm kernel cake 2	165	189	811	1.43	198	784	742
Palm kernel cake 3	177	250	751	1.70	266	760	681
Malt sprouts	328	532	390	6.90	671	842	524
Potato protein	835	243	757	2.23	324	887	836

[1] 0 h disappearance, not corrected; [2] Not soluble but potentially degradable fraction; [3] Fractonal rate of CP degradation; [4] Effective CP degradation at 5%/h fractional passage rate and corrected for particle losses; [5] Mobile bag total tract CP digestibility; [6] Calculated intestinal digestibility of rumen escape CP

References

Álvarez, C., Prestløkken, E., Nielsen, N., Volden, H., Klemetsdal, G., Weisbjerg, M.R. 2020. Precision and additivity of organic matter digestibility obtained via in vitro multi-enzymatic method. J. Dairy Sci. 103, 4880–4891. https://doi.org/10.3168/jds.2019-17778

Hvelplund, T. & Weisbjerg, M.R. 2000. In situ techniques for the estimation of protein degradability and postrumen availability. In: D.I. Givens, E. Oven, R.F.E. Axford and H.M. Omed (eds). Forage evaluation in ruminant nutrition. CAB International. 233-258.

Weisbjerg, M.R. & Hvelplund, T. 1993. Bestemmelse af nettoenergiindhold (FEK) i råvarer og kraftfoderblandinger. Forskningsrapport nr. 3, Statens Husdyrbrugsforsøg. 39 pp.

Åkerlind, M., Weisbjerg, M.R., Eriksson, T., Thøgersen, R., Uden, P., Olafsson, B.L., Harstad, O.M. & Volden, H. 2011. 5. Feed analysis and digestion methods. In: Volden, H. (ed.) Norfor – The Nordic feed evaluation system. EAAP publication No. 130. Wageningen Acedemic Publishers. 41-54.

Production of grass protein paste for monogastric livestock feeding

N. Ayanfe[1,2], Q. Wang[3], T. Stefański[1], A. Halmemies-Beauchet-Filleau[3], M. Franco[1], S. Perttilä[1], T. Kokkonen[3], A. Vanhatalo[3] & M. Rinne[1]
[1]*Natural Resources Institute Finland (Luke), Jokioinen, Finland*
[2]*Current address: Swedish University of Agricultural Sciences (SLU), Umeå, Sweden*
[3]*University of Helsinki, Finland*
Correspondence: marketta.rinne@luke.fi

Introduction

Grass is a sustainable feed material, but can be used in very limited amounts for monogastric livestock. In a Green Biorefinery concept, soluble proteins and other nutrients are mechanically liberated from the cell wall matrix, making them available for pigs and poultry (Keto et al., 2021). The aim of this study was to evaluate different additives in producing protein paste from a red clover (*Trifolium pratense* L.) dominated biomass in order to optimize the separation process. The paste could be used as such in liquid feeding systems for pigs or wet feeding of poultry providing an opportunity for on-farm production of a high quality protein supplement. This could promote organic production, where access to protein supplements is a serious bottleneck.

Materials and Methods

An organically grown sward (proportion of red clover 0.65 on dry matter (DM) basis) was harvested after a 48-day regrowth period in Humppila, Finland on 21 August 2024. Approximately 200 kg of biomass was immediately screw-pressed into liquid and solid fractions. The liquid fraction was carefully mixed and divided into buckets (5 litres per bucket). The additives in 4 replicates per treatment were mixed into the buckets as follows:

1. Control without additive
2. Lactic acid bacteria inoculant (LAB; Silomix wet, Vilomix Ltd., Paimio, Finland, at 1×10^6 colony forming units per g fresh matter)
3. Formic acid at 1.5 litres per ton (FA1; AIV 2 Plus Na, Eastman Ltd., Oulu, Finland)
4. Formic acid at 3.0 litres per ton (FA2)

The buckets were closed air-tight with lids and kept at room temperature. After 2 weeks, the grass "whey" or brown juice was removed from the top by vacuum, and the paste sedimented in the bottom of the buckets was collected. Both fractions were analysed for chemical composition using standard laboratory analyses (see Keto et al., 2021). The true protein (TP) was precipitated with the aid of trichloroacetic acid (TCA) precipitation, and amino acids (AA) were analysed by liquid chromatography (UPLC). The *in vitro* ileal nitrogen digestibility analysis for monogastrics was a slight modification of the method described by Boisen and Fernandez (1995). The statistical analysis was conducted using GLIMMIX procedure in SAS (SAS Institute Inc., 2002–2012, Release 9.4; Cary, NC, USA) using a model with additive as a fixed effect while replicate was treated as a random effect. The treatment effects were evaluated using contrasts as well as by Tukey's test.

Results and Discussion

The raw material composition can be considered typical for an unwilted organic red clover dominated crop (Table 1). On fresh matter basis, 0.44 of the raw material weight was recovered in the liquid, but 0.16 of DM, 0.18 of CP and 0.31 of ash was captured into liquid. It is

noteworthy that screw-pressing is not very efficient in concentrating the protein into the liquid fraction.

There were no differences in the protein paste or whey composition of the Control and LAB treatments (Table 2) indicating that the epiphytic flora effectively fermented the juice. The acidity caused by lactic acid formation precipitated TP so that 0.99 of it was captured into the paste in these treatments. Addition of FA resulted in less clear separation of paste and whey, and reduced the proportion on TP capture into the paste (0.93 and 0.83 for FA1 and FA2, respectively). This was somewhat unexpected as in a previous trial, FA increased the capture of TP into the paste, but also the pH levels achieved were much lower, already 3.70 at the lowest FA application (4 l/ton) in that experiment (Stefański *et al.,* 2024). The discrepancy between the trials may be caused by differences in the raw materials as red clover has much higher buffering capacity than grasses and thus may require higher acid addition, as Stefański *et al.* (2024) used timothy juice.

Although the TP yield was lower in FA treated samples, the *in vitro* ileal digestibility of N was higher than in Control and LAB treatments (Table 2). The AA profile of the paste was similar to the raw material (data not shown) and in line with Feed table values published for grass material (see e.g. www.luke.fi/feedtables), but somewhat constrained regarding the key AA such as lysine, methionine+cysteine and threonine for monogastric livestock nutrition.

Assuming an annual yield of 5000 kg grass DM per hectare and TP extraction according to the current experiment, 108 kg of TP could be harvested into the paste per hectare. This highlights the need to increase the efficiency of CP separation into the liquid fraction, which could be achieved by optimized technology and potential pretreatments of the biomass. Further, the seasonal production, storage and stability issues as well as technical solutions for the production and delivery steps of the moist grass paste to the animals still require elaboration. Drying of the paste would increase the costs and could denaturate proteins but would allow commercialization of the product. Using ensiled grass material would solve issues related to raw material and product stability, but precipitation of proteins is not possible from silage juice. Promising results were obtained from feeding pigs with intact silage juice (Keto *et al.,* 2022), but the high water and K contents limit inclusion levels of it.

Table 1 Composition of the clover raw material and the screw-pressed solid and liquid fractions of it, as well as the recovery of nutrients in the solid and liquid fractions.

	Raw material	Solid fraction	Liquid fraction	Recovery, g/g original material	
				In solid	In liquid
Dry matter (DM), g/kg	170	265	66	0.84	0.16
In DM, g/kg					
Ash	90	74	167	0.69	0.31
Crude protein	161	162	178	0.82	0.18
True protein	127	ND	126		
Neutral detergent fibre	444	525	ND		

ND = not determined

Conclusions

The production of grass paste was successful, and the high true protein and relatively low ash contents of it make it a potential protein supplement for monogastric livestock. Use of low DM paste could be practically feasible in liquid feeding of pigs or wet feeding of poultry. Practically feasible technical solutions to increase the yield and economic assessments would be needed to make pigs and poultry benefit from grass paste as a sustainable on-farm-produced protein supplement. A successful Green Biorefinery would have to find high added-value uses for the other fractions (solid fibre-rich fraction and grass whey) in addition to the protein paste to be viable.

References

Boisen, S & Fernandez, J.A., 1995. Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by in vitro analyses. Anim. Feed Sci. Technol. 51, 29-43. https://doi.org/10.1016/0377-8401(94)00686-4.

Keto, L., Perttilä, S., Särkijärvi, S., Immonen, N., Kytölä, K., Alakomi, H.-L., Hyytiäinen-Pabst, T., Tsitko, I., Saarela, M. & Rinne, M., 2021. Effect of silage juice feeding on pig production performance, meat quality and gut microbiome. Livest. Sci. 254, 104728. https://doi.org/10.1016/j.livsci.2021.104728.

Stefański, T., Ayanfe, N. & Rinne, M., 2024. Effect of formic acid treatment of grass liquid fraction on protein separation efficiency. Grassl. Sci. Eur. 29, 129-131. EGF 2024

Table 2 Composition of the grass pastes and wheys produced without any additive (Control), using lactic acid bacteria inoculant (LAB) or formic acid at 1.5 (FA1) or 3 (FA2) litres per ton.

	Additive treatment				SEM	Statistical significance		
	Control	LAB	FA1	FA2		LAB vs FA	FA Lin	FA Quad
Grass paste								
Yield, g/g	0.235	0.238	0.343	0.404	0.0111	<0.001	<0.001	0.075
Dry matter (DM), g/kg	103^{a}	104^{a}	82.0^{b}	76.0^{b}	1.73	<0.001	<0.001	0.005
pH	4.16	4.08	4.22	4.35	0.078	0.063	0.117	0.727
In DM, g/kg								
Ash	112^{b}	112^{b}	141^{a}	148^{a}	3.38	<0.001	0.133	<0.001
Crude protein (CP)	296^{a}	297^{a}	286^{a}	227^{b}	5.81	<0.001	<0.001	<0.001
True protein (TP)	238^{a}	248^{a}	233^{a}	167^{b}	4.57	<0.001	<0.001	<0.001
TP/CP	0.806^{a}	0.833^{a}	0.815^{a}	0.734^{b}	0.007	<0.001	<0.001	<0.001
Total amino acids/CP	0.829^{ab}	0.874^{a}	0.783^{b}	0.855^{a}	0.011	0.002	<0.001	0.012
In vitro N digestibility[1]	85.0^{c}	85.3^{c}	88.3^{b}	93.3^{a}	0.621	<0.001	<0.001	<0.001
Grass whey								
DM, g/kg	43.1^{b}	43.0^{b}	39.2^{b}	49.6^{a}	0.967	0.256	<0.001	<0.001
pH	4.22	4.10	4.30	4.46	0.078	0.034	0.211	0.917
In DM, g/kg								
Ash	239^{b}	240^{b}	268^{a}	219^{b}	6.55	0.672	0.050	<0.001
CP	106	100	116	123	7.74	0.067	0.148	0.863
TP	3.50^{b}	3.20^{b}	17.6^{ab}	34.7^{a}	7.09	0.022	0.115	0.007
Lactic acid	110^{a}	118^{a}	82.7^{b}	2.30^{c}	6.65	<0.001	<0.001	0.004
Acetic acid	57.8^{a}	52.9^{a}	58.3^{a}	3.18^{b}	3.98	<0.001	<0.001	<0.001

Means in rows with different superscript letters differ statistically significantly (P<0.05).
[1]*In vitro* ileal nitrogen digestibility of pigs

Evaluating the feed value of oat varieties using in vitro methods

T. Undheim, E. Prestløkken & A. Kidane
Norwegian University of Life Sciences. Department of Animal and Agricultural Sciences.
Correspondence: tiril.undheim@nmbu.no

Introduction

Oats (*Avena sativa*) are the third most important cereal crop in Norway, following barley and wheat, and the second most important cereal used for animal feed (Stabbetorp, 2023). Oats grow well in cool and humid climates and do not require high soil quality to grow, making them suitable for production in the Norwegian climate (Forsberg & Reeves, 1995; Givens et al., 2004). Compared with other cereals such as barley, oats typically have higher levels of indigestible neutral detergent fibre (iNDF), making them less digestible (Fant et al., 2020). However, chemical composition and degradability vary among oat varieties, which may influence rumen fermentation characteristics and overall feed value, and hence comparative characterization of oat varieties as ruminant feed is deemed vital.

The objective of this study was to evaluate the feed value of oat varieties using in vitro gas production and Ankom Daisy incubation methods.

Materials and Methods

Twelve oat varieties with differing fat and protein content were evaluated, of which four of the varieties were contaminated with mycotoxins. Fermentation characteristics were evaluated using the ANKOM RF Gas Production System. For this, 1.0 g dry matter (DM) sample material was incubated for 48h in sealed bottles containing 66 mL buffer solution and 33 mL rumen inoculum at 39°C. The buffer solution was prepared following the method of Goering and Van Soest (1970) and rumen fluid was collected from three rumen-fistulated, non-lactating Norwegian Red cows four hours after their morning feeding. Three batches were conducted, comprising a total of 119 bottles. Cumulative gas production was recorded throughout the incubation period. At the end of incubation, samples for volatile fatty acid (VFA) were collected, pH was measured and in vitro DM digestibility was calculated. The ANKOM Daisy incubation system was used for estimating organic matter (OM) loss after incubation for 12, 24, 48 and 96 hours in buffered rumen inoculum. The incubations were conducted in two rounds. Rumen inoculum was collected as for the gas production above.

Results were analyzed in R-studio (R version 4.5.3), using a linear mixed model. For gas production data, fixed effects were batch and treatment, with bottle as a random effect.

Results and Discussion

Fermentation pattern (Table 1) of the different oat varieties shows a similar increase in gas production in the first 6 hours. Variety 1000 (Oliver), as the only naked oat variety, shows a further increased gas production before reaching the maximum at around 200 ml/g DM after 48 h of incubation. The observed higher fermentation for this variety may be related to a lower fiber content, as naked oats contain lower fiber due to the absence of the hull, resulting in more readily fermentable carbohydrates (Biel et al., 2014). In contrast, variety 1003 (Fatima) which has high fat content, reached the lowest gas production with maximum production at just below 150 ml/ g DM. The other oat varieties, including the ones with mycotoxins, show a pattern

similar to each other, indicating that the mycotoxins had no or limited effect on in vitro rumen fermentation.

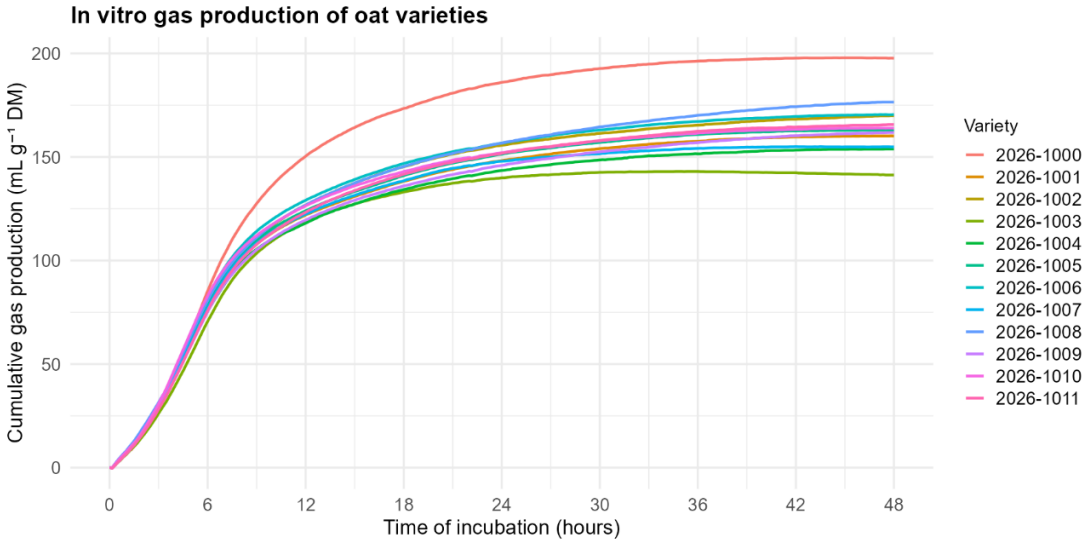


Figure 1 Cumulative gas production over 48 hours of in vitro incubation.

From table 1, variety 1000 consistently showed the highest fermentability with higher digestibility, higher total VFA production, lower pH, and a fermentation pattern with lower acetate and higher propionate. This pattern is consistent with diets high in rapidly fermentable carbohydrates, such as starch (McDonald et al., 2022).

Table 1 In vitro fermentation characteristics of oat varieties after 48h incubation.

Variety	DMd	pH	NH_4^+	TVFA	Ac	Pr	Bu
2026-1000	56.8^{b}	6.23^{a}	242^{a}	104.6^{b}	60.6^{a}	22.4^{b}	13.4
2026-1001	39.5^{a}	6.48^{bc}	265^{abc}	94.4^{ab}	62.2^{ab}	20.7^{ab}	13.3
2026-1002	48.6^{bc}	6.48^{bc}	289^{c}	88.1^{a}	63.2^{b}	19.4^{a}	13.4
2026-1003	40.1^{ab}	6.56^{c}	267^{abc}	83.3^{a}	60.8^{a}	22.3^{b}	13.3
2026-1004	43.7^{ab}	6.46^{b}	295^{c}	89.6^{a}	62.7^{b}	19.8^{a}	13.5
2026-1005	46.5^{ab}	6.44^{b}	252^{ab}	88.9^{a}	62.4^{ab}	20.8^{ab}	13.3
2026-1006	47.1^{ab}	6.46^{b}	275^{abc}	87.5^{a}	63.1^{b}	20.0^{ab}	12.9
2026-1007	43.4^{ab}	6.47^{bc}	267^{abc}	91.2^{a}	62.0^{ab}	21.3^{ab}	13.1
2026-1008	41.5^{ab}	6.47^{bc}	293^{c}	93.9^{a}	63.7^{b}	19.3^{a}	13.1
2026-1009	54.4^{ab}	6.47^{bc}	297^{c}	92.8^{a}	63.5^{b}	19.5^{a}	12.7
2026-1010	46.8^{ab}	6.50^{bc}	260^{abc}	89.3^{a}	63.3^{b}	20.3^{ab}	12.8
2026-1011	43.8^{ab}	6.49^{bc}	284^{bc}	89.4^{a}	63.5^{b}	19.8^{a}	12.9
SEM	0.02	3.326	8.3	2.28	0.39	0.56	0.25
p-value	***	**	***	***	***	***	ns

Means sharing at least one letter in a column are not significantly different (P<0.05). SEM is the average standard error of the estimated marginal means. ** P<0.01; *** P<0.001; ns = non-significant. DMd = dry matter digestibility; NH_4^+ = ammonium (mg/l); TVFA = total volatile fatty acids (mmol/l); Ac = acetate (molar, %); Pr = propionate (molar, %); Bu = butyrate (molar, %).

Figure 2 provides organic matter digestibility of the oat varieties based on Ankom Daisy system. The oat varieties show similar patterns to the gas production, with more rapid OM loss early in incubation for all varieties, and variety 1000 having the highest overall OM loss compared to the other varieties. This is consistent with the in vitro fermentation results. The varieties with mycotoxin show similar OM loss as most of the other varieties, further supporting that fermentation was not affected by mycotoxins.

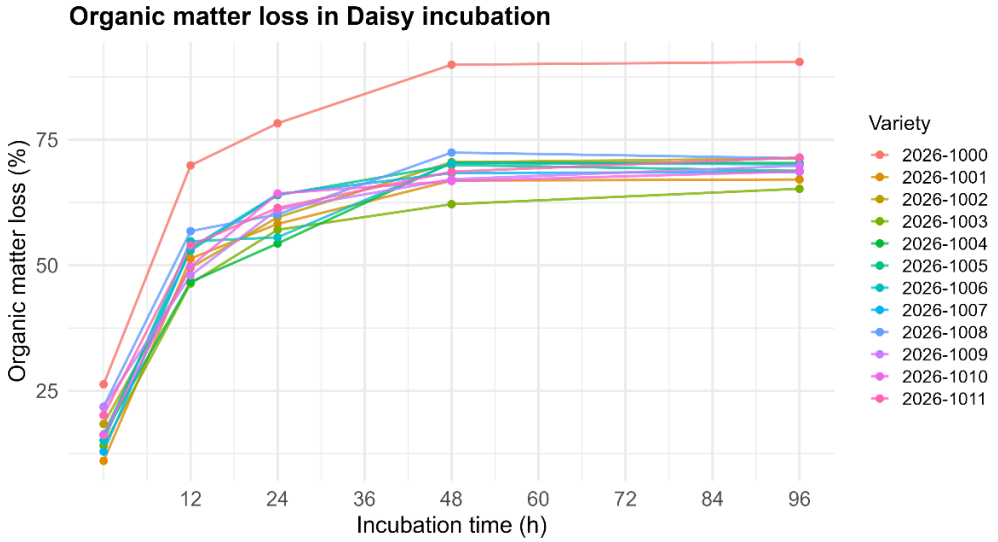


Figure 2 Organic matter disappearance over 96 hours of Daisy incubation.

Conclusions

In vitro methods showed differences in feed value among oat varieties, with the naked oat variety 1000 showing higher fermentability and degradability. Fermentation characteristics did not seem to be strongly affected by mycotoxin levels. These results indicate that oat variety influences rumen fermentation characteristics and also shows the usefulness of in vitro techniques for evaluating feed value for many feed sources.

References

Agriculture 2026, 16, 288 19 of 2030. Goering, H.K.; Van Soest, P.J. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications); Agriculture HandbookNo. 379; USDA-ARS: Washington, DC, USA, 1970.

Fant, P., Ramin, M., Jaakkola, S., Grimberg, Å., Carlsson, A. S., & Huhtanen, P. (2020). Effects of different barley and oat varieties on methane production, digestibility, and fermentation pattern in vitro. *Journal of Dairy Science*, *103*(2), 1404–1415. https://doi.org/https://doi.org/10.3168/jds.2019-16995

Forsberg, R. A., & Reeves, D. L. (1995). Agronomy of oats. In R. W. Welch (Ed.), *The Oat Crop: Production and Utilization* (pp. 223–251). Springer Netherlands. https://doi.org/10.1007/978-94-011-0015-1_8

Biel, W., Jacyno, E. & Kawęcka M. (2014). Chemical composition of hulled, dehulled and naked oat grains. *South African Journal of Animal Science*, 44(2), 189–197. https://doi.org/10.4314/sajas.v44i2.10

Givens, D. I., Davies, T. W., & Laverick, R. M. (2004). Effect of variety, nitrogen fertiliser and various agronomic factors on the nutritive value of husked and naked oats grain. *Animal Feed Science and Technology*, *113*(1), 169–181. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2003.11.009

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A. & Wilkinson, R. G. (2022). *Animal Nutrition*. 8th ed. Pearson Education.

Stabbetorp, H. (2023). Dyrkingsomfang og avling i kornproduksjonen. In *NIBIO Bok* (Vol. 10, pp. 18–29). NIBIO.

Rumen degradation kinetics of grass-clover pulp silage evaluated by in sacco and in *vitro* methods

G.Q. Khan[1], J. K. Sommerseth[2] & S. Adler[1]
[1] *Department of Grassland and Livestock, Division of Food Production and Society, Norwegian Institute of Bioeconomy Research, NO-1431 Ås, Norway*
[2] *Tine SA, R&D-dept, Chr. Magnus Falsens vei 18, 1432 Ås, Norway*
Correspondence: ghulam.khan@nibio.no

Introduction

Green biorefinery is gaining interest in Europe, particularly in the Nordic region, to increase local protein feed production. Green biorefining fractionates fresh grass-clover forage into a nutrient-rich press juice and a fibrous pulp fraction. The pulp represents a substantial share of the harvested dry matter, consists mainly of plant fibre and fibre-bound protein, and can be ensiled and used as a roughage source for ruminants (Gaffey et al., 2023). From a ruminant nutrition perspective, pulp silage is of interest because its chemical composition and physical structure differ from conventional silage. The removal of water-soluble components and the mechanical disruption of plant tissue may influence ruminal degradation properties (Volden, 2011). However, rumen degradation kinetics of pulp silage are still scarcely documented. Therefore, the present study aimed to evaluate rumen degradation characteristics of grass-clover pulp silage, using *in sacco* and *in vitro* gas methods.

Materials and Methods

Grass-clover silage (GS) and grass-clover pulp silage (PS) from a dairy cow feeding trial (OneTwo project) were used in the present study. Both silages were produced from a 1:1 mixture of first and second harvests in 2023 from the same grass-clover ley at the NIBIO research station Tuv, Steinkjer, Norway. The ley consisted of timothy, meadow fescue, and red clover (70:20:10, by seed weight). GS was prepared as conventional wilted bale silage, whereas PS consisted of the fibrous pulp obtained after mechanical pressing of forage and was subsequently preserved as bale silage. Dried samples of GS and PS were analysed for dry matter (DM), ash-corrected neutral detergent fibre (NDF), and crude protein (CP). Rumen degradation was determined by the *in sacco* technique as described in NorFor (Volden, 2011). Residues after rumen incubation were analysed for DM, NDF, and CP, and degradation parameters were estimated using the NLIN procedure of SAS (SAS, 2013). An exponential model was fitted to estimate the soluble fraction (*s*), potentially degradable fraction (*pd*), degradation rate (*kd*), and total degradable fraction (*d*). Effective degradability of DM, NDF, and CP was calculated assuming a rumen passage rate of 5%/h. *In vitro* gas production (IVGP) was determined using the ANKOM gas production system, following the general procedure of Muqier et al. (2023) but using buffered faecal inoculum instead of rumen fluid. Gas volume was calculated from pressure data using the ideal gas law. Gas production profiles were then fitted using the Groot model to estimate *A* (asymptotic gas production), *B* (time to produce half of the asymptotic gas volume), and *C* (shape parameter of the gas production curve), from which R_{max} (maximum fractional rate of gas production) and T_{max} (time to reach R_{max}) were derived. Data were analysed using the GLM procedure of SAS, with treatment and cow as fixed effects for the in sacco data and treatment only for the IVGP data. Results are presented as LS means with SEM.

Results and Discussion

Dry matter degradability was lower for PS than for GS, as reflected by lower *s*, lower *kd*, and lower effective degradability (Table 1), and a lower DM disappearance was seen for PS than for GS across incubation times (Figure 1). NDF degradability was similar between treatments, although PS tended to have lower effective degradability. Crude protein degradability showed a pattern broadly similar to that of DM, with lower *s* and lower effective degradability for PS than for GS. The lower DM and especially CP degradability of PS were as expected, reflecting the removal of soluble components during biorefining. However, the similar NDF degradability between treatments was somewhat unexpected, as mechanical fractionation could be expected to increase fiber accessibility in PS.

Table 4 In sacco degradation parameters and effective degradability of dry matter (DM), neutral detergent fiber (NDF) and crude protein (CP) for grass silage (GS) and pulp silage (PS), expressed as LS means (%).

Item	GS	PS	SEM	P-value
DM				
s, %	23.1	14.2	0.18	0.001
pd, %	62.2	69.7	0.56	0.011
kd, %/h	5.91	5.43	0.071	0.042
d, %	85.2	83.8	0.40	0.127
$EDDM_5$, %	56.7	50.4	0.20	0.002
NDF				
s, %	0.31	0.25	0.12	0.766
pd, %	84.0	84.3	0.04	0.030
kd, %/h	6.10	5.79	0.091	0.139
d, %	84.3	84.6	0.10	0.247
$EDNDF_5$	46.5	45.5	0.20	0.076
CP				
s, %	46.9	35.4	1.11	0.018
pd, %	35.0	44.2	2.35	0.109
kd, %/h	5.28	4.07	0.655	0.319
d, %	81.9	79.6	2.41	0.576
$EDCP_5$	64.7	54.5	0.49	0.004

s = soluble fraction; *pd* = potentially degradable fraction; *kd* = degradation rate; *d* = total degradable fraction. EDDM, EDNDF, and EDCP denote effective degradability of DM, NDF, and CP, respectively, calculated at passage rate of 5%/h.

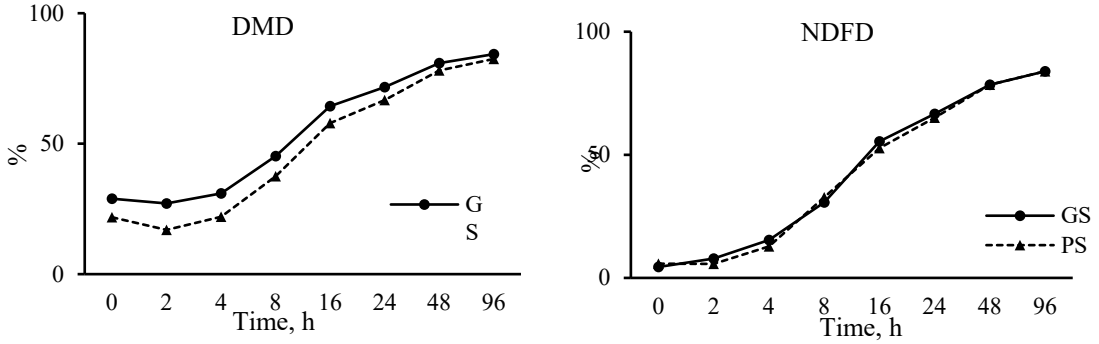


Figure 4 In sacco dry matter degradability (DMD) and neutral detergent fibre degradability (NDFD) of grass-clover silage (GS) and pulp silage (PS) at different rumen incubation times**.**

Broadly consistent with the *in sacco* results, IVGP kinetics showed no difference between GS and PS for the fitted parameters *A*, *B*, and *C* (Table 2). Likewise, R_{max} was similar between the treatments (Table 2; Figure 2). However, T_{max} tended to be higher for PS than GS, indicating that maximum fermentation activity occurred later in PS and suggesting slower fermentation.

Table 5 In vitro gas production kinetic parameters for grass-clover silage (GS) and pulp silage (PS).

Item	GS	PS	SEM	P-value
A, mL/g DM	198	191	10.9	0.660
B, h	15.2	17.1	0.57	0.114
C	1.92	2.18	0.15	0.283
R_{max}, h^{-1}	0.063	0.065	0.006	0.819
T_{max}, h	14.4	18.2	1.00	0.053

A, asymptotic gas production; *B*, time to produce half of the asymptotic gas volume); *C*, shape parameter of the gas production curve; R_{max}, maximum fractional rate of gas production; T_{max}, time to reach R_{max}.

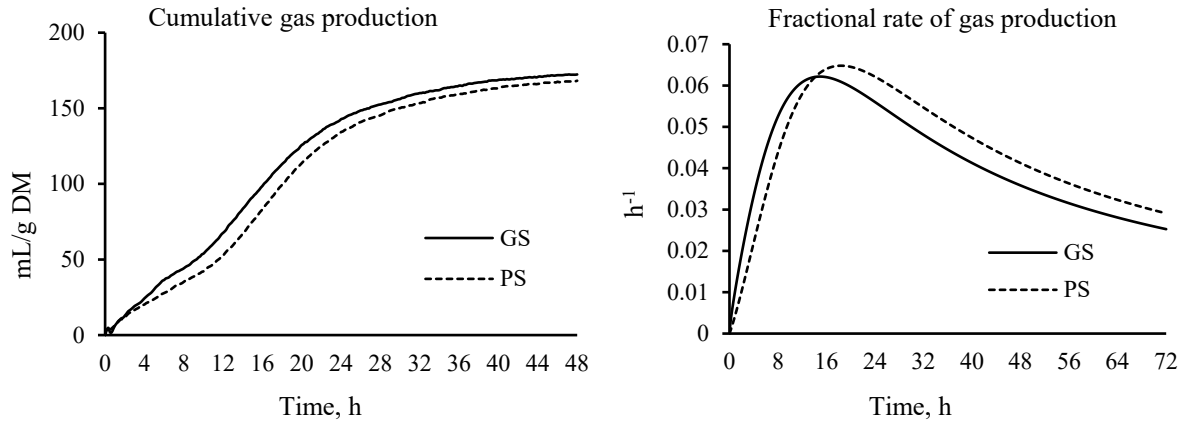


Figure 5 Cumulative gas production and fractional rate of gas production of grass-clover silage (GS) and pulp silage (PS) during incubation in the ANKOM gas production system.

As rumen degradation is affected by the physical integrity of feeds (Khan et al., 2022), grinding samples to 1 mm may have obscured expected differences in fiber degradation, suggesting a need to evaluate the rumen degradation of PS in a less processed form.

Conclusion

Overall, the results indicate that grass-clover pulp silage can serve as a forage resource for dairy cows, but its ruminal degradation characteristics differ from those of conventional silage, particularly for dry matter and crude protein.

References

Gaffey, J., Rajauria, G., McMahon, H., Ravindran, R., Dominguez, C., Ambye-Jensen, M., Souza, M.F., Meers, E., Aragonés, M.M., Skunca, D., Sanders, J.P.M., 2023. Green Biorefinery systems for the production of climate-smart sustainable products from grasses, legumes and green crop residues. Biotechnology Advances 66, 108168.

Khan, G.Q., Li, H., Prestløkken, E., 2022. Effects of expander and extruder processed concentrate pellets with specific physical functional properties on in sacco rumen degradation, In: Proceedings of the 11th Nordic Feed Science Conference, Swedish University of Agricultural Sciences., Uppsala, Sweden, pp. 116–119.

Muqier, X., Eknæs, M., Prestløkken, E., Jensen, R.B., Eikanger, K.S., Karlengen, I.J., Trøan, G., Vhile, S.G., Kidane, A., 2023. In Vitro Rumen Fermentation Characteristics, Estimated Utilizable Crude Protein and Metabolizable Energy Values of Grass Silages, Concentrate Feeds and Their Mixtures, Animals, p. 2695.

SAS, 2013. Base SAS 9.4 procedures guide: statistical procedures, SAS Institute, NC, USA.

Volden, H., 2011. NorFor-The Nordic feed evaluation system. EAAP Publication Report no 130. Wageningen Academic Publishers, Wageningen, The Netherlands.

EUnetHorse improves the resilience of horse farms – including grassland management

M. Rinne[1], A. Sironen[1], A. Yli-Viikari[1], R. Isomäki[2], E. Uusivuori[2], J. Vattulainen[1], M. Saastamoinen[1], L. Rantamäki-Lahtinen[2]
[1]*Natural Resources Institute Finland (Luke), Jokioinen, Finland*
[2]*University of Helsinki, Finland*
Correspondence: marketta.rinne@luke.fi

Introduction

Equine farms are an important part of EU's agriculture and rural development, but horse farmers face many challenges related to the economic performance, environmental impacts, welfare of horses as well as the social aspects (entrepreneur well-being, social license to operate). The EUnetHorse thematic network project (2023-2027) has identified the needs of horse farmers and searched for solutions to them to increase the resilience of the horse farms. The three thematic areas and specific topics within them are presented in Table 1.

EUnetHorse builds a European network for knowledge exchange and peer-to-peer learning between actors and stakeholders of the horse sector to improve the resilience of equine farms. The project is coordinated by the French Horse and Riding Institute (IFCE), and the other participating countries are Portugal, Spain, Romania, Switzerland, Belgium, Germany and Finland. For more information: www.eunethorse.eu.

Table 1 Topics included in the EUnetHorse project.

Thematic area	Topics
Socioeconomics	How can equine farm profitability be improved?
	How can working conditions be improved?
	How can problems associated with access to agricultural land be solved?
Environment	How can practices and resource management be adapted to climate change?
	How can biodiversity be managed while maintaining equine activities?
	How can pastures and grasslands be managed to face future challenges?
Horse health and welfare	What practices can be implemented to promote biosecurity measures and prevent emerging diseases?
	How can horse welfare conditions be improved?

Process to provide concrete solutions for horse farmers

Adapting good practices and solutions is a possibility for horse farms to address the current challenges. However, there is not enough validated data on the suitability of various solutions for equine farms. To evaluate potential solutions, an assessment methodology was created by using principles of multicriteria decision analysis. The method is based on simplified cost-benefit analysis, which is used to create assessment matrixes.

In the beginning of the project, horse farmers in all participating countries were interviewed for identification of their needs. After that, existing solutions were searched that matched the needs. The evaluation of solutions was then conducted in all countries in national workshops, where experts analysed them thoroughly. In the next step, the national evaluations were

validated in European level workshops where project partners as well as invited experts discussed the national assessments and decided which solutions will be further promoted. To collect feedback from the end users, training and demonstration days were held in all countries, where the solutions were demonstrated in real life sitations, and horse farm operators evaluated their usability. Further, an on-line survey was conducted to collect feedback about the solutions from the end users. Finally, the solutions are distributed to the end-users. The information produced in EUnetHorse is disseminated through various channels including an active Facebook. The main repository for the materials is the EU Farmbook, which store e.g. all solution technical sheets in open access format.

Grasslands as one of the key topics of EUnetHorse

Many important aspects in the equine sector are linked to grasslands and forages, so that solutions related to this topic are highlighted here. Forages are the main source of nutrition for horses, and adequate access to forages is a key factor ensuring the welfare of horses. Grasslands play a positive role in providing several ecosystem services such as soil carbon sequestration and improved soil structure, water and nutrient management, biological nitrogen fixation when forage legumes are used and improved biodiversity. On the other hand, forage production and grazing are challenged by the climate change and limited access to agricultural land. The solutions proven by the EUnetHorse assessment scheme can be accessed from the project website and EU Farmbook. Topics related to grassland management include:

- Crop rotation
- Reduced tillage cultivation
- Soil quality index
- pH corrections of soil
- Breeding/selection of new grass and legume species and varieties
- Multi-species grasslands to adapt to climate change
- Application of mycorrhizae to improve plant growth in pastures
- Pasture management to optimise land use
- Use of a grazing consumption grid to manage the pasture area
- Use of an application tool for plant identification in pasture management
- Avoid overgrazing by adapting the number of horses
- Grassmeter to evaluate forage mass in pastures
- Mixed grazing of horses and other animal species
- Pasture irrigation
- Collection and storage of manure
- Manure and compost valorization
- Use of side-products as fertilizers
- Maintaining open pastures and preventing forestration by slashing
- Drying hay in a barn/hay dryer

Conclusions

Throughout Europe, the horse sector is in a slight decline, and the same phenomenon is experienced in Finland. However, horses have a lot to offer to the society such as to balance the lifestyle of modern people by providing contact to large animals and nature, to bridge the gap between urban and rural lifestyles, and to contribute to the livelihoods in the countryside.

EUnetHorse aims to better utilize the existing information, but it also highlights areas where knowledge gaps exist. This serves to generate ideas for future projects. The equine sector is underpresented e.g. in EU Horizon projects, and would benefit from resources dedicated to its development. In Finland, Luke gave up the experimental horse pool in 2025, and there are no longer dedicated horse researchers at Luke, but expertize is gathered on project basis. This model requires support from national stakeholders and the international research community to continue development of the horse sector within Finland as well as at European and ultimately at global level.

Acknowledgements

Funding from European Research Executive Agency (REA) with a project number 101086551 is gratefully acknowledged. We wish to extend our gratitude to the whole EUnetHorse project team, participating stakeholders and farmers.

Figure 1 Logo of EUnetHorse.

Potential of sea buckthorn residues as ruminant feed: rumen degradability and fermentation profiles

D. Malenica[1*], M. Ramin[2], R. Bhat[3], Meelis Ots[1], R. Leming[1], A. Olt[1], M. Kass[1,4]
[1]*Chair of Animal Nutrition, Institute of Veterinary Medicine and Animal Science, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006 Tartu, Estonia*
[2]*Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden*
[3]*Chair in Food Science and Technology, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006 Tartu, Estonia*
[4] *Centre of Estonian Rural Research and Knowledge, Jõgeva, Estonia.*
**Correspondence:* dunja.malenica@emu.ee

Introduction

Sustainable use of agro-industrial by-products can improve livestock feeding, reduce reliance on conventional feeds, and lower environmental pollution (Singh et al., 2021). Ruminants are well suited to these fibre-rich resources due to their ability to utilize fibrous feedstuffs. In addition, growing global emphasis on reducing enteric methane (CH_4) emissions has increased interest in agro-industrial by-products as potential mitigation strategies (Marcos et al., 2022). However, because nutrient availability depends on rumen degradation and microbial fermentation, agro-industrial by-products must be carefully evaluated before incorporated into diets. Still, data on their ruminal behaviour and CH_4 mitigation potential are limited.

Materials and Methods

In vitro true digestibility of dry matter (IVTD), rumen degradability, and fermentation characteristics of three sea buckthorn by-products: leaves (SBL) and pomace with and without seeds (SBPS and SBPW, respectively) were evaluated. IVTD was determined using the ANKOM DAISY II Incubator. Briefly, 0.5 g samples were incubated in buffered rumen fluid, collected from two Holstein cows, at 39 °C for 48 h. Buffer was prepared following ANKOM methodology (ANKOM Technology, 2021) After the incubation, the bags were rinsed, subjected to NDF extraction and used to calculate digestibility. IVTD analyses was performed in triplicate. *In vitro* gas production was measured by incubating 1 g samples in 250-mL serum bottles for 48 h. Rumen fluid, collected from two Swedish Red Cows, was maintained under CO_2, filtered, and mixed with a buffered solution (20:80, v/v) containing peptone. Each bottle contained 60 mL of inoculum and was incubated at 39 °C. CH_4 concentration was analysed by gas chromatography, and gas yields were calculated according to Ramin and Huhtanen (2012). The presented data represent mean values derived from three replicates, while SBPW values were derived from seven replicates. *In sacco* degradability of dry matter (DM), crude protein (CP), and NDF was determined using polyester bags (38 µm pore size) incubated for 0 to 72 h in fistulated cows of Holstein breed. Corrections were applied for particle loss, following the method of Weisbjerg et al. (1990). Degradation kinetics were estimated according to McDonald (1981). Indigestible NDF (iNDF) was measured after 288 h rumen incubation using 12 µm bags (Huhtanen et al., 1994). All analyses and graphical outputs were performed using R statistical software.

Results and Discussion

Chemical composition of sea buckthorn by-products can be seen in Table 1.

Table 1 Chemical composition, indigestible neutral detergent fibre (iNDF) and model-derived undegradable neutral detergent fibre (C NDF) in sea buckthorn leaves (SBL) and pomace with (SBPS) and without seeds (SBPW).

Sample	DM, g/kg	CP, g/kg DM	NDF, g/kg DM	iNDF, g/kg DM	C NDF, g/kg DM
SBL	934	136	247	159	178
SBPS	922	225	358	254	246
SBPW	928	96	326	178	187

DM degradability was similar across the three treatments (Figure 1a). CP degradability differed considerably: SBPS showed the highest ruminal CP degradability, SBPW moderately lower values and SBL the lowest (Figure 1b). This is most likely due to leaf associated polyphenolics, particularly tannins, restricting microbial access (Marcos et al., 2022). This may suggest a higher rumen undegradable protein fraction in SBL; however, its intestinal digestibility warrants further investigation.

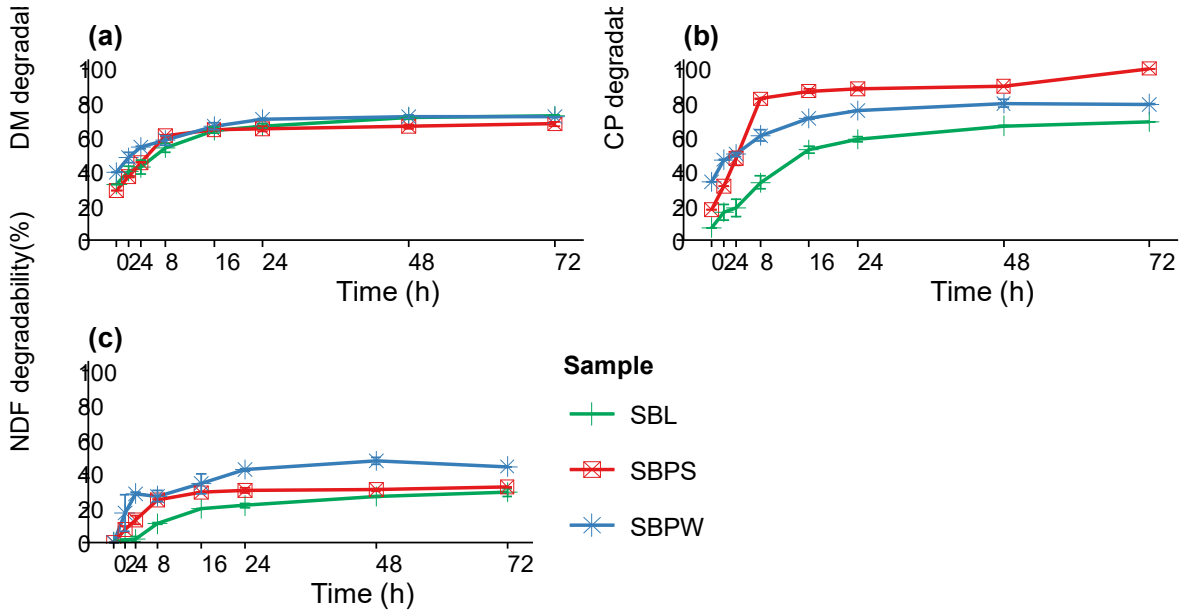


Figure 1 Dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF) degradability of sea buckthorn leaves (SBL) and sea buckthorn pomace with (SBPS) and without seeds (SBPW).

NDF rumen degradability was the lowest in SBL, followed by SBPS and SBPW (Figure 1c). All three by-products had substantial iNDF concentrations (Table 1). SBPW had the lowest iNDF/NDF ratio (0.55), indicating the most readily degradable fibre among the by-products, while SBPS had the highest, followed closely by SBL (0.71 and 0.63, respectively). The undegradable NDF (C NDF) estimated by the model matched the 288-h iNDF (Table 1). Interestingly, SBPS showed higher indigestible fibre fraction at 288 h than C NDF, likely due to model underestimation of C NDF.

Despite higher NDF degradability and a lower iNDF/NDF ratio, SBPW had the lowest IVTD (63% of DM). In contrast, IVTD values were similar for SBPS and SBL (79% and 80% of DM, respectively). Fermentation outcomes aligned with degradability patterns. After 48 h of

incubation, total gas production across the treatments averaged 188 mL/g DM, while CH_4 production averaged 22.6 mL/g DM (Figure 2).

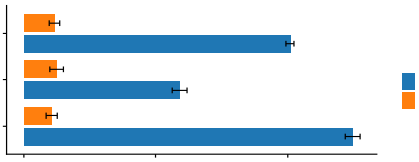


Figure 2 *In vitro* total gas and methane production of sea buckthorn leaves (SBL) and sea buckthorn pomace with (SBPS) and without seeds (SBPW).

The highest total gas yield was observed in SBPW, but conversely, it had the lowest CH_4 yield. In contrast, SBPS produced more CH_4 despite lower total gas production, suggesting that the presence of seeds may have influenced fermentation pathways; however, volatile fatty acids (VFA) analysis is needed to confirm this.

Conclusions

Sea buckthorn by-products show potential as supplementary feed ingredients for ruminants, but their nutritional contribution may be constrained by high iNDF fractions. Further studies, including in vivo trials and VFA analysis, are warranted.

Funding acquisition

This research work was funded by the ERA-Chair in Valortech (EU Commission Grant Number: 810630), and co-funded by the European Union and Estonian Research Council via project TEM-TA60.

References

Ankom Technology, 2021. Daisy II Incubator Operators Manual (2021) Available online: http://www.ankom.com (accessed on 21. February 2026).

Huhtanen, P., Kaustell, K., & Jaakkola, S. (1994). The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Animal Feed Science and Technology*, *48*(3-4), 211-227.

Marcos, C. N., Evan, T. D., Jiménez, C., & Carro, M. D. (2022). Potential of agroindustrial by-products to modulate ruminal fermentation and reduce methane production: in vitro studies. *Animals*, *12*(24), 3540.

McDonald, I. 1981. A revised model for the estimation of protein degradability in the rumen. J. Agric. Sci. Camb. 96, 251-252

Ramin, M., Huhtanen, P., 2012. Development of an in vitro method for determination of methane production kinetics using a fully automated in vitro gas system—A modelling approach. Anim. Feed Sci. Technol. 174 (3-4), 190–200.

Singh, R., Das, R., Sangwan, S., Rohatgi, B., Khanam, R., Peera, S. P. G., ... & Misra, S. (2021). Utilisation of agro-industrial waste for sustainable green production: a review. *Environmental Sustainability*, *4*(4), 619-636.

Weisbjerg, M. R., Bhargava, P. K., Hvelplund, T. & Madsen, J. 1990. Anvendelse af nedbrydningsprofiler i fodermiddelvurd- eringen. Beretn. 679 fra Statens Husdyrbrugsforsøg 33 pp.

Positive effect of sprouted grains on the fatty acid profile of intramuscular fat in bull meat

E. Meškinytė, Ž. Tarasevičienė, V. Jukna & O. Ilina
Animal Production Research and Innovation Center, Agriculture Academy, Bioeconomy Research Institute, Vytautas Magnus University, K. Donelaičio g. 58, LT-44248 Kaunas, Lithuania.
Correspondence: oksana.ilina@vdu.lt

Introduction

Beef provides a rich supply of amino acids, essential minerals, and vitamins, making it a valuable component of human nutrition (Vahmani et al., 2015). The level of intramuscular fat and its fatty acid (FA) composition, together with the biological value of the protein, as well as the content of trace elements and vitamins, are key factors that determine the nutritional value of beef. However, current dietary guidelines often recommend limiting the consumption of saturated fat–rich foods, including beef, due to concerns about cardiovascular health. Nevertheless, the FA profile of beef can be modified through dietary strategies (Scollan et al., 2014, Horcada et al., 2020).

This paper focuses on the use of specific feeding approaches for bulls aimed at reducing the proportion of saturated fatty acids (SFA) in meat. In particular, the review examines how feeding strategies such as the inclusion of sprouted grains can alter the FA composition of beef by increasing polyunsaturated fatty acids (PUFA) and decreasing SFA.

Materials and Methods

For the experiment, forty 7-month-old crossbred dairy–beef bulls with an average live weight of 423 ± 20.2 kg were selected and allocated into four groups: three experimental groups and one control group, with 10 bulls in each group. All bulls received the same basal diet consisting of 45% grass silage, 25% barley grain, and 30% alfalfa hay. In addition, bulls in the experimental groups were supplemented with 2 kg of sprouted seeds per animal per day, representing approximately 25% of the total ration. Bulls in experimental group 1 received sprouted alfalfa, those in experimental group 2 were fed a 1:1 mixture of sprouted wheat and barley, and animals in experimental group 3 received sprouted peas. Following an adaptation period to the new diet, the feeding trial was conducted over a period of 12 months. At the end of the experiment, the body weight of the bulls was 592 ± 17.0 in the control group, and 670 ± 32.0, 640 ± 29.0, and 651 ± 21.0 in the experimental groups, respectively. For analysis, meat samples were collected from the *longissimus dorsi* muscle. The intramuscular fat content of the beef samples was determined using the Soxhlet extraction method (Ostermann *et al.*, 2014). The FA composition of the intramuscular fat was determined using gas chromatography with a Shimadzu GC-2010 Plus system (Shimadzu Corporation, Japan), after converting the FA into their methyl esters (Ostermann *et al.*, 2014). The statistical analysis was performed by one-way ANOVA (Stat 1.1, StatSoft). Statistical significance was determined at p-values ranging from $p < 0.1$ to $p < 0.001$ in comparison with the control group.

Results and Discussion

The inclusion of sprouted seeds in bulls diets improved the FA profile of the meat by reducing the proportion of undesirable SFA. The FA composition evaluated included SFA, monounsaturated FA (MUFA), and PUFA.

Compared with the control group, the proportion of SFA was lower in all experimental groups, differing by 14.8 percentage units in bulls fed sprouted wheat and barley, 16.4 percentage units in bulls fed sprouted alfalfa, and 13.8 percentage units in bulls fed sprouted peas (Table 1).

Table 1 Intramuscular fat fatty acid content of the loin meat (*musculus longissimus dorsi*), g/100 g fatty acids

Diet	SFA*	MUFA*	PUFA*
Control	42.7	47.9	9.4
Wheat:barley	27.9	61.4	10.9
Alfalfa	26.3	62.1	11.5
Peas	28.9	61.9	9.15

*SFA -saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA -polyunsaturated fatty acids

An analysis of the SFA profile revealed that the content of decanoic acid ($p < 0.1$) was 2.5-2.6 times higher in control bulls intramuscular fat than in bulls fed sprouted seeds. An even greater reduction was observed for tetradecanoic acid ($p < 0.001$), which was 3.0-3.5 times lower in the experimental groups compared to controls. These changes are important, as the SFA are involved in energy metabolism and lipid processing and excessive intake may contribute to elevated blood lipid levels.

Significant differences were also observed for palmitic acid ($p < 0.05$). Bulls fed sprouted peas had 3.6 times lower levels of palmitic acid, while those fed sprouted wheat and barley or sprouted alfalfa had levels that were 1.8–2.3 times lower than those of the control group. High dietary intake of palmitic acid in humans is associated with vascular diseases such as atherosclerosis, heart attack, and stroke (Scollan *et al*., 2014)

Feeding bulls sprouted seeds led to a significant increase in MUFA content compared with the control group. The highest MUFA content was observed in bulls fed sprouted alfalfa seeds (14.3 g/100 g FA), slightly lower in those fed sprouted peas (14.1 g/100 g FA), and in the mixture of sprouted wheat and barley (13.7 g/100 g FA). The most pronounced changes were observed in the increased levels of *cis*-9-oleic ($p < 0.1$) and *trans*-9-elaidic acids ($p < 0.001$), particularly in bulls fed sprouted peas and the wheat-barley mixture. In addition, the meat of experimental animals contained higher amounts of omega-3 FA ($p < 0.001$), with the greatest concentration found in bulls fed sprouted alfalfa.

These changes can be explained by the rich biochemical composition of sprouted grains, which provides readily available lipids, enzymes, and metabolic precursors that stimulate the synthesis and accumulation of MUFAs in muscle tissue. Overall, the inclusion of sprouted seeds in the diet favorably modifies the FA profile of bull meat, enhancing its nutritional and functional value for human consumption.

Sprouted-seed supplementation favourably influenced PUFA - related markers in intramuscular fat, particularly by increasing α-linolenic acid ($p < 0.05$) concentrations in all experimental groups, with the greatest effect observed in bulls fed the wheat-barley mixture. Although ruminal biohydrogenation limits the transfer of dietary PUFA to muscle tissue, the observed increases in the α-linolenic acid fraction and its long-chain derivatives suggest that sprouted feeds can modestly enhance the nutritional quality of ruminant meat.

Conclusions

Incorporating sprouted seeds into the diet of fattening bulls reduces the content of harmful SFA, particularly palmitic acid, in meat. This improves the nutritional quality of the meat and may make it healthier for human consumption. Feeding bulls sprouted seeds improves the FA profile of meat by increasing beneficial monounsaturated (*cis*-9-oleic and *trans*-9-elaidic) and omega-3 FA**,** enhancing its nutritional value and making it healthier for human consumption.

References

Vahmani, P., Mapiye, C., Prieto, N., Rolland, D. C., McAllister, T. A., Aalhus, J. L., & Dugan, M. E. R. (2015). The scope for manipulating the polyunsaturated fatty acid content of beef: A review. *Journal of Animal Science and Biotechnology, 6,* Article 29. https://doi.org/10.1186/s40104-015-0026-z

Scollan, N. D., Dannenberger, D., Nuernberg, K., Richardson, I., MacKintosh, S., Hocquette, J.-F., & Moloney, A. P. (2014). *Enhancing the nutritional and health value of beef lipids and their relationship with meat quality*. *Meat Science, 97*(3), 384–394. https://doi.org/10.1016/j.meatsci.2014.02.015

Scollan, N. D., Dannenberger, D., Nuernberg, K., Richardson, I., & Moloney, A. P. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science, 74*(1), 17–33. https://doi.org/10.1016/j.meatsci.2006.05.002

Horcada, A., Polvillo, O., González-Redondo, P., López, A., Tejerina, D., & García-Torres, S. (2020). Stability of fatty acid composition of intramuscular fat from pasture- and grain-fed young bulls during the first 7 d postmortem. *Archives of Animal Breeding, 63*, 45–52. https://doi.org/10.5194/aab-63-45-2020

Ostermanna, A.; Müllera, M.; Willenberga, I.; Schebb, N.H. Determining the fatty acid composition in plasma and tissues as fatty acid methyl esters by gas chromatography – A comparison of different derivatization and extraction procedures. *Prostaglandins leukotrienes and essential fatty acids* 2014, 91, 235-241. https://doi.org/10.1016/j.plefa.2014.10.002

DISTRIBUTION:
Swedish University of Agricultural Sciences
Department of Applied Animal Science and Welfare
Box 7024
750 07 Uppsala

www.slu.se/thv