Proceedings of the 10th Nordic Feed Science Conference, Uppsala, Sweden

Sveriges lantbruksuniversitet
Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

Rapport 302
Report

Uppsala 2019
ISSN 0347-9838
ISRN SLU-HUV-R-302-SE

Proceedings of the 10th Nordic Feed Science Conference, Uppsala, Sweden

Sveriges lantbruksuniversitet
Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

Rapport 302
Report

Uppsala 2019

ISSN 0347-9838
ISRN SLU-HUV-R-302-SE

Published by:
Organising committee of the 10th Nordic Feed Science Conference
Department of Animal Nutrition and Management
Swedish University of Agricultural Sciences (SLU)
SE- 753 23 Uppsala, Sweden

Organising Committee:
Peter Udén
Rolf Spörndly
Bengt-Ove Rustas
Torsten Eriksson
Johanna Karlsson
Horacio Gonda

Edited by:
P. Udén
T. Eriksson
R. Spörndly
B-O. Rustas
J. Karlsson

Printed by:
SLU Repro
SE-75007 Uppsala, Sweden

Distributed by:
Department of Animal Nutrition and Management,
Box 7024,
SE-75323 Uppsala, Sweden
www.slu.se/animal-nutrition-management

These conference proceedings are available as updated PDF files (when applicable) at:
www.slu.se/nordicfeedscienceconference

Foreword

We celebrate the 10-year anniversary of our Nordic Feed Science Conference this year. I hope that we will be able to continue with this conference for a number of years to come. There are some foreseeable problems with this, which we will be a topic for the conference.

Early June is often a lovely time in Uppsala and, over the years, we have had mostly very pleasant weather. We hope that you will enjoy your stay and make the most of the 18.5-h day length during the conference as well as the scientific contributions.

We have 77 registered participants and a total of 26 written contributions this year, of which 11 are in the form of posters. In addition, there will be time for discussing protein evaluation and the future of the NFSC.

Global temperature is increasing and the future of coming generations is compromised. What can we do to change this? Everybody is talking about the weather, but nobody is doing anything about it, as Mark Twain once said. The Nordic countries, except Iceland, experienced a severe drought last year, which farmers had great problems in dealing with. We therefore, have sessions specifically devoted to climate and environment and non-traditional fibrous feeds (ICE – in case of emergency).

This year's conference also focuses on ruminant protein evaluation and, for that reason, Hélène Lapierre, Karl-Heinz Südekum, Pekka Huhtanen and Elisabeth Nadeau have graciously accepted to present their work on the conference. In addition, we look forward to a number of presentations on ruminant nutrition, models and forage conservation. During several of the sessions above, the NorFor model will be scrutinized.

Bioprocess Control Sweden AB will demonstrate their latest gas-in vitro system at the conference and, during the guided poster presentations, you will also be able to see our new research scale extruder at work.

You are all most welcome to the conference! For downloading proceedings of earlier conferences, please go to our homepage https://www.slu.se/en/departments/animal-nutrition-management/news/nordic-feed-science-conference-2019/.

Uppsala 2019-06-04

Peter Udén

Contents

Ruminant protein evaluation

Climate and environment

Fibre based products as feeds – ICE

Nutrition, Models & Methods

Conservation

Knowledge of amino acid metabolism helps to refine recommendations for dairy rations

H. Lapierre[1], R. Martineau[2] & D.R. Ouellet[1]
[1]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada, J1M 0C8*
[2]*Département des sciences animales, Université Laval, Québec, QC, Canada, G1V 0A6*
Correspondence: helene.lapierre@canada.ca

Introduction

In addition to decreasing feeding costs, improving the efficiency of utilization of nitrogen (N) directly addresses the general concern regarding the environmental footprint of animal production. Although emphasis is often put on the poor efficiency of N utilization for milk production (milk N / N intake) averaging below 30% (e.g., Huhtanen and Hristov, 2009), the transfer of human non-edible inputs into high-quality human-edible milk protein by the dairy cow should be acknowledged. In this context, dairy cows can make a valuable contribution to the human food chain with a protein efficiency (human-edible output/input) varying for example from 141 to 208%, depending of the production context (Broderick, 2018). Increased cost effectiveness and decreased pollution can be achieved through a lower input of dietary protein, provided productivity is not compromised.

It is acknowledged that improving the formulation of dairy rations requires accurate estimation of both supply and requirement of metabolizable protein (MP), far beyond the sole estimation of crude protein (CP). A further step involves moving estimations of supply and requirement from MP to those of individual essential amino acids (EAA). In recent years, many Europeans feeding systems have been revisited (e.g. NorFor, 2011; DVE/OEB system (Van Duinkerken *et al.*, 2011); INRA, 2018) and have included the latest knowledge in their estimation of requirements. The assumed linear relationship between MP supply and protein outputs arising from the use of a fixed efficiency has been progressively changed to a variable efficiency linked to both the supply of protein and energy. We will examine how the post-absorptive metabolism of EAA supports new changes adopted to estimate MP supply and requirement and suggest options for improving a factorial approach to determine recommendations of EAA supply in dairy cows.

Amino Acid Metabolism

To better understand how EAA are used to fulfil the needs of protein synthesis, we will follow the fate of AA from digestion into the small intestine to secretion into milk protein. To simplify the presentation, no change in body weight (BW) and no pregnancy are assumed. We will mainly follow the route of two EAA: 1) histidine (His) representing Group 1 AA (including also methionine (Met), phenylalanine (Phe) and tryptophan) and 2) leucine (Leu) representing Group 2 AA (also including isoleucine (Ile), lysine (Lys) and valine (Val)). At the end of this section, you will clearly see how the characteristic pattern of utilization of each group of AA differs. Data presented in Figure 1 are adapted from Raggio *et al.* (2004).

Portal-drained viscera

For the high MP supply treatment, net digestible flows of His and Leu were estimated at 64 and 213 g/d, respectively (Raggio *et al.*, 2004). Although the route seems fairly short and unidirectional between the small intestine and the portal vein, substantial utilization of EAA occurs between these two sites. Indeed, net portal absorption represented 83 and 74% of net digestible flow, for His and Leu respectively. In fact, blood portal circulation is not only collecting AA absorbed from the small intestine, but is also deprived of AA supplied from

arterial source and used by the portal-drained viscera (PDV). Comparisons of small intestinal disappearance of AA or net mesenteric appearance with net portal appearance in sheep (MacRae *et al.*, 1997) and dairy cows (Berthiaume *et al.*, 2001) confirmed that EAA are used by the PDV, but to different extent among the EAA. First, AA are used by the PDV to support protein synthesis. However, we have to remember that endogenous proteins that are secreted into the gut lumen, digested and reabsorbed, do not create a net demand on EAA as well as any protein turnover in the PDV if the gut is not growing. Therefore, only endogenous secretions that are not digested and are excreted in the faeces represent a net utilization of EAA. Second, EAA can be catabolized by the PDV. Although data are very limited on EAA catabolism by the PDV in dairy cows, indirect comparisons made by Pacheco *et al.* (2006) and direct measurements of oxidation in dairy cows (Leu only; Lapierre *et al.*, 2002) indicated very limited, if any, oxidation of Group 1 AA and oxidation of branched-chain AA (BCAA: Ile, Leu and Val). Although Lys belongs to Group 2 AA, there is so far no evidence of Lys oxidation by the PDV in ruminants. In sheep, Lys was not oxidized by the PDV (Lobley *et al.*, 2003) and there was no clear unaccounted usage of Lys by the PDV besides endogenous secretions according to Pacheco *et al.* (2006). In summary, for Group 1 AA, net usage by the PDV would be accounted for by undigested endogenous secretions excreted in the faeces whereas there is additional loss due to oxidation for the BCAA.

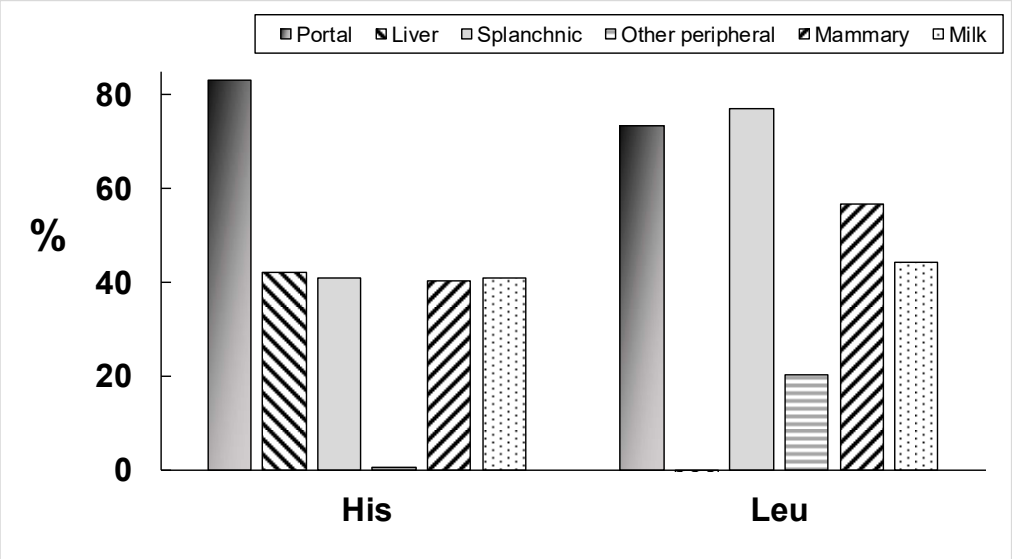


Figure 1 Net flows of histidine (His) and leucine (Leu) across tissues, as % of their respective net digestible flow; hatched bars represent a net uptake by tissues whereas solid bars represent a net release (adapted from the high MP supply treatment from Raggio *et al.*, 2004).

Liver

After absorption into portal circulation, AA are flowing directly into the liver. Initial studies with catheterized dairy cows were reporting large removal of AA by the liver: for example, between 38 to 47% of absorbed α-amino N was removed by the liver (Reynolds *et al.*, 1988). However, we have to be cautious because this generalization does not apply to every individual AA. As clearly depicted in Figure 1, there is indeed substantial removal of His by the liver, but literally none for Leu (Raggio *et al.*, 2004). This pattern is typical to what is reported in the literature (see review: Lapierre *et al.*, 2012): on average, 35, 31 and 51% of

net portal absorption of His, Met and Phe was removed by the liver, whereas there was no net measurable removal of Group 2 AA. Therefore, despite the fact that the liver is the major site of ureagenesis, not all of the EAA in excess are, on a net basis, extracted by the liver. They can be deaminated elsewhere in the body and the N returned to the liver through N-shuttles like alanine or glutamine prior to excretion of excess N as urea.

It has initially been suggested that liver removal of AA was related to their net portal absorption (e.g., Reynolds, 2006). Indeed, increased net portal absorption of AA usually increases plasma concentrations. Dissociation between these last two parameters has been achieved under physiological conditions, with cows investigated before and after initiation of lactation (Doepel *et al.*, 2009). Initiation of lactation increased intake and net portal absorption of AA, but the high demand of AA to support milk protein secretion reduced circulating concentrations of EAA and liver removal of EAA. It has been observed that liver removal was better correlated with total liver inflow rather than with net portal absorption (Hanigan, 2005; Lapierre *et al.*, 2005). Total inflow integrates both net portal absorption and arterial concentration, the latter including utilization of EAA by peripheral tissues. This indicates that hepatic extraction is not exclusively due to first-pass removal and that peripheral tissues have the opportunity over a short window of time to use absorbed AA before they are finally catabolized by the liver after a few passes across the splanchnic bed.

Other Peripheral Tissues

In dairy cows, AA metabolism in peripheral tissues other than the mammary gland has not been thoroughly studied. However, if estimated as the difference between release of AA by splanchnic tissues and mammary uptake, trends are very similar to what has been reported in growing animals (e.g. Harris *et al.*, 1992). Overall, net splanchnic flux was almost totally captured by the mammary gland, i.e. no peripheral tissue net removal, for His and the other Group 1 AA whereas net splanchnic flux of Leu and other Group 2 AA was greater than mammary uptake, indicating substantial removal of Group 2 AA by peripheral tissues (Figure 1).

Mammary Gland

In Figure 1, mammary uptake of His was equal to secretion into milk protein. Similarly, for Group 1 AA, the mammary uptake:output ratio in studies where samples have been analysed individually averaged 1.05 ± 0.05 and 1.01 ± 0.04 for His and Met, respectively; Phe +Tyr being used as markers to estimate mammary plasma flow were assigned a value of 1.0. Group 1 including Met, Phe+Tyr, and Trp has been proposed by Mepham (1982) for their stoichiometric transfer from blood into milk protein; His was later added to this group (Lapierre *et al.,* 2012). On the other hand, for Group 2 AA (BCAA and Lys), mammary uptake is in excess of the output in milk protein and this excess increases with increased supply (Lapierre *et al.,* 2012). This is in agreement with an increased mammary oxidation of Leu with increased MP supply (Raggio *et al.*, 2006). Lysine has also been reported to be oxidized within the mammary gland (Mabjeesh *et al.*, 2000). So overall, mammary uptake of Group 1 AA is adjusted to what is needed to cover milk protein secretion whereas, for Group 2 AA, it exceeds milk output. This excess can be used within the mammary gland as an energy source, a supply of N or carbon chain for the synthesis of non-EAA or as precursor for fat synthesis (Lapierre *et al*., 2012).

Whole Body

A last point regarding AA metabolism is their overall usage for protein synthesis. On a daily basis, a cow synthesizes approximately between 4 and 5 kg of proteins: this synthesis is distributed to muscles, skin, liver, gut and mammary gland with 15-20, 8-16, 4-15, 32-45 and 35-45% occurring in each tissue respectively (Lobley, 2003). From the synthesis of all these proteins, on a net basis, less than half of AA used for protein synthesis do not return to the pool of free AA, being secreted or becoming part of constitutive proteins which means that more than half will be degraded back to single AA into the pool of free AA (e.g., Lapierre *et al.*, 2002). The latter fraction does not represent a net demand on absorbed AA.

After following the fate of digested AA up to milk protein, let's see where this knowledge may impact concepts included in formulation models and help to refine them.

From Metabolism to Ration Formulation

Supply

Based on the understanding of PDV metabolism, it becomes clear that the endogenous protein duodenal flow does not constitute a net supply to the dairy cow because the AA used for its synthesis are supplied from arterial source, i.e. have been previously absorbed. Nevertheless, their presence must be acknowledged: the difference between total duodenal flow of CP and microbial CP flow is the sum of undegraded dietary protein and endogenous protein flow. Based on limited available data, daily endogenous duodenal CP flow has been estimated to: (15.4 + 1.21×dry matter intake ($DMI_{kg/d}$))×6.25 (Lapierre *et al.*, 2016a). The AA composition from rumen and abomasal isolates (Ørskov *et al.*, 1986) is currently the best estimation we have for this flow.

Recommendations

As presented above: 1) the sum of proteins secreted out of the cow represents less than half of the whole body protein synthesis; 2) the AA catabolism occurring in different tissues differs between groups of AA and 3) the catabolism of AA is not related to the intensity of protein synthesis in a tissue (e.g., no catabolism of Group 1 AA in the mammary gland). Based on these observations, it seems logical to assign an efficiency factor to protein synthesis which we are able to quantify, i.e. protein secretions (and accretion if present during growth and gestation), and not to the whole body protein secretion. Therefore, the first step in establishing recommendations of MP and AA is to quantify protein secretions and their AA composition whereas the second step will be to define an efficiency of utilization of MP and AA supply to support these functions.

Protein and amino acid secretions

Based on AA metabolism, protein secretions draining irreversibly AA from the available pool of AA and included in the recommendations are: scurf, endogenous urinary, undigested gut endogenous secretions and milk. Scurf represents a very small fraction of total secretions and the estimation from Swanson (1977) adjusted to yield true protein (TP) secretion, in g/d, becomes $0.2\times0.86\times BW^{0.60} = 0.17\times BW^{0.60}$, where 0.86 represents the TP/CP ratio of scurf based on its AA composition; here and through the text, BW is in kg. Endogenous urinary loss (EndoUri) is still based on Swanson's (1977) estimation in most models. We have revisited this estimation to better define its AA composition and obtained a daily loss (g TP/d) of 0.33×BW (Lapierre *et al.*, 2016b) - very close to the recent estimation of 0.31×BW from INRA

(2018). However, loss of EAA should be associated only to loss of endogenous urea (g TP/d: 0.063×BW), assuming that endogenous urea synthesis occurred from AA with the whole empty body composition); the other N-metabolites constituting EndoUri (creatinine, creatine, hippuric acid, endogenous purine derivatives) are synthesized from non-EAA and Arg, not strictly an EAA. Histidine excretion as 3-methyl-His [mg His/d = 7.82 + 0.55×BW] should be added to the His contribution to EndoUri. Undigested gut endogenous secretions corresponds to the metabolic faecal protein (MFP) output and should not include undigested microbial protein synthesized from recycled urea, either in the rumen or in the large intestine. Based on measurements of endogenous secretions in dairy cows (Ouellet *et al.*, 2002, 2007, and 2010) and sheep (Sandek *et al.*, 2001) and based on a meta-analysis of cattle studies (Marini *et al.*, 2008), daily TP secretion in MFP was evaluated as: TP excreted (g/d) = (8.5 + 0.1×$NDF_{\%DM}$)×DMI (kg/d) according to Lapierre *et al.* (2016b). The AA composition of MFP was based on the AA composition of ruminal and abomasal isolates from Ørskov *et al.* (1986), except for Leu for which only the rumen isolates was used and the endogenous flow at the ileum in pigs (Jansman *et al.*, 2002), assuming that 70% of the MFP is from undigested duodenal flow and the remaining 30% from the intestine (Ouellet *et al.,* 2002 and 2010). And finally, AA composition of MPY has also been recalculated based on its different protein fractions as reported in Lapierre *et al.* (2016b). The AA composition of the proteins detailed above were presented Lapierre *et al.* (2016b); note that those obtained from protein hydrolysis have been updated with correction factors proposed to take into account incomplete recovery of most AA with 24-h hydrolysis (Lapierre *et al.*, 2019).

Efficiency

Based on observations of AA metabolism, it has been proposed to use a combined efficiency assigned to all TP secretions: scurf, MFP and MPY (Lapierre *et al.*, 2007), assuming no BW change and no conceptus. Indeed, all Group 1 AA not used for protein secretions are removed by the liver, which is not the site of any protein export out of the cow. So why should we use different efficiencies for proteins synthesized by the gut (MFP) and the mammary gland (MPY)? Prediction of a variable efficiency of MP was improved when all protein secretions were combined and compared with a fixed efficiency applied to all non-productive functions and a variable efficiency assigned to MPY (Sauvant *et al.*, 2015). The contribution of MP and AA to EndoUri is, however, not included in secretions and removed from the supply: its efficiency is assumed to be 100%, as suggested by Sauvant *et al.* (2015), because these secretions are not TP but end-products of metabolic pathways.

As actually incorporated into the most recent European models for the efficiency of utilization of MP (Eff_{MP}), the efficiency of utilization of individual AA (Eff_{AA}) should also be considered to be variable. Initial work related the Eff_{AA} to the AA supply (Doepel *et al.*, 2004). This was evidenced by increased hepatic removal of Group 1AA and increased excess mammary uptake of Group 2 AA relative to milk protein when MP supply increased (Raggio *et al.,* 2004), thus reducing Eff_{AA}. However, more recent work is indicating that the relationship is improved when the Eff_{AA} is related to the ratio of AA/energy supplies (Lapierre *et al.*, 2016b). In fact, the ratio of MP to energy supplies is used to estimate a variable Eff_{MP} in Norfor (2011) and in the DVE/OEB system (van Duinkerken *et al.,* 2011). The new French system (INRA , 2018) predicts MPY based on both supplies as well. Work is in progress currently to improve predictions of Eff_{AA} based on AA and energy supplies.

Adequate predictions of Eff_{AA} could then be assigned to secretions of individual EAA described above to determine threshold recommendations of individual EAA supply.

Conclusion

Overall, a better knowledge of AA metabolism has improved quantification of daily amounts of exported AA, either as non-productive functions or MPY. In addition, knowledge of AA metabolism has suggested to: 1) use a combined efficiency for these functions (except endogenous urinary excretion) and 2) use a variable efficiency to convert these exported AA into recommendations. Although it was first suggested that Eff_{AA} was related to their respective digestible flow, it seems that the ratio of AA supply to energy supply is better related to efficiency: when the ratio AA/energy supplies increases, efficiency decreases. In a complete formulation model, determination of target efficiencies for the different EAA should allow to set thresholds for recommendations of EAA supply and a better prediction of MPY under predicted supply of EAA.

References

Berthiaume R., Dubreuil P., Stevenson M., McBride B.W. & Lapierre H., 2001. Intestinal disappearance and mesenteric and portal appearance of amino acids in dairy cows fed ruminally protected methionine. J. Dairy Sci. 84, 194-203.

Broderick G.A., 2018. Review: Optimizing ruminant conversion of feed protein to human food protein. Animal 12, 1722-1734.

Doepel L., Pacheco D., Kennelly J.J., Hanigan M.D., López I.F. & Lapierre H., 2004. Milk protein synthesis as a function of amino acid supply. J. Dairy Sci. 87, 1279-1297.

Doepel L., Lobley G.E., Bernier J.F., Dubreuil P. & Lapierre H., 2009. Differences in splanchnic metabolism between late gestation and early lactation dairy cows. J. Dairy Sci. 92, 3233-3243.

Hanigan M.D., 2005. Quantitative aspects of ruminant splanchnic metabolism as related to predicting animal performance. Animal Sci. 80, 23-32.

Harris P.M., Skene P.A., Buchan V., Milne E., Calder A.G., Anderson S.E., Connell A. & Lobley G.E., 1992. Effect of food intake on hind-limb and whole-body protein metabolism in young growing sheep: chronic studies based on arterio-venous techniques. Br. J. Nutr. 68, 389-407.

INRA, 2018. Feeding system for ruminants. Wageningen Academic Publishers, Wageningen, The Netherlands.

Huhtanen P. & Hristov A.N., 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk n N efficiency in dairy cows. J. Dairy Sci. 92, 3222-3232.

Jansman A.J.M., Smink W., van Leeuwen P. & Rademacher M., 2002. Evaluation through literature data of the amount and amino acid composition of basal endogenous crude protein at the terminal ileum of pigs. Anim. Feed Sci. Tech. 98, 49-60.

Lapierre H., Blouin J.P., Bernier J.F., Reynolds C.K., Dubreuil P. & Lobley G.E., 2002. Effect of supply of metabolizable protein on whole body and splanchnic leucine metabolism in lactating dairy cows. J. Dairy Sci. 85, 2631-2641.

Lapierre H., Berthiaume R., Raggio G., Thivierge M.C., Doepel L., Pacheco D., Dubreuil P. & Lobley G.E., 2005. The route of absorbed nitrogen into milk protein. Animal Sci. 80, 11-22.

Lapierre H., Lobley G.E., Ouellet D.R., Doepel L. & Pacheco D., 2007. Amino acid requirements for lactating dairy cows: reconciling predictive models and biology, in Proc. Cornell Nutrition Conference for Feed Manufacturers, Cornell University, NY, USA, pp. 39-59.
Lapierre H., Lobley G.E., Doepel L., Raggio G., Rulquin H. & Lemosquet S. 2012. Triennial lactation symposium: Mammary metabolism of amino acids in dairy cows. J. Anim. Sci. 90, 1708-1721.
Lapierre H., Hanigan M.D. & Ouellet D.R., 2016a. Estimation of duodenal endogenous protein flow in dairy cattle: a regression approach, in Skomiał, J., Lapierre, H. (Eds.), Energy and protein metabolism and nutrition. EAAP publication No. 137, Wageningen Academic Publishers The Netherlands, pp. 131-132.
Lapierre H., Ouellet D.R., Martineau R. & Spek J.W., 2016b. Key roles of amino acids in cow performance and metabolism - considerations for defining amino acid requirement, in Proc. Cornell Nutrition Conference for Feed Manufacturers, Cornell University, NY, USA, p. 205-219.
Lapierre H., Binggeli S., Sok M., Pellerin D. & Ouellet D.R., 2019. Estimation of correction factors to determine the true amino acid concentration of protein after a 24-hour hydrolysis. J. Dairy Sci. 102, 1205-1212.
Lobley G.E., 2003. Protein turnover - What does it mean for animal production? Can. J. Anim. Sci. 83, 327-340.
Lobley G.E., Shen X., Le G., Bremner D.M., Milne E., Calder A.G., Anderson S.E. & Dennison N., 2003. Oxidation of essential amino acids by the ovine gastrointestinal tract. Br. J. Nutr. 89, 617-629.
Mabjeesh S.J., Kyle C.E., Macrae J.C. & Bequette B.J., 2000. Lysine metabolism by the mammary gland of lactating goats at two stages of lactation. J. Dairy Sci. 83, 996-1003.
MacRae J.C., Bruce L.A., Brown D.S., Farningham D.A.H. & Franklin M., 1997. Absorption of amino acids from the intestine and their net flux across the mesenteric- and portal-drained viscera of lambs. J. Anim. Sci. 75, 3307-3314.
Marini J.C., Fox D.G. & Murphy M.R., 2008. Nitrogen transactions along the gastrointestinal tract of cattle: A meta-analytical approach. J. Anim. Sci. 86, 660-679.
Mepham T.B., 1982. Amino acid utilization by lactating mammary gland. J. Dairy Sci. 65, 287-298.
NorFor 2011. The Nordic feed evaluation system. Wageningen Academic Publishers, The Netherlands.
Ørskov E.R., MacLeod N.A, & Kyle D.J., 1986. Flow of nitrogen from the rumen and abomasum in cattle and sheep given protein-free nutrients by intragastric infusion. Br. J. Nutr. 56, 241-248.
Ouellet D.R., Demers M., Zuur G., Lobley G.E., Seoane J.R., Nolan J.V. & Lapierre H., 2002. Effect of dietary fiber on endogenous nitrogen flows in lactating dairy cows. J. Dairy Sci. 85, 3013-3025.
Ouellet D.R., Valkeners D., Holtrop G., Lobley G.E. & Lapierre H., 2007. Contribution of endogenous secretions and urea recycling to nitrogen metabolism, in Proc. Cornell Nutrition Conference for Feed Manufacturers, Cornell University, NY, USA, p. 1-24
Ouellet D.R., Berthiaume R., Holtrop G., Lobley G.E., Martineau R. & Lapierre H., 2010. Effect of method of conservation of timothy on endogenous nitrogen flows in lactating dairy cows. J. Dairy Sci. 93, 4252-4261.

Pacheco D., Schwab C.G., Berthlaume R., Raggio G. & Lapierre H., 2006. Comparison of net portal absorption with predicted flow of digestible amino acids: Scope for improving current models? J. Dairy Sci. 89, 4747-4757.
Raggio G., Pacheco D., Berthiaume R., Lobley GE., Pellerin D., Allard G., Dubreuil P. & Lapierre H., 2004. Effect of level of metabolizable protein on splanchnic flux of amino acids in lactating dairy cows. J. Dairy Sci. 87, 3461-3472.
Raggio G., Lemosquet S., Lobley G.E., Rulquin H. & Lapierre H., 2006. Effect of casein and propionate supply on mammary protein metabolism in lactating dairy cows. J. Dairy Sci. 89, 4340-4351.
Reynolds C.K., Huntington G.B., Tyrrell H.F. & Reynolds P.J., 1988. Net portal-drained visceral & hepatic metabolism of glucose, L-lactate, and nitrogenous compounds in lactating Holstein cows. J. Dairy Sci. 71, 1803-1812.
Reynolds, C .K., 2006. Splanchnic amino acid metabolism in ruminants, in Sejrsen, K., Hvelplund, T., Nielsen, M.O. (Eds.), Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress. Wageningen Academic Publishers, Wageningen, the Netherlands, p. 225-248
Sandek A., Krawielitzki K., Kowalczyk J., Kreienbring F., Schöenhusen U., Gabel M., Żebrowska T., Hagemeister H. & Voigt J., 2001. Studies on N-metabolism in different gastro-intestinal sections of sheep using the digesta exchange technique. 2. Passage of endogenous nitrogen. J. Anim. Feed Sci. 10, 605-618.
Sauvant D., Cantalapiedra-Hijar G., Delaby L., Daniel J.B., Faverdin P. & Nozière P., 2015. Actualisation des besoins protéiques des ruminants et détermination des réponses des femelles laitières aux apports de protéines digestibles dans l'intestin. INRA Prod. Anim. 28, 347-368.
Swanson E.W., 1977. Factors for computing requirements of protein for maintenance of cattle. J. Dairy Sci. 60, 1583-1593.
Van Duinkerken G., Blok M.C., Bannink A., Cone J.W., Dijkstra J., Van Vuuren A.M. & Tamminga S., 2011. Update of the Dutch protein evaluation system for ruminants: the DVE/OEB$_{2010}$ system. J. Agr. Sci. 149, 351-367.

Evaluation of the NorFor, Finnish (FIN) and 2001 NRC protein systems

P. Huhtanen
[1]*Swedish University of Agricultural Sciences (SLU), Department of Agriculture for Northern Sweden, 90183 Umeå, Sweden*
Correspondence: pekka.huhtanen@slu.se

Introduction

Accurate estimation of protein value of feeds and diets is important for both optimizing production economy and minimizing negative environmental effects from overfeeding protein. New feed protein evaluation systems which started to evolve in 1980's take into account degradable N requirements of rumen microbes and absorbed amino acid requirements of the host animals. Milk protein yield responses are much better related to the intake of metabolisable protein (MP) than crude protein (CP) or digestible CP (DCP). Actually, intake of metabolisable energy (ME) or dry matter (DM) has predicted milk protein yield better than CP or DCP both within and among experiments (Huhtanen, 2005).

Several feed protein evaluation systems, differing in complexity, have been developed since 1980's. After introduction of the *in situ* (nylon bag) method in determining effective ruminal protein degradability (EPD), the main focus in ruminant feed protein evaluation research has been on the determination of the rumen undegraded protein (RUP) contribution to the MP supply. Microbial protein which is quantitatively much more important than RUP or feed MP has attained less attention. Although the *in situ* method has several weaknesses, it has been used in feed protein evaluation almost without any criticism. Even nowadays, studies investigating *in situ* degradability are frequently published. However, already almost 30 years ago Voigt and Piatkowski (1991) published an equation demonstrating that microbial protein and RUP are non-additive. Several reviews and meta-analysis (Santos et al., 1998; Ipharraguerre and Clark, 2005; Huhtanen and Hristov, 2008; Huhtanen et al., 2009) demonstrated that RUP is clearly overvalued. Using a constant EPD, rather than variable *in situ* values in calculating MP supply, predicted milk protein yield better (Tuori et al. 1998) indicating that differences *in situ* determined EPD values were of little value.

In the development of feed protein evaluation systems, models are seldom evaluated against data from production studies, even though production responses are the final test of a feed evaluation system. For optimisation the economy of milk production (milk income over feed costs), predicted feeding values should describe the productive values of feeds and diets accurately. Some evaluations, mostly in single studies, have been made by comparing observed and MP allowable yields. This can, however, be misleading in ranking of diets. Average MP allowable and observed yields can have a small prediction error, whereas ranking of the diets is inconsistent (low R^2). On the other hand, MP allowable and observed yield can have a large prediction error at the same time as ranking of the diets is consistent (high R^2). In the first case, the problem is caused by errors in the estimated input (MP supply) and in the latter case, animal requirements are wrongly estimated. In the latter case MP supply and observed yield are better correlated, and the difference between MP allowable and observed yield can be adjusted by changing the requirements (feeding recommendations). It is also important to evaluate the relationship between the diets within experiments using mixed models with random study effect rather than global relationships using fixed regression models.

The objectives of this paper was to evaluate three protein evaluation systems differing in complexity: NorFor (Volden, 2011), NRC (2001) and FIN (LUKE, 2018) in predicting milk protein yield.

Material and Methods

The data sets represented typical Nordic dairy cow diets consisting mainly of forages (mainly grass silage, but also grass hays, legume and whole crop silages) with cereal grains and by-products as energy supplements and different protein supplements. Diets without protein supplements were also included in the dataset. The supply of MP in each system was estimated using the same input data (intake of dietary ingredients, body weight, and chemical composition of ingredients). For the NRC system and FIN systems, tabulated EPD values and intestinal digestibility of RUP (in the FIN system it was a constant of 0.82) were used. Intake of total digestible nutrients (TDN) in the NRC system was estimated from determined in vivo or in vitro organic matter digestibilities of forages and from tabulated values for concentrate ingredients. For the NorFor system, nutrient supplies were calculated from intake and feed composition data by the NorFor team.

The relationships between MP supply and milk protein yield (MPY) were estimated by mixed model regression analysis according to St-Pierre (2001). The models were run using a random intercept or a random intercept and slope. Using a random slope reduces residual variance as it takes into account variation in the slope among studies. This variation can result from differences among studies in the stage of lactation (earlier - better responses), the genetic potential of cows (high potential cows could respond better?), level of protein supplementation (smaller responses at high inputs) and random errors in output data. In addition, random slope variance can increase due to errors in input data. If the differences in MP supply within a study are overestimated, the slope of MPY on MP supply will be underestimated, and *vice versa*, underestimation of difference in input will overestimate the slope. In addition to MP supply, DM and ME intake were used as input variables to evaluate how well MPY can be predicted from simple input data.

The evaluations were made using three different datasets including a total of 339 treatment means. The NorFor and FIN systems were compared using two versions of NorFor system (2007 and 2017) with a smaller dataset (N=152). The NRC and FIN models were also compared with a larger dataset (N = 986 diets).

Results and Discussion

When only the intercept was used as random factor, the NorFor model had the greatest residual variance and adjusted root mean squared error (Table 1). Interestingly, even DMI predicted MPY better than MPI, calculated according to the NorFor system. No negative quadrative effect was significant in NorFor (P = 0.65), but there was a tendency (P = 0.11) in the FIN system. Quadratic terms did not improve the models in terms of reduced AICC or RMSE. Performance of the NRC model was equal to DMI but inferior to MEI adjusted for feeding level and associative effects (interaction between diet composition and feeding level).

Including a random slope effect in the NorFor model gave the greatest improvement in performance of the model as a result of the large random slope variance. Random slope variance was 1.7 and 2.9 times greater than in the NRC and FIN systems, respectively. Because of the strong negative correlation between intercept and slope, variance components of intercept and covariance intercept × slope were considerably greater the NorFor than for

the other MP models. This indicates greater errors in the estimates of MP supply in the NorFor system compared with the other systems. Other possible factors contributing to random slope variance were similar among the systems. When ranges in the supply are overestimated, the slope of MPY on MPI decreases, and when the ranges in the supply are underestimated, the slope increases. As feed MP in both the NRC and NorFor systems rely on *in situ* determined EPD, passage rates of feed particles and intestinal digestibility of RUP, the greater slope variance in the NorFor system is likely related to greater errors in estimating microbial MP. It seems that equations predicting ruminal digestibility and different ATP value of fermentable substrates does not improve predictions of microbial MP. The NRC (2001) system predicts microbial MP from TDN: digestible nutrients in the total tract + higher (2.25) coefficient for digested fat intake in estimating microbial protein.

Table 1 Predicting milk protein yield from intake of dry matter (DMI), metabolizable energy (MEI) and metabolizable protein (MPI) estimated by three protein evaluation systems (N = 339 diets)

			Variance components						
	Intercept	Slope	Inter-cept	Int × Slope	Slope	Residual	AICC[1]	Adj. RMSE[2]	Adj. R^2
Random intercept									
DMI, kg/d	-65	49.5	7537			802	3448	25.9	0.957
MEI, MJ/d	-59	4.54	5900			606	3360	22.5	0.962
NRC, kg/d	288	338	10150			796	3458	25.8	0.922
NorFor, kg/d	405	298	8763			1044	3535	29.4	0.911
FIN, kg/d	191	389	7482			522	3155	20.9	0.957
Random intercept and slope									
DMI, kg/d	-96	50.9	51990	-3014	187	739	3434	24.3	0.964
MEI, MJ/d	-133	4.87	43535	-203	1.04	552	3350	20.9	0.971
NRC, kg/d	311	323	33926	-15442	9791	641	3436	22.3	0.936
NorFor, kg/d	419	292	66918	-30992	16950	660	3459	22.1	0.945
FIN, kg/d	189	393	26698	-10779	5756	405	3271	17.7	0.969

[1] Akaike's information criteria, corrected (smaller is better); [2] Root mean squared error, adjusted for random effects.

The supply of fermentable organic matter (OM) is estimated using rather simply by NRC as total digestible nutrients (TDN) at production level. In the FIN system, it is calculated from digestible OM at maintenance – rumen undegraded protein. In the NorFor model, fermentable OM is estimated by (semi)mechanistic equations for each dietary component. In evaluation of the NorFor digestion model using sheep digestibility data, prediction errors of OMD were about 2-fold higher as compared to estimates of in vivo OMD from in vitro OMD (Huhtanen, unpublished). In the NorFor NDF digestion sub-model, selective retention is taken into account twice (passage rate model based on rumen evacuation derived passage rate estimates of potentially digestible NDF that are further divided to retention in rumen non-escapable and escapable pools. Using *in situ* based degradation kinetic data for starch can lead to greater errors in estimating total fermentable OM than total digestibility due to particle losses and other shortcomings of the *in situ* method. The NorFor system discounts for silage fermentation acids in estimating fermentable OM for microbial OM. Theoretically this is correct, but discounting for fermentation acids in estimating MP supply increased prediction error of MPY compared with MP calculated without discounts (Rinne et al., 2008). It is possible that fermentation of silage to lactic acid increases glucose supply to the cow, which

could improve efficiency of amino acid utilization for milk protein synthesis. If a discount for fermentation acids is made, then, also possible effects of increased supply of glucose with extensively fermented silages should be taken into account. Reduced silage DM intake accounted entirely for the adverse effect of extensively fermented silages on MPY without any specific effect of silage total acid concentration, which supports the previous speculation (Huhtanen et al., 2003). It is also possible that the feeding level effect on MP is too strong in the NorFor system. Calculated dietary MP concentration increases about 30% when feeding level increases from 8 to 20 kg DM/d. According to analysis of omasal sampling data, the corresponding increase in efficiency of microbial protein synthesis was about 20% (Broderick et al., 2010), whereas NorFor predicts about 35% increase (equation 7.28). It is possible that the effects of rapidly degradable carbohydrates on efficiency of microbial protein synthesis is too large. At DMI of 20 kg/d and optimal level of rapidly fermentable carbohydrates (235 g/kg DMI) predicted efficiency microbial protein synthesis is 35% greater than at zero concentration. AAT values reported in NorFor feed tables do not well reflect observed MPY responses. For example, tabulated AAT20 values for the most important protein supplements - soybean meal, untreated rapeseed meal and heat-treated rapeseed meal are 209-220, 198 and 148 g/kg DM, respectively, but observed MPY responses in a meta-analysis were 98, 133 and 136 g/kg incremental CP intake, respectively (Huhtanen et al., 2010). On a DM basis, these responses were equal. Another practical example of disagreement between tabulated *in situ* based MP (AAT) values is underestimation of hay compared with silage in NorFor feed tables. At the same energy concentration, MP concentration of hay is about 20% greater than for silage. This is in contrast to production studies (Bertilsson, 1983) and duodenal flow studies (Jaakkola and Huhtanen, 1993) which indicated at least similar protein values for silage and hay harvested at the same time from the same ley. Constant EPD values are used for forages as before in Sweden leading to rather constant MP/ME ratio in forages.

The NCR system use *in situ* based estimates for predicting MP supply from feed protein. Smaller slope variance in the NRC system compared with the NorFor system suggest that either NRC tabulated values reflect the true supply of feed MP better than NorFor, or more likely, other factors discussed above increase variability in MP supply that is not reflected in MPY responses. Indeed, standard deviation in dietary MP concentration was greater for NorFor (9 g/kg DM) compared NRC (7) and FIN (5) systems. In the Finnish feed tables, degradability values are based on situ measurement, but inconsistencies between *in situ* data vs. duodenal/omasal flow measurements and production studies have been taken into account. If ruminal protein degradability is manipulated by chemical or physical treatments, the manufacturer should demonstrate that that treatment is realized as improved performance

The NorFor and FIN systems were compared using a smaller dataset in 2007 (Table 2). In terms of residual variance, AIC, and adjusted RMSE and R^2, the 2007 version of NorFor performed better, especially when slope was assumed fixed. MPI estimated according to NorFor 2007 version predicted MPY responses better than DMI, whereas the reverse was true for the 2017 version. All parameters describing the model performance were the best for the FIN model.

Ranking of DMI, MEI and MPI estimated according to the NRC and FIN systems remained similar in a larger dataset (N = 986) compared to a smaller dataset (Table 3). MEI was a better predictor of MPY compared with MPI estimated according to NRC. This was also the case for the North American data (Huhtanen & Hristov, 2009). Random slope variance was

about 2-fold in the NRC system compared with the FIN system, indicating that production responses per unit of MP were more variable among studies when MP was estimated according to the NRC (2001) system.

Table 2 Predicting milk protein yield from DMI and MPI estimated by three protein evaluation systems (N = 152 diets)

			Variance components						
	Intercept	Slope	Inter-cept	Int × Slope	Slope	Residual	AICC	Adj RMSE	Adj. R^2
Random intercept									
DMI, kg/d	-316	62.1	3290			831	1507	27.2	0.933
NorForA[1], kg/d	215	384	2052			774	1486	26.3	0.931
NorForB[2], kg/d	374	332	3335			1226	1555	33.1	0.863
FIN, kg/d	162	423	1690			426	1402	19.5	0.955
Random intercept and slope									
DMI, kg/d	-220	57.6	79344	-3458	157	723	1504	24.8	0.951
NorForA[1], kg/d	179	401	37274	-15479	6810	654	1484	23.5	0.940
NorForB[2], kg/d	328	350	132507	-58567	26656	721	1524	24.3	0.913
FIN, kg/d	156	425	12915	-5638	2824	378	1402	17.9	0.962

[1]NorFor evaluation 2007; [2] NorFor evaluation 2017.

Table 3 Predicting milk protein yield from DMI, MEI and MPI estimated by the NRC and FIN systems (N = 986 diets)

			Variance components						
	Intercept	Slope	Inter-cept	Int × Slope	Slope	Residual	AICC	Adj. RMSE	Adj. R^2
Random intercept									
DMI, kg/d	-20	46.6	6409			875	10197	26.4	0.961
MEI, MJ/d	-45	4.37	5350			715	10007	23.9	0.965
NRC, kg/d	292	307	9603			840	10242	25.8	0.931
FIN, kg/d	171	383	5919			548	9810	20.9	0.967
Random intercept and slope									
DMI, kg/d	-37	47.4	29870	-1801	126	752	10127	23.8	0.969
MEI, MJ/d	-72	4.49	29811	-156	0.94	593	9922	21.1	0.974
NRC, kg/d	256	329	27794	-14499	10288	641	10124	21.5	0.957
FIN, kg/d	155	392	17450	-8365	5662	461	9749	18.4	0.976

Simple models

The simplest way of estimating MPI is to predict microbial MP from intake of digestible OM or ME by assuming that all digestible components have the same energy value for rumen microbes, and feed MP from CP intake. For PY = DOMI + CPI model the values of residual variance, AICC and adjusted RMSE were 483, 3339 and 19.5 when both intercept and slope were random, and 584, 3362 and 22.1 when only intercept was random, respectively. These values are considerably smaller than the values for the NRC and NorFor models (Table 1), especially for the Norfor model with only random intercept. For the NRC model, the difference are likely from errors in feed MP, since microbial MP is estimated simply from TDN intake. Part of the greater error is due a lower efficiency of feed MP compared with

microbial MP. The regression coefficient of MPY on microbial MP was 5-fold compared with feed MP in the meta-analysis of a North American and a North European dataset (Huhtanen and Hristov, 2009). In addition, microbial MP and feed MP may not be additive, i.e. increased RUP intake decreases the efficiency of microbial synthesis (see Huhtanen et al., 2018).

Table 4 Predicting MPY (g/d) when microbial MP was estimated from DOM intake at maintenance (kg/d) or according to NorFor system and feed MP from CP intake or according to NorFor (N = 337)

Microbial MP	Feed MP	Intercept	Slope1	Slope2	Intercept variance	Residual	AICC	Adj. RMSE
DOM_m[1]	CP	83	45.8	58.7	6654	593	3536	22.1
DOM_m	NF-FMP[2]	99	54.1	145	5871	572	3516	21.7
NF-MMP	CP	213	290	99	8128	762	3618	25.1
NF-MMP	NF-FMP	303	403	211	7599	954	3678	28.1

[1]Digestible OM intake (kg/d) estimated at maintenance intake; [2]Feed MP estimated according to NorFor (kg/d).

In the NorFor system, supply of energy for rumen microbes is calculated using semi-mechanistic equations for ruminally digested dietary components, which have variable coefficients for estimating microbial MP. When MPY were predicted using different combinations of simple model (intake of DOM and CP) and by the NorFor model, the greatest residual variance and RMSE values were observed when both microbial MP and feed MP were estimated according to the NorFor system. With NorFor microbial MP, performance of the model improved when feed MP was estimated from CP intake rather than according to the NorFor system. When microbial MP was predicted directly from DOM intake, feed MP estimates according to the NorFor system slightly improved performance of the model compared with predicting feed MP from CP intake. This analysis indicates that complicated equations in the NorFor model clearly worsen MPY predictions compared with a simple model predicting microbial MP from DOM intake (in vitro OMD for forages and tabulated digestibility coefficients for concentrate ingredients) and feed MP from CP intake (assumes constant degradability and intestinal digestibility of RUP). This agrees with the analysis of Schwab et al. (2004), in which the German system based on ME and urea-free CP intakes performed better than most of the other models. Based on indirect comparison with NRC, German and FIN models, performance of the Danish version of the Nordic AAT-PBV model was superior to the NorFor model in the current evaluation.

Assuming an average ruminal CP degradability of 0.70 and a digestibility of RUP of 0.82 (original AAT-PBV system), observed milk protein yield response to increased CP intake (58.7 g/kg; Table 4) results in a marginal efficiency of 0.235 which is close to the 0.25 found in a meta-analysis of casein infusion studies (Martineu et al. 2017).

Conclusions

In the current evaluation, the most complicated model (NorFor) was the poorest in predicting MPY. The better performance of the NRC (2001) model compared with the NorFor model is likely related to the complicated equations predicting microbial protein synthesis in the NorFor system. One reason for the poor performance of complicated models is the lack of reliable analytical methods for estimating important parameter values, especially ruminal degradation kinetics of feed protein, NDF and starch. The weaknesses of the *in situ* methods have been reported, but rather than taking this criticism seriously, focus has been on developing correction methods. An *in situ* method could possibly rank feeds according to

ruminal degradability, but a reliable feed evaluation needs quantitatively accurate data. Most likely, our feed protein systems could have been further developed if the *in situ* method had never been invented. At least, the better prediction of MPY using a constant EPD and intestinal digestibility supports this suggestion. It would have forced researchers to develop to something else. If this was not been successful, using constant degradability values for all feeds would have been a better option as the data in Table 4 demonstrates. Predictions may be slightly improved by adjusting the constant values according to digesta flow and/or production studies. It should also be important to realize that productive value microbial and feed MP are not additive. This is partly due to the variable association with ME intake, but also because reduced ruminal CP degradability decreases efficiency of microbial protein synthesis. According to the authors knowledge, this has not been taken into account in any modern feed protein systems even though Voigt and Piatkowski published already in 1991 an equation, in which reduced ruminal protein degradability decreased microbial protein synthesis more than the supply of fermentable energy. "Academic" feed protein evaluations systems have not been vigorously tested using data from production experiments and new elements have been included without testing if performance of the model justifies the inclusions. It can be that the model is sensitive only to changes in some parameter values, e.g. the proportion of soluble N in total N. However, more important would be if also the cows are sensitive to this parameter. From farmers' point of view, it is important that tabulated feeding values are in good agreement with observed production responses in order to optimize the economy. Improving current complicated protein systems would be difficult, because some errors compensate each other. Also, as long as *in situ* based degradability data is used, the potential for improvements is limited to simplifying calculations of microbial MP.

References

Bertilsson, J., 1983. Effects of conservation method and stage of maturity upon the feeding value of forages to dairy cows. Ph. D. thesis, Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, Report 104.

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. Dairy Sci. 93, 3216–3230.

Huhtanen, P., 2005. Critical aspects of feed protein evaluation systems for ruminants. J. Anim. Feed Sci. 14, Suppl. 1, 145-170.

Huhtanen, P., Ahvenjärvi, S. & Broderick, G. A., 2018. Microbial protein and rumen undegraded protein are not additive. Proc. of the 9th Nordic Feed Science Conference, Uppsala, Sweden, Pages 75-82.

Huhtanen, P., Hetta, M. & Swensson, C., 2011. Evaluation of canola meal as a protein supplement for dairy cows: a review and meta-analysis. Can. J. Anim. Sci. 91, 529-543.

Huhtanen, P. and Hristov, A.N., 2009. A meta-analysis of the effects of protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. J. Dairy Sci. 92, 3222–3232**.**

Huhtanen, P., Nousiainen, J.I., Khalili, H., Jaakkola, S., Heikkilä, T., 2003. Relationships between silage fermentation characteristics and milk production parameters: analyses of literature data. Livest. Prod. Sci. 81, 57-73.

Ipharraguerre, I.R. & Clark, J.H., 2005. Impacts of the source and amount of crude protein on the intestinal supply of nitrogen fractions and performance of dairy cows. J. Dairy Sci. 88 (E Suppl.), E22-E37.
LUKE, 2018. Finnish Feed Tables and Feeding Recommendations. https://portal.mtt.fi/portal/page/portal/Rehutaulukot/feed_tables_english
Martineau, R., Ouellet, D.R., Kebreab, E. & Lapierre, H., 2016. Casein infusion rate influences feed intake differently depending on metabolizable protein balance in dairy cows: A multilevel meta-analysis. J. Dairy Sci. 99, 2748–2761.
NRC (National Research Council), 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy of Science, Washington, DC.
Rinne, M., Huhtanen, P. & Nousiainen, J., 2009. Effects of silage effective protein degradability and fermentation acids on metabolizable protein concentration: a meta-analysis of dairy cow production experiments. J. Dairy Sci. 92, 1633–1642.
Santos, F.A.P., Santos, F.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.
St-Pierre, N.R., 2001. Integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci. 84, 741–755.
Schwab, C.G., Huhtanen, P., Hunt, C.W. & Hvelplund, T., 2005. Nitrogen Requirements of Cattle. In Interactions between Cattle and the Environment (Eds. R. Pfeffer and A. N. Hristov). CAB International. pp. 13-70.
Tuori, M., Kaustell, K. & Huhtanen, P., 1998. Comparison of the protein evaluation systems of feed for dairy cows. Livest. Prod. Sci. 55, 33–46.
Voigt, J. & Piantkowski, B., 1991. Models for estimation of non-ammonia nitrogen supply to the small intestine and to the balance of nitrogen in the rumen of dairy cows. In. Proc. 6th Int. Symp. Prot. Metab. Nutr. Vol 2, 364-366.
Volden, H., 2011. NorFor – The Nordic Feed evaluation system. EAAP Publication No. 130. Wageningen Academic Publishers.

The Hohenheim gas test for evaluating protein to ruminants

K.-H. Südekum & C. Böttger
University of Bonn, Institute of Animal Science, Endenicher Allee 15, 53115 Bonn, Germany.
Correspondence: ksue@itw.uni-bonn.de

Introduction

It is unquestionable that dairy cows and other ruminants, like all non-ruminant species, have a requirement for essential amino acids and in addition to that, α-amino-N to fulfil the requirements for non-essential or dispensable amino acids. Considerable research in the past decade has addressed the issue of which diet types may not match the cow's requirements based on the typical proportions (and amino acid patterns) of microbial protein and ruminally undegraded feed protein. This topic will not be addressed here, but even when the above objective can be satisfactorily addressed, utilisation of absorbed amino acids may also vary. Lack of reliable data on this variation was one major reason for the Committee for Requirement Standards (AfBN) of the Society of Nutrition Physiology (GfE) in Germany to establish a protein evaluation system which to date focuses on the flow of crude protein (CP) to the small intestine – termed "utilisable CP at the duodenum" (uCP) instead of considering individual amino acids. A brief outline only of the system is given below followed by considerations on how uCP and other variables such as ruminal microbial CP (MCP) synthesis or ruminally undegraded feed CP (RUP). These variables are also key elements of other protein evaluation systems for ruminants and are estimated from incubations by an in vitro system based on the protocol of the Hohenheim gas test (HGT).

The uCP, in German nXP ["nutzbares Rohprotein am Duodenum"] as key factor or variable of the German protein evaluation system (GfE, 2001) is calculated as (Lebzien & Voigt, 1999):

uCP (g/day) = (non-ammonia nitrogen (NAN) flow at the duodenum) × 6.25 - endogenous CP.

The endogenous CP at the duodenum (g/day) is estimated from duodenal dry matter (DM) flow (DMF) as (3.6 × kg DMF) × 6.25 (Brandt *et al*., 1980). The RUP (g/day) is then calculated as:

6.25 × (g NAN at the duodenum - g microbial N) - g endogenous crude protein.

In the GfE (2001) database, MCP at the duodenum was estimated based on either ^{15}N or RNA. A data set of 327 individual cow experiments was then used to derive regression equations to estimate uCP from feed characteristics. Best estimates were obtained from combinations of the variables metabolizable energy (ME), CP and RUP or digestible organic matter, CP and RUP. This system is widely used throughout Germany and Austria and has also shown "excellent performance" when compared with other European and the NRC (2001) protein evaluation systems in terms of predicted supply of metabolizable protein and resulting milk protein yield (Schwab *et al*., 2005).

In vitro procedures may offer alternatives to animal dependent experiments which use in situ or in vivo methods. The present paper presents a simple, substrate-specific, and labor-efficient in vitro method of analyzing feed protein value which bypasses the need to estimate RUP altogether.

The Modified Hohenheim Gas Test

The modified Hohenheim gas test (modHGT) was developed by Steingaß *et al.* (2001) and applies a modification (Raab *et al.*, 1983) to the standard HGT (Menke and Steingaß, 1988) whereby ammonia is measured after incubation with rumen fluid. The NAN concentration at the end of the incubation forms the basis for calculating uCP, which, as already mentioned above, is defined at the sum of MCP and RUP at the duodenum. The procedure also shows potential for calculating 'effective uCP' to represent selected rates of ruminal passage, which would provide a more suitable uCP value for animals fed at various levels. Principles of the modHGT have been outlined by Steingaß & Südekum (2013). The modHGT has been applied and described in detail, e.g. by Edmunds *et al.* (2012) and, more recently, by Böttger & Südekum (2017a, 2017b) and Wild *et al.* (2019). The procedure has also been applied to prediction of omasal flow of NAN and milk protein yield from in vitro determined uCP values (Gidlund *et al.*, 2018). Already about a decade ago, studies from the Nordic countries have reported application of the modHGT to estimate ruminal CP protein degradation of protein supplements (Karlsson et al., 2009) or recycling of microbial N and CP degradation (Lorenz et al., 2011). In the 9th Nordic Feed Science Conference, Udén (2018) reviewed techniques to measure ruminal CP degradation, and made constructive comments and critique also on the modHGT procedure. This paper tries to elucidate the procedure in more detail than has been done previously, which is hoped to stimulate further considerations of its strengths and weaknesses.

General Outline of the Procedure and Basal Calculations

The modHGT follows procedures of the regular HGT (Menke & Steingaß, 1988) with a chemical alteration of 2 g/l increase in NH_4HCO_3 and 2 g/l decrease in $NaHCO_3$ in the buffer solution. This modification prevents N from becoming a limiting factor in microbial biomass production. Recommended incubation times are 8 and 24 h for concentrates and 8 and 48 h for forages (Leberl *et al.*, 2007). Terminating the incubation at 24 h is unsuitable for forages due to a similar level of ammonia (after blank correction) at both 8 and 24 h, which confounds uCP results from subsequent calculation to assumed passage rates (B. Edmunds, Inst. of Animal Science, University of Bonn, Germany; unpublished results).
Rumen fluid is normally collected from two or three fistulated sheep or cattle receiving a mixed ration twice daily. The rumen fluid is extracted before morning feeding and transported in a pre-warmed thermos, which is completely filled, and immediately sealed. The rumen fluid is filtered through two layers of cheesecloth into a warm flask and then added to the reduced buffer solution. After allowing 15 min to acclimatize, 30 ml of the solution is added to a pre-warmed syringe containing 200 ± 30.0 mg substrate. Syringes are immediately placed in a rotary incubator which had been pre-warmed to 39°C. Starting time of the incubation is recorded after all syringes have been filled. Each feedstuff is analyzed at least in duplicate (analytical replicates) and over two runs using different batches of rumen fluid (statistical replicates). At the end of each incubation time (8 h and 24 h or 48 h) gas volume is recorded and syringes put on ice to stop microbial activity. Syringes remain in the ice slurry for a minimum of 2 h until required for ammonia analysis. Gas production (GP) is also recorded at 24 h for use in calculation of ME. At both the 8 h and 24 h readings, the plunger is set back to 30 ml (not for the blank). A blank, containing rumen fluid/buffer solution without added substrate is also incubated in duplicate alongside the samples for 8 and 48 h. Ammonia-N (mg NH_3-N/30 ml) from both the blank (NH_3-N_{blank}) and from the

syringes containing substrate (NH_3-N_{sample}) is measured by distillation or any other suitable method and used in the following calculation:

$$uCP\ (g/kg\ DM) = \frac{NH_3\text{-}N_{blank} + N_{sample} - (NH_3\text{-}N_{sample})}{\text{weight (mg DM)}} \times 6.25 \times 1000$$

where, N_{sample} is N added to the syringe from the measured amount of feedstuff (mg), weight is the amount of sample weighed into the syringe and calculated to DM and other variables are as previously described. Figure 1 depicts a schematic representation of the procedure.

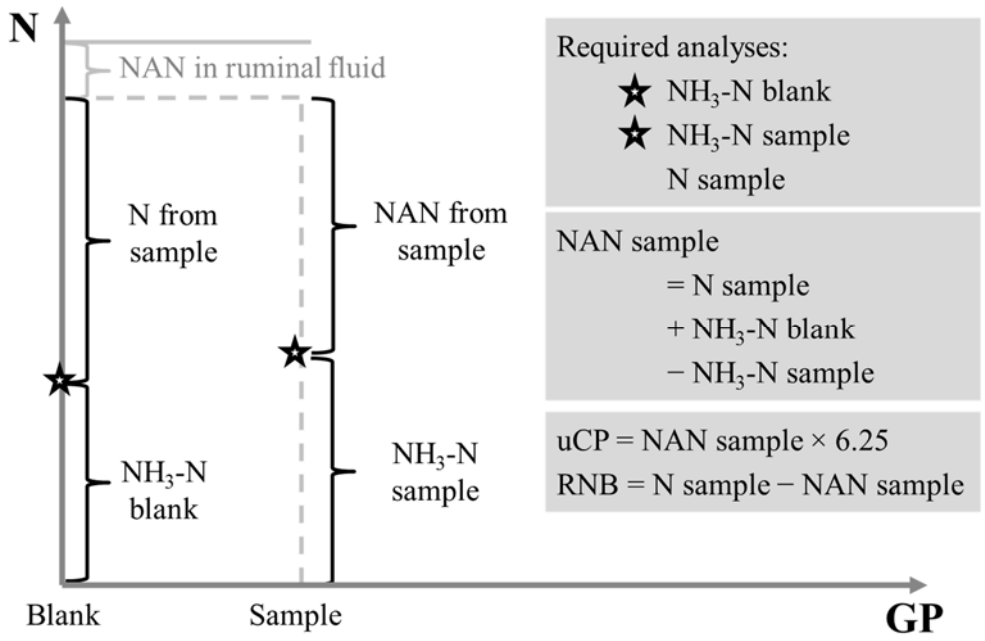


Figure 1 Schematic representation of the procedure to determine the utilisable crude protein at the duodenum (uCP) using a modified Hohenheim gas test procedure adapted from Steingaß & Südekum, 2013); GP = Gas production; NAN = non- ammonia nitrogen; RNB = ruminal N balance.

When using a live rumen fluid, small biological fluctuations among runs are inevitable. To correct for this, a protein standard provided by the University of Hohenheim is analyzed in every run. The 'standard' is a concentrate mixture of (per kg DM) 450 g rapeseed meal, 300 g faba beans and 250 g molassed sugar beet pulp, and has a CP content of about 250 g/kg DM. The correction follows the same method as for gas production (Menke & Steingaß, 1988) whereby the mean uCP value for the standard for 8, 24 or 48 h, is divided by the recorded value of the standard for that run and all other samples are then multiplied by the resulting correction factor. Whole runs are repeated if the correction factor for either incubation time, lay outside the range of 0.9 to 1.1. The hay and concentrate standards typically used for correcting gas production are also included in the incubation not only to correct gas production values, but to ensure the rumen fluid solution followed typical fermentation.

Diagrammatic Representation of the Estimation of Protein Characteristics from in vitro Incubation

An attempt can be made to calculate effective uCP. As with effective CP degradability, effective uCP should represent various rates of digesta flow through the rumen. Following

corrections using the protein standard, uCP values from the two incubation time points of one run are plotted against the time scale, where 'Time' is the time of incubation. The resulting regression equation is then used to calculate effective uCP at assumed passage rates (K_p) of 2, 5 and 8%/h (or other assumed passage rates depending, e.g., on the type of feed ration) using the formula:

$$\text{Effective uCP} = y + a \times \ln(1/K_p)$$

where, y is the intercept and a is the slope. Among run regression equations will differ slightly due to methodological error, however variations to the slope and intercept balance out to provide effective uCP values that can be used as replicates. Effective uCP should only be calculated if the correction factor of the standard is within the range of 0.9-1.1. The assumption of a linear decrease in uCP with ln time was demonstrated using soybean meal incubated at several time points spanning 4-48 h (H. Steingaß, unpublished results) and using grass silage and the protein standard at time points spanning 2-48 h. Another example of the drafted procedure to estimate effective uCP is presented in Figure 2 for rapeseed meals.

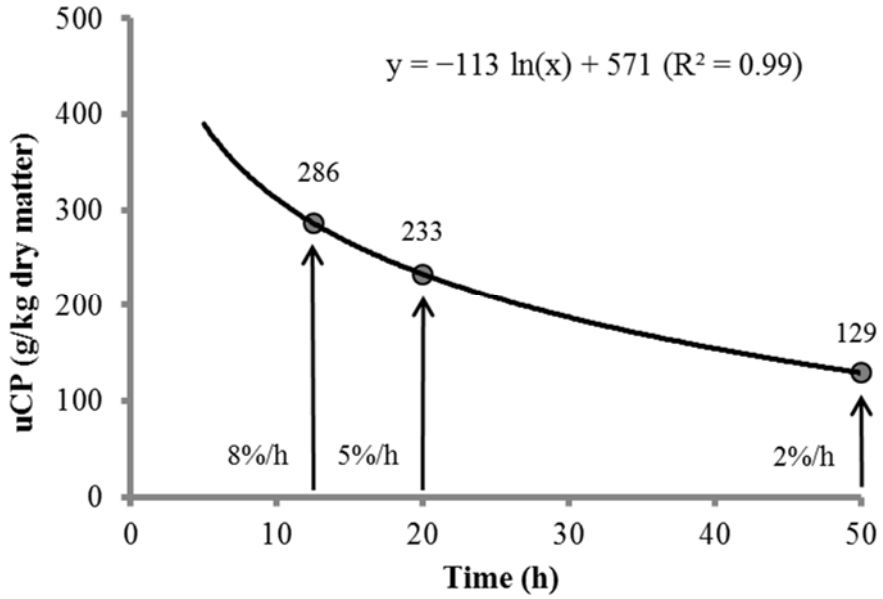


Figure 2 Modified Hohenheim gas test: Determination of the effective utilisable crude protein at the duodenum (uCP; g/kg dry matter on the y-axis) using the example of a solvent-extracted rapeseed meal (H. Steingass, unpublished); percentage values correspond to assumed passage rates according to the respective retention time (adapted from Steingaß & Südekum, 2013).

In addition to a direct estimation of uCP, the two constituting uCP fractions, namely RUP and MCP, can also be estimated from in vitro incubations using the same general procedure. As a first step, the total feed or sample N is separated into ruminally degraded and undegraded fractions. This is achieved by incubating feeds with and without addition of a carbohydrate mixture consisting of cellulose, maize starch, wheat starch and sucrose in a ratio of 40:20:20:20.

To estimate ruminal feed CP degradability, a linear regression between NH_3-N and GP is calculated from the respective values for incubations of a sample with and without added carbohydrates:

$$NH_3\text{-N} = a + b \times \text{GP}$$

In this regression equation, the theoretical point of zero GP implies that no energy would be available to microbes and thus, only feed CP degradation but no microbial protein synthesis would occur. Subtracting NH_3-N_{blank} from the intercept *a* yields N solely originating from the feed (ruminally degraded N, RDN).

Finally, MCP can be estimated as illustrated in Figure 3 which can also be done using different incubation times and thus, yield effective MCP values as for uCP and RUP.

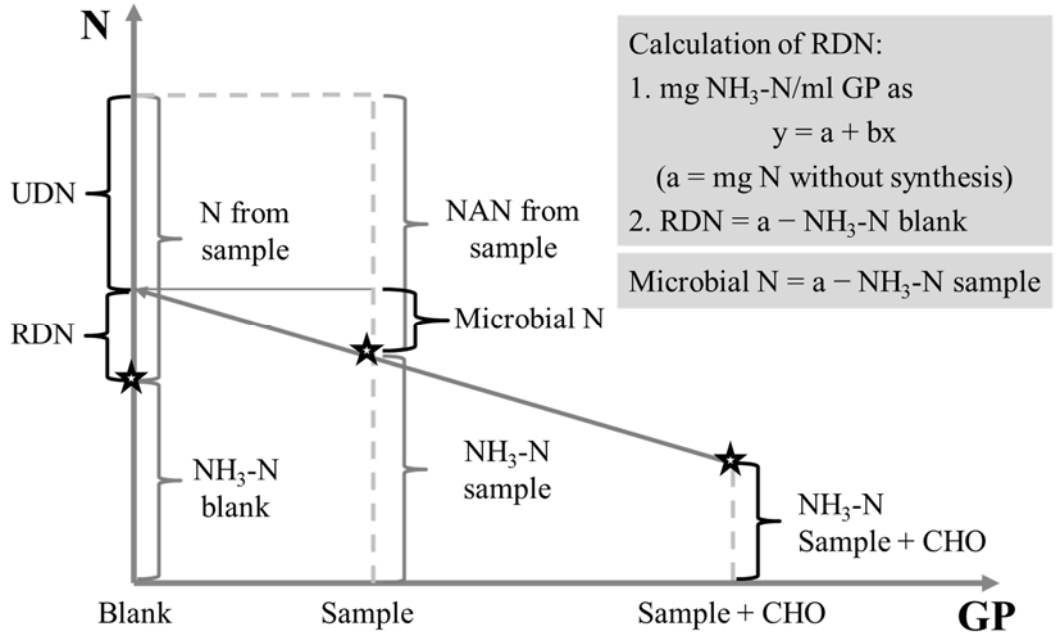


Figure 3 Schematic representation of the procedure to distinguish the utilisable crude protein at the duodenum (uCP) into ruminally undegraded crude protein (expressed as N, i.e. UDN) and microbial N using a modified Hohenheim gas test procedure (adapted from Steingaß & Südekum, 2013); CHO = carbohydrate mixture; GP = Gas production; RDN = ruminally degraded N.

Conclusion

The modHGT offers an in vitro method that simplifies the estimation of protein value of ruminant feeds with the potential to eliminate some methodological inaccuracies of modern protein evaluation systems. The method involves incubation of feeds with rumen fluid, after which NH_3-N is measured. The NAN content is then used to calculate uCP, which corresponds to ruminal MCP and RUP flowing to the duodenum. Indirect validations of forage protein values against the German feed protein evaluation system (GfE, 2001) have indicated that the method has high potential for estimating uCP (Edmunds *et al.*, 2012). Theoretically, the problems of the in situ method (particle loss, soluble N, microbial contamination) should be smaller in the modHGT method, which also takes into account possible effects on microbial N synthesis though this also involves assumptions.

References

Böttger, C. & Südekum, K.-H., 2017a. European distillers dried grains with solubles (DDGS): Chemical composition and in vitro evaluation of feeding value for ruminants. Anim. Feed Sci. Technol. 224, 66-77.

Böttger, C. & Südekum, K.-H., 2017b. Within plant variation of distillers dried grains with solubles (DDGS) produced from multiple raw materials in varying proportions: Chemical composition and in vitro evaluation of feeding value for ruminants. Anim. Feed Sci. Technol. 229, 79-90.
Brandt, M., Rohr, K. & Lebzien, P., 1980. Bestimmung des endogenen Protein-N im Duodenalchymus von Milchkühen mit Hilfe von ^{15}N. Z. Tierphysiol. Tierernährg. Futtermittelkde. 44, 26 (Abstr.).
Edmunds, B., Südekum, K.-H., Spiekers, H., Schuster, M. & Schwarz, F.J., 2012a. Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for ruminants, from forages using a modified gas test. Anim. Feed Sci. Technol. 175, 106-113.
GfE (Gesellschaft für Ernährungsphysiologie), 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt/Main, Germany.
Gidlund, H., Vaga, M., Ahvenjärvi, S., Rinne, M., Ramin, M. & Huhtanen, P., 2018. Predicting omasal flow of nonammonia N and milk protein yield from in vitro-determined utilizable crude protein at the duodenum. J. Dairy Sci. 101, 1164–1176.
Karlsson, L., Hetta, M., Udén, P. & Martinsson, K., 2009. New methodology for estimating rumen protein degradation using the *in vitro* gas production technique. Anim. Feed Sci. Technol. 153, 193-202.
Leberl, P., Gruber, L., Steingaß, H. & Schenkel, H., 2007. Comparison of the methods modified Hohenheimer Futterwerttest (moHFT) and Cornell system for determination of nXP-content of concentrates, in: Kapun, S., Kramberger, B., Ceh, T. (Eds.), 16th Intern. Science Symp. Nutr. Domest. Anim. Radenci, Slovenia, p. 171-176.
Lebzien, P. & Voigt, J., 1999. Calculation of the utilizable crude protein at the duodenum of cattle by two different approaches. Arch. Anim. Nutr. 52, 363-369.
Lorenz, M.M., Karlsson, L., Hetta, M. & Udén, P, 2011. Recycling of microbial N and estimation of protein degradation by in vitro gas production. Anim. Feed Sci. Technol. 170, 111-116.
Menke, K.H. & Steingaß, H., 1988. Estimation of the energetic feed value from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev. 28, 7-55.
NRC (National Research Council), 2001. Nutrient Requirements of Dairy Cattle. 7th. ed. National Academy Press, Washington, DC, USA.
Schwab, G.C., Huhtanen, P., Hunt, C.W. & Hvelplund, T., 2005. Nitrogen requirements of cattle, in: Pfeffer, E., Hristov, A.N. (Eds.) Nitrogen and Phosphorus Nutrition of Cattle. CABI Publishing, Wallingford, UK, p. 13-70.
Steingaß, H., Nibbe, D., Südekum, K.-H., Lebzien, P. & Spiekers, H., 2001. Schätzung des nXP-Gehaltes mit Hilfe des modifizierten Hohenheimer Futterwerttests und dessen Anwendung zur Bewertung von Raps- und Sojaextraktionsschroten. VDLUFA-Kongress 113, Berlin, Kurzfassungen der Vorträge, 114 (Abstr.).
Steingaß, H. & Südekum, K.-H., 2013. Proteinbewertung beim Wiederkäuer – Grundlagen, analytische Entwicklungen und Perspektiven. Übers. Tierernährg. 41, 51-73.
Udén, P., 2018. Techniques to measure ruminal protein degradation – a review, in: Udén, P., Spörndly, R., Rustas, B.-O., Eriksson, T., Karlsson, J. (Eds.), Proceedings of the 9th Nordic Feed Science Conference, Uppsala, Sweden. Report 298. Departm. Anim. Nutr. Managm., Swedish University of Agricultural Sciences, Uppsala, Sweden, pp. 67-73.

Wild, K.J., Steingaß, H. & Rodehutscord, M., 2019. Variability of in vitro ruminal fermentation and nutritional value of cell-disrupted and nondisrupted microalgae for ruminants. GCB Bioenergy 11, 345-359.

Forage protein quality as affected by wilting, ensiling and the use of silage additives

E. Nadeau[1,2], D. O. Sousa[3] & H. Auerbach[4]
[1]Department of Animal Environment and Health, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences (SLU), BOX 234, 532 23 Skara, [2]The Rural Economy and Agricultural Society Sjuhärad, Rådde Gård, 514 05 Länghem, Sweden, [3]Department of Animal Science, University of São Paulo, Luiz de Queiroz College of Agriculture, Piracicaba, 13418900, Brazil & [4]International Silage Consultancy,06193 Wettin-Löbejün, Germany,
Correspondence: Elisabet.Nadeau@slu.se

Introduction

Forage is an important locally produced protein source for ruminants and plays a major role in replacing soy-based concentrates as it has less effect on the climate compared to annual crops as protein sources. Forages catch more sunlight for photosynthesis and is, therefore, a more efficient carbon sink than annual crops (Solati et al., 2018). However, forage protein utilization by ruminants remains a challenging topic as about 75% of forage crude protein (CP) is rumen degradable protein (Merchen and Bourquin, 1994) of which non-protein nitrogen (NPN) comprises 50 to 60% of the CP in silage (Muck and Hintz, 2003). NPN is lost as urea in the urine when rapidly fermented carbohydrates are not available for microbial protein synthesis (Jardstedt et al., 2017). Consequently, energy concentration of forages is at least as important as its CP concentration as a majority of the metabolizable protein (MP) from forage originates from microbial protein (Merchen and Bourquin, 1994). Proteolysis occurs both during wilting and ensiling of forages and rapid wilting under favourable weather conditions and a quick pH drop during ensiling have been shown to decrease these processes (Broderick, 1995; Charmley, 2000). Also, high nitrogen fertilization rates can increase the NPN content of forages (Tremblay et al., 2005). Recently, Johansen et al. (2017) concluded that the MP concentration in grass-clover silage is improved by wilting as a result of increased amino acid digestion in the small intestine and a higher duodenal flow of amino acids in dairy cows. Furthermore, use of silage additives can decrease proteolysis during ensiling by direct acidification or by lactic acid formation causing a decrease of pH close to 4.0 (Auerbach et al., 2012; Fijalkowska et al., 2015). To evaluate the protein utilization of forages, both before and after ensiling, it is important to investigate possible changes in the true protein (TP) fractions, which vary in rumen degradability (Sniffen et al., 1992). The aim of this paper is to give an overview of the effects of wilting, ensiling and the use of silage additives on potential changes in the NPN and TP fractions of forage protein.

Material and Methods

Results on forage protein quality from Swedish experiments presented in this paper are based on analyses of freeze-dried samples according to Licitra et al. (1996) and evaluated by the Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992). Five different CP fractions; A, B_1, B_2, B_3 and C are presented. Fraction A is the non-protein nitrogen (NPN), whereas the B and C fractions are the TP. The NPN is the nitrogen passing into the filtrate after precipitation with tungstic acid. B_1 is soluble in borate-phosphate buffer at rumen pH and is degraded rapidly in the rumen, B_2 is insoluble in borate-phosphate buffer, but soluble in the neutral-detergent (ND) solution. Fraction B_2 means the protein within the plant cell with high molecular weight and has variable degradation. The B_3 is the protein insoluble in the ND solution but soluble in the acid-detergent (AD) solution. This protein is normally cell

wall-bound, digestible, but slowly degradable of which most occurs post-ruminally. The ND solution was used without sodium sulfite to avoid reduction of the protein content in NDF. Fraction C is the protein insoluble in the AD solution and is regarded as indigestible. This fraction, named ADIN (acid-detergent insoluble nitrogen) is associated with lignin, Maillard products or non-enzymatic browning reaction caused by heating and drying (Licitra et al., 1996). Rumen undegraded protein (RUP) at 5 and 8% passage rate was calculated according to Kirchhof et al. (2010). Dry matter, ammonia-N and water-soluble carbohydrates were analysed according to conventional methods. The experimental design was a randomized complete block using three field blocks per treatment for the effect of wilting and nitrogen fertilization (Table 2). For the other experiments, a completely randomized design using three replicates per treatment was used. The experiments were done at The Rural Economy and Agricultural Society Sjuhärad, Länghem and at Lantmännen Dairy Research Farm Nötcenter Viken, Falköping.

Results and Discussion

Effect of wilting

Wilting for 5 hours from 16 to 28% DM of grass-clover forage in the second cut decreased the proportions of fractions B_1 and B_2 while fractions B_3 and C increased, resulting in an improved RUP at 5% passage rate (Table 1).

Table 1 Effects of wilting for 5 hours in sunny weather during second cut in 2013 on contents of dry matter (DM), water-soluble carbohydrates (WSC), crude protein (CP), true protein (TP), ammonia-N (NH_3-N), CP fractions and rumen undegraded protein of grass-clover forage (n=6)[1]

	Fresh forage	Wilted forage	SEM	*P*-value
DM, g/kg	158	275	5.6	<0.001
WSC, g/kg DM	116	106	4.1	ns
CP, g/kg DM	177	181	4.8	ns
TP, g/kg DM	152	150	4.4	ns
NH_3-N, % of tot N	2.8	2.1	0.11	0.001
A, % of CP[2]	14.1	16.8	1.15	ns
B_1, % of CP[2]	15.4	9.1	0.7	<0.001
B_2, % of CP[2]	52.3	44.3	1.25	0.002
B_3, % of CP[2]	15.4	25.8	0.83	<0.001
C, % of CP[2]	2.7	4.0	0.15	<0.001
RUP_5, % of CP[3]	26.4	29.4	0.62	0.009

[1]The grass-clover forage contained 33% red clover, 7% white clover, 55% grass of timothy, perennial ryegrass and meadow fescue and 5% weeds of DM. [2]A, non-protein nitrogen; B_1, buffer-soluble protein; B_2, neutral detergent-soluble protein; B_3, acid detergent-soluble protein; C, acid detergent-insoluble protein. [3]RUP_5, rumen undegraded protein at a passage rate of 5% / h.

Wilting of grass forage, containing perennial ryegrass, meadow fescue and timothy, for 6 hours from 20 to 34% DM resulted in decreased ammonia-N and fraction B_2 while fraction B_3 increased (Table 2). Nitrogen fertilization rate affected the effect of wilting on fraction B_3 with an increase in the fertilized grass only. Likewise, Nadeau et al. (2016) reported conversion of soluble TP (fraction B_1) to cell-wall bound protein (fraction B_3) during a 6-h wilting to 40% DM of a lucerne (90%)/white clover (10%) mixture in the second cut. No increase in the A fraction indicates that a high wilting rate in sunny weather minimizes proteolysis (Edmunds et al, 2014), confirming own results on grass wilted for 2 days (unpublished data). However, longer wilting for 4 days increased fraction A and reduced all the B fractions, resulting in a decline in RUP_5 from 26.3% in fresh grass to 19.3% of CP in wilted herbage. Conversions of soluble protein fractions to cell-wall bound proteins might be

related to oxidation of *o*-diphenol to *o*-quinone by polyphenol oxidase (PPO). The *o*-quinone can react with functional groups of proteins, forming protein-bound phenolics (PBP). It is plausible that PBP also can be formed by other pathways than PPO activity (Lee et al., 2014).

Table 2 Effects of wilting (W), nitrogen (N) fertilization rate (0, 100 and 200 kg N/ha) and their interactions on contents of dry matter (DM), water-soluble carbohydrates (WSC), crude protein (CP), true protein (TP), ammonia-N (NH_3-N), CP fractions and rumen undegraded protein of grass forage in first cut averaged over 2 years (n=6)

	Fresh Grass			Wilted Grass			*P*-value		
	0	100	200	0	100	200	W × N	W	N
DM, g/kg	248^{c}	185^{d}	161^{d}	331^{b}	340^{ab}	362^{a}	<0.001	<0.001	<0.001
WSC, g/kg DM	344	233	209	394	240	220	ns	0.082	<0.001
CP, g/kg DM	78	113	164	86	125	163	ns	ns	<0.001
TP, g/kg DM	52	82	113	56	90	112	ns	ns	<0.001
NH_3-N, % of tot N	3.1	3.5	2.9	2.7	2.4	1.8	ns	<0.001	0.096
A, % of CP[1]	33.0	27.4	31.0	34.9	27.3	31.2	ns	ns	0.033
B_1, % of CP[1]	5.6	9.3	10.0	7.8	9.9	9.4	ns	ns	0.020
B_2, % of CP[1]	42.3	46.5	45.8	38.3	42.5	40.7	ns	0.003	0.040
B_3, % of CP[1]	15.6^{ab}	14.2^{b}	11.3^{c}	16.2^{ab}	17.9^{a}	16.6^{ab}	0.007	<0.001	0.007
C, % of CP[1]	3.6	2.6	1.9	2.8	2.4	2.1	0.064	ns	<0.001
RUP_5, % of CP[2]	30.3	28.4	22.4	28.7	27.2	23.1	ns	ns	<0.001

[1]A, non-protein nitrogen; B_1, buffer-soluble protein; B_2, neutral detergent-soluble protein; B_3, acid detergent-soluble protein; C, acid detergent-insoluble protein. [2]RUP_5, rumen undegraded protein at a passage rate of 5% / h.

Effects of ensiling and silage additives

Most of the proteolysis resulting in breakdown of especially the TP fractions B_1 and B_2 and of B_3 to a lesser extent, to NPN occurred during the first 30 days of ensiling (Figure 1). Thereafter, fraction B_2 continued to decrease while fraction B_3 increased. This conversion of the B_2 fraction to cell-wall bound protein (fraction B_3) under anaerobic conditions at pH around 4 is difficult to explain but is most likely related to microbial activity.

Results from our previous experiment on lucerne/white clover silage showed decreases in all of the TP fractions (B_1, B_2 and B_3), while the NPN fraction increased during 90 days of storage (Nadeau et al., 2016). Fresh lucerne contains more soluble TP (fraction B_1) than red clover (Kirchhof et al., 2010) and is, consequently, more susceptible to proteolysis during ensiling.

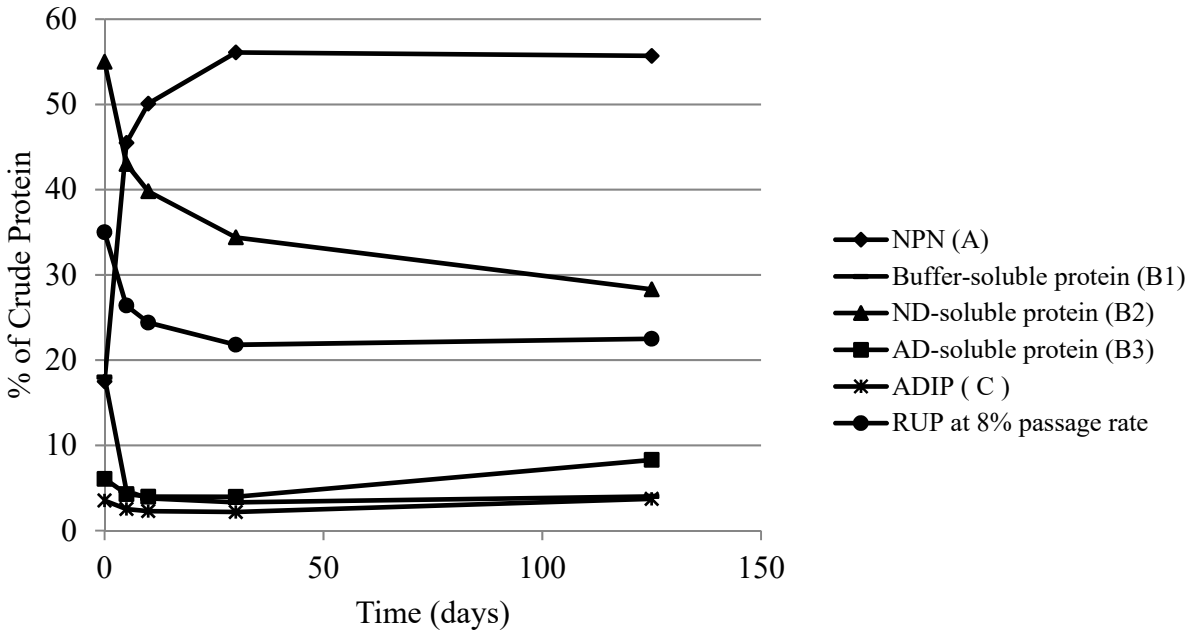


Figure 1 Effect of time of fermentation on the crude protein fractions and calculated rumen undegraded protein of grass (77%)-legume (23%) silage at 35% DM.

Table 3 Effects of silage additives on contents of dry matter (DM), water-soluble carbohydrates (WSC), crude protein (CP), true protein (TP), ammonia-N (NH_3-N), CP fractions and rumen undegraded protein of chopped grass silage stored in 1.7-L laboratory silos for 105 days (n = 6)[1]

	First cut 2015				First cut 2016			
	Con[2]	Acid[3]	SEM	*P*-value	Con[2]	Salt[4]	SEM	*P*-value
DM, g/kg	313	314	10.8	ns	359	361	1.2	Ns
WSC, g/kg DM	278	307	16.6	ns	194	214	1.9	<0.001
CP, g/kg DM	121	122	2.5	ns	122	123	0.9	ns
TP, g/kg DM	46.1	49.1	2.96	ns	56.2	59.4	0.60	0.002
NH_3-N, % of tot N	5.4	4.4	0.39	0.09	5.3	3.4	0.3	0.009
A, % of CP[5]	62.6	60.1	1.63	ns	53.2	50.3	0.83	0.03
B_1, % of CP[5]	1.5	2.2	0.42	ns	3.7	3.6	0.59	ns
B_2, % of CP[5]	27.9	28.0	0.81	ns	30.6	31.3	0.37	ns
B_3, % of CP[5]	5.3	7.6	0.76	0.05	8.7	11.0	0.44	0.003
C, % of CP[5]	2.8	2.1	0.16	0.008	3.7	3.9	0.15	ns
RUP_5, % of CP[6]	20.4	20.4	0.42	ns	23.6	24.4	0.28	0.08

[1]80% perennial ryegrass, 14% meadow fescue and 6% timothy of DM. [2]Con=untreated control silage. [3]Acid=formic acid, propionic acid and salt of organic acids at 3.0 L/t forage. [4]Salt=sodium nitrite, hexamine and sodium benzoate in liquid form at 2.0 L/t forage. [5]A, non-protein nitrogen; B_1, buffer-soluble protein; B_2, neutral detergent-soluble protein; B_3, acid detergent-soluble protein; C, acid detergent-insoluble protein. [6]RUP_5, rumen undegraded protein at a passage rate of 5% / h.

Use of silage additives can decrease proteolysis during ensiling. Grass silage treated with an acid (formic, propionic, salts of organic acids) at 3 L/tonne had a greater proportion of fraction B_3, which is the cell wall bound protein, a lower proportion of ADIN and tended to have a lower ammonia-N content than the untreated control silage after 105 days of ensiling in 1.7-L laboratory silos (Table 3). When the grass silage was treated with a salt-based additive (sodium nitrite, hexamine, sodium benzoate) at 2 L/tonne, contents of ammonia-N and NPN decreased while the content of fraction B_3 increased, resulting in a tendency to increased RUP compared to the control silage (Table 3).

When chopped grass silage was ensiled in hard-pressed round bales, addition of a salt-based additive (sodium nitrite, hexamine, sodium benzoate, potassium sorbate) at 2 L/tonne decreased the content of NPN but increased the cell-wall bound protein (fraction B_3) and the content of WSC compared to the control silage (Table 4). When a bacterial inoculant, containing both homofermentative and heterofermentative lactic acid bacteria was used, ammonia-N decreased while fraction B_3 increased compared to the control silage. Decreased proportions of ammonia-N and NPN and increased proportion of fraction B_3, as observed in silages treated with acids, salt or inoculants, show that additives are effective in reducing proteolysis in the silage (Auerbach et al., 2012; Fijalkowska et al., 2015).

Table 4 Effects of silage additives on contents of dry matter (DM), water-soluble carbohydrates (WSC), crude protein (CP), true protein (TP), ammonia-N (NH_3-N), CP fractions and rumen undegraded protein of chopped grass silage stored in hard-pressed round bales for 120 days (n = 3)[1]

	Con[2]	Inoculant[3]	Salt[4]	SEM	*P*-value
DM, g/kg	261[c]	366[a]	332[b]	4.9	<0.001
WSC, g/kg DM	10.1[b]	14.7[b]	21.3[a]	1.67	0.009
CP, g/kg DM	163[a]	153[b]	162[a]	1.8	0.013
TP, g/kg DM	76.3[b]	75.9[b]	83.6[a]	1.22	0.0072
NH_3-N, % of tot N	10.3[a]	8.7[b]	8.9[ab]	0.34	0.032
A, % of CP[5]	53.3[a]	50.4[ab]	48.4[b]	1.09	0.049
B_1, % of CP[5]	3.8	2.1	3.9	1.07	ns
B_2, % of CP[5]	30.4	33.7	34.0	1.26	ns
B_3, % of CP[5]	7.9[c]	10.0[a]	9.4[b]	0.13	<0.001
C, % of CP[5]	4.6	3.8	4.2	0.33	ns
RUP_5, % of CP[6]	20.4	22.0	21.6	0.67	ns

[1]Grass silage contained 70% grass, 15% red clover and 10% white clover of DM. [2]Con = untreated control silage. [3]Inoculant=bacterial inoculant containing *Lactobacillus plantarum* and *Lactobacillus buchneri* at 200 000 cfu/g forage. [4]Salt=sodium nitrite, hexamine, sodium benzoate and potassium sorbate in liquid form at 2.0 L/tonne forage. [5]A, non-protein nitrogen; B_1, buffer-soluble protein; B_2, neutral detergent-soluble protein; B_3, acid detergent-soluble protein; C, acid detergent-insoluble protein. [6]RUP_5, rumen undegraded protein at a passage rate of 5% / h.

Conclusions

Rapid wilting improves forage quality by limiting proteolysis and converting soluble protein to cell wall protein of which most is rumen undegradable. As cell-wall protein is less prone to proteolysis than soluble protein during ensiling, wilting will improve the protein quality of silage, which is further enhanced by the use of silage additives.

References

Auerbach, H., Weiss, K. & Nadeau, E., 2012. Benefits of using silage additives, in: Auerbach, Luckstadt, C. & Weissbach, F. (Eds.), Proc. 1st Int. Silage Summit, Leipzig, Saxony, Germany, Anytime Publishing Services, Worthington, UK., p. 75-144.

Broderick, G.A., 1995. Desirable characteristics of forage legumes for improving protein utilization in ruminants. J. Anim. Sci. 73, 2760-2773.

Charmley, E., 2001. Towards improved silage quality – A review. Can. J. Anim. Sci. 81, 157-168.

Edmunds, B., Spiekers, H., Südekum, K.-H., Nussbaum, H., Schwarz, F.J. & Bennett, R., 2014. Effect of extent and rate of wilting on nitrogen components of grass silage. Grass Forage Sci. 69, 140-152.

Fijalkowska, M., Pysera, B., Lipinski, K. & Strusinska, D.,2015. Changes of nitrogen compounds during ensiling of high protein herbages - A review. Annals Anim. Sci. 15, 289-305.
Jardstedt, M., Hessle, A., Nørgaard, P., Richardt, W. & Nadeau, E., 2017. Feed intake and urinary excretion of nitrogen and purine derivatives in pregnant suckler cows fed alternative roughage-based diets. Livest Sci. 202, 82-88.
Johansen, M., Hellwing, A.L.F., Lund, P. & Weisbjerg, M.R., 2017. Metabolisable protein supply to lactating dairy cows increased with increasing dry matter concentration in grass-clover silage. Anim. Feed Sci. Technol. 227, 95-106.
Kirchhof, S., Eisner, I., Gierus, M. & Südekum, K.-H., 2010. Variation in the contents of crude protein fractions of different forage legumes during the spring growth. Grass Forage Sci. 65, 376-382.
Lee, M. 2014. Forage polyphenol oxidase and ruminant livestock nutrition. Frontiers in Plant Sci. 5, 694, 9 pp.
Licitra, G., Hernandez, T.M. & Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractions of ruminant feeds. Anim. Feed Sci. Technol. 57, 347-358.
Merchen, N.R. & Bourquin, L.D., 1994. Processes of digestion and factors influencing digestion of forage-based diets by ruminants. Fahey, G.C., Collins, M., Mertens, D.R., Moser, L.E. (Eds.), Forage, quality, evaluation, and utilization, p. 564-612. ASA, CSSA, SSSA Inc. Madison, WI, USA.
Muck, R. E., Hintz, R.W., 2003. Effects of breeding for quality on alfalfa ensilability. Trans ASAE. 46, 1305-1309.
Nadeau, E., Hallin, O., Richardt, W. & Jansson, J., 2016. Protein quality of lucerne – a comparison to red clover and effects of wilting and enisling. Grassl. Sci. Europe 21, 372-375.
Nadeau, E., Richardt, W., Murphy, M. & Auerbach, H., 2012. Protein quality dynamics during wilting and preservation of grass-legume forage, in: Kuoppala, K., Rinne, M. & Vanhatalo, A. (Eds.), Proc. XVI Int. Silage Conf., Hämeenlinna, Finland, 2-4 July, 2012, p. 56-57.
Sniffen, C.J., O'Connor, J.D., Van Soest, P.J., Fox, D.G. & Russell, J.B., 1992. A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability, J. Anim. Sci. 70, 3562-3577.
Solati, Z., Jørgensen, U., Eriksen, J. & Søegaard, K., 2018. Estimation of extractable protein in botanical fractions of legume and grass species. Grass Forage Sci. 73, 572-581.
Tremblay, G.F., Bélanger, G. & Drapeau, R., 2005. Nitrogen fertilizer application and developmental stage affect silage quality of timothy. Grass Forage Sci. 60, 337-355.

Application of three laboratory methods to estimate the protein value of rapeseed meal for ruminants

C. Böttger, T. Weber, F. Mader & K.-H. Südekum
Institute of Animal Science, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany.
Correspondence: cboe@itw.uni-bonn.de

Introduction

In dairy cow nutrition, precise diet formulation requires information on feed protein value, including ruminal degradability of crude protein (CP) to estimate supply of ruminally undegraded feed CP (RUP). The in situ method is often regarded as a reference method to estimate RUP but its suitability with regard to large sample numbers or routine analysis is limited. It will thus become more important to estimate the protein value of feedstuffs using laboratory methods. This is particularly important for protein supplements, like rapeseed meal (RSM), which is commonly used in Europe including the Nordic countries. Feeding RSM may be advantageous in situations where use of soybean meal is debated regarding GMO or attention is paid to utilisation of regionally available protein sources. In dairy cow rations, RSM (or canola meal) can successfully replace soybean meal (e.g. Huhtanen et al., 2011). However, like other protein supplements, RSM may be subject to variability in ruminal CP degradability depending on raw material and production plant (Broderick et al., 2016). Processing in the oil mill affects protein value as it generally involves heat. In addition, RSM may undergo specific treatments to decrease ruminal CP degradability. The aim of this study was to compare RUP estimates of RSM by three specific laboratory methods including chemical analysis, simulation of enzymatic digestion in the intestinal tract or rumen fluid incubations.

Materials and Methods

Samples and General Analyses

Rapeseed meal commodities (n = 9; labelled A to I) were obtained from two commercial feed manufacturers. Samples included RSM as obtained directly from oil mills as well as RSM which underwent additional thermal and/or xylose treatments. All samples were ground to pass a 1-mm screen in a centrifugal mill (ZM 200, Retsch, Haan, Germany) and analysed for CP concentration using Kjeldahl digestion. All analyses were conducted at the Institute of Animal Science, Bonn University, except that CP fractionation data of samples G, H and I were provided by a commercial laboratory.

Modified Hohenheim Gas Test

A modified Hohenheim gas test (modHGT) was applied to estimate uCP from ammonia release upon in vitro incubation in rumen fluid-buffer solution. The modHGT was extended to estimate feed CP degradability (Raab et al., 1983). Principles of the modHGT have been outlined by Steingaß and Südekum (2013). The modHGT has been applied and described in detail, e.g. by Edmunds et al. (2012a) and Wild et al. (2019). Briefly, approximately 130 mg of each sample was weighed into 100-mL glass syringes with and without addition of a carbohydrate mixture (approximately 130 mg) consisting of cellulose, maize starch, wheat starch and sucrose (40:20:20:20). Samples were incubated in a rumen fluid-buffer solution for 8 and 24 h and gas production (GP) was recorded. In addition, blank samples containing only rumen fluid-buffer solution were incubated. Rumen fluid was taken from two ruminally cannulated sheep (steers for samples G to I) prior to morning feeding. The animals received a

mixture of grass hay and compound feed corresponding to their maintenance energy requirements. After incubation, syringes where put on ice and ammonium-N was analysed in each syringe containing RSM (ammonium-N_{RSM}; mg) and blanks (ammonium-N_{blank}; mg) using steam distillation. Contents of ammonium-N in syringes containing RSM without added carbohydrates and blanks were used to calculate uCP (g/kg DM) at 8 and 24 h as follows:

$$uCP = ((N_{RSM} - (\text{ammonium-}N_{RSM} - \text{ammonium-}N_{blank})) / \text{weight}_{RSM}) \bullet 6.25 \bullet 1000$$

where, N_{RSM} = total N from RSM (mg), weight_{RSM} = RSM incubated (mg DM) and other variables are as described above. To estimate feed CP degradability, a linear regression between ammonium-N and GP was calculated from the respective values for RSM with and without added carbohydrates:

$$\text{ammonium-N} = a + b \bullet GP$$

In this regression equation, the theoretical point of zero GP implies that no energy would be available to microbes. It was thus assumed that only feed CP degradation, but no microbial protein synthesis would occur. Subtracting ammonium-N_{blank} from the intercept *a* yielded N solely originating from the feed (rumen degradable N, RDN, in mg). Ruminally undegraded feed CP was calculated at both 8 and 24 h as follows:

$$RUP_{HGT} = (N_{RSM} - RDN) / N_{RSM} \bullet 1000$$

Linear regression of uCP values at 8 and 24 h to ln of time allowed for the calculation of effective uCP for assumed K_p of 0.05/h ($uCP_{0.05}$) through calculating the function value of ln (20). Analogously, RUP_{HGT} was calculated for K_p 0.05/h.

Crude Protein Fractionation

Crude protein was fractionated according to Licitra et al. (1996), comprising analysis of true protein (TP), buffer insoluble CP, neutral detergent insoluble CP, and acid detergent insoluble CP (ADICP). From these analytical fractions, five subfractions (i.e., A, B1, B2, B3 and C) of CP were estimated (Licitra et al., 1996). Finally, RUP was calculated from results of chemical CP fractionation assuming a K_p of 0.05/h as follows (Shannak et al., 2000):

$$RUP_{CHEM} = -189.682 - 304.721 \bullet CP / PNDF + 0.0030 \bullet CP \bullet B2 - 0.0263 \bullet CP \bullet C + 0.0038 \bullet CP \bullet (A + B1) + 0.0002 \bullet CP \bullet C^2 - 0.0022 \bullet PNDF \bullet B1 + 0.0038 \bullet (B3 + C) \bullet B2$$

where, RUP_{CHEM} and CP are in g/kg DM, CP fractions are in g/kg CP and PNDF (g/kg DM) refers to neutral detergent fibre estimated from the residue after boiling in neutral detergent solution according to Licitra et al. (1996).

Three-Step Enzymatic Procedure

A three-step enzymatic method was conducted to estimate RUP from degradation in a *Streptomyces griseus* protease solution (RUP_{ENZ}) with a further incubation of the residues from this step in a pepsin/pancreatin solution to estimate IPD (Irshaid, 2007). The procedure was conducted as described by Böttger and Südekum (2017) except for the duration of the protease incubation. In short, samples were incubated in borate-phosphate buffer containing

S. griseus protease corresponding to 41 U/g TP for 18 h before filtering contents through a 'FibreBag' (30-µm pore size, Gerhardt, Königswinter, Germany). Residues were freeze dried, weighed and analysed for N and RUP_{ENZ} (g/kg CP) was calculated as the amount of CP in the residue divided by incubated amount of CP, multiplied by 1000. To estimate IPD, residues were consecutively incubated in pepsin-HCl and pancreatin solutions for 1 h and 24 h, respectively. Afterwards, trichloroacetic acid (TCA) was used to stop enzymatic action and to precipitate undigested protein. After centrifugation and filtration, the residue was analysed for TCA-insoluble N. To calculate IPD, N soluble in TCA was divided by N incubated in pepsin/pancreatin.

Results and Discussion

The pattern of CP fractions (Table 1) differed considerably between RSM commodities, presumably due to varying effects of different treatments. Fraction B2 and B3 accounted for a high proportion of CP. High concentrations of CP fraction C, as observed for sample A, indicate potential heat damage. This may lead to reduced IPD, yet IPD of sample A was not particularly low (Table 2), possibly due to the fact that ADICP created by the Maillard reaction during heat treatment is partly digestible (Waters et al., 1992).

Table 1 Concentrations of crude protein (CP; g/kg DM) and CP fractions (g/kg CP) in different rapeseed meal commodities

Commodity	CP	A	B1	B2	B3	C
A	338	79	31	261	406	223
B	369	46	136	625	134	58
C	381	68	40	525	297	70
D	368	41	130	628	149	61
E	348	49	95	445	339	72
F	312	48	42	362	397	151
G	370	115	50	662	106	67
H	356	123	26	654	123	74
I	340	141	1	662	119	77

Values of $uCP_{0.05}$ (Table 2) corresponded well to previously reported values (Hippenstiel et al., 2012). However, RUP values (Table 2) were higher than generally assumed for RSM (Hippenstiel et al., 2012) for most samples regardless of method of estimation, which may be related to the additional treatment some samples underwent. All three methods resulted in similar rankings (Table 2, Figure 1). The similarity of RUP values was more pronounced between the two methods based on enzymatic degradation – either by a specific addition of *S. griseus* protease or by microbial activity in rumen fluid. Values for RUP_{CHEM} were generally higher compared to RUP_{HGT} and RUP_{ENZ}. Estimation of RUP from CP fractions yielded almost 2000 g/kg CP for sample A which is twice the theoretical maximum.

Table 2 Concentrations of utilisable crude protein at the duodenum (uCP; g/kg DM), ruminally undegraded feed crude protein (RUP; g/kg crude protein) estimated from the modified Hohenheim gas test (RUP_{HGT}), chemical crude protein fractionation (RUP_{CHEM}) and *Streptomyces griseus* protease incubation (RUP_{ENZ}) as well as intestinal digestibility of RUP (IPD; fraction of RUP) estimated from pepsin/pancreatin incubation in different rapeseed meal commodities

Commodity	uCP[1]	RUP_{HGT}[1]	RUP_{CHEM}[1]	RUP_{ENZ}[2]	IPD
A	284	578	1987	583	0.81
B	183	302	476	296	0.72
C	229	407	670	542	0.86
D	218	382	506	269	0.69
E	248	461	538	534	0.84
F	272	632	963	691	0.82
G	219	347	540	452	0.70
H	216	387	590	402	0.69
I	210	393	600	406	0.65

[1]Assumed ruminal passage rate of 0.05/h; [2]18-h incubation in protease solution.

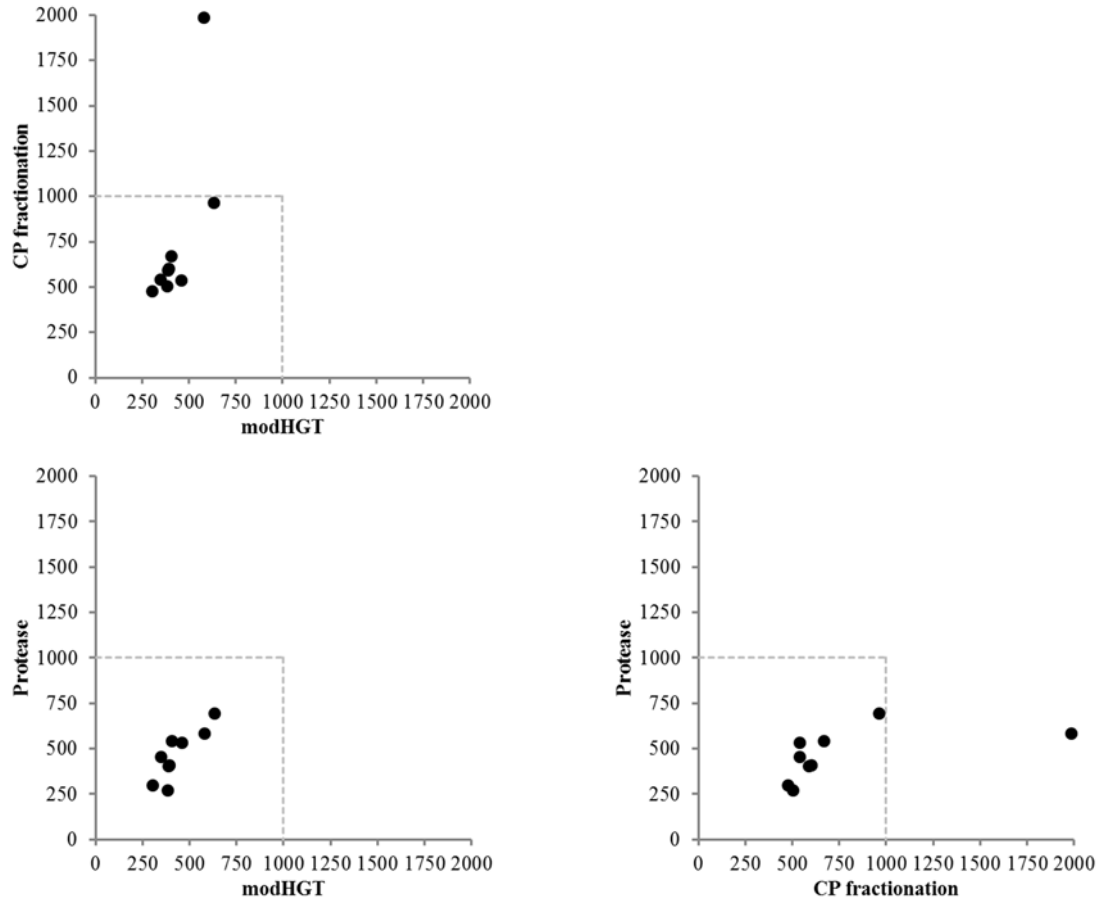


Figure 1 Comparison of ruminally undegraded feed crude protein (g/kg crude protein) estimated using three different methods, i.e. a modified Hohenheim gas test ('modHGT'), chemical crude protein fractionation ('CP fractionation') and incubation in *Streptomyces griseus* protease solution ('Protease').

Although below 1000 g/kg CP, also sample F displayed an unrealistically high RUP_{CHEM} value. These high values were presumably due to the very high concentrations of CP fraction C, which enters the prediction equation as a quadratic term. Obviously, the quality of prediction equations is essential. It has been shown that estimating RUP from CP fractionation requires specific equations for different types of feedstuffs (Edmunds et al.,

2012b). Moreover, it may not be sufficient to use separate equations for forages and concentrate feeds, as there are differences also within these categories. For example, equations of Shannak et al. (2000) could not successfully be applied to distillers dried grains with solubles (Westreicher-Kristen et al., 2012). It has to be noted that the data set from which the equation used in this study was derived from included samples of RSM, yet the majority of feed samples were concentrate feeds other than RSM.

Estimating RUP from incubation in *S. griseus* protease solution does not require regression equations but standardisation seems to be an issue. Complex investigations have been published with regard to incubation time and enzyme concentration (e.g. Licitra et al., 1999; Gallo et al., 2018). The procedure applied to RSM in the current study was modified in that no filter paper but a nylon bag was used for filtration to facilitate recovery of residues for the estimation of IPD. Other authors have replaced filtration with centrifugation (Wild et al., 2019). No reference values were available for RSM samples included in the current study and 'true' values of RUP were not known. For a set of ten different RSM commodities, Steingaß et al. (2011) found that the relationship between RUP estimated from the modHGT (x) and in situ RUP (y) was $y = 0.98 x + 3.7$ ($R^2 = 0.62$). As a rumen fluid-based method, the modHGT requires ruminally cannulated animals but offers the advantage that estimation of uCP and its differentiation into microbial CP and RUP can be achieved in the same procedure (Steingaß and Südekum, 2013). The potential of the approach of Raab et al. (1983) to evaluate RUP has been discussed by Udén (2018). The modHGT is also treated in greater detail by Südekum & Böttger in the current proceedings.

Conclusions

The comparison among estimates of RUP for RSM commodities resulting from the three different methods showed that absolute values (g/kg CP) differed, but ranking of the samples was similar among methods. Particular attention should be paid to choice of regression equations when RUP is estimated from chemical CP fractionation. Calibration of laboratory methods remains a challenge. Eventually, laboratory methods that can be used in diet formulation to predict animal response to feed protein value will be required.

References

Böttger, C. & Südekum, K.-H., 2017. European distillers dried grains with solubles (DDGS): chemical composition and in vitro evaluation of feeding value for ruminants. Anim. Feed Sci. Technol. 224, 66-77.

Broderick, G.A., Colombini, S., Costa, S., Karsli, M.A. & Faciola, A.P., 2016. Chemical and ruminal in vitro evaluation of Canadian canola meals produced over 4 years. J. Dairy Sci. 99, 7956-7970.

Edmunds, B., Südekum, K.-H., Spiekers, H., Schuster, M. & Schwarz, F.J., 2012a. Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for ruminants, from forages using a modified gas test. Anim. Feed Sci. Technol. 175, 106-113.

Edmunds, B., Südekum, K.-H., Spiekers, H. & Schwarz, F.J., 2012b. Estimating ruminal crude protein degradation of forages using in situ and in vitro techniques. Anim. Feed Sci. Technol. 175, 95-105.

Gallo, A., Fortunati, P., Bruschi, S., Giuberti, G. & Masoero, F., 2017. Use of central composite design to optimize working conditions of *Streptomyces griseus* enzymatic method in estimating in vitro rumen undegraded crude protein of feedstuffs. J. Agric. Sci. 156, 100-109.

GfE, 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt/Main, Germany.
Hippenstiel, F., Südekum, K.-H., Meyer, U., Flachowsky, G., 2012. Co-products from biofuel production for farm animals – an EU perspective, in: Makkar, H.P.S. (Ed.), Biofuel Co-Products as Livestock Feed – Opportunities and Challenges. FAO, Rome, Italy, pp. 209-227.
Huhtanen, P., Hetta, M. & Swensson, C., 2011. Evaluation of canola meal as a protein supplement for dairy cows: A review and a meta-analysis. Can. J. Anim. Sci. 91, 529-543.
Irshaid, R., 2007. Estimating Intestinal Digestibility of Feedstuffs for Ruminants Using Three-step in Situ-in Vitro and in Vitro Procedures. Dissertation. Christian-Albrechts-Universität, Kiel, Germany.
Licitra, G., Hernandez, T.M. & Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347-358.
Licitra, G., Van Soest, P.J., Schadt, I., Carpino, S. & Sniffen, C.J., 1999. Influence of the concentration of the protease from *Streptomyces griseus* relative to ruminal protein degradability. Anim. Feed Sci. Technol. 77, 99-113.
Raab, L., Cafantaris, B., Jilg, T. & Menke, K.H., 1983. Rumen protein degradation and biosynthesis 1. A new method for determination of protein degradation in rumen fluid in vitro. Br. J. Nutr. 50, 569-582.
Shannak, S., Südekum, K.-H. & Susenbeth, A., 2000. Estimating ruminal crude protein degradation with in situ and chemical fractionation procedures. Anim. Feed Sci. Technol. 85, 195-214.
Steingass, H., Kneer, G., Lehnen, S., Wischer, G. & Rodehutscord, M., 2011. Ruminaler Abbau des Rohproteins und der Aminosäuren sowie Verdaulichkeit des unabgebauten Futterrohproteins bei Rapsextraktionsschroten. Report. Union zur Förderung von Oel- und Proteinpflanzen e.V. (Ed.), Berlin, Germany.
Steingaß, H. & Südekum, K.-H., 2013. Proteinbewertung beim Wiederkäuer – Grundlagen, analytische Entwicklungen und Perspektiven. Übers. Tierernährg. 41, 51-73.
Udén, P., 2018. Techniques to measure ruminal protein degradation – a review, in: Udén, P., Spörndly, R., Rustas, B.-O., Eriksson, T., Karlsson, J. (Eds.), Proc. 9th Nord. Feed Sci. Conf., Uppsala, Sweden. Report 298. Departm.Anim. Nutr. Managem., Swedish Univ. Agric. Sci., Uppsala, Sweden, p. 67-73.
Waters, C.J., Kitcherside, M.A. & Webster, A.J.F., 1992. Problems associated with estimating the digestibility of undegraded dietary nitrogen from acid-detergent insoluble nitrogen. Anim. Feed Sci. Technol. 39, 279-291.
Westreicher-Kristen, E., Steingass, H. & Rodehutscord, M., 2012. Variations in chemical composition and in vitro and in situ ruminal degradation characteristics of dried distillers' grains with solubles from European ethanol plants. Arch. Anim. Nutr. 66, 458-472.
Wild, K.J., Steingaß, H. & Rodehutscord, M., 2019. Variability of in vitro ruminal fermentation and nutritional value of cell-disrupted and nondisrupted microalgae for ruminants. GCB Bioenergy 11, 345-359.

Effect of rumen-protected amino acid supplementation of dairy cows fed a grass silage and by-product based diet

J. Karlsson[1], M. Lindberg[1] & K. Holtenius[1]
[1]*Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Box 7024, 750 07 Uppsala.*
Correspondence: johanna.karlsson@slu.se

Introduction

Ruminants can produce meat and milk from fibrous feeds and by-products not suitable for human consumption. However, high yielding dairy cows are generally fed high proportions of cereal grains and pulses, products that often can feed humans directly. Another sustainability aspect of dairy production is to minimize nitrogen leakage, which can lead to eutrophication. Feeding dairy cows diets low in crude protein (CP) decreases the risk of nitrogen leakage. In low CP diets deficiencies of some amino acids (AA) can often limit milk production in high yielding dairy cows. Methionine and lysine are considered the first limiting AAs for synthesis of milk in high yielding dairy cows (Schwab *et al*., 1992). The aim of this study was, therefore, to investigate the effects on feed intake, production and efficiency from diets based on grass/clover silage and a concentrate containing mainly human inedible by-products with or without supplementation of rumen protected lysine and methionine.

Table 1 Ingredients in the two concentrate types

Ingredient, g/kg DM	With synthetic amino acids (WithAA)	No synthetic amino acids (NoAA)
Sugar beet pulp[1]	566	566
Wheat bran	120	120
Wheat flour[2]	100	100
Rapeseed meal[3]	70	70
Distillers grain[4]	70	70
Feed fat	25.0	25.8
Molasses	22.1	28.3
Salt	10.7	10.6
Limestone, ground	7.4	7.4
Rumen protected lysine[5]	5.0	N.I.[7]
Rumen protected methionine[6]	1.9	N.I.
Premix	2.0	2.0

[1]Dried with no inclusion of molasses (Nordic Sugar AB, Eslöv, Sweden); [2]Feed quality; [3]Solvent-extracted and heat-moisture treated and with low levels of glucosinolates and erucic acid (ExPro, AAK Sweden Ab, Karlshamn, Sweden); [4]Fiber and yeast cells from ethanol manufacturing (Agrow Drank 90, Lantmännen Agroetanol, Norrköping, Sweden); [5]LysiPearl (Kemin, Herentals, Belgium); [6]MetaSmart Dry (Adisseo, Antony, France). [7]N.I. = not included.

Materials and Methods

Thirty-seven multiparous Holstein (n = 13) and Swedish Red (n = 24) dairy cows were followed from calving until lactation week 22. A 2x2 factorial arrangement of treatments were used with two concentrate levels and two concentrate types. A low concentrate diet of maximum 6 kg per day was fed to 27 cows, while 10 cows were fed a high concentrate diet of maximum 12 kg per day. All cows were then allotted to either the concentrate with rumen protected amino acids (WithAA; n = 19) or to a similar concentrate without rumen protected amino acids (NoAA; n = 18). The concentrate was pelleted and fed in automatic concentrate

stations. Concentrate compositions are in Table 1. Grass/clover silage was offered *ad libitum*. Chemical composition of silage and concentrates are in Table 2. Individual feed intake (CRFI, BioControl Norway As, Rakkestad, Norway), body condition score (BCS; 3D camera, DeLaval International AB, Tumba, Sweden) and body weight (BW; AWS100, DeLaval International AB, Tumba,, Sweden) were recorded automatically. Energy balance (EB) was calculated according to the NorFor system as EB = net energy $(NE)_{intake}$ – ($NE_{maintenance}$ + $NE_{lactation}$). The cows were milked in an automatic milking station and milk samples were collected every other week. Blood plasma was collected in lactation week 2, 4, 6 and once in lactation week 19-21.

Feed and milk samples were analysed as described by Karlsson *et al*. (2018). Plasma was analysed for glucose, insulin, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB) and insulin-like growth factor 1 (IGF-1) concentrations.

Statistical analyses were performed using PROC MIXED in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The model was:
Y = μ + parity + breed + concentrate level + concentrate type + lactation week + cow, with lactation week as repeated measurement and cow as random variable. Interactions were calculated, but removed from the final model, if not significant ($P<0.05$). All residuals were tested for normality and log transformations were used for those that did not follow normal distribution. Statistical significance was set at $P < 0.05$, with tendencies noted at $P < 0.10$.

Table 2 Chemical composition of the grass/clover silage and two concentrate types used in the diets to dairy cows (g/kg of DM, unless otherwise stated)

		Concentrate type[2]	
	Grass/clover silage[1]	With synthetic amino acids (WithAA)	No synthetic amino acids (NoAA)
Dry matter, g/kg	440 ± 31	870 ± 9	876 ± 7
Ash	85.8 ± 4.7	67.5 ± 2.6	65.3 ± 2.0
Crude protein	154 ± 7	155 ± 7	149 ± 4
Crude fat	N.A.[3]	25.6 ± 3.5	22.3 ± 1.9
Neutral detergent fiber	450 ± 14	359 ± 12	365 ± 11
Starch	N.A.	59.9 ± 15.4	47.3 ± 3.7
Water soluble carbohydrates	N.A.	5.28 ± 1.2	6.25 ± 2.1
VOS[4]	90.4 ± 1.7	N.A.	N.A.
Ammonia-N	0.53 ± 0.07	N.A.	N.A.
pH	4.3 ± 0.1	N.A.	N.A.
Metabolisable energy, MJ/kg DM	11.5 ± 0.3[5]	8.0[6]	8.0[6]
Net energy of lactation, MJ/kg DM	6.2[6]	6.0[6]	5.9[6]

[1]Silage samples were taken five days a week and pooled into three-week samples. For the data in the present study 19 pooled samples were analysed; [2]Concentrate samples were taken weekly and pooled into four-week samples. For the data in the present study 11 pooled samples were analysed; [3]N.A. = not analysed; [4]In vitro organic matter digestibility (%); [5]Calculated as ((0.16 x VOS)-1,91) x (1000-Ash)/1000; [6]Calculated in NorFor based on chemical composition and estimates where analytical data were lacking.

Results and Discussion

There were no difference in intake of total dry matter (DM), forage or concentrates between WithAA or NoAA. However, it has been shown in a meta-analysis, that cows fed rumen protected methionine (Smartamine, Adisseo, Antony, France), similar to the present study, had higher DM intake (Zanton *et al*., 2014).

Unintentionally, the starch content in the NoAA concentrate was lower than in the WithAA concentrate (Table 2), which explains why starch intake was lower in cows fed the NoAA concentrate (Table 3). However, a difference as low as approximately 10 g/kg DM or 0.07 kg DM/d in starch is not likely to have caused any effects on production. There was no difference in intake of other feed composition parameters as organic matter, CP, crude fat or NDF between cows fed WithAA and NoAA.

Regarding milk yield, ECM, milk composition and yield of fat, protein and lactose from milk, there was no overall difference between cows fed WithAA and NoAA concentrate. There was also no effect of concentrate type WithAA or NoAA on BW, BCS or N and feed efficiency.

Cows fed WithAA had an overall higher BHB plasma concentration than cows fed NoAA (Table 3). There was also a concentrate type x lactation week interaction (P = 0.001) where cows fed WithAA had higher plasma concentrations of BHB in early lactation (1.29 mmol/L) compared to mid lactation cows (0.75 mmol/L) and cows fed NoAA concentrate (0.84 mmol/L). No other plasma metabolites, insulin or estimated EB were affected, which could have support changes in EB or adipose fat mobilization from supplementation of rumen protected methionine and lysine. Apparently, methionine (MetaSmart) does not affect plasma concentration of BHB in lactating cows (Osorio *et al.*, 2013). Thus, it might be speculated that the increased BHB content was related to the lysine supplementation. We are not aware of any studies investigating the effect of lysine on ketogenesis. However, it has been shown in mice that dietary supplementation with lysine stimulates liver β-oxidation by activating carnitine palmitoyltransferase 1a (CPT1) (Sato *et al*., 2018). In ruminants, this enzyme is a key enzyme facilitating transport of NEFA into the mitochondria for β-oxidation and ketogenesis (Herdt 2000). Potentially, this would increase ketogenesis and decrease esterification of NEFA to form triglycerides, lowering the risk of fat infiltration in the liver in early lactation dairy cows.

Methionine is the first limiting AA in typical North American diets (Chen *et al*., 2011), which are often based on corn silage, alfalfa and soybean meal. Diets with high inclusion of corn by-products, which are low in protein, have previously shown positive effects on milk production when supplemented with rumen protected lysine (Broderick, 2018). It has been suggested that histidine, not lysine or methionine, is the first limiting AA in milk production of dairy cows fed grass silage-cereal based diets (Vanhatalo *et al*., 1999; Kim *et al*., 1999; Korhonen *et al*., 2000). This could partly explain the lack of effect on milk production in cows WithAA in the present study. Another possible explanation could be that both diets contained sufficient amounts of essential AA, and that the total CP level of the diets (15.2-15.5%) were not low enough to create a need for supplementing with rumen protected lysine or methionine. Other studies investigating supplementation of essential AA used both lower (Lee *et al*., 2018; Doepel and Lapierre, 2010) and higher (Chen *et al*., 2011; Socha *et al*., 2005) total dietary CP content compared to the concentration in the present study.

Conclusions

A by-product and forage based diet to early and mid-lactating dairy cows supplemented with rumen protected methionine and lysine (WithAA) gave no effect on feed intake, milk production and milk composition. Cows fed WithAA concentrate had a higher plasma concentration of BHB compared with cows fed NoAA concentrate. Other blood metabolites, insulin, and EB showed no difference between cows independent of concentrate type.

Table 3 Treatment effects on daily intake, milk yield, milk components, body weight (BW), body condition score (BCS), feed and N efficiency for the two concentrate types (LSM with SEM and P-value)

	With synthetic amino acids	No synthetic amino acids	SEM	P-value
Total dry matter intake (DMI), kg DM/d	26.0	26.4	0.58	0.58
Forage intake, kg DM/d	18.8	19.2	0.55	0.69
Concentrate intake, kg DM/d	7.19	7.31	0.065	0.19
Organic matter intake, kg DM/d	20.9	21.3	0.50	0.55
Crude protein intake, kg DM/d	4.05	4.05	0.090	0.84
Crude fat intake, kg DM/d	0.79	0.78	0.018	0.85
Neutral detergent fiber intake, kg DM/d	11.0	11.2	0.25	0.52
Starch intake, kg DM/d	0.62	0.55	0.008	<.001
Milk yield, kg/d	37.6	37.7	0.97	0.97
ECM[1] yield, kg/d	39.1	39.7	0.99	0.67
Fat, kg/d	1.74	1.66	0.045	0.24
Protein, kg/d	1.30	1.24	0.030	0.16
Lactose, kg/d	1.88	1.81	0.053	0.38
Fat, g/kg	43.1	44.5	0.08	0.23
Protein, g/kg	33.0	33.5	0.05	0.49
Lactose, g/kg	48.0	47.9	0.02	0.66
BW, kg	734	732	12.7	0.89
BW change, kg/week	-0.449	0.792	0.7516	0.23
BCS	3.28	3.19	0.065	0.29
BCS change, BCS/week	-0.020	0.021	0.0047	0.91
Energy balance, MJ NEL[2]/d	-0.95	-1.20	3.223	0.95
Glucose, mmol/L	2.95	2.99	0.084	0.77
Insulin (log10)	-0.88	-0.96	0.067	0.38
Insulin antilog, µg/L	0.13	0.11	N.A.	N.A.
NEFA[3] (log10)	-0.52	-0.54	0.033	0.62
NEFA, mmol/L	0.30	0.29	N.A.	N.A.
BHB[4] (log10)	0.05	-0.08	0.029	0.002
BHB antilog, mmol/L	1.13	0.84	N.A.	N.A.
IGF-1[5] (log10)	1.86	1.91	0.030	0.26
IGF-1 antilog, ng/ml	73.0	81.4	N.A.	N.A.
Feed efficiency, kg ECM/kg DMI	1.55	1.54	0.044	0.91
N-efficiency, g/kg	333	310	11.0	0.15

[1]ECM = Energy corrected milk yield. [2]NEL = net energy of lactation. [3]NEFA = non-esterified fatty acids. [4]BHB = beta-hydroxybutyrate. [5]IGF-1 = insulin-like growth factor 1.

References

Broderick, G.A., 2018. Review: Optimizing ruminant conversion of feed protein to human food protein. Animal 12, 1722-1734.

Chen, Z.H., Broderick, G.A., Luchini, N.D., Sloan, B.K. & Devillard, E., 2011. Effect of feeding different sources of rumen-protected methionine on milk production and N-utilization in lactating dairy cows. J. Dairy Sci. 94, 1978-1988.

Doepel, L. & Lapierre, H., 2010. Changes in production and mammary metabolism of dairy cows in response to essential and nonessential amino acid infusions. J. Dairy Sci. 93, 3264-3274.

Herdt, T.H., 2000. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. Vet. Clin. North Am. Food Anim. Pract. 16, 215-230.

Karlsson, J., Spörndly, R., Lindberg, M. & Holtenius, K., 2018. Replacing human-edible feed ingredients with by-products increases net food production efficiency in dairy cows. J. Dairy Sci. 101, 7146-7155.

Kim, C.H., Choung, J.J. & Chamerlain, D.G., 1999. Determination of the first-limiting amino acid for milk production in dairy cows consuming a diet of grass silage and a cereal-based supplement containing feather meal. J. Sci. Food Agric. 79, 1703-1708.
Korhonen, M., Vanhatalo, A., Varvikko, T. & Huhtanen, P., 2000. Responses to graded postruminal doses of histidine in dairy cows fed grass silage diets. J. Dairy Sci. 83, 2596-2608.
Lee, C., Hristov, A.N., Cassidy T.W., Heyler, K.S., Lapierre, H., Varga, G.A., de Veth, M.J., Patton, R.A. & Parys, C., 2018. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. J. Dairy Sci. 95, 6042-6056.
Osorio, J.S., Ji, P., Drackley, J.K., Luchini, D. & Loor, J.J., 2013. Supplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. J. Dairy Sci. 96, 6248-6263.
Sato, T., Muramatsu, N., Ito, Y., Yamamoto, Y. & Nagasawa, T., 2018. L-Lysine attenuates hepatic steatosis in senescence-accelerated mouse prone 8 mice. J. Nutr. Sci. Vitaminol 64, 192-199.
Schwab, C.G., Bozak, C.K., Whitehouse, N.L. & Mesbah, M.M.A., 1992. Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation. J. Dairy Sci. 75, 3486-3502.
Socha, M.T., Putnam, D.E., Garthwaite, B.D., Whitehouse, N.L., Kierstead, N.A., Schwab, C.G., Ducharme, G.A. & Robert, J.C., 2005. Improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen-protected methionine and lysine. J. Dairy Sci. 88, 1113-1126.
Vanhatalo, A., Huhtanen, P., Toivonen, V. & Varvikko, T., 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. J. Dairy Sci. 82, 2674-2685.
Zanton, G.I., Bowman, G.R., Vázquez-Añón, M. & Rode, L.M., 2014. Meta-analysis of lactation performance in dairy cows receiving supplemental dietary methionine sources or postruminal infusion of methionine. J. Dairy Sci. 97, 7085-7101.

Alternative crops as feed sources during the drought in Sweden 2018

G. Bergkvist[1] & R. Spörndly[2]
[1]Department of Crop Production Ecology, Swedish University of Agricultural Sciences (SLU), BOX 7043, 750 07 Uppsala. [2]Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, BOX 7024, 750 07 Uppsala, Sweden. Correspondence: Goran.Bergkvist@slu.se

Introduction

The summer of 2018 was exceptionally dry in northern Europe and in Sweden. Yields of cereals and grain legumes were 43% and 35% lower than the five-year mean, respectively (Statistiska Meddelanden, 2018). Yields of grass-clover as forage crops was 26% lower than 2017, and they were particularly small in southern Sweden. Amounts harvested were low already at the first harvest and through the second harvest, due to high temperatures and rapid crop development, combined with severe water deficit in many places. The total yield was to some extent compensated for by high yields in late summer and autumn as rain came in late July or early August.

Due to poor growth and rapid development of annual crops, they were harvested early, which opened for the possibility to establish a second crop during summer. Some cereal crops intended for grain harvest were harvested prematurely as whole crops to replace the failing grass-clover forage. Since farmers fertilised for an expected higher yield, much soil N remained at harvest of the annual crops. On top of that, mineralisation of nitrogen was high during late summer. Growth of microbes responsible for mineralisation increases exponentially with temperature up to about 40°C and is at its maximum when about 60% of the pores are filled with water (Linn & Doran, 1984; Kätterer et al., 1998). Thus, when rain came in late summer, there was a boost of mineralisation, which increased concentration of available N in the soil.

The low yields of forage crops created a need to find alternative fodder. One option was to establish cover crops after harvest of the main crops, which could take advantage of the early harvest, rains and the high concentrations of available N during late summer and autumn. Subsidiary crops, such as cover crops, are crops that are mainly grown for their ecological services rather than to produce an economic yield (Schmidt et al. 2017). Such services include increased water infiltration, decreased soil evaporation, soil protection, nutrient cycling, N fixation, weed competition, sequester carbon for increased long-term soil fertility, and to reduce pest and diseases in adjacent or subsequent crops (Snapp et al., 2005; Sharma et al., 2018). Their use are limited by cost of sowing, prevention of other weed control measures and because they can become weeds themselves and contribute to spreading or maintaining weeds, pests and diseases.

The aim of this paper is to present options for use of subsidiary crops to produce fodder.

Results and Discussion

Pro-Active Use of Subsidiary Crops

Pro-active uses of subsidiary crops consider options available when planning the cropping system. Subsidiary crops can be under-sown in a main crop and have their main growth period after harvest of the main crop or be sown in conjunction with or after harvest of the main crop. The first option is suitable as a pro-active method to adapt to situations when fodder production is not sufficient from other sources or as part of a strategy to produce forage. The most suitable species for under-sowing are perennial grasses and legumes as they grow slowly and have less effect on main crop growth than annual species (Känkänen and Eriksson, 2007). The method of establishment are the same as for rotational leys and the forage they can produce in autumn is of similar quality as in rotational leys. Therefore, the implementation of this adaption strategy is easy for farmers. Another benefit is the flexibility of the system. If more leys need to be replaced than usual, they can remain to the next year as a rotational ley. The disadvantage compared to post-harvest sowing is that their establishment can be affected by drought in the same way as regular fodder crops. As would have been the case in 2018, that they can reduce yield of the main crops by competition and affect possibilities of weed control in the main crop.

It is possible to affect the disadvantages of using under-sown subsidiary crops by adjusting management practices. A safe establishment could be achieved by under-sowing in conjunction with sowing of a preceding autumn sown main crop like winter wheat. At high latitudes, establishment of legumes in the autumn is difficult due to the risk of frost kill during the winter. This was only successful for white clover in one experiment of five conducted in southern Sweden (Bergkvist et al., 2002), while over-wintering is less of a problem further south in Europe (Heyland and Merkelbach, 1991). Many grasses, such as perennial ryegrass, stem-elongate during approximately the same time period as winter wheat and, therefore, compete strongly with wheat (Bergkvist et al., 2002), as well as produce seeds that could lead to a weed problem. Red fescue sown at a seeding rate lower than 4 kg/ha could be an option. There are varieties that do not produce seeds under wheat and the negative effect of competition is much less than with ryegrass (Bergkvist et al., 2002). Red fescue produced about 1.5 ton DM/ha until harvest of wheat in the experiment by Bergkvist et al. (2002). The biomass was not harvested in the experiments, thus the additional increase in biomass during autumn was quite small, but if the biomass was harvested in the summer, the crop could probably have been grazed during autumn. Red fescue is mostly used for grazing and the nutritive quality generally decreases rapidly with stage of development. But, when harvested in vegetative stage before shooting, the energy content is equivalent to meadow fescue. Mixing with clover, where autumn sowing is possible, increases quality and growth under N limited conditions.

It would be more feasible to do under-sowing in spring in winter wheat than in autumn (Bergkvist et al., 2002; Bergkvist et al., 2011; Amosse et al., 2013). Under conventional practices, it is important for successful establishment to do under-sowing early spring (Bergkvist et al., 2002). Later, the crop is more competitive and soil surface denser. In organic farming, more light is generally penetrating the crop due to lower nutrient availability and larger row spacing, particularly if row hoeing is used, which benefits legumes in particular. Legumes are more limited by light than by nutrient competition since they can fix N from the air. Under-sowing in spring is not likely to affect grain yield of wheat even if the

biomass of legumes can be high at low N fertilizer doses (Bergkvist et al., 2002; Bergkvist et al., 2011; Amosse et al., 2013), but the protein content of the wheat could be reduced (Amosse et al., 2013). The biomass of under-sown clover and grass is generally only a few hundred kg/ha at harvest of wheat, except at low N availability when legume growth could be vigorous under the wheat. The above ground biomass typically increases about one ton DM/ha during autumn in southern Sweden, but in years with good growth, yields of up to two tonnes DM/ha have been recorded with Italian ryegrass and red clover in the seed mixture. Perennial ryegrass grow less. There is a strong positive correlation between precipitation in late summer and growth during autumn (Schröder et al, 1997). The nutritive quality of perennial ryegrass is considered to be the best of grasses in terms of energy content when harvested in time. It develops fast and digestibility decreases rapidly when harvested after its peak.

When under-sowing is done in spring cereals, it is possible that the biomass becomes quite large already at harvest of the main crop. The biomass of perennial grass is mainly determined by species, seeding rate, and time of under-sowing. If using the competitive species Italian ryegrass, it is important to reduce seed rate or delay under-sowing in relation to the cereal to avoid yield loss. In investigations by Ohlander et al. (1997), the biomass of Italian ryegrass under-sown in spring barley was about 0.8 ton DM/ha at harvest of barley when under-sown on the same day, but only half of that if under-sown at emergence of barley and the biomass was not significantly affected by dose of N fertilizer. The biomass of the less competitive perennial ryegrass was about half in both treatments, while red clover was not much affected by relative time of under-sowing as long as there were water enough for germination which limited production when sowing after emergence of barley. Instead, the amount of red clover was determined by dose of N fertilizer and was twice as large with 80 as with 40 kg/ha. For safe establishment, it is best to do the under-sowing in the same operation as the sowing of barley but at an appropriate depth (Bergkvist et al., 1995) and regulate biomass production with the seed rate. On soils with relatively course soil structure and sufficient water during establishment, seed rates down to a few kg/ha would be enough to achieve maximum increase in biomass during autumn (Bergkvist et al., 1995).

Re-Active Use of Subsidiary Crops

The reactive use of subsidiary crops include crops that are sown when opportunity and need appear during summer. Such crops are generally called cover crops. Seeds could be broadcasted a few weeks before expected harvest of the main crop or, more commonly, in conjunction with main crop harvest. Moisture availability is crucial for success of the establishment. Thus, sowing in combination with soil tillage immediately after harvest, before the soil dries out, is considered the safest method. Barbibar et al. (2018) found that , total weed biomass did not increase when cover crops were established after tillage, which could be expected as germination of weed seeds are stimulated by tillage, probably because perennial weeds were damaged by the tillage operation. Another factor that is crucial for the possibility to produce significant amount of fodder from cover crops is that they must be established early, particularly under Nordic condition where radiation and temperature decreases rapidly during autumn. A third crucial factor is that there is N available for the cover crop. Without sufficient soil mineral N or application of N fertilizer, legumes or mixtures with legumes are the best options as cover crops.

Cover crops for animal fodder can be harvested and served fresh to the animals, be harvested for silage or be grazed. The intended purpose determines choice of species, but to be at all relevant, they need to grow fast. The fastest growing species of 36 tested potential cover crops in experiments in Switzerland and France conducted between August and October was sunflower (*Helianthus annuus*), which increased its shoot biomass by 5.6 kg DM/ha per growing degree days (GDD = ∑ ((maxtemp+mintemp)/2-base temperature); °C*d] when water and nutrients were supplied in abundance. Brennan and Boyd (2012) found a strong correlation between growing degree days and biomass of cover crops in experiments conducted during winter in California. Rye and white mustard (*Sinapis.alba*) required about 500 and 600 growing degree days (base temperature = 4°C), respectively, to reach three tonnes DM/ha shoot biomass. In the Nordic countries, growth in autumn is not only limited by temperature, water and nutrients. Frost terminates growth and low light levels restricts the maximum biomass that can be sustained even if temperature is sufficient for growth. Olsson (2009) found that both White mustard and oilseed radish (*Raphnus sativus*) required 50 days with temperatures above 9°C to reach about one ton DM/ha. According to Aronsson et al. (2012), such conditions would occur in four years of five in the southernmost landscapes of Sweden and less often further north. This means that, under average conditions, cover crops for fodder production is probably not economical. Aurell (2018) measured the amount of cover crops in several fields and in demonstration experiments during 2018 when conditions for cover crops were exceptionally good during autumn. She did not record more than three tonnes DM/ha for any cover crop established after harvest of the main crop.

Cereals and annual ryegrass

The fastest growing cereals are spring barley and oats and the quality is similar to forage grasses. However, they need high seed rates and are susceptible to many fungal diseases that influence quality and quantity, particularly barley is much affected. Rye is a good option if the intended use is for grazing. It needs a period of vernalisation under low temperatures before stem elongation, but produces many tillers with leaves during autumn and suits grazing well. Mildly grazed rye could be left over winter to produce a cereal crop next year. During the drought summer 2018, oats, barley, rye and annual ryegrass (*Lolium westerwoldicum*) were sown after harvest of winter wheat and cultivated for 86 days until 25 October in an experiment in southern Sweden (Löfquist, unpublished data). Among the cereals, DM yield was highest for barley, but the energy value was lowest due to lignification of the fiber. Annual ryegrass yielded more than all cereals and maintained a high nutritional value. The crops were cultivated with or without N fertilization and in this case, residual N after the preceding crop was insufficient (Table 1). Annual ryegrass needs to be sown earlier than cereals to produce enough biomass to be harvestable, but can be sown with a lower seed rate, is likely to be less affected by diseases than spring cereals and can both be harvested for silage and grazed. Similar alternatives are *Sorghum sudanense* and *Avena strigosa*.

The family *Brassicaceae*

Many species in the family *Brassicaceae* grow fast during autumn. They all have a developed taproot and, depending on species, more or less biomass is allocated to this taproot. They generally compete well with weeds, but can propagate diseases to the disadvantage of related crops, e.g. *Plasmodiophora brassicae*, *Verticillium* and *Sclerotinia sclerotiorum*. Thus, farmers must be careful if oilseed rape (*Brassica napus*) is in the rotation. Good options are oilseed radish or radish that needs to be sown in early August to produce sufficient biomass in Sweden. An alternative for grazing is forage turnip (*Brassica rapa ssp. rapa*), where both

shoot and roots can be grazed. In the experiments by Löfquist from 2018 (unpublished), oilseed radish and turnip yielded better than the grasses and particularly the turnip had as high energy value but lower NDF content compared to grasses (Table 1).

The family *Fabaceae*

Legumes (family *Fabaceae*) are of obvious interest since they can fix N, but with late sowing, they cannot compare in growth with species from other families. For early sowing, pea (*Pisum sativum*), common vetch (*Vicia sativa*) and blue lupine (Lupinus angustifolius) could be options. Hairy vetch (*Vicia villosa*) and crimson clover (*Trifolium incarnatum*) can survive winter under Nordic conditions and have their major growth period in early spring before sowing of a late sown non-legume like maize. Legumes generally do well in mixtures with non-legumes. Legumes like common vetch, hairy vetch and crimson clover contributed substantially to the DM yield and CP content when sown with barley, oats or rye in treatments without application of N fertilizer in experiments 2018 (Löfquist, unpublished; Aurell, 2019).

Table 1 Estimated nutritive value for ruminants of crops harvested 25th of October as forage after being sown 31 of July (998 degree days; base temperature = 5°C) in South of Sweden. Values, except in brackets, are quoted from Löfquist (unpublished), analyzed by Eurofins Sweden using NIR method for grasses and wet chemistry for others

Species	DM, %	Crude protein, % of DM	Fiber, NDF, % of DM	Metabolis. energy, MJ/kg DM	Yield, kg DM/ha 50 kg N	Yield, kg DM/ha, 0 kg N
Rye	21.2	14.3	43.2	11.5	1250	79
Oats	24.6	11.9	44.7	10.8	1549	182
Barley	24.4	11.8	66.6	9.8	1823	1084
Westerwolths ryegrass (*Lolium multiflorum westerwoldium*)	23.6	12.5	38.8	11.8	2333	699
Oilseed radish (*Raphanus sativus*)	16.6	13.2	(19.2) [1)]		3333	676
Rapeseed (*Brassica napus*)	21.9	12.6	16.9	9.6	1180	1379
Turnip (*Brassica rapa*)	14.2				5670	6390
-shoot		10.6	(17.0) [1)]			
-root		5.6	11.1	11.3		

[1)] Samarappuli et al, 2014.

Others

Phacelia (*Phacelia tanacetifolia*), Sunflower and fodder beet (*Beta vulgaris*) are other species that grow well. They have the advantage of not being closely related to crops grown on large areas in Sweden, but farmers still need to check if they propagate any pest or disease on crops in the rotation.

Conclusions

Subsidiary crops are generally grown to support the cropping system, but could also be used as fodder. The choice of subsidiary crops for fodder purposes should consider effects on other crops in the rotation and how the fodder should be utilized. There are technical challenges as well as challenges related to conserving and using fodder from most subsidiary crops. Suggested crops are typically low in DM content and autumn weather makes wilting before ensiling difficult and are better suited for strip-grazing. The low fiber content of some crops should also be considered if the main purpose is to replace shortage of normal roughages with

high fiber contents. High yielding roots and tubers could be considered as concentrate feeds due to their high content of rapidly available carbohydrates.

References

Amosse, C., Jeuffroy, M-H, & David C., 2013. Field Crops Research 145, 78 - 87.
Aurell, A. 2018. Utvärdering av eftergrödor/grönfodergrödor 2018. Växa Sverige. Falkenberg. (*in Swedish)*
Baraibar, B., & Mortensen, D.A., Hunter, M.C. et al.., 2018. Agron. Sustain. Developm. 38, 65.
Bergkvist, G. Ohlander, L. & Nilsdotter-Linde, N., 1995. Undersown catch crops in cereals - establishment methods and their effect on cereal yield and catch crop growth. In: The Use of
Brennan, E.B and Boyd, N.S., 2012. Agron. J. 104, 684–698.
Catch or Cover Crops to Reduce Leaching and Erosion (ed. B. Lindén). NJF-utredning/rapport nr 99. 33-42. ISSN 0333-1350.
Bergkvist, G., Ohlander, L. & Rydberg, T., 2002. Insådd av mellangrödor i höstsäd. Sveriges lantbruksuniversitet, Institutionen för ekologi och växtproduktionslära. Rapport nr 4, 54 pp. ISSN 1404-2339.
Bergkvist G., Stenberg M., Wetterlind J. et al., 2011. Clover cover crops under-sown in winter wheat increase yield of subsequent spring barley—Effect of N dose and companion grass. Field Crops Res. 120, 292-298.
Heyland, K.U. & Merkelbach, H. 1991. Die Möglichkeiten des Einsatzes von Untersaaten zur Unkrautunterdrückung sowie Konkurrenzwirkungen von Unkraut und Untersaat auf die Ertragsbildung des Winterweizens. Bodenkultur 42, 347-359.
Känkänen, H. & Eriksson, C.,, 2007. Eur. J. Agric. 27, 25–34.
Linn, D.M. & Doran, J.W., 1984. Soil Sci. Soc. Am. J. 48, 1267-1272.
Löfquist, I., 2019. Sensommarsådda grönfoderblandningar avsedda för höstskörd. Hushållningssällskapet, Kristianstad.
Kätterer, T., M. Reichstein, M., Andrén, O. & Lomander, A., 1998. Biol. Fertil. Soils 27, 258–262.
Ohlander L., Bergkvist G., Stendahl F. & Kvist M., 1996. Acta Agric. Scand, Section B, Soil Plant Sci. 46, 161-168.
Olsson, Å. 2009. Sanering av betcystnematoder med resistenta mellangrödor. Nordic Beet Res. Slutrapport 409-2006-2009.
Samarappuli, D., Johnson, B., Kandel, H & Berti, M., 2014. Field Crops Res. 167, 31-39
Schmidt, J.H., Bergkvist, G., Campiglia, E. et al., 2017. Annals of Applied Biology 171, 477–489.
Schröder, J., Tenholte, L. & Janssen, B.H. 1997. Neth.s J. Agric. Sci. 45, 231–248.
Sharma, P., Singh, A., Kahlon, C.S. et al., 2018. Am. J. Plant Sci. 9, 1935-1951.
Snapp, S.S., Swinton, S.M., Labarta, R. et al., 2005. Agron. J. 97, 322-332.
Statistiska Meddelanden 2018a. Skörd av spannmål, trindsäd, oljeväxter och slåttervall 2018. Sveriges officiella statistik JO 19 SM 1802.
http://www.jordbruksverket.se/webdav/files/SJV/Amnesomraden/Statistik,%20fakta/Vegetabilieproduktion/JO19/JO19SM1802/JO19SM1802.pdf
Tribouillois, H., Fort, F., Cruz, P. et al., 2015. PLoS ONE 10, e0122156.

Feeding by-products to dairy cows – is it good for the environment and profitable for the farmer?

M. Lindberg[1], M. Henriksson[2], S. Bååth Jacobsson[3] & M. Berglund[2]
[1]Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, P.O. Box 7024, 750 07 Uppsala. [2]Hushållningssällskapet Halland, Lilla Böslid 146, 305 96 Eldsberga. [3]Växa Sverige, Lilla Böslid 146, 305 96 Eldsberga.
Correspondence: Mikaela.Lindberg@slu.se

Introduction

Agriculture faces several challenges globally including a growing population that requires increased food production. Today, agriculture accounts for about 20% of global greenhouse gas emissions (IPCC, 2015), and 38% of global land area is used for agriculture (Foley et al., 2011) of which about 70% for feed production (FAO, 2010). Measures to reduce environmental impact from milk production may be to use large proportions of by-products from food- and biofuel production in diets for dairy cows. By-products do not compete for land when it comes to food production and thus, environmental impact as well as resource use can be allocated to more than one crop. Sustainable economy in the food chain is crucial and any changes in a production system should not decrease profitability for farmers. The aim of this study was to evaluate environmental impact and feed costs by use of by-product-based concentrates in combination with grass/clover silage to lactating dairy cows. Earlier life cycle assessments (LCA) on milk production rely on information from surveys with farmers, databases or other large records of compiled data, but the novelty of the present study is that the LCA was performed using data from actual feeding trials.

Materials and Methods

The LCA was based on the International Dairy Federation (IDF) Guidelines for Dairy Product LCA (IDF, 2015) and two Product Environmental Footprint Category Rules (PEFCR), namely methods for feed for food-producing animals and milk production and dairy products (EDA, 2018; PEFCR, 2018). The principle for the LCA was attributional, e.g. a product's total environmental impact is calculated and all environmentally relevant flows to and from a product's entire life cycle are accounted for. Economic allocation was used for concentrate ingredients. The system boundary was set to farm-gate, the functional unit was 1 kg energy corrected milk (ECM) and results are presented without allocation of environmental burdens to meat. Environmental categories presented in this paper are climate change (Global Warming Potential; GWP_{100}) (kg CO_2e), land requirement (m^2), potential eutrophication (CML 2; re-calculated to g NO_3e) and use of non-renewable energy (MJ). Economic calculations were performed with focus on feed costs. Concentrate prices were obtained from the manufacturer of the experimental feeds (Lantmännen, Malmö, Sweden) and the price of forage was obtained from calculation of farm production costs. Other costs than feed costs were derived from collected data from real farms within the association European Dairy Farmers (2018).

The feeding trials A and B described below (see also Table 1) were conducted under different conditions, and comparisons can therefore only be made within each trial. Total feed consumption and milk yields were re-calculated to a full year, regardless of length of the actual experiment.

Trial A: Full lactation experiment where the cows were fed a concentrate based on by-products (beet fibre in combination with rapeseed meal and distillers' grains) in a "low" (5 kg) or "high" (9.5 kg) amount (mean value over the entire lactation) in addition to *ad libitum* access to grass/clover silage of a 1st cut harvest.

Trial B: Mid-lactation change-over experiment were the cows were fed concentrates based on by-products (beet fibre in combination with rapeseed meal and/or distillers' grains) or a control concentrate based on cereals and soybean meal at a flat rate (11 kg). In addition to the concentrate, a mixture of 1st and 2nd cuts of grass silage was fed *ad libitum*.

Table 1 Dry matter intake (DMI), proportion of roughage, proportion of feed components in the concentrate (in % of DMI if not stated otherwise) and milk yield based on data from the different feeding trials A and B re-calculated to 305 days of lactation and 60 days of dry period

Feeding trial	A. By-products		B. Control vs. by-products			
Concentrate level/type	Low	High	Control	RS[a]	DG[b]	RS[a] + DG[b]
DMI, kg	7 908	8 606	7 403	7 406	7 406	7 407
Roughage (% of DMI)	83	70	60	60	60	60
Cereals	-	-	28	-	-	-
Other, non-BP[c]	1.2	1.8	3.1	1.8	2.0	1.7
Cereal by-products[d]	3	6	-	3	1	3
Beet fibre	10	17	-	21	20	20
Distillers' grains	1	2	-	-	14	6
Rapeseed meal[e]	1	2	-	12	-	7
Soy products[f]	-	-	8	-	-	-
Other by-products[g]	0.6	1.0	1.2	2.0	2.0	2.4
Milk yield						
Produced, kg ECM	9 882	10 675	10 065	10 065	10 065	10 065
Delivered[h], kg ECM	9 190	9 928	9 360	9 360	9 360	9 360

[a]RS, rapeseed; [b]DG, distillers' grains; [c]Rapeseed, corn kernel, green pellet, fat, minerals; [d]Bran and middlings; [e]Incl. expro, i.e. heat treated rapeseed meal; [f]Soybean meal, soybean expeller, soybean; [g]Ingredients representing <5 %, i.e. molasses, palm expeller, sunflower meal, pea residues; [h]93 % of produced milk was assumed to be delivered to the dairy plant.

The mean values of feed intake and milk yield over the lactation were used in the software program IndividRAM to perform calculations according to the NorFor feed evaluation system (Volden, 2011). Theoretical values for the dry period and for the rearing period of recruitment heifers, 27.3 months, were calculated by using the software program and results were added to calculations of total environmental impact and feed costs. Numbers were scaled up to represent a herd of 100 dairy cows with 38% recruitment, and with the assumptions that all bull calves were sold at the age of eight weeks and that no recruitment heifers were sold.

Results and Discussion

Trial A, "Low and High Concentrate Level".

The low concentrate level resulted in a higher intake of silage, but lower total feed intake and lower milk yield compared with the high concentrate level. However, feed efficiency (kg ECM/kg DMI) was slightly higher on the low concentrate level (data not shown). This was believed to be the reason why overall climate impact (Figure 1) was somewhat lower for the low concentrate level. Enteric methane per kg ECM was higher in the low concentrate group due to a higher intake of silage. Energy use was higher on the high concentrate level due to the energy demanding drying of by-products. Land requirement was higher for the low

concentrate level compared to the high concentrate level, due to a higher intake of silage and, hence, larger areas required for cultivation of ley. Land required to produce the by-product-based concentrate was low because most of the environmental impact of crop cultivation was allocated to the main product. The same argument was valid for eutrophication. In other words, overall environmental impact of concentrate level in this trial varied for each environmental parameter.

Economically, calculations showed no difference in total feed cost for the two concentrate levels. Despite the fact that the alternative with low concentrate level resulted in lower milk yield, higher feed efficiency combined with the replacement of concentrate with roughage, which is a cheaper feed, meant that total feed cost per kg ECM was similar (Figure 2). The production cost for roughage becomes an extra important factor. This cost has large variations depending on e.g. equipment, location of fields, yields, losses and land prices.

Trial B, "Concentrates with and Without By-Products".

The effects on environmental impact was highest for the control concentrate in all categories except for energy use. The control concentrate showed a large climatic impact mainly due to use of soybean meal and, thus, inclusion of emissions from direct land use change. A larger area of arable land was required for the control because of cultivation of cereals and soybeans and because feed ingredients carried all or much of the environmental impact of cultivation of these crops. Land requirement is a minor item for by-products as the allocation puts a larger share on the primary products (sugar beets, cereals and rapeseed). Results of eutrophication showed the same pattern as land requirement, and the reason for this was the same as above. For energy use, the picture was the opposite, due to the large energy consumption required to dry the by-products, especially products with low DM content such as beet fibre and distillers' grains. In the by-product based concentrates, beet fibre constituted the largest proportion of the feed ingredients, thus having a large impact on the results.

The economic evaluation showed no differences in feed cost depending on which concentrate that was used in trial B. The price of the concentrates was set to market price. The by-products used are common on the feed market today, and the price was balanced by the existing market for soy and cereals as alternatives, rather than related to the production cost of the by-products.

With the feed rations included in these calculations, and assuming the assumptions made, the feed costs per kg of ECM do not increase, neither by using more by-products in the concentrate, nor by using low amounts of concentrate (Figure 2). Differences that appeared in the feed costs are marginal and mostly depend on variations in feed utilization that did not show significance in the underlying feed trials. The by-products used in the trials were all included in dry concentrate mixtures manufactured at the feed factory. This means that they can be used without changes to the farm's infrastructure or internal farm mechanics. If using wet by-products, completely different needs may arise for investing in machinery and buildings in order to be able to handle the feed material on the farm. Costs for feed usually amount to around 50 % of the total costs of a dairy farm. Other costs that occur in production are largely unaffected by choice of feeding strategy or milk yield within the ranges included in this paper. With unchanged costs at farm level, other costs will thus be higher per kg ECM at lower milk yield. Costs for work and buildings all varies greatly between farms. In order to evaluate the economic effect of a certain feeding strategy on an individual farm, it is always required that production cost for roughage is carefully calculated for the specific case. This

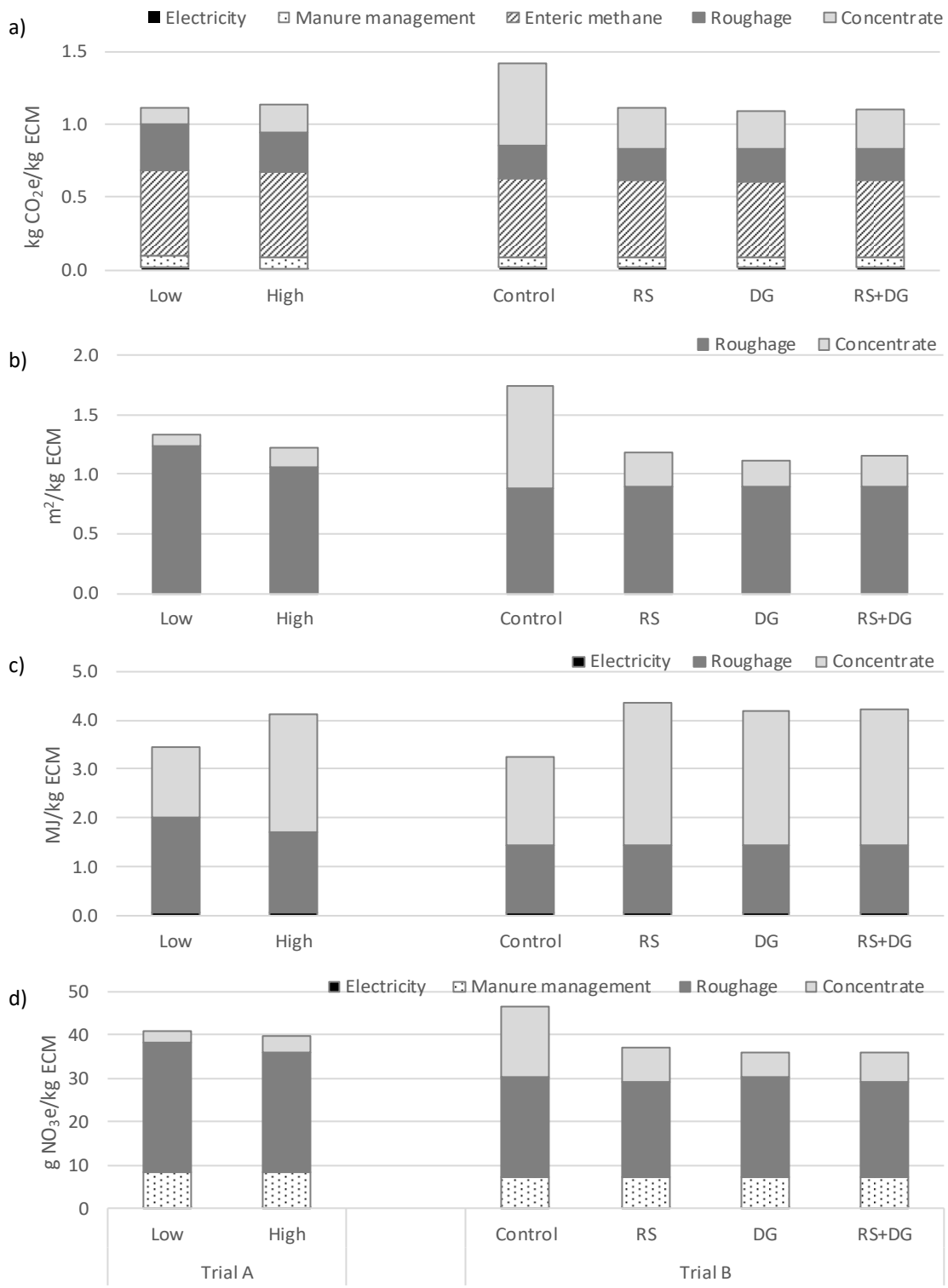


Figure 1 Environmental impact categories expressed per kg energy corrected milk (ECM). Emissions include recruitment heifers. a) Climate change including emissions from direct land use change (soybean and palm oil production). b) Land requirement. c) Non-renewable energy use. d) Eutrophication. Results are presented without allocation of environmental impact to meat. Bars named "low" and "high" (trial A) refers to diets of different levels of concentrate based on by-products (sugar beet pulp, rapeseed meal=RS and distillers'

grains=DG). Control refers to a diet with concentrate based on soybean and cereals. The other concentrates were based on by-products, where the main components were sugar beet pulp, RS and/or DG (trial B).

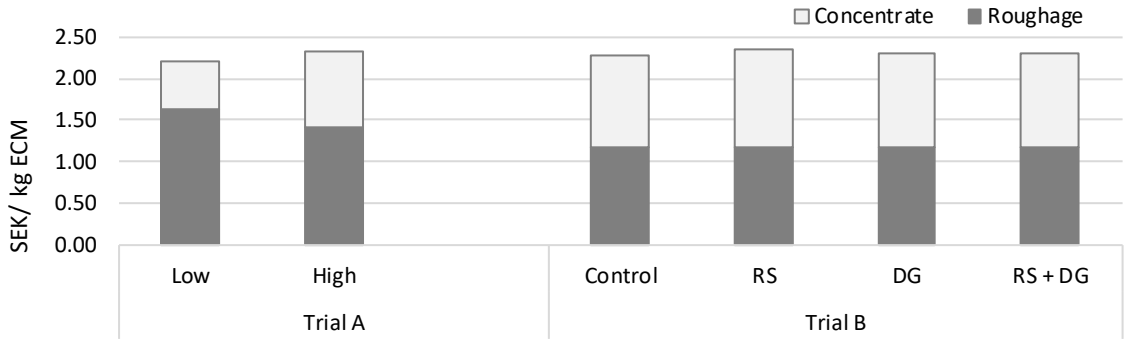


Figure 2 Cost of feed per kg energy corrected milk (ECM), calculated as production costs on-farm of roughage and purchase of concentrate. Bars named "low" and "high" (trial A) refers to diets of different levels of concentrate based on by-products (sugar beet pulp, rapeseed meal=RS and distillers' grains=DG). Control refers to a diet with concentrate based on soybean and cereals. The other concentrates were based on by-products, where the main components were sugar beet pulp, RS and/or DG (trial B).

becomes even more important in alternatives with low amounts of concentrates. In farms that can keep a low production cost for roughage, this alternative can prove to be economically advantageous.

Conclusions

The environmental impact when using by-product concentrates varies depending on type of environmental category. Climate impact, land requirement and eutrophication potential was lower when by-product based concentrates were used, whereas energy use was higher due to the need for drying of wet by-products. If it would be possible to use largely renewable energy sources in the future, advantages of using by-product based concentrates would increase. This study did not reveal any large differences in feed costs per kg ECM when by-products were the basis of concentrates to dairy cows compared with a concentrate based on cereals and soybean meal. Feeding a high vs. low amount of by-product based concentrate did not affect feed costs either when current market prices were used in the calculations.

References

EDA, 2018. Product Environmental Footprint Category Rules for Dairy Products. Version 1.0.25 April 2018. The European Dairy Assoc., Bruxelles, Belgium. http://ec.europa.eu/environment/eussd/smgp/pdf/PEFCR-DairyProducts_2018-04-25_V1.pdf
European Dairy Farmers, 2018. Cost of production comparison 2015-2018.
FAO, 2010. Livestock in the balance: The state of food and agriculture 2009. FAO, Rome, Italy. http://www.fao.org/3/a-i0680e.pdf.
Foley, J.A., Ramankutty, N., Brauman, K., et al., 2011. Solutions for a cultivated planet. Nature, 478, 337-342.
IDF, 2015. A common carbon footprint approach for the dairy sector - The IDF guide to standard life cycle assessment methodology, Bull. Internat. Dairy Fed., Bruxelles, Belgium.
IPCC, 2015. Introductory Chapter. In Climate Change 2014: Mitigation of Climate Change: Working Group III Contribution to the IPCC Fifth Assessment Report. Cambridge University Press, Cambridge, UK, p. 111-150.

PEFCR, 2018. PEFCR Feed for food producing animals. First public version 4.1, April 2018. Bruxelles, Belgium. http://ec.europa.eu/environment/eussd/smgp/pdf/PEFCR_feed.pdf
Volden, H., 2011. NorFor - The Nordic Feed Evaluation System. EAAP publication No 130. Wageningen Academic Publishers, The Netherlands, 180 pp.
doi: 10.3920/978-90-8686-718-9.

Estimating and optimizing carbon footprint of milk in NorFor

N. I. Nielsen
Livestock Innovation, SEGES & NorFor, Agro Food Park, 8200 Aarhus N, Denmark.
Correspondence: ncn@seges.dk

Introduction

The dairy sector has a huge challenge when it comes to significantly reduce greenhouse gas emissions (GHGE) from the production of milk and meat. Nevertheless, the biggest dairy and meat companies in Denmark, Arla and Danish Crown, respectively, launched in March 2019 at statement with the ambition that their products will be close to neutral in terms of GHGE in 2050 and that a significant reduction in GHGE will have taken place before 2030. Reduction in GHGE on farm necessitates that the farmer must know the overall effect of a given action on GHGE. Estimation of enteric methane emissions from a given ration has been available in the NorFor system for more than 5 years. However, methane is only approximately half of the total GHGE connected to production of milk while another major part of GHGE is due to feed production, i.e. growing forages and crops in the field and bought in feed (Henriksson et al., 2019). Thus, enteric methane emissions and GHGE related to feed production are the major hotspots in cattle production. The interactions between herd, feed production and fertilizer management and the various emissions related to it, makes it difficult to predict an overall climate impact of a given action. Therefore, tools are needed that can estimate and optimize/reduce GHGE from animals and crop production when rations are planned or evaluated.
The present paper describes how NorFor has been updated with a climate module that integrates GHGE from animals and feed production. Further, examples are given on how much carbon footprint (CFP) on milk can be reduced from different rations/feedstuffs to lactating cows.

Materials and Methods

NorFor feedstuff table
The FeedStuff Table (FST) in NorFor was expanded to include additive carbon footprints (CFP) of forages and raw materials expressed in grams of CO_2-equivalents per kg dry matter (DM). Presently, CFP for typical Danish forages and raw materials are available in the FST and because raw material composition of compound feeds is mostly unknown, no CFP is available for compound feeds from the feedstuff companies, at this stage. The CFP-values, were extracted from Mogensen et al. (2018) where a Life Cycle Assessment (LCA) methodology had been applied for individual feedstuff in order to estimate CFP.

Carbon footprint on feedstuffs
The methodology is described in detail by Mogensen et al. (2018) but some main principles will be presented here:

The LCA covers all stages of production of a feed, until it is available for feeding to the animal at the farm. This included cultivation (e.g. seeds, fertilizer, fuel, machinery), processing (e.g. heating, rolling, extraction, electricity, machinery) and transportation (e.g. fuel, machinery) are included. The level of fertilizer was assumed to be according to official Danish standards and average net yields across Denmark were used for individual crops. For imported raw materials, e.g. sunflower and soybean products, transportation to Denmark and transportation to the farmer within Denmark are included. If more products are produced

from the same crop, e.g. rapeseeds that are processed to obtain oil and cake or meal, then GHGE must be divided between oil and cake/meal. Economic allocation has been used by Mogensen et al. (2018), i.e. the price and the amounts for main and the by-product(s) are used to allocate the total GHGE. The GHGE includes direct N_2O emission from artificial fertilizer and from crop residues and indirect N_2O emission from NH_3 emissions and NO_3 leaching. Emission coefficients used for the different sources are mainly derived from Danish research but emission coefficients for N_2O from artificial fertilizer are from IPCC (2006). All N_2O emissions were converted to CO_2-eq. by multiplying with a factor of 265 in order to achieve a 100-year perspective (IPCC, 2013). Table 1 gives examples of CFP values for selected raw materials and forages.

Table 1 Carbon footprint (CO_2 eq./kg DM) and nutritional values of selected protein supplements and forages in the NorFor feedstuff table

Parameter	Unit	0038-002 Distillers dried grains, wheat based	0042-002 Rapeseed meal	0053-003 Soybean meal	0226-001 Clover grass silage, high OMD	0307-005 Maize silage, high OMD
Protein						
Crude protein	g/kg DM	347	385	487	157	74
Fat						
Crude fat	g/kg DM	68	40	29	46	22
NDF						
NDF	g/kg DM	257	279	135	419	322
Starch						
Starch	g/kg DM	35	26	62	10	347
Standard feed values						
Net energy lactation 20 kg DM	MJ/kg DM	7,54	6,63	8,35	6,45	6,45
AAT 20 kg DM	g/kg DM	143	148	209	79	86
PBV 20 kg DM	g/kg DM	139	172	219	27	-61
Other parameters						
CO2e	g/kg DM	839	545	631	414	265

At this stage, Land Use Change (LUC) and carbon sequestration in soil (C_soil) have not been implemented in NorFor, although values have been estimated by Mogensen et al. (2018) using different methods. Neither has Carbon Opportunity Cost (COC; Searchinger et al., 2018) been taken into account in CFP values in NorFor as COC is a debatable way of looking at LUC and needs more work, understanding and alignment in order to handle difficult time dependent aspects in an LCA perspective (Rosa, 2016), as does estimation of C_soil. This means that presently, CFP values in NorFor are generally underestimated.

Enteric methane emission

Enteric methane is estimated by the following equation in NorFor based on Nordic research data (Nielsen et al., 2013):

CH_4 (MJ/d) = 1.39 * DMI (kg DM/d) – 0.091 * fatty acids (g/kg DM),
where DMI=dry matter intake.

Enteric CH_4 emissions were converted to CO2-eq. by multiplying with a factor of 25 in order to achieve a 100-year perspective (IPCC, 2013).

Carbon footprint on milk

NorFor has been updated to express CO_2 equivalents from feedstuffs in the ration (feed) and from enteric methane (animal) and to show total CFP as CO_2 equivalents per cow per day or expressed per kg energy corrected milk yield (ECM), see Figure 1.

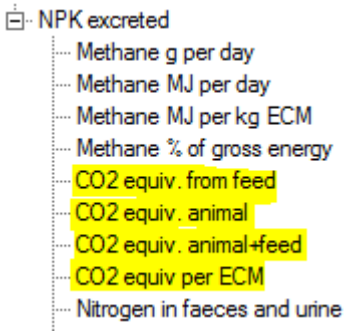


Figure 1 Methane and carbon footprint variables in NorFor where, 'animal' refers to enteric methane and 'feed' refers to greenhouse gas emissions related to production of the total feed ration in terms of cultivation, processing and transportation.

Results and Discussion

In order to investigate what the CFP for milk is and how much it can be changed, using the updated climate module in NorFor, a standard ration for a Holstein cow was planned and compared with different types of rations. A total of 6 rations were defined (Table 2):

- 'Standard', a typical Danish ration used on commercial farms
- 'Maize', a ration with more maize and less grass silage than 'Standard'
- 'Fat', a ration with more fatty acids than 'Standard'
- 'Byproduct', a ration with brewers grain and HP-sugar beet pulp
- 'Forage', a ration with more forage than 'Standard'
- 'Reduced', a ration optimized by NorFor to reduce CFP as much as possible

All rations were planned to supply a herd yield of 11.000 kg ECM per cow per year (average milk yield in Denmark for Holstein cows) and should meet basic recommendations according to NorFor in terms of energy (100%), absorbed amino acids (AAT) (min. 15 g/MJ; variation: 15.9-17.4 g/MJ), rumen protein balance (PBV; min. 10 g/kg DM; variation: 10-14 g/kg DM), fatty acids (min. 20 g/kg DM, see Table 2), rumen load index (max. 0.6; variation: 0.38-0.44) and fill value balance (variation: 99-103%). All rations used a 1st cut clover-grass silage (OMD=77%; NEL20=6.19 MJ/kg DM) and an average quality of maize silage in terms of organic matter digestibility (OMD=75%; NEL20=6.24 MJ/kg DM). All rations were planned with 14 kg DM forage except 'Forage', where it was assumed that cows could consume 15.5 kg DM forage. 'Byproduct' was planned to include 2 kg DM of both brewers grain and HP-sugar beet pulp.

In general, CFP per kg ECM in Table 2 might seem low compared to other studies, which are typically close to 1000 g CO_2 eq./kg ECM (Henriksson et al., 2019; Knudsen et al., 2019), but in those studies, heifers, electricity, and emissions from manure in the stable has been included as opposed to the current version in NorFor. Although the level of CFP is of interest, it is more relevant to investigate what can be done by choosing different feedstuffs and composing different rations in order to reduce CFP of milk.

Table 2 shows that inclusion of more maize silage ('Maize') or more forage ('Forage') had very limited effect on CFP. Although maize silage has lower CFP than clover-grass silage, inclusion of more maize silage also requires more protein supplements which have higher

CFP than maize silage (Table 1). Therefore, it more or less levels out to the same CFP per kg ECM for the 'Maize' and 'Forage' rations.

Table 2 Carbon footprint (CFP) of rations planned in NorFor to Holstein cows in herds with 11.000 kg ECM. The titles of the different rations indicate how they have been changed compared with a typical Danish 'standard' ration

	Ration (kg DM/d)[a]					
Feedstuff	Standard	Maize	Fat	Byproduct	Forage	Reduced[b]
Barley	3.5	3	2.5	2.8	2.3	
Wheat						3.6
Rapeseed meal	2	2.5		2	2	
Rapeseed cake	1	1	3		1	
Soybean meal	2	2	2	2	2	4.4
Sugarbeet pellets	2	2	2		2	
Clovergrass silage	5	3	5	5	5.5	5
Maize silage	9	11	9	9	10	9
Brewers grain				2		
HP sugarbeet pulp				2		
Fat (saturated)			0.4			0.5
	Ration parameters					
DMI (kg/day)	24.5	24.5	23.9	24.8	24.8	22.5
Fatty acids (g/kg DM)	20	20	40	22	20	37
	CFP					
Methane (kg CO2 eq./day)	14.5	14.5	13.3	14.6	14.7	12.5
Feed (kg CO2 eq./day)	10.5	10.2	10.6	8.8	10.3	9.8
Methane & feed (g CO2 eq./kg ECM)	651	644	621	609	652	582
Change (%)[c]		-1	-5	-7	0	-11

[a]See text for explanation of the different rations; [b]This ration was optimized in NorFor in order to have as low CFP as possible within same constrains as 'Standard'; [c]Change in percentage compared to the 'standard' ration.

Byproducts such as brewers grain and HP-sugar beet pulp have rather low CFP (Mogensen et al., 2018) and therefore, 2 kg DM of each of these feedstuffs were included in a 'Byproduct' ration, which reduced feed CFP markedly (8.8 vs 10.5 kg CO_2 eq./day) and also total CFP per kg ECM with 7%, compared to the 'Standard'. However, it should be kept in mind that these byproducts are only available in rather limited amounts and therefore cannot help the dairy sector to a sustainable reduction in CFP.

The two fat-rich rations ('Fat' and 'Reduced') leads to lower DMI and, as higher inclusion of fatty acids and lower DMI, they both decrease enteric methane emission (Nielsen et al., 2013). The 'Fat' and the 'Reduced' rations were estimated to reduce CFP with 5 and 11% compared to the 'Standard', respectively. The amounts of fat and soybean meal included in the 'Reduced' ration is debatable and very much dependent on the methodology for LUC as these feedstuffs are from areas in the world (Malaysia & South America) where rainforests are cut down. Due to the present debate on how to include LUC, it has not yet been implemented in NorFor. The 'Reduced' ration is 10% more expensive than the 'Standard' and it may seem far away from what is current practice. But never the less, it does contain a standard amount of forage. Reducing forage is also a potential mitigation option, although not investigated here. Furthermore, the 'Reduced' ration illustrates the potential for what can be obtained by optimizing feed rations in order to reduce CFP of milk. It is therefore clear that other mitigation options are wanted and necessary in order to achieve dairy and meat products with zero net CFP.

Conclusions

Table values for CFP of forages and raw materials including cultivation, processing and transportation have been implemented in the NorFor FST and it is now possible to estimate CFP per kg ECM as both GHGE from feed production and the cow is included. Thus, this update of NorFor makes it possible to formulate economic rations that are nutritionally sound and, at the same time, reduces CFP of milk. Different ration scenarios have so far shown that CFP of milk can only be reduced with 5-10% and that there are relatively big costs associated with it. It is therefore clear that other mitigation options are wanted and necessary in order to achieve significant reductions in CFP of milk.

References

IPCC, 2006. IPCC Guidelines for national greenhouse gas inventories. Available online at: http://www.ipcc-nggip.iges.or.jp/public/2006gl/vol4.html

IPCC, 2013. Supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Available online at: http://www.ipcc-nggip.iges.or.jp/home/wetlands.html

Henriksson, M., Jacobsson, S.B., Lindberg, M., & Lundberg, M.B., 2019. Mjölk på gräs och biprodukter – miljö och ekonomi. Rapport från Hushållningssälskapet, 58 pp.

Knudsen, M.T., Dorca-Preda, T., Djomo, S.N., Peña, N., Padel, S., Smith, L.G., Zollitsch, W., Hörtenhuber, S., & Hermansen, J., 2019. The importance of including soil carbon changes, ecotoxicity and biodiversity impacts in environmental life cycle assessments of organic and conventional milk in Western Europe. J. Clean. Prod. 215:433-443.

Mogensen, L., Knudsen, M.T., Dorca-Preda, T., Nielsen, N.I., Kristensen, I.S., & Kristensen, T. 2018. Bæredygtighedsparametre for konventionelle fodermidler til kvæg. DCA-rapport nr. 116. Aarhus University, 129 pp.

Nielsen, N.I., Volden, H., Åkerlind, M. Brask, M., Hellwing, A.L.F., Storlien, T., & Bertilsson, J., 2013. A prediction equation for enteric methane emission from dairy cows for use in NorFor. Acta Agri. Scand. 63, 126-130.

Rosa, M.D., 2016. Land-use changes in life cycle assessment. PhD-thesis. Aarhus University. 167 pp.

Searchinger, T.D., Wirsenius, S., Beringer, T., & Dumas, P., 2018. Assessing the efficiency of changes in land use for mitigating climate change. Nature. 564, 249-253.

In vitro evaluation of different dietary methane mitigation strategies

J. Chagas[1], M. Ramin[1] A. Jafari[2] & S. Krizsan[1]
[1]*Swedish University of Agricultural Sciences (SLU), Skogsmarksgränd 90183 Umeå, Sweden*,
[2]*Lorestan University, Department of Animal Science, 465 Khorramabad, Iran*
Correspondence: juana.chagas@slu.se

Introduction

Agriculture accounted for 14% of total GHG emissions in Sweden in 2017, but as much as almost 90% was from livestock, where more than 30% was derived from enteric CH_4 fermentation by ruminants (Anderson *et al.*, 2010). The ruminant CH_4 emission is a concern since it has a 25-fold greater global warming potential than CO_2 (Salom *et al.*, 2007), and that the additional CH_4 production during fermentation represents a loss of feed energy. The Rumen Census project sequenced a wide variety of rumen and camelid foregut microbial communities in many samples from a wide variety of animal species and countries, to estimate what caused the greatest variation of diet, host species, or geography (Henderson *et al.*, 2015). Rumen archaeal diversity was similar irrespective of host or diet, and a core rumen bacterial population occurred irrespective of host or diet, which made up 67% of the community. Diet caused the main diversity changes in other bacteria present and not host genetics (Henderson *et al.*, 2015). Although dietary mitigating strategies of enteric CH_4 production in ruminating animals have been intensively studied, there is still no conclusive answer to the most efficient dietary strategy for dairy cows on forage-based diets.

Methane losses of typical dairy cow diets are 6-7% of gross energy intake. Comparably lower losses of approximately 3% in feedlot situations promote feeding high concentrate diets to reduce CH_4 (Johnson & Johnson, 1995). Relatively new data have indicated that the effect on CH_4 production of feeding more grain in diets to dairy cows is small (Ramin & Huhtanen, 2013) and the strategy should therefore be questioned. Use of antimethanogenics or plant inhibitory compounds in ruminant diets can also reduce GHG emission, and have sometimes been suggested as effective and feasible strategies in the livestock sector (Knapp *et al*., 2014). This study aimed to rank different dietary CH_4 mitigating strategies using an automated gas *in vitro* system, to provide background for future evaluation of dietary manipulation of CH_4 production in dairy cows.

Materials and Methods

Two *in vitro* experiments were conducted. In Trial 1 the potential of several dietary CH_4 mitigating strategies were tested with six chemicals at two levels, three plants at two levels, nine different potentially low-CH_4 diets, and two silage fermentation acids at two levels to mimic different silage fermentation qualities (Table 1). In Trial 2, the macroalgae *Asparagopsis taxiformis* (AT) and 2-nitro ethanol (2-NE) were tested in a dose-level experiment where five different treatment levels were obtained by repeatedly halving the dose, starting at the lowest level used in Trial 1.

Experimental diets were composed from a basal diet of timothy grass, rolled barley and rapeseed meal in the ratio 545:363:92 g/kg of diet dry matter (DM). Replacements in the low-CH_4 diets were made so that the forage:concentrate ratio was kept constant between treatments and to sustain 160 g crude protein (CP)/kg of diet DM. In diets with two levels of protein supplementation, the second level aimed to provide 180 g CP/kg of diet DM. Further,

urea was added to achieve appropriate CP concentration in the diet where the forage component were composed of maize silage and grass.

Rumen fluid was collected from the same lactating Swedish Red cows fed *ad libitum* on a diet of 600 g/kg grass silage and 400 g/kg concentrate on DM basis for all incubations. The experimental diets were subjected to *in vitro* incubations in which gas production was automatically recorded and corrected to normal atmospheric pressure (101.3 kPa). Dietary ingredients had previously been dried at 60ºC for 48 h and thereafter ground to pass a 1-mm screen using a Retsch SM 2000 cutting mill (Retsch GmbH, Haan, Germany). Diet samples of 1000 mg of DM were incubated in 60 ml of buffered rumen fluid for 48 h. The volume ratio of rumen fluid to buffer was 1:4. All samples were incubated to give three replicates from consecutive runs in Trial 1, while Trial 2 was performed with all replicates in a single batch. All runs included triplicate bottles with blanks, and samples were randomly allocated to the *in vitro* incubation bottles and never incubated in the same bottle in more than one run. Mean blank gas production within run was subtracted from sample gas production.

Gas samples were drawn from each bottle by a gas tight syringe (Hamilton, Bonaduz, Switzerland) at 2, 4, 8, 24, 32 and 48 h of incubation. Concentration of CH_4 was determined by injecting 0.2 ml of gas into a Varian Star 3400 CX gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA) equipped with a thermal conductivity detector. Peaks were identified by comparison with standard mixture of CO_2 and CH_4 (100 mmol/mol) prepared by AGA Gas (AGA Gas AB, Sundbyberg, Sweden). Predicted *in vivo* CH_4 production was calculated as described by Ramin & Huhtanen (2012). Liquid samples of 0.6 ml were collected at 8, 24 and 48 h of incubation from the bottles and immediately stored at -20°C until processed for analysis of NH_3-N and volatile fatty acids (VFA). Samples for NH_3-N and VFA analysis from the replicated treatments between runs were pooled before analysis. Individual and total VFA productions were calculated by subtracting mean blank VFA concentration from sample concentration. *In vitro* true organic matter digestibility (TOMD) and neutral detergent digestibility (NDFD) were determined for all samples in all runs by analysing ash free neutral detergent fibre (NDF) concentrations in the residues after the 48 h incubations using 07-11/5 Sefar Petex (Sefar AG, Heiden, Switzerland) *in situ* bags according to Krizsan *et al.* (2015). Mean blank true *in vitro* digestibility within run was subtracted from sample *in vitro* digestibility.

Concentrations of DM, ash, NDF and CP in individual experimental dietary ingredients were determined according to Franco *et al.* (2017). Individual VFA concentrations in rumen fluid samples were determined using a Waters Alliance 2795 UPLC system as described by Puhakka *et al.* (2016), and NH_3 according to the method provided by SEAL Analytical (Method no. G-102-93 multitest MT7) using AutoAnalyzer 3.

Data from Trial 1 was analysed using the MIXED procedure (SAS Inc. 2002-2003, Release 9.2; SAS Inst., Inc., Cary, NC, USA) by a model correcting for random effect of bottle, and fixed effect of run and treatment. Orthogonal contrasts were included for evaluation of basal diet vs. treatment and of linear responses to level of treatment. Data on VFA and NH_3 measurements were evaluated in a repeated measurements model using the Toeplitz function in MIXED procedures (level within treatment was used as subject). The model accounted for effects of treatment and time, and interactions between treatment and time. Least square means across all time points) are reported for VFA in Trial 1. Data from Trial 2 was subjected to linear and quadratic regression analysis using the REG procedure (SAS Inc.

2002-2003, Release 9.2; SAS Inst., Inc., Cary, NC, USA). Best fit was judged from lowest root mean square error and highest adjusted R^2.

Results and Discussion

Chemical composition of the basal diet and the low-CH_4 diets are in Table 2. Treatments 2-NE and AT showed the greatest inhibition ($P < 0.01$) of CH_4 production among all experimental treatments (Table 3).

Table 1 Experimental treatments evaluated for methane mitigating potential in Trial 1

Treatments	Levels	
Chemical additives to basal diet		
2-nitro ethanol	5 mM	10 mM
Nitrate	Urea:$CaCO_3$[a]	$Ca(NO_3)_2$ x $4H_2O$[b]
Propionic acid	2 mM	4 mM
Ferulic acid	10 mM	20 mM
p-Coumaric acid	10 mM	20 mM
Bromoform	1.5 mg/g DM	3 mg/g DM
Plant additives to basal diet		
Fireweed	50 g/kg DM	100 g/kg DM
Asparagopsis taxiformis	10 g/kg OM	20 g/kg OM
Rapeseed oil	40 g/kg DM	80 g/kg DM
Potentially CH_4-reducing diets		
Dried distillers' grain	90 g/kg DM	180 g/kg DM
Barley:Oat	175:175 g/kg	0:350 g/kg
Maize silage:Grass	275:275 g/kg[c]	0:545 g/kg[d]
Red clover:Grass	275:275 g/kg	- - -
Lactic acid	60 g/kg DM	120 g/kg DM
Lactic acid + Acetic acid	80 + 30 g DM	80 + 60 g DM

[a]3.5% of urea + 5.1% of $CaCO_3$ of DM basis included in basal diet in comparison with nitrate treatment; [b]8.9% $Ca(NO_3)_2$ x $4H_2O$ of DM basis; [c]Urea was add to correct CP at 16% (0.0094 g); [d]Urea was add to correct CP at 16% (0.0186 g).

Supplementation of 2-NE and AT did not affect ($P \geq 0.27$) any other *in vitro* traits except the pattern of fermentation. The 2-NE only tended to ($P = 0.10$) decrease TVFA, but both 2-NE and TA treatments displayed lower ($P < 0.01$) molar proportion of acetate, and greater ($P < 0.01$) proportions of propionate and butyrate compared to the basal diet. Bromoform, propynoic acid and nitrate, in declining order, also decreased ($P < 0.01$) CH_4 production compared to the basal diet. These treatments decreased ($P \leq 0.04$) TOMD (bromoform) and NDFD (propynoic acid and bromofrom), and nitrate increased ($P \leq 0.01$) NH_3 concentration compared to the basal diet. Additionally, propynoic acid and bromoform clearly decreased ($P \leq 0.01$) TVFA. Further, propynoic acid and bromoform affected molar proportions of volatile fatty acids in a similar way as 2-NE and AT ($P \leq 0.09$). CH_4 production of treatments 2-NE, AT and bromoform was not different ($P \geq 0.70$) between levels, while the higher level of propynoic acid decreased ($P = 0.01$) CH_4 production in comparison with the lower level. Noteworthy was that none of the low-CH_4 diets, or lactic acid and acetic acid treatments affected ($P \geq 0.27$) CH_4 production in this study. Many CH_4 inhibitors, increase propionate with a concomitant decrease in the proportion of acetate. The *in vitro* study of Zhang & Yang (2011) suggested a high potential of 2-NE to mitigate CH_4. However, it negatively affected *in vitro* digestibility in their study, something that was not observed in this trial. A 2% inclusion of AT to grass hay in a 72-h *in vitro* fermentation study decreased CH_4 production, and did not alter digestibility or TVFA (Machado *et al*., 2016), supporting the results in Trial 1.

In Trial 2, CH_4 production decreased curvilinearly with level of both AT and 2-NE (Figure 1). The change in CH_4 production for both treatments was accompanied by changes in TVFA at 48 h ranging between 4.71 and 4.33 mmoles for AT and between 5.35 and 3.00 mmoles for 2-NE from lowest to highest level of supplementation (the response was linear at 24 h and curvilinear at 8 and 48 h; results not presented) and molar proportion of acetate, while propionate and butyrate proportions increased curvilinearly with all time points included (Figure 1).

Table 2 Chemical composition of basal and potentially low-CH_4 diets evaluated *in vitro*

Treatment	Level	DM[1]	OM[2]	CP[2]	NDF[2]
Basal diet	-----	446	944	160	387
Rapeseed oil	40 g/kg DM	430	906	154	372
Rapeseed oil	80 g/kg DM	414	869	149	356
Dried distillers' grain	90 g/kg DM	446	946	161	378
Dried distillers' grain	180 g/kg DM	491	946	181	366
Barley:Oat	175:175 g/kg	490	944	165	385
Barley:Oat	0:350 g/kg	534	944	170	383
Maize silage:Grass	275:275 g/kg	476	954	160	355
Maize silage:Grass	0:545 g/kg	505	963	160	323
Red clover:Grass	275:275 g/kg	414	932	171	345
Lactic acid	60 g/kg DM	422	887	151	364
Lactic acid	120 g/kg DM	398	831	143	341
Lactic acid + Acetic acid	80 + 30 g DM	402	840	144	345
Lactic acid + Acetic acid	80 + 60 g DM	390	812	140	333

[1]g/kg; [2]g/kg DM.

There were no linear or curvilinear relationships between digestibility and NH_3 concentration and level of supplementation for any of the treatments (results not presented). Machado *et al*. (2015), testing AT at dose levels between 0 to 16.7% of OM suggested an optimal dose of 2% to decrease CH_4 production by 99% compared to a control diet. However in Trial 2, CH_4 production was inhibited almost completely by AT already at 0.5% of OM.

In addition, Li *et al*. (2018) used AT *in vivo* added to sheep diets, which supported consistent reduction of CH_4 production at inclusion levels lower than 2%, but with less acetate and more propionate produced even for the lowest inclusions of 0.5% of OM intake. Bioactives, produced naturally by the macroalgae *Asparagopsis taxiformis*, in the form of haloforms and dihalomethanes, acting the same way as bromochloromethane, enabled *Asparagopsis* to be a potential natural CH_4 inhibitor (Machado *et al*., 2016). However, few trials have been conducted and effects on and magnitude of CH_4 reductions *in vivo* have not yet been related to any specific bioactive compound.

Conclusions

Asparagopsis taxiformis inclusion showed a strong dose dependent CH_4 mitigating effect with the least impact on rumen fermentation parameters. The natural antimethanogenic *Asparagopsis* agent was judged the best potential inhibitor at a low dose for further research *in vivo.*

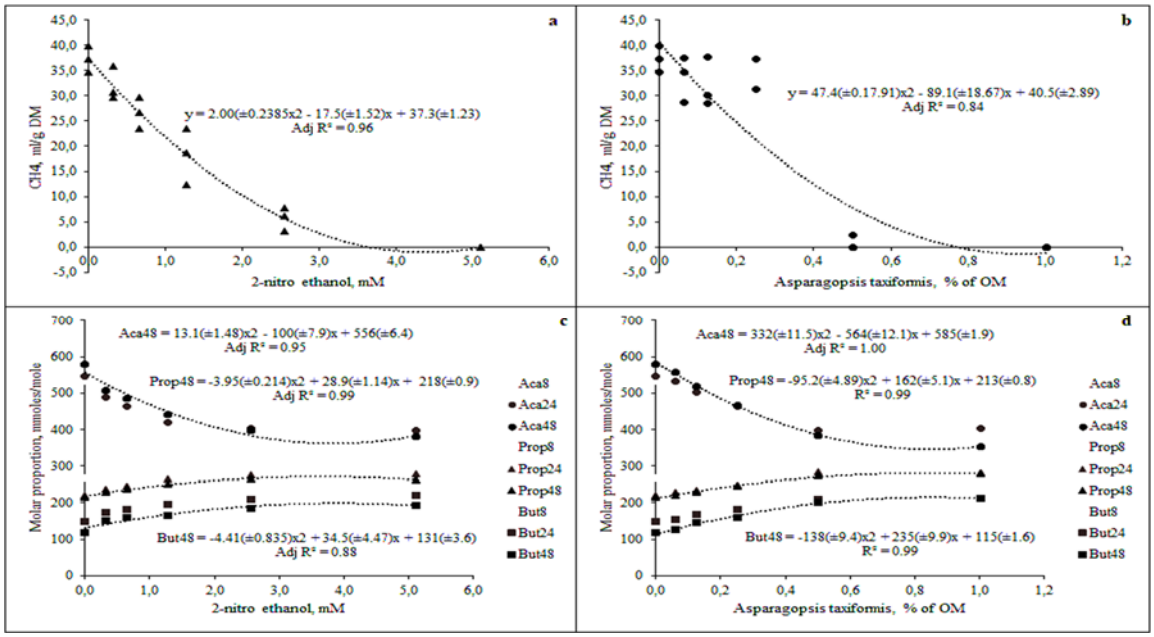


Figure 1 Methane production after 48h rumen *in vitro* incubation of a basal diet with inhibitors 2-nitro ethanol (a) and *Asparagopsis taxiformis* (b); molar proportions of acetate (Aca), propionate (Prop) and butyrate (But) at the different time points with 2-nitro ethanol (c) and *Asparagopsis taxiformis* (d). Treatments supplied in different doses (each level was replicated three times *in vitro).*

References

Andersson, R., Bång, M., Frid, G. & Paulsson R., 2010. Minskade växtnäringsförluster och växthusgasutsläpp till 2016-förslag till handlingsprogram för jordbruket. Rapport 2010, 10. Board of Agriculture, Jönköping, Sweden.

Franco, M.O., Krizsan, S.J., Ramin, M., Spörndly, R. & Huhtanen, P., 2017. *In vitro* evaluation of agro-industrial by-products replacing soybean meal in two different basal diets for ruminants, in: 8th Nord. Feed Sci. Conf., June 13-14, 2017, Uppsala, Sweden.

Henderson, G., Cox, F., Ganesh S., Jonker A., Young W., Janssen, P.H., *et al.,* 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci. Rep. 5, 14567.

Johnson, K.A. & Johnson, D.E. 1995. Methane emissions from cattle. J. Anim. Sci. 73, 2483–2492.

Knapp, J.R., Laur, G.L., Vadas, P.A., Weiss, W.P. & Tricarico, W., 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97, 3231–326.

Krizsan, S.J., Rinne, M., Nyholm, L. & Huhtanen, P., 2015. New recommendations for the ruminal in situ determination of indigestible neutral detergent fibre. Anim. Feed Sci. 205, 31-41.

Li, X., Norman, H.C., Kinley, R.D., Laurence, M., Wilmot, M., Bender, H., Nys, R. & Tomkins, N., 2018. *Asparagopsis taxiformis* decreases enteric methane production from sheep. Anim. Prod. Sci. 58, 681–688.

Machado, L., Magnusson, M., Paul, N.A., Kinley, R., Nys, R. & Tomkins, N., 2016. Identification of bioactives from the red seaweed Asparagopsis taxiformis that promote antimethanogenic activity in vitro. J. Appl. Phycol. DOI: 10.1007/s10811-016-0830-7.

Machado, L., Magnusson, M., Paul, N.A., Kinley, R. de., Nys, R. & Tomkins, N., 2015. Dose–response effects of *Asparagopsis taxiformis* and *Oedogonium* sp. on *in vitro* fermentation and methane production. J. Appl. Phycol. DOI: 10.1007/s10811-015-0639-9.
Puhakka, L., Jaakkola, S., Simpura, I., Kokkonen, T. & Vanhatalo, A., 2016. Effects of replacing rapeseed meal with fava bean at two concentrate crude protein levels on feed intake, nutrient digestion, and milk production in cows fed grass silage-based diets. J. Dairy Sci. 99, 7993-8006.
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, 190-200.
Solomon, S., Qin, D. & Manning, M., 2007. Technical summary, in: Climate change 2007: The physical science basis, Solomon S, Qin D, Manning, M., Marquis, M., Averyt, K., Tignor, M.M.B., Miller, H.L., Chen, Z.L., (Eds.), Cambridge Univ. Press, Cambridge, p. 749-844.
Zhang, D.F. & Yan, H.J., 2011. *In vitro* ruminal methanogenesis of a hay-rich substrate in response to different combination supplements of nitrocompounds; pyromellitic diimide and 2-bromoethanesulphonate. Anim. Feed Sci. Technol. 163, 20–3.

Table 3 Effect of 48h rumen *in vitro* incubation on digestibility and production of methane, volatile fatty acids and ammonia. Experimental treatment vs. control

Treatment	TOMD,	NDFD,	CH_4,	TVFA,	Acet,	Prop,	But,	NH_3-N ,	P-value[a]							
	g/kg	g/kg	ml/ g DM	mmoles		mmoles/ mol TVFA		mg/l	C_{TOMD}	C_{NDFD}	C_{CH4}	C_{TVFA}	C_{Ace}	C_{Prop}	C_{But}	C_{NH3}
2-nitro ethanol	847	626	1.6	3.01	440	309	211	282	0.43	0.27	<0.01	0.1	<0.01	<0.01	<0.01	0.39
Nitrate	879	777	5.6	2.91	619	250	107	436	0.45	0.15	<0.01	0.78	0.25	0.43	0.35	<0.01
Propynoic acid	807	546	13.6	2.57	476	297	209	270	0.15	0.16	<0.01	0.01	<0.01	<0.01	<0.01	0.25
Ferulic acid	835	724	27.8	3.54	597	229	109	311	0.54	0.19	0.33	0.83	0.67	0.68	0.35	0.98
p-Coumaric acid	764	563	22.7	3.01	492	176	121	320	0.04	0.34	0.43	0.1	0.01	<0.01	0.83	0.81
Bromoform	812	552	3.2	2.3	436	270	261	263	0.02	<0.01	<0.01	<0.01	<0.01	0.09	<0.01	0.17
Fireweed	868	719	31.3	3.71	586	241	117	304	0.74	0.22	0.81	0.8	0.94	0.82	0.65	0.78
A. taxiformis	858	658	1.5	3.61	418	327	184	289	0.66	0.69	<0.01	0.98	<0.01	<0.01	<0.01	0.81
Rapeseed oil	909	739	37.5	4.04	600	217	128	354	0.03	0.06	0.27	0.25	0.61	0.29	0.84	0.25
DDG	869	683	30.8	3.74	549	241	152	319	0.24	0.43	0.55	0.73	0.31	0.83	0.12	0.85
Barley:Oat	870	671	38.5	3.66	596	225	124	301	0.99	0.81	0.92	0.9	0.69	0.52	0.98	0.75
Maize:Grass	866	628	35	3.71	577	240	136	359	0.94	0.52	0.87	0.8	0.86	0.88	0.51	0.2
Red clover:Grass	902	731	46.5	3.16	599	234	129	281	0.87	0.38	0.3	0.28	0.66	0.88	0.85	0.45
LA	853	672	36.2	3.65	516	271	158	306	1	0.64	0.96	0.93	0.06	0.08	0.06	0.86
LA+AC	883	693	34.4	3.34	598	224	135	323	0.2	0.31	0.53	0.44	0.65	0.5	0.55	0.77

DDG = distillers dried grains; LA = lactic acid; AC = acetic acid;TOMD = true organic matter digestibility; NDFD = neutral detergent fibre digestibility; TVFA = total volatile fatty acids; Acet = acetate; Prop = propionate; But = butyrate; NH3 = ammonia. [a]Orthogonal contrasts of basal diet vs. treatment of the different in vitro traits in column order with SEM of TOMD of 10.06 g/kg, NDFD of 9.56 g/kg, CH4 of 3.76 ml/g DM, TVFA of 0.12 mmoles, acetate of 7.59 mmoles/mol TVFA, propionate of 4.37 mmoles/mol TVFA, butyrate of 4.87 mmoles/mol TVFA, NH3-N 12.44 mg/l.

Rapeseed lipids to decrease saturated fatty acids in milk and ruminal methane emissions of dairy cows

A. Halmemies-Beauchet-Filleau[1], S. Jaakkola[1], T. Kokkonen[1], A.M. Turpeinen[2], D.I. Givens[3] & A. Vanhatalo[1]

[1]University of Helsinki, Department of Agricultural Sciences, P.O. Box 28, 00014 Helsinki, Finland. [2]Valio Ltd., R&D, Meijeritie 6, 00370 Helsinki, Finland. [3]Institute for Food, Nutrition and Health, University of Reading, RG6 6AR, Reading, United Kingdom. Correspondence: anni.halmemies@helsinki.fi

Introduction

Ruminants are often criticized for the production of enteric methane that is a potent greenhouse gas. It is estimated that 14 to 17% reduction in greenhouse gas emissions of dairy cows is achievable in developed OECD countries by improving energy efficiency and including lipid supplements into the diet (Mottet *et al.,* 2017). Unsaturated lipids like oil from domestic rapeseeds (*Brassica napus*) have also great potential to modify lipid composition of ruminant meat and milk by decreasing the proportion of saturated fatty acids and increasing that of unsaturated fatty acids inherent to lipid supplements. This is noteworthy as milk and dairy products contribute significantly to human 12:0, 14:0 and 16:0 consumption, excessive intake of these saturated fatty acids being associated with increased risk for cardiovascular disease as well as lowered insulin sensitivity (review by Shingfield *et al*., 2013). The form of lipid inclusion in the dairy cow diet affects bioavailability and final product composition. Milling of rapeseeds was necessary to make lipids within seeds available to animals (Kairenius *et al*., 2009). Furthermore, milled rapeseeds in the diet resulted in a similar fatty acid profile in bovine milk as free rapeseed oil with the exception of lower increase in *trans* fatty acids. The aim of this study was therefore to examine the effects of milled rapeseed on milk fat composition and ruminal methane emissions of dairy cows on grass silage based diets of high digestibility.

Materials and Methods

The study was conducted at the University of Helsinki, Viikki research farm in Finland from the beginning of September to mid November 2018. The whole Finnish Ayrshire herd in milk was fed a control diet for 3 weeks (Period 1) followed by rapeseed lipid-rich diet of 4 weeks (Period 2). After this, all cows were switched back to the control diet (3 weeks, period 3). The dairy herd of Helsinki University was mainly autumn-calving and the number of cows in milk was 49, 52, 50 and 59 at the beginning of the experiment and of sampling Period 1, 2 and 3, respectively. Days in milk were on average 176, 153, 141 and 117 at the beginning of the experiment and of sampling periods 1, 2 and 3, respectively. Forage-rich dairy cow total mixed rations (TMR) based on high quality grass silage were fed *ad libitum* (Table 1). The pre-wilted grass silage was of 1st cut and ensiled with formic acid based additive in big bales. Concentrates in TMR comprised of home-grown cereals, rapeseed feeds as protein supplement, molassed sugar beet pulp and vitamins and minerals. Rapeseed protein was isonitrogenously supplied either as a lipid extracted meal (control diet) or full-fat seeds milled using an ordinary hammer mill (sieve pore size 6 to 8 mm) (test diet). The amount of additional rapeseed lipids in the test diet was adjusted to ca. 50 g/kg diet dry matter (DM). The experimental rapeseed was cultivated in southern Finland and contained 432 total fat, 240 crude protein and 164 neutral detergent fibre (analysed with amylase and expressed inclusive of residual ash) per g/kg DM. Cereal in the control diet was barley and in the test

diet oats. The feeding troughs (Insentec RIC, Marknesse, The Netherlands) registered TMR intakes automatically and individually. When visiting the milking-robot, cows producing less than 30, between 30 and 40 and over 40 kg of milk per day at the beginning of the trial received 3, 4 or 5 kg of standard concentrate (Maituri 10000, Raisioagro Ltd, Raisio, Finland) per day throughout the study.

Table 1 The composition of total mixed rations

Ingredient, kg/t dry matter	Control	Test
Grass silage*	600	600
Barley	189	-
Oats	-	136
Rapeseed meal	120	34
Milled rapeseeds	-	139
Molassed sugar beet pulp	70	70
Minerals and vitamins	17	17
Propylene glycol	4	4
Total	1000	1000

*Digestible organic matter 696 g/kg DM

Milk from 13 multiparous dairy cows was individually sampled during the last week of each experimental period for analysis of major milk constituents. Of these 13 cows, 10 were in late lactation (days in milk from 153 to 308 at the beginning of the experiment) and 3 were in early lactation (days in milk from 13 to 27 at the beginning of the experiment). All cows in the herd freely visited a milking robot (Lely Astronaut A3, Lely, Maassluis, The Netherlands) equipped with GreenFeed system (C-Lock Inc., Rapid City, SD, USA) that measures ruminal methane, carbon dioxide and hydrogen emissions. Only records of cows (n=23) that were in milk during all 3 experimental periods and had on average 10 or more accepted reads from GreenFeed in the last week of each experimental period were used for statistical testing. In addition, tank milk was analysed at the end of periods 1 and 2 for fatty acid composition. Chemical composition of feeds and milk were determined as described by Lamminen *et al.* (2019).

Data were analysed by ANOVA for linear and quadratic responses using the Mixed procedure of SAS (version 9.4, 2012). The statistical model contained period as fixed and cow as random effect. The results were considered as statistically significant when $P < 0.05$.

Results and Discussion

The animals had no health concerns when fed the test diet. Animal performance of 13 multiparous dairy cows individually sampled for milk is presented in Table 2. Dry matter intake was decreased by 0.9 kg in test diet relative to control diet ($P < 0.05$ for quadratic response). This is not unexpected as, lipid supplementation often suppresses DM intake at high inclusion rates (Huhtanen *et al*., 2008; Halmemies-Beauchet-Filleau *et al*., 2017). Milk yield linearly decreased throughout the experiment ($P < 0.01$). This can be attributed to advances in the lactation stage of animals as 10 out of 13 were in the late lactation at the beginning of the trial and thus on the descending part of the lactation curve. Indeed, after that effect of time on milk yield was taken into account, no difference in milk yield was seen between test and control diets (mean response of milk yield to test diet just above 0 kg/d). The same was true for energy corrected milk production. Milk fat concentration and fat yield were unaffected by dietary changes. Milk protein yield was linearly decreased during the experiment ($P < 0.01$), but in the absence of quadratic responses, this was most probably

related to decreased milk yield due to advances in lactation stage rather than changes in energy and protein status and utilization due to dietary change. Overall, the test diet had no major effect on milk production and major milk constituents. This is in line with previous studies with milled rapeseeds (Kairenius *et al.*, 2009; Brask *et al.,* 2013).

Table 2 Animal performance (n=13)

	Treatment			Mean		Significance	
	Control $diet_{1per}$	Test $diet_{2per}$	Control $diet_{3per}$	response to test diet*	SEM	Lin	Quad
Dry matter intake, kg/d	21.9	21.2	22.2	-0.9	0.70	0.582	0.027
Milk							
Yield, kg/d	31.4	29.7	27.6	0.2	2.66	<0.001	0.638
Energy corrected milk, kg/d	32.4	30.8	29.0	0.1	2.61	0.025	0.796
Fat yield, g/d	1317	1265	1181	16	113.0	0.123	0.727
Fat content, g/kg	42.8	43.3	43.6	0.1	2.27	0.726	0.996
Lactose yield, g/d	1413	1342	1231	20	132.6	<0.001	0.418
Lactose content, g/kg	44.5	44.7	44.1	0.4	0.60	0.216	0.156
Protein yield, g/d	1129	1049	1027	-29	82.8	0.007	0.488
Protein content, g/kg	36.4	36.3	38.1	-1.0	1.03	0.002	0.010
Urea content, mg/dL	27.3	27.0	24.9	0.9	1.37	0.028	0.254

* Test $diet_{2per}$ – (Control $diet_{1per}$ + Control $diet_{3per}$)/2, per = experimental period.

Test diet altered milk fat composition (Table 3). The total saturated fatty acid content of milk fat from the test diet was 17% lower than from the control diet (Table 4). Moreover, the 10- to 16-carbon saturated fatty acids, regarded as the key blood cholesterol-increasing fatty acids in humans, were 31 to 49% lower in milk from the test than in the milk from the control diet. This was expected as increased supply of long-chain fatty acids is known to inhibit de novo synthesis of medium-chain saturates in the mammary gland (review of Shingfield *et al.,* 2010). The total *cis*-monounsaturated fatty acids were 58% higher in milk fat from the test diet than the control diet. These changes principally originate from oleic acid (*cis*-9 18:1) that is the predominant fatty acid in rapeseed. The increase in *trans* fatty acids was marginal and the major *trans* isomers in milk were vaccenic acid (*trans*-11 18:1) and rumenic acid (*cis*-9,*trans*-11 18:2) with potentially beneficial effects on human health (reviews of Field *et al.,* 2009 and Koba *et al.,* 2012).

Ruminal methane, carbon dioxide and hydrogen emissions were substantially decreased from cows on the test diet ($P < 0.01$ for quadratic response, Table 4). For methane and hydrogen, the decrease on the test diet was substantial being 18 and 36% relative to control diet, respectively, whereas for carbon dioxide, the decrease was much smaller (5%). Thus milled rapeseeds seemed to decrease hydrogen load in the rumen. The decrease in methane production in response to rapeseed lipids was slightly higher in magnitude compared to the study of Brask *et al.* (2013, 2-3% added lipids, minus 14%), whereas Martin *et al.* (2011, 3% added lipids) reported no effect. A meta-analysis by Beauchemin *et al.* (2008) showed a linear relationship between percentage of lipid added and reduction in ruminal methane production. Therefore, the high lipid inclusion rate (ca. 5% added lipid) in the present study together with a high-forage diet may at least in part explain the big decrease in ruminal methane emissions in the current study.

Table 3 Composition of tank milk collected from the whole herd at the end of experimental periods 1 and 2

Fatty acid, g/100 g total fatty acids	Control diet	Test diet	Change in %
4:0	3.2	3.4	+7
6:0	2.2	1.8	-20
8:0	1.5	1.0	-34
10:0	3.9	2.0	-49
12:0	4.6	2.2	-52
14:0	13	8.5	-35
16:0	31	21	-31
18:0	9.7	18	+82
cis-9 18:1	16	28	+70
trans-11 18:1	1.1	1.2	
total *trans* 18:1	2.4	3.8	
cis-9,*cis*-12 18:2	1.3	1.1	
cis-9,*trans*-11 18:2 (CLA)	0.4	0.5	
cis-9,*cis*-12,*cis*-15 18:3	0.4	0.4	
Total saturated fatty acids	74	61	-17
Total monounsaturated fatty acids	23	36	+58
Total polyunsaturated fatty acids	2.6	2.3	
Total *trans* fatty acids	3.6	5.0	

Table 4 Ruminal gas production (n=23)

	Treatment			Mean		Significance	
	Control $diet_{1per}$	Test $diet_{2per}$	Control $diet_{3per}$	response to test diet*	SEM	Lin	Quad
Methane, g/d	456	378	468	-84	16.9	0.783	<0.001
Carbon dioxide, g/d	12447	11870	12575	-641	352.8	0.735	<0.001
Hydrogen, mg/d	653	399	598	-227	49.6	0.157	<0.001

* Test $diet_2$per – (Control $diet_1$per + Control $diet_3$per)/2 , per = experimental period

Conclusions

Replacing rapeseed meal with milled rapeseed in a dairy cow diet had no adverse effects on milk production, but improved milk fat profile by decreasing the proportion of medium-chain saturated fatty acids. In addition, milled rapeseeds substantially reduced ruminal methane and hydrogen production.

Acknowledgements

This study was financed in part by EIT Food (EIT Food Project 18095: Dairy products with reduced saturated fatty acids, tasks A1802 and A1903). The authors thank the contribution of the staff at the University of Helsinki Viikki research farm and laboratory of Animal Science. Valued contributions of MSc students Milja Korjus, Kasperi Ojala, Vappu Tauriainen and Tuire Tapola for practical experimental work at the dairy barn is much appreciated.

References

Beauchemin, K.A., Kreuzer, M., O'Mara, F. & McAllister, T.A., 2008. Nutritional management for enteric methane abatement: A review. Aust. J. Exp. Agric. 48, 21–27.
Brask, M., Lund, P., Weisbjerg, M.R., Hellwing, A.L.F., Poulsen, M., Larsen, M.K. & Hvelplund, T., 2013. Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. J. Dairy Sci. 96, 2356-2365.
Field, C.J., Blewett, H.H., Proctor, S. & Vine, D., 2009. Human health benefits of vaccenic acid. Appl. Phys. Nutr. Met. 34, 979-991.

Halmemies-Beauchet-Filleau, A., Shingfield, K.J., Simpura, I., Kokkonen, T., Jaakkola, S., Toivonen, V. & Vanhatalo, A., 2017. Effect of incremental amounts of camelina oil on milk fatty acid composition in lactating cows fed diets based on a mixture of grass and red clover silage and concentrates containing camelina expeller. J. Dairy Sci. 100, 305-324.
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, 942-953.
Kairenius, P., Toivonen, V., Ahvenjärvi, S., Vanhatalo, A., Givens, D.I. & Shingfield, K.J., 2009. Effects of rapeseed lipids in the diet on ruminal lipid metabolism and milk fatty acid composition in cows fed grass silage based diets. In Ruminant Physiology: Digestion, Metabolism and Effects of Nutrition on Reproduction and Welfare: Proc. XIth Intern. Symp. Rumin. Physiol. (p. 232). Wageningen Academic Pub.
Koba, K. & Yanagita, T., 2014. Health benefits of conjugated linoleic acid (CLA). Ob. Res. Clin. Pract. 8, e525-e532.
Lamminen, M., Halmemies-Beauchet-Filleau, A., Kokkonen, T., Jaakkola, S. & Vanhatalo, A., 2019. Different microalgae species as a substitutive protein feed for soya bean meal in grass silage based dairy cow diets. Anim. Feed Sci. Technol. 247, 112-126.
Martin, C., Pomiès, D., Ferlay, A., Rochette, Y., Martin, B., Chilliard, Y., Morgavi, D.P. & Doreau, M., 2011. Methane output and rumen microbiota in dairy cows in response to long-term supplementation with linseed or rapeseed of grass silage- or pasture-based diets. Proc. N.Z. Soc. Anim. Prod. 71, 243–247
Mottet, A., de Haan, C., Falcucci, A., Tempio, G., Opio, C. & Gerber, P., 2017. Livestock: On our plates or eating at our table? A new analysis of the feed/food debate. Global Food Sec., 14, 1-8.
Shingfield, K.J., Bernard, L., Leroux, C. & Chilliard, Y., 2010. Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4, 1140-1166.
Shingfield, K.J., Bonnet, M. & Scollan, N.D., 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal 7, 132-162.
SAS, 2012. SAS 9.4. SAS institute Inc. Cary. NC. USA.

Wood products as emergency feed for ruminants

E. Prestløkken[1] & O.M. Harstad
Norwegian University of Life Sciences, Faculty of Biosciences, Department of Animal and Aquacultural Sciences, P.O. Box 5003, 1432 Ås, Norway.
Correspondence: egil.prestlokken@nmbu.no

Introduction

The summer of 2018 was the driest for more than 60 years, reducing Norwegian production of grass for feed with around 25% compared to the last 10 years average (SSB, 2019), whereas Norwegian production of cereal grains for feed were almost halved compared to an average year (Norske Felleskjøp, 2019). During the summer of 2017, parts of Norway (South-West and West coast) experienced the opposite situation with heavy rainfall and wet conditions not allowing crops to be harvested. Lack of cereal grains and ingredients for concentrate feeds had to be solved by import. With respect to grass and roughage, import is not a simple matter and alternatives are needed. Inspired by research conducted in the US 50 years ago, wood or wood-based products came up as a potential substitute for this part of the diet. In a summary, Baker et al. (1975) pointed out sawdust of aspen as a material of particular interest for replacing roughages in diets for lactating cows. With this background, a feeding test with sawdust of aspen were carried out in the period 15th of August until 5th of September 2018. The intention was also to perform an initial test to evaluate animal acceptance of aspen sawdust, and if accepted, evaluate if sawdust from aspen can replace parts of grass silage in diets to lactating cows. This paper gives a brief overview of the work conducted and the main findings.

Materials and Methods

The sawdust was produced by chopping whole aspen logs with bark using a rotating drum fitted with chain saw bands in a screw configuration. The equipment was originally produced by a local entrepreneur for production of bedding material. In a test production, particle size was considered to be too fine for the purpose. By modifying the chain saw bands, particle size was increased. Dry sieving revealed that 10 and 53% of the particles were retained on 2 and 1 mm screen, respectively. Later, during production, these values changed to 33 and 35%, respectively. The test started with the first quality and changed to the second quality mid-way through the experimental period. The feeding test included six lactating cows kept in tie-stalls and milked twice daily. The cows were taken without an adaptation period from summer pasture and put on a diet consisting of 10 kg concentrate and 6 kg dry matter of an early cut grass silage (289 g DM/kg, 133 g CP/kg DM and 414 g NDF/kg DM). The cows were in mid to late lactation and averaged 23.9 kg of milk when the test started. During the first days of the test, the concentrate was ‘Drøv Energirik Låg’ (Norgesfôr Mysen mølle, Mysen, Norway). After a few days, the concentrate was changed to ‘Drøv Energirik Høg’ that had a higher content of protein and 2% of sodium bicarbonate. The silage diet was offered at 07:00 and 18:00 h in two equal portions. The concentrate and sawdust were offered simultaneously at 07:30, 14:00 and 18:30 h in three equal portions.

The test started 15th of August 2018 with selecting three cows for an initial acceptance test. The sawdust was introduced by mixing it into the concentrate by hand, starting with a small amount. The amount of sawdust in the diet was then gradually increased, whereas the amount of silage was gradually decreased. When it was clear that the animals were readily eating the sawdust, three more cows were included 22nd of August. Intake of feeds (sawdust, silage and

concentrate), milk yield and rumination activity (RumiWatch, Itin+Hoch GmbH, Liestal, Switzerland) were monitored daily. Samples of morning and evening milk for chemically analyses were frequently taken throughout the test period until the test stopped at 5th of September. In one cow fitted with a rumen fistula, pH was measured every 10 minutes in periods of three days using an adapted pH electrode attached to a portable pH-meter. Samples for organoleptic quality of milk were collected twice during the test.

Results and Discussion

Results are presented for the period 22nd of August until 5th of September 2018 when all cows were included in the test. The setup of the test was simple with no control period or changeover of animals. Thus, the setup did not allow statistical evaluation and results presented are the arithmetic means of the observations. When the test started, all animals received 6 kg DM silage and 8.7 kg DM concentrate daily. When the test ended, the animals where consuming 2.5 kg DM silage, 11.3 kg DM concentrate and 5.4 kg DM sawdust (9 kg). For the first three cows, the adaptation to sawdust was done over a 10 to 12 day period. As they rapidly adapted to the sawdust, the three last cows were adapted over a 7 to 8 day period.

Milk production together with fat and protein concentration of the milk are in Figure 1.

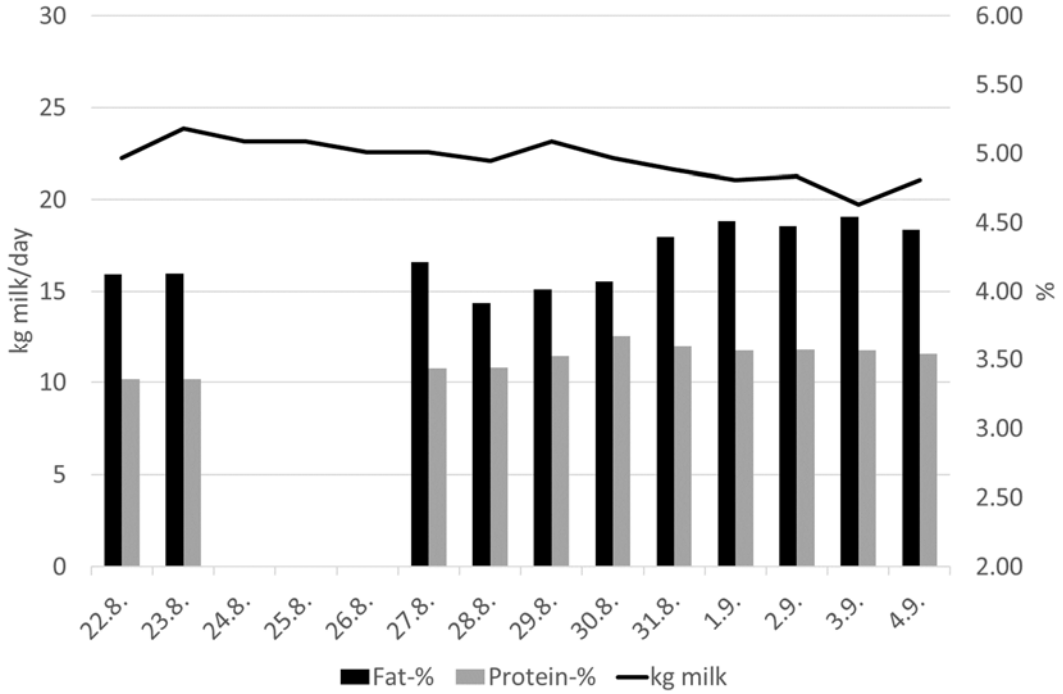


Figure 1 Average milk yield, and fat and protein content of the milk in the days between 22nd of august and 4th of September 2018 for the six cows used in the experiment.

At the end of the test, average milk yield was 21.9 kg, a decrease of only two kg compared to the start of the test. This indicates that the increased concentrate allowance compensated for the reduced intake of energy from grass silage. Moreover, no major changes in milk fat and milk protein concentration was observed. This indicates that a normal rumen function and environment was maintained. For a short period, silage offered was reduced to 2 kg DM. At that level, some animals showed reduced feed intake and reduced fat content in the milk, indicating rumen acidosis. Increasing the silage proportion by 0.5 kg DM/day removed that

tendency, indicating a minimum requirement of feed structure in the rumen. A minimum requirement of roughage is in line with Satter et al. (1973) who concluded that sawdust can replace a substantial part of roughage in the diet, but a minimum supply of long hay is needed.

Average rumination, eating and chewing time are in Figure 2. Replacing grass silage with sawdust reduced rumination time. Assuming a chewing time of 60 min/kg DM silage, and 4 min/kg DM concentrate, chewing time at the level of 350 min/day indicates a chewing time of sawdust of approximately 150 min/day. Dry matter concentration in sawdust was around 60%. Assuming intake of 5 kg DM/day, chewing time of sawdust was approximately 30 min/kg DM. Compared to grass silage, this is low. During daytime, when animals were resting, visual observations showed rumination activity to be low. Nevertheless, although rumination was low, milk yield and milk fat concentration was maintained. Moreover, in the cow with rumen fistula, ruminal pH dropped to below 5.5, but increased to a value around 6.8 before morning feeding (Figure 3). This indicates that the sawdust aided in maintaining normal digestion of nutrients even when feeding only 2.5 kg DM grass silage.

The last two days of the experiment a third production lot of sawdust with longer particles was used. Towards end of the experiment, rumination time increased, indicating that structure of sawdust is important, something that needs to be further explored.

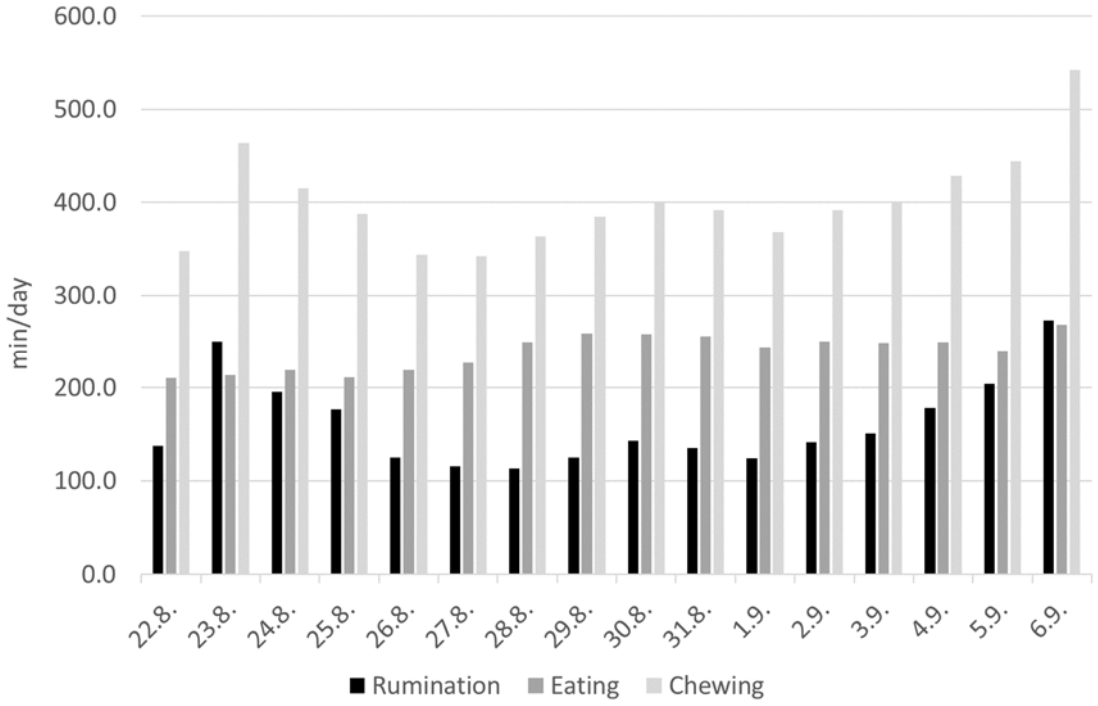


Figure 2 Average rumination, eating and total chewing activity

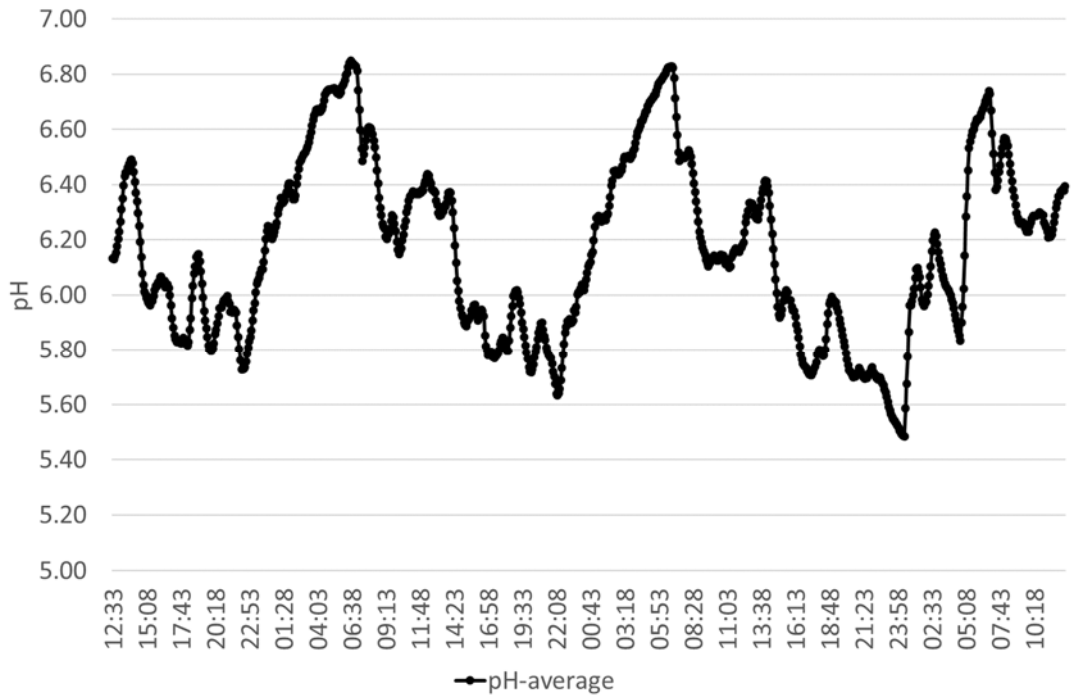


Figure 3 Rumen pH of cow 6303 in the days 31st of August to 3rd of September 2018. Feeding of silage at 07:00 and 18:00 h, feeding of concentrate and sawdust at 07:30, 14:00 and 18:30 h.

Although results from the milk evaluations are not presented, no indication in reduced quality was observed and all samples had scores of 4 or better on a scale where 5 is best. No increase in water intake was observed during the experimental period.

Conclusions

Aspen sawdust can replace a substantial part of grass silage in diets for lactating cows without affecting milk production and milk composition and quality. A minimum requirement of grass silage, or other feed giving structure to the diet appears to be needed. Further studies on aspen sawdust, or other wood products are needed.

References

Baker, A., J., Millet, M.A. & Satter L.S., 1975. Wood and wood-based residues in animal feeds. Aspen Bibliography. Paper 5153, Utah State University, USA.

Norske Felleskjøp, 2019. Prognose norsk kornproduksjon. https://www.fk.no/nyheter/maiprognosen

Satter, L.D., Lang, R.L., Baker, A.J. & Millett. M.A., 1973. Value of Aspen Sawdust as a Roughage Replacement in High- Concentrate Dairy Rations. J. Dairy Sci., 56, 1291-1297.

SSB (Statistisk sentralbyrå), 2019. Potet- og grovfôravling. Statistics Norway. https://www.ssb.no/jord-skog-jakt-og-fiskeri/statistikker/jordbruksavling

Feeds for ruminants from forests?

M. Rinne & K. Kuoppala
Natural Resources Institute Finland (Luke), Tietotie 2 C, FI-31600 Jokioinen, Finland
Correspondence: marketta.rinne@luke.fi

Introduction

Currently, there is a great interest towards more efficient use of various by-products and novel biomaterials as feeds (e.g. Rinne *et al*., 2014, Halmemies-Beauchet-Filleau *et al*., 2018). The proportional area of forests in Finland is the highest in Europe (Forest Europe, 2015; Table 1), which makes it natural to consider forests for many different uses. With the increasingly sophisticated bioeconomy, novel uses of wood materials are actively developed in e.g., plastic replacers, textiles, antimicrobial uses (wood protection, plant protection), biochar applications, food ingredients, medicine etc.

Historically, feed materials originating from trees have played a substantial role in feeding of ruminant livestock. Until the beginning of 20th century, it was common that arable fields were fenced to keep livestock out of them and cattle as well as small ruminants grazed freely in meadows and forests. Ruminants consumed mostly grasses and herbs, but would also browse shrubs and leaves from trees. Small branches of deciduous trees were collected and dried to be used as winter feed, and leaves from trees can even be ensiled (Smith *et al*., 2014). Wild ruminants are also known to utilize coniferous trees as winter feed (Stolter *et al*., 2009).

In search for novel feed resources for ruminants, wood by-products provide an intriguing possibility due to their vast abundance. Although they are not used to large extent, it should be possible to include such feeds without major biological, ethical or legal obstacles into ruminant diets because ruminants naturally utilize plant fibre as their main source of energy (Van Soest, 1994). In the case of commercial use, the manufacturer of a feed material is responsible for the safety of the product.

Fibre in wood materials is so highly lignified that the unprocessed wood is virtually indigestible even for ruminants. It is possible to process wood by e.g. pulping, using steam-explosion or extraction to improve digestibility. The characteristics of different wood derived feed materials vary widely based on composition of the parent material and processing methods used. Different types of wood based products have successfully been used in a number of studies without deleterious effects on animal health, productivity or animal products, which indicates that there are no biological obstacles to incorporating them into ruminant diets. It is also noteworthy that the topic has been studied quite intensively although practical applications are scarce.

Table 1 Forest area in selected European countries (Forest Europe, 2015)

Country	Proportion of forests from land area, %
Finland	76
Sweden	74
Norway	46
Germany	33
Denmark	16
Ireland	12
The Netherlands	11

Wood chemistry and digestibility

The principal components of wood are cellulose [400 to 450 g/kg dry matter (DM)], hemicelluloses (200 to 300 g/kg DM) and lignin (200 to 300 g/kg DM) (Sjöström, 1993). These three compounds account for more than 90 % of wood, the residual comprising of protein, minerals and other components. Cellulose is a homopolymer of β-(1-4)-linked D-glucose units whereas hemicelluloses are heteroglycans of several different neutral and acidic monosaccharides (Sjöström, 1993).

Untreated wood in a diet is very poorly utilized by many animals including ruminants. The *in vitro* digestibility of DM of various woods has a range from 0.02 to 0.35 (Millett *et al*., 1970). Hardwood species (aspen, birch, maple) were more digestible than softwood species (pine and spruce) which showed hardly any digestibility. The bark had slightly higher digestibility than the wood of given species. Twigs and leaves were more digestible than the stem in the experiments of Millett et al. (1970). For example, maple buds and twigs had a digestibility of 0.35 compared with 0.20 of maple wood.

Processing methods to enhance nutritional use of wood

Most pulping and papermaking residues have undergone at least partial delignification and, as a result of that, wood carbohydrates become more available for rumen microbes as an energy source. The aim of pulping is to break down the bulk structure of the fibre source into the constituent fibres by degrading the lignin and hemicellulose into small, water-soluble molecules which can be washed away without depolymerizing the cellulose fibres.

Depending on the process, the residual product from pulping may contain only hemicelluloses or hemicelluloses and cellulose with or without lignin. The amount of lignin in the residue affects utilization of these products. Digestibility of pure cellulose is rather high and corresponds to that of e.g. barley grain. Digestibility is mainly affected by the removal of lignin. Saarinen *et al*. (1959) determined *in vivo* digestibility of 40 wood pulps produced by various pulping methods and reported a range in digestibility from 0.27 to 0.90 depending on lignin content. *In vivo* digestibility of bleached (lignin erased and the pulp whitened) chemical pulp fines from mixed hardwood was 0.78 for DM and 0.86 for carbohydrates (Millett *et al*., 1973) indicating that the materials have high energy values for ruminants. In the data set of Näsi (1984), various wood based products from whole short rotation trees to wood pulp were evaluated *in vivo* using sheep. A large variation in digestibility was observed with the highest values being obtained for dissolving pulp (0.75 of organic matter).

Hemicelluloses occur in primary and secondary cell walls of all plants and are the second most abundant polysaccharides after cellulose (Schädel *et al*., 2010). Hemicelluloses are not homogeneous compounds but a group of mixed polysaccharides. There are different types of hemicelluloses including xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan. Spruce and pine (softwood) contain somewhat less hemicelluloses than birch (hardwood) and composition differs among these species (Saarinen *et al*., 1959). Glucomannans and galactomannans are the principal hemicelluloses of coniferous trees (spruce and pine) and xylans in deciduous trees (birch) while β-glucans are restricted to grasses. Norway spruce contained hemicelluloses ca. 25% and about 15% was

galactoglucomannan (Willför and Holmbom, 2004). Birch contained mainly xylans (226 g/kg DM) at a higher level than in aspen, oak, poplar, wattle and beech (Willför *et al.*, 2005).

A relatively novel method for extraction of hemicelluloses from spruce was introduced by Leppänen *et al*. (2011). In a pressurized hot water extraction (PHWE), temperature is above 100°C but below 374 °C and water is kept in a liquid form by keeping the system under sufficiently high pressure. This method was used to produce various hemicellulose extracts, and digestibility was studied in an *in vitro* gas production system (Rinne *et al*., 2016). Based on the *in vitro* analysis, galactoglucomannan extracted from spruce using PWHE was chosen for an *in vivo* digestibility trial using sheep. The *in vivo* organic matter (OM) digestibility of galactoglucomannan was 0.591 (Rinne *et al.*, 2016). In PHWE, the hemicellulose is provided in a very dilute water solution. Delivering the feed to animals in the liquid form would make drying unnecessary, but on the other hand, logistics would be a problem. Use of low DM spruce and birch hemicellulose extracts was demonstrated by Kautto *et al.* (2014), and after some initial intake problems, the extracts could successfully be used for dairy cows.

Hemicelluloses in liquid form are in the literature often called wood molasses or wood sugar concentrates. After extraction of cellulose, remaining liquid contains free sugars from hemicelluloses and may also contain variable amounts of lignin depending on the pre-treatment. Composition and feed value of wood molasses depends on the method of processing and should be taken into account when feeding to livestock. Wood molasses contains only traces of other constituents than sugars. According to the old Finnish Feed tables (Salo *et al*., 1982), hemicelluloses or wood molasses (600 g DM/kg) contain 10 g CP, 10 g ether extracts, 975 g nitrogen free extracts and 5 g ash per kg DM and no crude fibre with an OM digestibility of 0.850.

The effect of wood molasses on ration digestibility depends on the amount of lignosulphonates and may also depend on level of inclusion in the diet. Using wood molasses decreased total tract digestibility of DM and OM when wood and cane molasses were fed at a dietary level of 10.5 % of DM (Zinn, 1990). When the proportion of those molasses was 4 % of diet DM, no digestibility difference was found between these two types of molasses (Zinn, 1993). Zelenak *et al*. (1979) reported no difference between diets containing wood or cane molasses at 12% of daily ration, in digestibility, energy balance or weight gain of lambs. The wood molasses used was derived from waste water of fibre board production from mainly spruce and pine containing 587 g/kg DM total carbohydrates, the majority of which was mannose and glucose.

Wood molasses has been investigated as a preservative of high moisture cereal grains by Huhtanen (1984a,b,c). It was derived from spent sulphite liquor of birch trees by decreasing the amount of lignosulfonates to one third of the initial content by ultrafiltration. The liquid was evaporated to a DM content of 550 g/kg. Wood molasses contained 520 g/kg sugars of which 700 g/kg was xylose (Salo, 1978). Huhtanen (1984a) concluded that wood molasses can be used as a preservative of high moisture barley and that a level of 8-12% of barley DM was adequate to prevent deterioration during storage. Wood molasses was also more effective than a formic acid based solution in preventing aerobic deterioration after opening of the silo. When the ration of sheep contained 8-12% wood molasses replacing barley DM (daily ration of 300 g hay and 700 g barley), ration OM digestibility was not decreased but at an inclusion level of 16%, digestibility decreased (Huhtanen, 1984b). Wood molasses also tended to

increase protein utilization. With growing cattle, a similar feeding did not affect average daily gain, feed conversion or carcass characteristics (Huhtanen, 1984c).

Recently, two dairy cow experiments were conducted at Luke to evaluate wood based high fibre feed components. One experiment used microcrystalline cellulose (AaltoCell™; Savonen *et al*., 2019) while pine bark meal was used in a second study (unpublished). The wood-based feeds replaced cereal grains in the diets, and resulted in decreased milk production (Figure 1). It is obvious that such feeds do not support high milk yields, but could be included in diets without problems. The dairy cow is an excellent model for evaluating novel feeds due to the high nutrient requirements and quick responses to changes in nutrient supply in terms of feed intake and milk production, which can be analysed statistically using efficient cross-over designs.

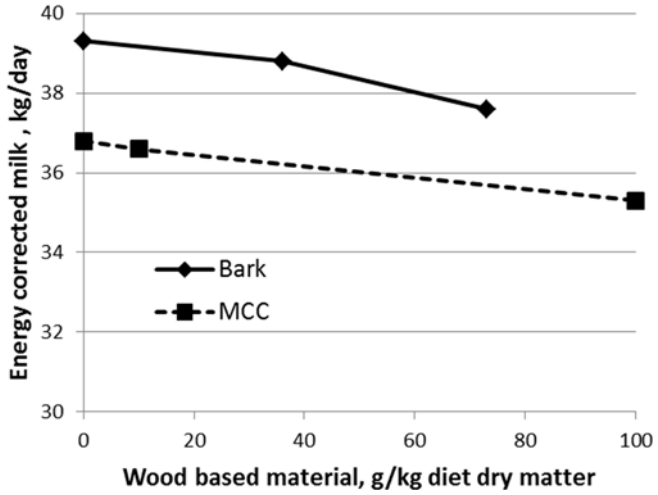


Figure 1 Milk production responses of dairy cows to increased bark meal (Unpublished) or microcrystalline cellulose (MCC) inclusion (Savonen *et al.*, 2019) in the diet.

Bioactive effects of wood based materials

Another approach to bulk feed use is to utilize the bioactive properties of wood based products. Wood, bark and resin are known to contain a variety of compounds with potential to modulate animal responses. In this respect, monogastric animals may also be an interesting target group, but that is beyond the scope of this article.

The 'Tall' (*Pinus sylvestris*) oil based product Progres® (Hankkija, 2019) is an example of a wood derived bioactive compound, which is used as a commercial feed material for pigs, poultry and ruminants. Recently, indications of positive effects were detected in early lactation cows (Kairenius *et al*., 2019).

No positive responses in rumen fermentation could be detected when microcrystalline cellulose (AaltoCell™) was fed to dairy cows (Savonen *et al*., 2019) although *in vitro* studies indicated some benefits (Stefanski *et al.*, 2018).

Agro-forestry

An additional way of using wood-based materials in ruminant diets is the extensive grazing practiced around the Mediterranean. Earlier, similar grazing of ruminants was also applied throughout Europe. Recently, agroforestry applications have been very limited in North-Western Europe, although the concept of e.g. combining energy and grassland production by use of strips of willow and grazed grass has been demonstrated (Smith *et al.,* 2012). Pastoral and agro-forestry systems may fit well with tropical and semi-arid agricultural systems, but they are not included in this article.

Agro-forestry systems may provide ecosystem services such as increased biodiversity and carbon sequestration as well as buffering changes in feed supply and improving the microclimate. Recently, loss of biodiversity has raised serious concerns and grazing of natural woody areas has a potential to maintain the biodiversity. Benefits, other than those related to livestock productivity, need to be taken into account.

Quantitative and economic aspects of wood by-products

The main factors that will determine the use of wood by-products as feeds are the price and availability of suitable products. With an increasingly sophisticated bio-economy, ruminants may play a role in utilizing wood-products such as hemicelluloses which have little value for other uses e.g. in paper production or as an energy source. For example, if hemicellulose residues from wood pulping for paper in Finland could be utilized for ruminant feeds, it would account for 1.5 million tons. Wood derived feeds may also provide a source of feed in case of crisis situations (see e.g. https://www.slu.se/en/faculties/vh/research/forskningsprojekt/not/huv-managing-forage-shortage-crises-with-forest-by-products/).

Table 2 Approximate price estimates for crude economic comparisons of various biomasses

Material	Price per ton dry matter, €
Timber for pulping	125
Wood chips for heating	150
Cellulose pulp	900
Barley grain	165
Grass silage	75

Based on the prices of various wood based materials and corresponding traditional ruminant feeds (Table 2), it is obvious that the potential to use wood based materials as bulk feeds is very limited even if they would be suitable as such. When accounting for the heavy processing needed to make them digestible for ruminants, this alternative becomes even more unrealistic. In order to be applicable, there needs to be some other benefits than just providing energy to the animals.

Summary and conclusions

Carbohydrates from wood are available in large quantities, but because of very low digestibility, heavy processing is required to improve digestibility if they are to be used as feeds. It is likely that wood based feeds are most suitable for animal groups that do not have particularly high energy requirements.

The feasibility of using wood derived carbohydrates as energy sources in ruminant diets depends on the cost of processing, preservation and logistics as well as supply chain

acceptance. However, there are no legal or biological obstacles in using them. Currently, it seems that using wood based fibre as an energy source for ruminants is not economically feasible, but there may be specific cases when wood based materials may show potential in ruminant feeding:

- Use as an emergency feed in case of seasonal lack of feed, which may become more common with a changing climate
- Modulation of rumen fermentation, gut microbiota or animal physiology by providing bioactive compounds to improve gut health, decrease enteric methane production or to have other physiological or metabolic benefits
- Antimicrobial activities in improving feed hygienic quality
- Diluting the diet with respect to e.g. nitrogen and phosphorus in order to decrease environmental load
- Agro-forestry concepts providing ecosystem services such as increased biodiversity on top of simply the provision of feeds

References

Forest Europe, 2015. State of Europe's Forests 2015. Available at: https://www.foresteurope.org/docs/fullsoef2015.pdf

Halmemies-Beauchet-Filleau, A., Rinne M., Lamminen, M., Mapato, C., Ampapon, T., Wanapat, M. & Vanhatalo, A., 2018. Alternative and novel feeds for ruminants: nutritive value, product quality and environmental aspects. Animal 12, s295-s309. http://dx.doi.org/10.1017/S1751731118002252

Hankkija, 2019. Progres®. Available at: http://www.suomenrehu.fi/en/products/progres/

Huhtanen, P., 1984a. Wood molasses as a preservative for high moisture barley .1. Preservation and digestibility in pigs. J. Agric. Sci. Finl. 56, 255-263.

Huhtanen, P., 1984b. Wood molasses as a preservative for high moisture barley. 2. Ration digestibility and rumen fermentation in sheep. J. Agric. Sci. Finl. 56, 265-274.

Huhtanen, P., 1984c. Wood molasses as a preservative for high moisture barley. 3. Feeding value for growing cattle. J. Agric. Sci. Finl. 56, 275-282.

Kairenius P., Mäntysaari, P., Lidauer P., Franco M., Frantzi, M., Kettunen H. & Rinne, M., 2019. The effects of in-feed resin acid inclusion on milk production responses of dairy cows. Submitted to Int. Symp. Ruminant Phys., Leipzig, Germany, 3-6 September 2019.

Kautto, O., Sairanen, A., Kuoppala, K., Byman, O., Ilvesniemi, H. & Rinne, M., 2014. Koivusta ja kuusesta kuumavesiuutetun hemiselluloosaliuoksen maittavuus lypsylehmille Maataloustieteen Päivät 2014. Available at: https://journal.fi/smst/article/view/75394/36818

Leppänen, K., Spetz, P., Pranovich, A., Hartonen, K., Kitunen, V. & Ilvesniemi, H., 2011. Pressurized hot water extraction of Norway spruce hemicelluloses using a flow-through system. Wood Sci. Technol. 45, 223-236.

Millett, M.A., Baker, A.J., Feist, W.C., Mellenberger, R.W. & Satter, L.D., 1970. Modifying wood to increase its *in vitro* digestibility. J. Anim. Sci. 31, 781-788.

Millett, M.A., Baker, A.J., Feist, W.C., Mellenberger, R.W. & Satter, L.D., 1973. Pulp and papermaking residues as feedstuffs for ruminants. J. Anim. Sci. 37, 599-607.

Näsi, M., 1984. Evaluation of various types of forest biomass and wood processing residues as feed for ruminants. J. Agric. Sci. Finl. 56, 205-212.

Rinne, M., Dragomir, C., Kuoppala, K., Smith, J. & Yáñez-Ruiz, D., 2014. Novel feeds for organic dairy chains. Org. Agric. 4, 275–284. DOI 10.1007/s13165-014-0081-3.

Rinne M., Kautto, O., Kuoppala, K., Ahvenjärvi, S., Willför, S., Kitunen, V., Ilvesniemi, H. & Sormunen-Cristian, R., 2016. Digestion of wood-based hemicellulose extracts as screened by *in vitro* gas production method and verified *in vivo* using sheep. Agric. Food Sci. 25, 13-21. Available at: http://ojs.tsv.fi/index.php/AFS/article/view/46502.
Saarinen, P., Jensen, W. & Alhojärvi, J., 1959. On the digestibility of high yield chemical pulp and its evaluation. Acta Agr. Fenn. 94, 41-64.
Salo, M.-L., 1978. Wood molasses as a preservative for high moisture grains) J. Agric. Soc. Finl. 50, 206-211.
Salo, M.-L., Kiiskinen, T. & Tuori, M., 1982. Feed Tables and Feeding Recommendations (in Finnish). Available at: https://portal.mtt.fi/portal/page/portal/Rehutaulukot/Tietosiilo/Arkisto/vanhat_rehutaulukot/6ED260485048EDEDE040A8C0033C4D6F
Savonen, O., Kairenius, P., Mäntysaari, P., Stefanski, T., Pakkasmaa, J. & Rinne, M., 2019. The effects of microcrystalline cellulose as a dietary component for lactating dairy cows. Proc of the 10th Nordic Feed Sci. Conf., Uppsala, Sweden, June. 2019 (in press).
Schädel, C., Richter, A., Bloechl, A. & Hoch, G., 2010. Hemicellulose concentration and composition in plant cell walls under extreme carbon source-sink imbalances. Phys. Plant. 139, 241-255.
Sjöström, E., 1993. Wood Chemistry: Fundamentals and Applications. Academic Press. USA, 293 pp.
Smith, J., Leach, K., Rinne, M., Kuoppala, K. & Padel, S., 2012., Integrating willow-based bioenergy and organic dairy production – the role of tree fodder for feed supplementation. VTI Agriculture and Forestry Research, Special issue 362. p. 417-420. Available at: http://orgprints.org/21758/1/Smith_2OAHC%20proceedings_2012.pdf
Smith, J., Kuoppala, K., Yáñez-Ruiz, D., Leach K. & Rinne M., 2014. Nutritional and fermentation quality of ensiled willow from an integrated feed and bioenergy agroforestry system in UK. Maataloustieteen Päivät 2014, 9 pp. available at: https://journal.fi/smst/article/view/75342/36788
Stefanski, T., Välimaa, A-L., Kuoppala, K. Jalava, T., Paananen, P. & Rinne, M., 2018. *In vitro* ruminal degradation rate and methane production of different fraction of microcrystalline cellulose (MCC). Proc. 9th Nord. Feed Sci. Conf., Uppsala, Sweden, 12-13 June 2018, p. 87-93. Availabe at: https://www.slu.se/globalassets/ew/org/inst/huv/nfsc/nfsc2018/nfsc-2018_proceedings_corr_e-version.pdf.
Stolter, C., Niemelä, P., Ball, J.P., Julkunen-Tiitto, R., Vanhatalo, A., Danell, K., Varvikko, T. & Ganzhorn, J.U., 2009. Comparison of plant secondary metabolites and digestibility of three different boreal coniferous trees. Basic and Applied Ecology 10, 19-26.
Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. Cornell University Press. New York, USA, 476 pp.
Willför, S. & Holmbom, B., 2004. Isolation and characterisation of water soluble polysaccharides from Norway spruce and Scots pine. Wood Sci. Technol. 38, 173-179.
Willför, S., Sundberg, A., Hemming, J. & Holmbom, B., 2005. Polysaccharides in some industrially important softwood species. Wood Sci. Technol. 39, 245-258.
Zelenak, I., Boda, K., Bucko, J., Jalc, D., Walko, T., Beseda, I., 1979. Wood Molasses in the Nutrition of Ruminants. Ann. Recherch. Veter. 10, 457-459.
Zinn, R.A., 1990. Feeding value of wood sugar concentrate for feedlot cattle. J. Anim. Sci. 68, 2598-2602.

Zinn, R.A., 1993. Comparative feeding value of wood sugar concentrate and cane molasses for feedlot cattle. J. Anim. Sci. 71, 2297-2302.

Feeding dairy cows with no or reduced amounts of forage

C.F. Børsting[1], A.L.F. Hellwing[1], M.R. Weisbjerg[1], S. Østergaard[1], B.L. Raun[2], B.A. Røjen[3] & N.B. Kristensen[3]
[1]Department of Animal Science, Aarhus University, AU Foulum, P.O. Box 50, DK 8830 Tjele, Denmark; [2]DLG, Vesterbrogade 4a, DK 1620 Copenhagen V, Denmark; [3]SEGES, Agro Food Park 15, DK 8200 Aarhus N, Denmark.
Correspondence: Christian.borsting@anis.au.dk

Introduction

In Northern Europe the production of forage was severely impaired by drought in 2018. In Norway sawdust was tested as an alternative fiber source for dairy cows (Harstad & Prestløkken (2018). In earlier Danish studies Kristensen et al. (2003) demonstrated that it was possible to feed dairy cows chopped ammonia treated straw as the only roughage. However, the energy utilization was lower than for diets with forage. In late July 2018, there were a potential risk that some Danish dairy farms would have very limited amounts of forage. Therefore, it was decided to study how straw could be included in diets of high yielding dairy cows. The aim of this study was to examine the effects of replacing all or half of the forage (50% of dry matter (DM)) in a standard diet with straw and concentrate. The two concentrate mixtures used were based either on non-gene modified (NonGM) locally grown ingredients or mainly on gene modified (GM) ingredients.

Materials and Methods

Expected daily intakes of each feedstuff in the experimental diets are in Table 1.

Table 1 Planned daily dry matter intake (kg DMI) of each feedstuff in 5 diets

	Diet				
Pct. forage (excl. straw)	50	25	25	0	0
Concentrate source	Standard	NonGM	GM	NonGM	GM
Feedstuff:					
Chopped barley straw	0.3	1.22	1.22	2.13	2.13
1. cut clover grass silage	3.0	1.5	1.5	0	0
3. cut clover grass silage	3.8	1.9	1.9	0	0
Maize silage	6.5	3.25	3.25	0	0
Barley	3.0	3.10	1.50	3.19	0
Wheat, NaOH treated	0	1.88	0	3.75	0
Maize, finely grinded	0	0	3.36	0	6.72
Soybean meal	1.43	0.72	2.31	0	3.18
Soy hulls	0	0	3.66	0	7.31
Sunflower meal, dehul.	0	0	0.56	0	1.12
Molasses	0	0.23	0.29	0.46	0.58
Palm fatty acids distil.	0	0.02	0.16	0.04	0.32
Dried distillers grain	0	1.68	0	3.36	0
Rape seed cake	2.15	2.71	1.08	3.26	0
Dried beet pulp	3.29	4.07	1.65	4.85	0
Ca-carbonate	0.018	0.084	0.099	0.150	0.180
Salt	0.054	0.097	0.092	0.140	0.130
Na-bicarbonat	0.045	0.068	0.078	0.090	0.110
Mineral and vit.mixture	0.126	0.138	0.148	0.150	0.170
AMS Concentrate mix	2.6	2.6	2.6	2.6	2.6

GM: gene modified; NonGM: non-gene modified.

In the Standard diet grass-clover silage, maize silage and straw constituted 26, 24 and 1% of DM, respectively. In the diets with Zero forage, 9% of DM was straw and 91% was concentrate, whereas the 25% forage diets were produced by mixing 50% (DM basis) of the Standard diet and 50% of the Zero forage diets. Cows were housed in a stable with automatic milking systems and all cows were offered 2.6 kg DM of a concentrate mixture in the milking robot, whereas all other feedstuffs were mixed in a partial mixed ration (PMR) for each dietary treatment.

Barley straw was baled with a Krone baler with 'Opticut' equipment for cutting the straw. The risk of sorting was reduced by cutting the straw further by knives placed on the "vertical tower" mixing the feed in the JF mixer wagon. Water was added to all PMR diets to a DM content of 40% to reduce sorting in the PMR. The PMR diets with Zero forage were mixed for 20 minutes, the Standard diet for 28 minutes and the 25% forage diets were mixed for another 6 minutes.

The diets were planned using the NorFor feed evaluation system for cows with an expected ECM yield of 10500 kg. All diets were planned to give the same daily intake of net energy when fed *ad libitum*. The content of all nutrients were within normal recommendations. Despite large variation in the proportion of concentrate, the crude protein concentration was similar whereas starch and NDF content in the diets differed more.

Crude protein was 175 g/kg DM in Standard and GM diets, and 169 g/kg DM in the Zero forage NonGM diet. NDF content was 302 g/kg DM in the Standard diet, 283 in the Zero forage NonGM diet and 356 in the Zero forage GM diet. The higher NDF content in the GM diet compared to the Standard diet was mainly due to the high content of soy hulls. Due to the maize grain in the Zero forage GM diet (224 g starch/kg DM) and barley and wheat in the Zero forage NonGM diet (214 g starch/kg DM), starch content was higher in these diets compared to the Standard diet (172 g/kg DM).

Two groups of 45 Holstein cows were used in the trial. All cows were at least 4 weeks postpartum at the start of the experiment. In the first group, 9 cows were allocated to the Standard diet and 18 cows to each of the NonGM diets with 25% and Zero forage, respectively. In the second group, 9 cows were allocated to the Standard diet and 18 cows to each of the GM diets with 25% and Zero forage, respectively. In a three week pre-experimental period, all cows were fed the Standard diet. Thereafter, cows were gradually adapted to the experimental diets over one week, followed by a 3 week adaptation period. The following 4 weeks constituted the actual experimental period from which data were used.

Rumen fluid was sampled from intact animals by an oro-ruminal FLORA sampling device (FLORA®; Profs products, Wittibreut, 73 Germany). Energy corrected milk was calculated according to Sjaunja et al. (1991). Data were analyzed as repeated measurements using the Mixed-procedure of SAS 9.4. Treatment (50%, 25% or 0% forage), parity (primi- or multiparity) and experimental week (1, 2, 3 or 4) were included as fixed effects. The pre-experimental data was used to calculate a covariate, which was included in the model. The covariance structured was first-order autoregressive (AR(1)). Data in tables are LSmeans. Milk price was based on the milk composition and actual milk price in November 2018. The price of milk with the same composition was 0.075 DKK higher for milk from cows fed the two NonGM diets due to a premium for NonGM milk (Arla). The price of grass-clover and maize silage was 1.27 DKK per Scandinavian Feed Unit (SFU) and 1.10 DKK per SFU, respectively according to standard values from SEGES (Højholdt, 2018), whereas the price of

all other feed ingredients were the actual prices when the feeds were bought in August 2018. The price of the mix of dry ingredients (excl. AMS concentrate) for the GM diet was 2.28 DKK/kg and the price of the mix of dry ingredients (excl. AMS concentrate and NaOH treated wheat) for the NonGM diet was 2.18 DKK/kg.

Results and Discussion

The DMI was on average 23.3 kg and not (P>0.05) affected by diet (Table 2). Nor was daily energy intake (MJ/day) affected (P>0.05). This shows that the energy intake in the diets with Zero forage must have been regulated by energy density of the diets and not by gut fill because the fill factor of the Zero forage NonGM and GM diets were only 5.28 and 5.76 per kg DM, respectively, compared to 8.18 in the Standard diet.

Table 2 Dry matter intake (DMI), energy intake (MJ NEL), milk production and acetate:propionate proportion in rumen fluid

	Diet					
Pct. forage (excl. straw):	50	25	0	50	25	0
Concentrate source:	Standard	GM	GM	Standard	NonGM	NonGM
DMI, kg/day	23.1	24.3	23.9	23.4	22.8	22.4
MJ NEL/day	152	155	146	154	150	148
Milk, kg/day	34.6	34.6	33.9	35.3	34.9	33.2
Fat, %	4.27[a]	3.89[a]	3.27[b]	3.99[a]	3.55[b]	2.79[c]
Protein, %	3.8	3.74	3.88	3.65	3.72	3.72
ECM[1], kg/day	35.8[a]	34.4[a]	31.8[b]	36.5[a]	34.7[a]	29.7[b]
Acetate/propionate[2]	2.9	2.7	2.2	2.9	2.5	1.9

[1]Energy corrected milk (Sjaunja et al. 1991). [2]Molar proportions of acetate:propionate in rumen fluid. a, b, c: Values with different superscript letters within gene modified (GM) and non-gene modified (NonGM) treatments, respectively, are significantly different (P<0.05).

Daily milk yield was not affected by diet and was on average 34.4 kg. Protein percentage was on average 3.75 and unaffected by diet.

Fat percentage in milk was influenced by diet (P<0.05). Feeding GM with diet Zero forage decreased fat percentage to 3.27 compared to 4.27 with the Standard diet (P<0.05). This led to a decrease in ECM yield from 35.8 to 31.8 kg/d (P<0.05). Feeding the NonGM diet without forage decreased fat percentage to 2.79 compared to 3.99 in the Standard diet (P<0.05). This caused a decrease in yield from 36.5 to 29.7 kg ECM/d (P<0.05).

The dramatic decrease in fat percentage was in accordance with changes in rumen VFA proportions since both GM and NonGM diets without forage led to large decreases in the acetate:propionate ratio. Feeding GM diets led to a decrease in the acetate:propionate ratio from 2.9 in the Standard diet to 2.7 and 2.2 in the 25% and Zero forage GM diets , respectively (P<0.05). For the NonGM diets, this ratio fell from 2.9 down to 2.5 and 1.9 for the 25% and Zero forage GM diets, respectively (P<0.05). Therefore, it is obvious that rumen fermentation was challenged by the Zero forage diets, with extreme effects on both VFA ratios and milk fat percentage, when the Zero forage NonGM diet was fed. As mentioned above, the content of starch was similar in the GM and nonGM diets. However, the source of starch was very different, since most of the starch in the GM diet came from the slowly degradable maize grain starch, whereas in the Zero forage NonGM diet starch sources were finely ground barley and NaOH treated wheat kernels. Furthermore, the higher NDF content of 356 g/kg DM in the GM diet versus 283 g/kg DM in the NonGM diet probably alleviated the negative effect of the lack of forage on rumen fermentation.

The negative impact of the Zero forage diets on ECM yield and VFA ratios are in line with Kristensen et al. (2003), who demonstrated a lower utilization of the theoretically calculated energy content of diets with a lower roughage organic matter digestibility , when diets with barley straw, barley whole crop silage and grass silage were compared. They ascribed this negative effect on energy utilization to the higher proportion of concentrate needed in the diet to compensate for the low energy content of straw.

The value of the milk was reduced especially when the Zero forage diets were fed, due to the lower milk price per kg, caused by the lower fat percentage as shown in Table 3. Feed costs were much higher for these diets, because the price of the concentrate was higher per unit energy than forage in the Standard diet. This led to marked differences in "net revenue" (milk revenue – feed cost). With actual prices of feed and milk, there was a loss of 34 DKK per cow per day when the Standard diet was replaced by the Zero forage GM diet and a loss of 32 DKK when replaced with the Zero forage NonGM diet.

Table 3 Net revenue from milk production (DKK per cow per day)

	Diet					
Pct. forage (excl. straw):	50	25	0	50	25	0
Concentrate source:	Standard	GM	GM	Standard	NonGM	NonGM
Milk revenue	103	98	91	100	98	85
Feed cost	34	46	56	34	43	51
Net revenue (milk – feed)	69	52	35	66	55	24
Loss compared to standard		17	34		11	32

GM: gene modified, NonGM: non-gene modified

The actual price to produce forage varies a lot among farms and, in many farms, it is higher than the standard values from SEGES used in the calculations of feed costs. Therefore, sensitivity of net revenue to feed costs was examined. Net revenue was calculated with a doubled price per MJ to produce forage, which might be the case in extreme years like in 2018. However, even with a doubled forage price, the revenue per cow per day was still 23 and 20 DKK lower for the Zero forage GM and NonGM diets, respectively, compared to the Standard diet with 50% forage. Also prices of concentrates can vary a lot. However, even with a 50% reduction in the price for all ingredients in the concentrate, the net revenue per cow per day was still 18 and 19 DKK lower for the Zero forage GM and NonGM diets, respectively, compared to the Standard diet.

These sensitivity calculations show that even with extremely high prices for forage or low prices for concentrates, the economic net revenue is still markedly reduced when diets without forage are fed. The negative effect on ECM yield was not linear but was more severe when reducing from 25% to Zero forage than reducing from 50% to 25% forage, therefore also the loss of net revenue was largest for the reduction from 25% to Zero forage.

When considering reducing the proportion of forage in the diet due to draught or other reasons, also other factors than production and economy are important. One important factor is animal health. The large increase in propionate:acetate ratio and low fat percentage when Zero forage diets were fed strongly indicate an increased risk for acidosis. We did not have cows with clinical acidosis, but we did have more cases of cows going off feed when they were fed the NonGM diet. Probably, this problem might be more severe if cows were also fed the Zero forage diets immediately after calving. The experiment was performed in a system with free cow traffic to milking robots, and it was necessary to fetch more cows for milking in the 4 groups fed only 25% or Zero forage. This indicates a good acceptability of these

PMRs compared to the Standard PMR because cows fed the experimental diets were more reluctant to go to the robots for concentrates indicating a higher preference for the experimental diets.

Conclusions

Milk fat percentage, ECM yield and economic net revenue were reduced when Zero forage was fed irrespective of the choice of concentrate mixture. The decrease in fat percentage and increase in propionate:acetate ratio strongly indicate that there is a higher risk for rumen acidosis when diets without forage are fed. Even with extremely high prices for forage or low prices for concentrates, the economic net revenue is still markedly reduced when diets without forage are fed.

References

Harstad, O.M. & Prestløkken, E., 2018. Kan sagflis erstatte surfor til mjølkekyr? Buskap 8, 66-67. https://www.buskap.no/issue/2018/8/m-1998/Kan_sagflis_erstatte_surf%C3%B4r_til_mj%C3%B8lkekyr?

Højholdt, M., 2018. De vejledende grovfoderpriser for 2018, 2019 og 2020 er klar.

Kristensen, V.F., Weisbjerg, M.R., Børsting, C.F. Aaes, O & Nørgaard, P., 2003. Malkekoens energiforsyning og produktion. In: DJF Rapport no 54, 73 – 112. Danmarks Jordbrugsforskning. Foulum, Danmark.

Sjaunja L.O., L. Baevre., L. Junkkarinen, J. Pedersen & J. Setälä J. 1991. A Nordic proposal for an energy corrected milk (ECM) formula, in Gaillon P., Charbert Y. (Eds.), Performance recording of animals: state of the art, EAAP publication no. 50, Pudoc Wageningen, p.156-157.

The effects of microcrystalline cellulose as a dietary component for lactating dairy cows

O. Savonen[1], P. Kairenius[1], P. Mäntysaari[1], T. Stefanski[1], J. Pakkasmaa[2] & M. Rinne[1]
[1]Natural Resources Institute Finland (Luke), Tietotie 2 C, FI-31600 Jokioinen, Finland
[2]XAMK/FiberLaboratory, Vipusenkatu 10, FI-57200 Savonlinna, Finland
Correspondence: marketta.rinne@luke.fi

Introduction

Cellulose and hemicellulose that are major components of feeds for ruminants typically originate from grasses (Van Soest, 1994). However, wood and wood derived products, which also mainly consist of cellulose and hemicellulose, can be used as feed materials according to the legislation in EU (Feed Materials Register, 2019). Structurally parent cellulose has crystalline regions and one form of that is microcrystalline cellulose (MCC). It has been studied as a functional ingredient in foods and has been shown to provide positive effects on gastrointestinal physiology and for being hypolipidemic (Nsor-Atindana et al., 2017).

In order to be capable of rumen digestion, wood based feed materials must be processed. Several studies have been conducted to improve their utilization over recent decades. Pulping, steam explosion (Kaustell and Tuori, 1993) and pressurized hot water extraction (Rinne *et al.,* 2016) have shown to improve the feeding value of wood. Aalto University has developed and patented a cost-efficient method to produce MCC AaltoCell^{TM} from soft wood (Vanhatalo, 2017).

The effects of MCC on health and performance of monogastric animals has been studied to some extent. However, the authors found no published reports where MCC would have been used as a feed component for ruminants except our previous *in vitro* study (Stefanski *et al*., 2018), where some alterations of rumen fermentation were found. Thus, there is a need for *in vivo* experiments using wood based MCC. The present study was conducted to examine dairy cow responses to dietary MCC inclusion rates of 10 (as a feed additive) and 100 (as a feed material) g per kg dry matter (DM) on feed intake, rumen fermentation, diet digestion and milk production.

Materials and Methods

The production of MCC AaltoCell™ was based on two patented inventions of Aalto University (Dahl et al., 2011a, 2011b). As raw material was used unbleached softwood kraft pulp taken after the digester stage at pulp mill's chemical pulping line. Pulp was acidified to a pH level of 1.8. After that pulp was fed to a continuous digester, where it was hydrolysed at 165°C for 30 minutes. The final steps were washing the resulting MCC in order to increase pH to 3.5 and thickening with a belt thickener to a DM concentration of 270 g/kg. Experimental MCC was produced at the XAMK FiberLaboratory in Savonlinna, Finland in one production run and transported to Luke, Jokioinen for the feeding trial.

Twenty four multiparous (average parity 3.3 ± 1.13) Nordic Red cows were divided into four blocks of six cows each according to parity and calving date. The experimental treatments in the current study were: Total mixed rations (TMRs) containing no MCC (control, MCC0), MCC inclusion in diet DM of 10 g per kg diet DM (MCC10) or 100 g per kg diet DM (MCC100) (Table 1). The aim in MCC100 was to replace rolled barley with MCC, while in MCC10, MCC was given in addition to the basal diet. Crude protein (CP) concentration of the MCC100 diet was maintained by increasing the proportion of rapeseed meal and by adjusting the amounts of the other cereals. At the beginning of the trial, the cows averaged

176 ± 59.3 days in milk (DIM) and their average milk yield was 38.9 ± 5.59 kg/d. The average live weight at the beginning of the experiment was 682 ± 63.9 kg. Cows were kept in a free-stall barn and equipped with transponder collars for identification in the feeding area, milking parlour and scale.

The experimental measurements, sampling and analytical methods were the same as in the feeding trial of Savonen et al. (2018) except for rumen fluid sampling. Rumen fluid was sampled during the 3rd day in the last week of both periods from 6 cows fitted with rumen cannulas. Samples were taken between 06.00 and 16.30 h at 1.5-hour intervals.

The experimental cows were fed TMR which contained a primary growth grass silage of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) grown at Jokioinen, Finland (60˚49´N, 23˚28´E) and other components presented in Table 1. The silage was slightly pre wilted, precision chopped and preserved in a silo using a formic acid based additive (AIV 2 Plus, Eastman Chemical Company, Oulu, Finland) at a target rate of 5 l/ton. The MCC0 and MCC10 treatments had the same TMR and MCC for cows on MCC10 treatment was top-dressed onto the TMR portion twice daily. For cows on MCC100 treatment, separate TMR was prepared which included fresh MCC.

The description of calculations was reported earlier (Savonen et al., 2018). In the current study, the metabolizable energy (ME) intake was calculated in three different ways. By the basal method, ME values (MJ/kg DM) of the feeds, calculated on the basis of digestible nutrients, were multiplied by their intake. The "Corr" refers to correcting the ME intake by using the equation presented in Luke (2019) in order to account for the negative associative effects of feeding level on diet digestion. The third method to estimate ME intake was based on measured organic matter (OM) digestibility (Dig) by using AIA as an internal marker. A value of 16 MJ ME per kg organic matter digested was used.

The data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability with dietary treatment as fixed effect and cow as random effect. The model used was $Y_{ijkl} = \mu + B_i + C(B)_j + P_k + T_l + \varepsilon_{ijkl}$, where μ is the overall mean, B_i represents the block, $C(B)_j$ the cow within block, P_k the period, T_l is the treatment effect and ε_{ijkl} is the residual effect. Sums of squares for treatment effects were further divided into single degree-of-freedom contrasts to test for significance of linear (P_L) and quadratic (P_Q) effects of MCC inclusion. The contrast coefficients were adjusted to match the uneven inclusion levels on MCC. Tukey's test was used for pairwise comparisons.

Results and Discussion

The chemical composition and feed values of the experimental feeds are in Table 2. Grass silage was of good quality based on energy value (D-value 682 g/kg DM), fermentation quality (pH < 4) and ammonia N (54 g of kg total N). The neutral detergent fibre (NDF) and acid detergent fibre contents of MCC were very high but ash and CP concentrations were notably low. Also *in vitro* digestibility of MCC was low, which resulted in a low calculated energy value for it. Crude protein concentration of experimental diets decreased slightly when MCC proportion increased (171, 169 and 166 g/kg DM; P_L<0.001) while NDF concentration increased clearly with increasing MCC inclusion (375, 381 and 451 g/kg DM; P_L<0.001).

Feed intake of the cows on different treatments are in Table 3. Total DM intake was high on all treatments being on average 25.6 kg/d ± 0.40). There were no differences in feed intake,

although numerically it was highest at the highest MCC inclusion. The NDF intake increased linearly (P_L<0.001) with increasing MCC inclusion, which was expected as MCC replaced barley in the diets, but there was no difference in CP intake.

There were no differences between control *vs*. MCC inclusion of 100 g/kg DM on digestibility of DM (0.701 *vs* 0.697) or OM (0.721 *vs* 0.717) but digestibility of CP (0.678 *vs* 0.662) decreased (P<0.05) and that of NDF (0.603 *vs* 0.669) increased (P<0.001) with MCC inclusion. There was no difference between the treatments in energy intake. The total CP intake was similar among the treatments but in MCC100, much greater proportion of protein was from rapeseed meal compared to barley.

No effects on rumen pH, ammonia concentration or rumen fermentation pattern could be detected (Figure 1). Furthermore, no interactions were detected between treatment and sampling time. At 10 g/kg inclusion level, no differences in production or rumen fermentation parameters could be detected. Our hypothesis was that MCC inclusion could stabilize rumen fermentation by replacing barley starch with digestible fibre in the diet. Thus, MCC inclusion could prevent subacute rumen acidosis especially in intensive dairy production (Krause & Oetzel, 2006). However, we could not confirm this stabilizing effect. It can be speculated that such an effect could have been visible under more challenging dietary conditions and without the feeding of TMR to dairy cows.

Dietary MCC supplementation linearly decreased (PL<0.05) daily yields of energy corrected milk (ECM), fat and protein (Table 3). Milk concentrations of protein and urea also decreased (PL<0.05). The amount of energy available is the major determiner of milk production of the dairy cow (Huhtanen & Nousiainen, 2012) but the methods used to estimate ME intake in the current study showed no differences among treatments. Rapeseed meal was used to balance the CP intake from the diets so that the proportion of it was highest on MCC100. Because rapeseed protein is known for its good production responses, it is unlikely that protein intake would have limited milk production on Treatment MCC100 in comparison to the other treatments. Further, nitrogen use efficiency tended (P_L=0.062) to decrease in response to MCC inclusion.

Economic calculations were not conducted, but it is obvious that replacing barley with MCC resulting in reduced milk output would not be economically profitable. Although feed grade MCC is not commercially available, it would be more expensive than barley. In long term, other reasons for using MCC as feed component may be considered such as potential shortage and elevated prices of traditional feeds. Also growing needs to use non-human edible feed components in animal production would draw attention to wood based feed materials. Further, if rumen acidosis (subacute or acute) could be alleviated by use of MCC as a feed component, benefits in animal health and herd productivity could be gained, but that could not be demonstrated under the conditions of the current study.

Table 1 Recipes (g/kg dry matter (DM)) of the total mixed rations and the concentrate given at the milking parlour (MPC)

	Total mixed rations			MPC
	MCC0[1]	MCC10[2]	MCC100[3]	
Microcrystalline cellulose	-	10	100	-
Barley	110	109	-	310
Oats	90	89	68	-
Wheat	50	50	30	190
Sugar beet pulp	60	59	75	120
Rapeseed meal	177	175	214	355
Minerals[4]	13	13	13	25
Grass silage	500	495	500	

[1]MCC0 = Control without microcrystalline cellulose (MCC) inclusion
[2]MCC10 = diet containing 10 g MCC per kg dry DM top-dressed onto TMR twice daily
[3]MCC100 = diet containing 100 g MCC per kg DM
[4]Mineral premix (Lypsykivennäinen Tiineys+, Suomen Rehu Ltd., Hyvinkää, Finland) declared as containing Ca (210 g/kg), P (15 g/kg), Mg (90 g/kg), Na (95 g/kg), Selenium (3bE8, 20 mg/kg; 3b8.11, 10 mg/kg), Vitamin E (3a700; 2000 mg/kg) and biotin (3a880; 30 mg/kg).

Table 2 Chemical composition of the experimental feeds

	Grass silage[1]	Concentrates			
		MCC0[2], MCC10[3]	MCC100[4]	MPC[5]	MCC[6]
Dry matter (DM), g/kg	217	880	877	876	286
In DM, g/kg					
Ash	92	74	93	74	1.2
Crude protein	133	207	243	213	12.5
Crude fat	40	34	34	30	-
Neutral detergent fibre (NDF)	513	244	263	216	937
Acid detergent fibre (ADF)	301	-	-	-	892
Acid detergent lignin (ADL)	27	-	-	-	23
Hemicellulose (NDF – ADF)	212	-	-	-	45
Cellulose (ADF - ADL)	274	-	-	-	869
Indigestible NDF	76	79	97	61	-
In vitro organic matter digestibility, g/g	0.751	-	-	-	0.404
D-value, g/kg DM	682	-	-	-	404
Feed values					
ME[7], MJ/kg DM	10.9	11.8	11.8	12.2	6.5
MP[8], g/kg DM	81	127	126	123	41
PBV[9], g/kg DM	12	50	47	40	-50

[1]Silage fermentation quality: pH 3.99, ammonia N (g/kg total N) 54, lactic, acetic, propionic and butyric acid, ethanol and water soluble carbohydrates 84, 18, 1.0, 0.05, 8.8 and 58 g/kg DM.
[2]MCC0 = control without MCC inclusion
[3]MCC10 = diet containing 10 g MCC per kg dry matter
[4]MCC100 = diet containing 100 g MCC per kg dry matter
[5]MPC = milking parlour concentrate
[6]MCC = Microcrystalline cellulose
[7]ME = Metabolizable energy
[8] MP = Metabolizable protein (amino acids absorbed from the small intestine)
[9] PBV = Protein balance in the rumen

Table 3 Intake, milk production and efficiency of dairy cows fed microcrystalline cellulose (MCC)

	Proportion of MCC in the diet			SEM	Stat. significance[1]	
	0	10	100		Lin	Quad
Intake, kg/d						
Total dry matter (DM)	25.4	25.2	26.0	0.43	0.197	0.65
Silage DM	12.4	12.2	12.8	0.22	0.139	0.391
MCC DM	0	0.252	2.557	0.0400	<0.001	0.919
Total concentrate +MCC	12.9	13.0	12.2	0.22	0.273	0.963
Organic matter (OM), kg/d	23.3	23.1	23.9	0.40	0.214	0.68
Crude protein (CP), kg/d	4.33	4.25	4.32	0.073	0.863	0.469
Neutral detergent fibre, kg/d	9.52[b]	9.59[b]	11.7[a]	0.187	<0.001	0.574
Metabolizable energy (ME) intake						
MJ ME (Not corrected)	291	287	280	4.8	0.146	0.698
MJ ME (Corr[2])	270	267	263	4.1	0.018	0.434
MJ ME (Dig[3])	294	291	299	4.7	0.317	0.564
Metabolizable protein (g/d)	2563	2526	2541	42.8	0.897	0.57
Protein balance in the rumen (g/d)	636[ab]	611[b]	670[a]	11.3	0.006	0.092
Production per day						
Milk (kg)	34.7	35.6	34.7	0.40	0.074	0.885
Energy corrected milk (ECM; kg)	36.8	36.6	35.3	0.45	0.024	0.977
Fat (g)	1499	1500	1442	20.2	0.039	0.818
Protein (g)	1290	1290	1231	15.5	0.008	0.783
Lactose (g)	1575	1548	1518	20.2	0.094	0.477
Milk composition (g/kg)						
Fat	42.0	42.3	41.8	0.45	0.615	0.703
Protein	36.2	36.5	35.7	0.13	0.003	0.129
Lactose	44.0	43.5	43.7	0.02	0.554	0.072
Total solids	13.3	13.3	13.2	0.50	0.117	0.654
Urea (mg/100ml)	25.3	25.3	23.6	0.50	0.015	0.888
Efficiency of milk production						
Nitrogen use efficiency[2]	0.297	0.304	0.286	0.0054	0.062	0.329
ECM/ME intake (Corr[3]) (kg/kg)	0.136	0.167	0.135	0.0022	0.517	0.650
ECM/ME intake (Dig[4]) (kg/kg)	0.125[ab]	0.126[a]	0.119[b]	0.0019	0.010	0.519
ECM/DM intake (kg/kg)	1.45[ab]	1.46[a]	1.36[b]	0.025	0.010	0.604

[1]Significance of quadratic (Quad) and linear (Lin) effects of MCC inclusion in diet
[2]Nitrogen use efficiency = (kg N in milk / kg N intake)
[3]Corr refers to the correction equation presented in Luke (2018)
[4]Dig refers to MEI calculated from digestibility measured individually for each cow using AIA (mean of MCC0 used for cows fed MCC10).

Conclusions

This study demonstrated that MCC can be included in dairy cow diets and that it is readily consumed. However, it decreased milk production and no beneficial effects on ruminal digestion could be demonstrated under the conditions of the current experiment.

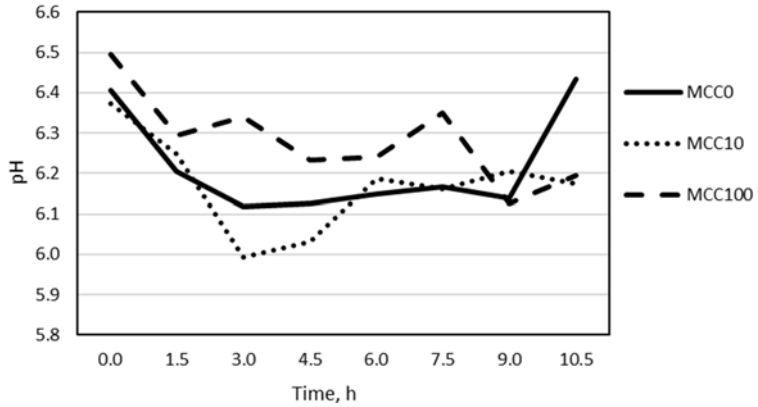


Figure 1 Ruminal pH of cows fed microcrystalline cellulose at 0 (MCC0), 10 (MCC10) or 100 (MCC100) g/kg diet dry matter measured over the day (first sampling at 6 am). There were no statistical differences in diet or diet × sampling time interaction.

References

Dahl, O., Vanhatalo K. & Parviainen, K., 2011a. A novel method to produce microcellulose. FI patent 2011050526.

Dahl, O., Vanhatalo, K., Parviainen, K. & Svedman, M., 2011b. A novel method to produce microcellulose. FI patent 2011050527.

Huhtanen, P. & Nousiainen, J., 2012. Production responses of lactating dairy cows fed silage-based diets to changes in nutrient supply. Livest. Sci. 148, 146–58.

Krause, K.M. & Oetzel, G.R., 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim. Feed Sci. Technol. 126, 215-236.

Kaustell, K. & Tuori, M., 1993. The effect of steam explosed birch wood to milk yield and composition and energy balance of dairy cows given grass silage ad libitum. World Conf. Animal Prod., Edmonton, Canada. p. 348-349.

Luke, 2019. Feed tables and Nutrient Requirements. Available at: www.luke.fi/feedtables.

Nsor-Atindana, J., Chen, M., Goff, H. D., Zhong, F., Sharif, H. R. & Li, Y., 2017. Functionality and nutritional aspects of microcrystalline cellulose in food. Carbohyd. Polym. 172, 159–174.

Rinne, M., Kautto, O., Kuoppala, K., Ahvenjärvi, S., Willför, S., Kitunen, V., Ilvesniemi, H. & Sormunen-Cristian, R., 2016. Digestion of wood-based hemicellulose extracts as screened by in vitro gas production method and verified in vivo using sheep. Agric. Food Sci. 25, 13-21. Available at: http://ojs.tsv.fi/index.php/AFS/article/view/46502.

Savonen, O., Franco, M., Stefanski, T., Mäntysaari, P., Kuoppala, K. & Rinne, M., 2018. Grass silage for biorefinery – dairy cow responces to diets based on solid fraction of grass silage. Proc. 9th Nordic Feed Sci. Conf., Uppsala, Sweden. 12-13 June 2018. SLU, HUV Report 298, p. 55-60.

Stefanski, T, Välimaa, A.-L., Kuoppala, K., Jalava, T., Paananen, P & Rinne. M., 2018. In vitro ruminal digestion and methane production of different products of microcrystalline cellulose. Proc. 9th Nordic Feed Sci. Conf., Uppsala, Sweden. 12-13 June 2018. SLU, HUV Report 298, p. 153-158.

Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant. Cornell university Press. New York, USA, 476 pp.

Vanhatalo, K., 2017. A new manufacturing process for microcrystalline cellulose (MCC). Aalto University publication series Doctoral Dissertations, 152/2017.

A method for measuring energy content in compound feeds in the NorFor system.

Álvarez C.[1,2]; Nielsen N.I.[3], Weisbjerg M.R.[4]
[1]Norwegian University of Life Sciences, Department of Animal and Aquaculture Sciences.[2] TINE SA, Langbakken 20, 1430 Ås, Norway. [3]Livestock Innovation, SEGES, 8200 Aarhus N, Denmark, [4]Department of Animal Science, AU Foulum, Aarhus University, 8830 Tjele, Denmark
Correspondence: clementina.alvarez@tine.no

Introduction

Net energy of lactation (NEL) is the energy expression used in the NorFor Feeding system for energy concentration of feeds and requirements of the dairy cow. Feedstuffs do not have a fixed NEL value, because NorFor takes into consideration interactions among feedstuffs in the ration and feed intake level. However, for trading purposes and feedstuff selection, comparison among energy levels is essential (Åkerlind & Volden, 2011). For this, standard feed values were created in NorFor. For NEL, net energy of lactation at 20 kg of dry matter intake (NEL20) is the most used standard value. NEL20 has standardized fixed values for input parameters, as for example dry matter intake (20 kg DM), concentrate proportion of the diet (50%), and passage rates for crude protein, starch and neutral detergent fibre.

A challenge in the estimation of NEL20, is that degradation rates for each feedstuff are required for the calculation. These degradation rates are obtained for each feedstuff through the in sacco technique, converting it into an expensive and time-consuming procedure. Although degradation rates and indigestible fractions of nutrients are known (table values) for ingredients, these values are unknown for commercial compound feeds. For these types of feedstuffs, NEL20 is calculated by the sum of the NEL20 of the ingredients weighted by the proportion in the mixture. The main issue is that at present it is, in the industry, unlikely to measure NEL20 concentration directly in compound feeds. Therefore, an alternative calculation of NEL20 for compound feedstuffs, replacing the use of degradation rates and indigestible fractions, will be of great use when the ingredients and compound compositions are not available for NEL estimation.

The hypothesis of this study was that organic matter digestibility (OMD) of compound feeds is a good predictor of NEL20.Therefore, the objective was to develop a model to estimate NEL20 directly from compound feeds, using OMD and chemical composition of the feed to estimate NEL20.

Materials and Methods

A total of 118 feed samples were collected from feed companies in Denmark, Sweden and Norway. Of these, 49 were compound samples and 69 ingredients of these compound feedstuffs. Compound samples had a known composition of ingredients. All samples were analysed for organic matter digestibility (OMD), measured by the EFOS method developed by Weisbjerg and Hvelplund (1993). Further, all samples were analysed for dry matter (DM), ash, crude fat (CFat) according to EU152/2009 (European Commission, 2009), crude protein (CP) by the Dumas method (Dumas, 1831), neutral detergent fibre (NDF) by the aNDF method (Mertens, 2002) and starch (ST) as suggested by spectrophotometric method (NorFor, 2007). A summary of the composition of all samples are in Table 1.

Net energy of lactation at 20 kg of DM (NEL20) values for the compound feed samples were calculated according to NorFor, by adding the NEL20 values of each ingredient according to

their proportion in the mixture. In order to calculate NEL20 for ingredients, table values, based on experimental data, were used for degradation rates and indigestible fractions (NorFor, 2018). Summary of the NEL20 values for all samples are also in Table 1.

Table 1 Variation in chemical composition and energy content in compound feeds (mix) and ingredients

Type	N	DM %	DOM %DM	Ash %DM	NDF %DM	CFat %DM	CP %DM	ST %DM	NEL20 MJ/kg DM
Mix	49	84.1-89.8 (88.2)	70.6-91.9 (82.6)	4.2-14.4 (7.3)	13.4-36.2 (23.1)	3.5-12.5 (6.0)	16.3-42.7 (24.0)	1.6-46.9 (22.0)	6.1-8.6 (7.2)
Ingre-dients	69	82.4-99.8 (89.2)	45.7-94.4 (78.1)	0.2-26.5 (5.3)	0-71.6 (25.8)	0.98-98.9 (9.1)	0.1-66.7 (22.8)	0.5-72.6 (22.4)	4.1-20.3 (7.5)

N: number of samples, DM: percentage of DM, DOM: Digestible organic matter (%DM) measured by EFOS, NDF: Nutrient detergent fibre (%DM), CFat: Crude fat (%DM), ST: Starch (% DM), CP: Crude Protein (%DM), NEL20: Net energy of lactation at 20 kg DMI (MJ/kg DM), calculated by weighed sum of NEL20 of ingredients. () mean values.

Model pre-selection was performed by selecting the variables presented in Table 1 (DM was not included in the selection) through stepwise forward selection by Akaike information criterion (AIC) using 'stepAIC' function from MASS package (Venables and Ripley, 2002) using R software (R Core Team, 2012). Models were created with feed company as random effect, resulting in linear mixed models analysed through lme4 package (Bates et al., 2015). AIC measures the quality of the model for a given set of data, evaluating the goodness of fit while considering overfitting by penalizing for the inclusion of more parameters.

Models were evaluated by multicollinearity with variance inflation factor (VIF) (Zuur et al., 2010). If VIF higher than 5 were recorded, the variable with highest VIF was discarded until VIF for all variables met this criterion. Standard regression and residual analysis were used to compare and evaluate models for linear and mean bias. Residual plots centered the predicted values of each model by the mean to make them slope and intercept independent to evaluate linear and mean bias (St-Pierre, 2003). Root mean square error of prediction (RMSEP) was used as error index for comparison of models. The RSR (RMSEP-Standard deviation of observations Ratio) was also calculated and selected as a dimensionless index. This indicator is a normalized statistic index that quantifies the relative magnitude of the residual variance compared to variance of the observed values. Moreover, comparison was also done by AIC (Zuur et al., 2009).

Results and Discussion

Compound feed samples displayed a large variation in NEL20 values (standard deviation of 0.54 MJ/ kg DM). This is of great importance, not only for modelling but also because these samples were a representation of variability in the market.
Table 2 shows the summary of the preselected models and Table 3 shows the estimated coefficients and standard errors. Four models were pre-selected. Model 1 included the first two variables included by the stepwise forward procedure - NDF and CFat. Model 2, also included digestible organic matter (DOM; % of DM) and in Model 3, ST was added to Model 2. These additions were based on the stepwise forward ranking. Based on the high and negative correlation between ST and CP (-0.77), Model 4 was created by including CP and excluding ST. Moreover, CP is more commonly analysed in compound feeds compared to ST, which supports the creation of Model 4.

Table 2 Statistical evaluation of the preselected models to predict NEL20 of compound feeds and ingredients

Model	Equation	Std Error[1]	RMSEP[2]	RSR[3]	AIC[4]
1	NEL20 = NDF + CFat	0.20	0.19	0.35	13.90
2	NEL20 = NDF + CFat + DOM	0.16	0.15	0.27	1.63
3	NEL20 = NDF + CFat + DOM + ST	0.14	0.13	0.24	2.87
4	NEL20 = NDF + CFat + DOM + CP	0.15	0.14	0.25	5.91

NEL20: NEL20 reference value of the mixes (MJ/kg DM), DOM: Digestible organic matter (% in DM) measured by EFOS, CFat: Crude fat (%DM), NDF: Nutrient detergent fiber (%DM), ST: Starch (%DM), CP: Crude Protein (%DM), Ash (%DM).
[1]Standard error of the models, [2]Root mean square error of prediction, [3]RMSEP/sd observed values, [4]Akaike information criterion by maximum likelihood.

Table 3 Coefficient estimates (Standard Errors) for four models predicting NEL20 of 49 compound feeds

Variable	Model			
	1	2	3	4
Fixed effects				
Intercept	7.56 (0.18)	3.29 (0.83)	2.52 (0.78)	2.20 (0.88)
NDF	-0.07 (0.01)	-0.05 (0.01)	-0.06 (0.01)	-0.05 (0.01)
CFat	0.17 (0.01)	0.16 (0.01)	0.13 (0.02)	0.13 (0.02)
DOM	--	0.05 (0.01)	0.07 (0.01)	0.06 (0.01)
ST	--	--	-0.01 (0.003)	--
CP	--	--	--	0.01 (0.005)
Random effect (Feed company)				
Standard deviation	0.17	0.15	0.14	0.13

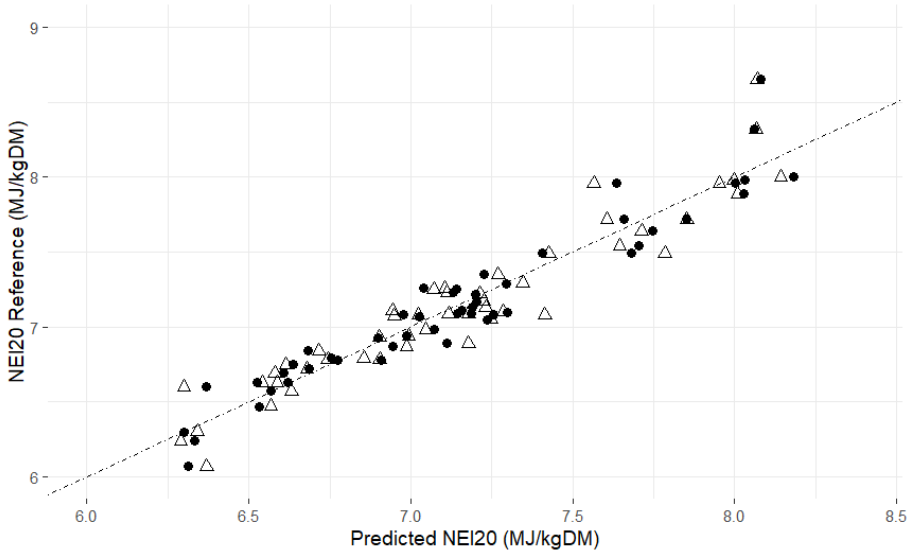
DOM: Digestible organic matter (% DM) measured by EFOS, CFat=Crude fat (% DM), NDF= Nutrient detergent fiber (%DM), ST= Starch (%DM), CP= Crude Protein (%DM), Ash (%DM).

Table 2 shows an RMSEP and RSR lower for Model 2 than Model 1, signifying a better prediction capacity for Model 2. This indicates an improvement of the prediction by including OMD (as DOM) in the prediction of NEL20.

Model 3 showed the lowest RMSEP and RSR, indicating a better prediction ability. However, Model 4 resulted in the second best performance. Observed vs. predicted plot and standard regressions for Model 3 and Model 4 are in Figure 1. Both models show similar dispersion along the X=Y line with slopes = 1, which differed from 0 and with an intercept not different from 0 ($P<0.05$). Residual plots and residual regressions for both models are in Figure 2. Residuals for both models seemed to have a random dispersion along the horizontal line, supporting the assumption of homoscedasticity. Residual regressions for both models had slopes and intercepts not different from zero. This means that both models have no linear or mean bias. Although these are desirable characteristics in models, no decision can be made with respect to model superiority. However, the better prediction indicators together with a lower AIC, it suggests a better predicting ability of Model 3 than Model 4.

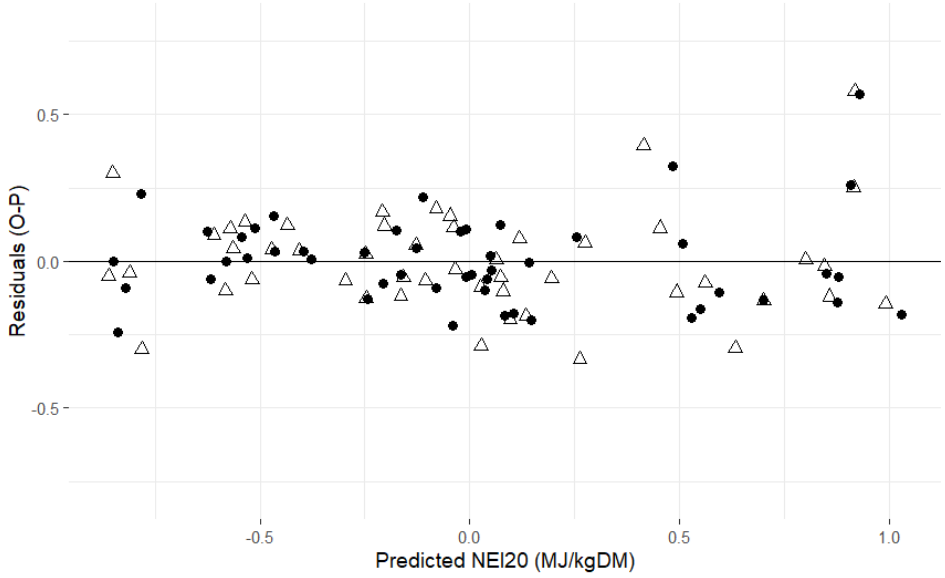
The error for future prediction (calculated as percentage of the average NEL20) was estimated to be a maximum of 3.6 and 3.9% for Model 3 and 4, respectively. These limits are lower than the suggested 4% latitude in Denmark using the Scandinavian Feed Unit System (Fødevarestyrelsen, 2018).

Despite results indicating a better performance of Model 3, Table 3 shows that ST had a negative effect (negative coefficient) on NEL20 for Model 3, indicating a reduction of NEL20 with increase of ST. This is contrary to what is expected, as ST, together with the



• Model 3: NEL20= DOM + CFat + NDF + ST.
Δ Model 4: NEL20= DOM + CFat + NDF + CP
- - - - X=Y

Figure 1 Observed vs. predicted scatter plots of the two models predicting NEL20 for compound feeds.



• Model 3: NEL20= DOM + CFat + NDF + ST.
Δ Model 4: NEL20= DOM + CFat + NDF + CP
—— Predicted=Observed, residual=0

Figure 2 Residual scatterplot of the two models predicting NEL20 for compound feeds. Predicted values in the x axis are centered around the mean.

other carbohydrates, is a source of rapidly available energy, although with lower value than protein and fat. It is difficult to state a reason for the negative effect of ST. A possible explanation could be the high negative correlation between ST and CP already mentioned.

It is important to point out that while Model 3 had better performance indicators, Model 4 includes a coefficient for CP with the expected direction. Also, the inclusion of CP in Model 4 agrees with the higher impact of CP on NEL20. Moreover, the practicality of analysing CP instead of ST makes the Model 4 useful.

Conclusions

The inclusion of OMD expressed as DOM (predicted by EFOS) resulted in improvement of the prediction of NEL20 in compound feeds. A model including DOM, NDF, CFat and ST predicted energy content (NEL20) in compound feed the best. However, the model including DOM, NDF, CFat and CP fits expectations and practical use better without a substantial reduction of the prediction accuracy. Both models show lower prediction errors as what has been previously reported by other energy estimation models for compound feeds.

References

European Commission, 2009. No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed.
Bates, D., Mächler, M., Bolker, B. & Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software; Vol 1, Issue 1.
Dumas, J., 1831. Procedes de l'analyse organique. Ann. Chim. Phys, 247, 198-205.
Fødevarestyrelsen., 2018. Tolerancer for hovednæringsstoffer og makromineraler i fodermidler og foderblandinger. Markedsføringsforordningen, bilag IV, del A, jf. ændringsforordning 2017/2279 til markedsføringsforordningen.
Mertens, D. R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J AOAC Int, 85, 1217-1240.
NorFor., 2007. Starch analysis by enzymatic colorimetric method. Accesed: 30 March 2019. http://www.norfor.info/files/pdf-dokumenter/pdf_lab/analyses/starch_method_spectrophoto.pdf
St-Pierre, N. R., 2003. Reassessment of biases in predicted nitrogen flows to the duodenum by NRC 2001. J. Dairy Sci., 86, 344-350. doi:https://doi.org/10.3168/jds.S0022-0302(03)73612-1
Venables, W. N. & Ripley, B. D., 2002. Modern Applied Statistics with S (Fourth ed.). New York: Springer. Accesed: 4 April 2019. http://www.stats.ox.ac.uk/pub/MASS4
NorFor. 2018. NorFor Feed Ration Optimizer 2018.13.0.217 ed. NorFor, Denmark.
Weisbjerg, M. R. & Hvelplund, T., 1993. Bestemmelse af nettoenergiindhold (FEK) i råvarer og kraftfoderblandinger. Forskiningsrapport nr. 3. Statens Husdyrbrugsforsøg. Denmark, 39 pp.
Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A. & Smith, G. M., 2009. Mixed Effects Models and Extensions in Ecology with R: Springer New York. doi: 10.1007/978-0-387-87458-6
Zuur, A. F., Ieno, E. N. & Elphick, C. S., 2010. A protocol for data exploration to avoid common statistical problems. Meth. Ecol. Evol., 1, 3-14. doi:10.1111/j.2041-210X.2009.00001.x
Åkerlind, M. & Volden, H., 2011. Standard feed value. In: Volden, H. (Ed.), NorFor-The Nordic Feed Evaluation System, EAAP Publ. no. 130, Wageningen Acad. Publ., Wageningen, p. 41-54. https://doi.org/10.3920/978-90-8686-718-9

How to use NorFor feed values when analysing and reporting experimental results?

M.R. Weisbjerg[1], M.Ø. Kristensen[2], N.I. Nielsen[2], T. Kristensen[1], M. Johansen[1], P. Lund[1] & M. Larsen[1]
[1]*Aarhus University, Department of Animal Science, AU Foulum, 8830 Tjele, Denmark.*
[2]*SEGES, Agro Food Park 15, 8200 Aarhus N, Denmark.*
Correspondence: Martin.weisbjerg@anis.au.dk

Introduction

Calculated energy intake is by tradition typically reported when publishing results from production trials with cattle, which also allows assessment of energy efficiency. Calculation of energy intake is relatively simple and straightforward when classical additive energy systems are used; however, the calculation is certainly not trivial with recent non-additive systems as e.g. NorFor (Volden, 2011) and INRA (2018).

Energy intakes and efficiencies based on non-additive systems should better reflect what animals truly experience. However, a number of problems arise when using non-additive systems on observed and individual data. Therefore, in a non-additive system it is important to consider and decide which 'level' to use for feed value calculations when reporting experimental production results. The aim of this paper is to give some examples of problems and dilemmas, and to open for a discussion on how to calculate and present non-additive feed values from NorFor.

Materials and Methods

Data used as examples in this paper are from experiments performed at AU Foulum. Data presented in Table 1 are from experiments performed in late nineties (Kristensen et al., 2003), and comprise two repeated randomized block design experiments, each with 63 cows. In each experiment, there were 7 cows on each of 9 treatments (rations), which were combinations of 3 forages and 3 concentrate:forage ratios for each forage. In one experiment, 3 out of 7 cows and in the other, 1 out of 7 cows per treatment were primiparous. The two experiments were similar, but conducted in two different years whereby different feeds were used. NorFor calculations were made using 'Development tool' by Jensen et al. (2012; 2015), and therefore, NorFor calculations were based on the NorFor model 2012.

Data presented in Table 2 are from Alstrup et al. (2016). The full experiment was two 4x4 Latin squares with 24 cows performed in 2009-2010. The treatments (rations) consisted of 4 types of grass-clover silage combined with two different concentrate:forage ratios (50 and 80 % forage on dry matter (DM) basis). Forage consisted of 33% maize silage and 66% of the above mentioned grass-clover silages on DM basis. Only results from multiparous cows fed the rations with 80% forage (n = 8) are presented and used in the present study. NorFor values were calculated using the Development Tool version 1.93.

Examples

In Table 1, examples based on the Kristensen et al. (2003) experiment are shown. Data for NEL, AAT and PBV per kg of DM intake using either individual cow input data or based on average group data are presented per parity (first or older) and for the whole experimental period. Data in Table 1 shows that averages per treatment are very robust, and the differences between 'individual' and 'group' average are minimal. However, the variation is also given as min-max and as deviance between max and min in % of average (Table 1). This reveals that the variation in most situations was much higher for 'individual' vs. 'group'.

Table 1 Average values for NEL, AAT and PBV per kg of DM intake when based on individual cow data (per animal whole exp. period) or group data (per parity (first or older) whole exp. period). Beside averages, also min-max and deviance between max and min in % of average are given in brackets. For individual input data this is min-max for 14 individual cows of different parities in two experiments (N=14). For input data averaged per parity, this is based on 4 groups consisting of 2 parities in two experiments (N=4). Based on experimental data (Kristensen et al., 2003) from a repeated production trial with nine treatments (3 forage types x 3 Concentrate:Forage (C:F) ratios)

	NEL MJ /kg DM		g AAT/kg DM		g PBV/kg DM	
C:F ratio	Individual input data	Input data averaged per parity	Individual input data	Input data averaged per parity	Individual input data	Input data averaged per parity
			Grass/clover silage			
Low	6.41 (6.21-6.61; 6.2%)	6.40 (6.28-6.56; 4.34%)	96.0 (78.5-119; 42.2%)	95.7 (92.8-99.3; 6.8%)	55.7 (25.7-93.0; 120.8%)	55.3 (28.3-84.9; 102.4%)
Medium	6.56 (6.39-6.76; 5.6%)	6.57 (6.43-6.71; 4.3%)	104.6 (93.6-122; 27.2%)	104.0 (99.4-104; 4.4%)	47.2 (24.8-105; 169.9%)	47.8 (28.9-75.6; 97.7%)
High	6.74 (6.53-7.08; 8.2%)	6.72 (6.54-6.96; 6.3%)	117.1 (95.2-150; 46.8%)	117.1 (108-128; 17.1%)	42.5 (25.8-57.7; 75.1%)	41.0 (29.7-58.5; 70.7%)
			Barley whole crop silage			
Low	6.13 (6.06-6.21; 2.5%	6.11 (6.06-6.24; 3.78%)	87.6 (72.9-100; 30.9%)	87.6 (78.6-99.4; 23.7%)	30.7 (23.7-36.2; 40.7%)	29.6 (25.0-37.7; 43.9%)
Medium	6.37 (6.24-6.54; 4.8%)	6.39 (6.31-6.56; 3.9%)	98.6 (87.1-117; 30.3%)	97.9 (89.2-101; 12.1%)	28.2 (22.8-34.7; 42.2%)	28.9 (24.5-41.2; 57.8%)
High	6.64 (6.50-6.88; 5.7%)	6.62 (6.54-6.74; 3.0%)	115.9 (100-140; 25.9%)	115.5 (108-137; 25.1%)	23.1 (15.3-35.9; 89.2%)	21.8 (18.1-28.7; 48.6%)
			NH_3 straw			
Low	5.96 (5.81-6.16; 5.9%)	5.94 (5.84-6.07; 3.9%)	98.3 (90.0-107; 17.3%)	98.5 (95.7-105; 9.4%)	4.87 (0.47-13.4; 265.5%)	4.07 (2.20-10.3; 199.0%)
Medium	6.26 (6.00-6.46; 7.3%)	6.24 (6.10-6.43; 5.3%)	103.9 (89.8-115; 24.3)	104.0 (97.3-111; 13.2%)	13.9 (0.66-22.6; 157.8%)	12.6 (0.66-21.6; 166.2%)
High	6.52 (6.28-6.77; 7.5%)	6.51 (6.40-6.72; 4.9%)	112.5 (87.0-131; 39.1%)	112.6 (106-117; 9.8%)	21.3 (7.63-35.2; 129.4%)	20.6 (14.9-32.1; 83.5%)

In Table 2, examples based on Alstrup et al. (2016) are shown. Selected data are for older cows fed 80% forage. Also for this dataset, averages per treatment are very robust to group vs. individual calculations, but there is an enormous variation among individuals in the feed values per kg DM as evidenced by the min and max values.

Table 2 Data from Alstrup et al. (2016) for older cows fed 80% grass-clover silage from early spring (ESP), late spring (LSP), early summer (ESU) and late summer cuts (LSU). Calculation on group (Group) and individual (Ind.) basis with individual minimum (Min) and maximum (Max) data shown

Treatment	N		NEL MJ /kg DM	g AAT/kg DM	g PBV/kg DM
ESP	1	Group	6.58	97.7	36.1
	8	Ind.	6.58	97.8	36.0
		Min	6.5	95.0	31.7
		Max	6.66	100.8	40.0
LSP	1	Group	6.20	93.9	22.0
	8	Ind.	6.21	93.9	21.9
		Min	6.10	89.8	16.9
		Max	6.35	97.4	27.9
ESU	1	Group	6.35	96.4	80.9
	8	Ind.	6.35	96.4	80.8
		Min	6.27	92.1	75.8
		Max	6.48	99.8	87.1
LSU	1	Group	6.18	98.1	62.8
	8	Ind.	6.18	98.1	62.8
		Min	6.06	94.3	56.8
		Max	6.30	102.5	68.0

Discussion

In classical feed/energy evaluation systems, a feed will have a unique and additive energy value (Weisbjerg et al., 2010), but net energy (NE) can possible differ between different production types (e.g. milk production vs. growth). In the non-additive system NorFor, the energy value is affected by (in the case of dairy cows) live weight, dry matter (DM) intake, and ration composition. The effect of ration composition is an extra challenge if cows are not fed TMR, as concentrate:forage ratios will vary from day to day and among cows when fed forage ad libitum together with a fixed amount of concentrate. In the equations in the NorFor model (Volden, 2011), live weight affects efficiency of microbial protein synthesis in the rumen (eq. 7.28) and passage rates (eq. 7.1, 7.2, 7.3, 7.5). Dry matter intake also affects passage rates (eq. 7.1, 7.2, 7.3, 7.4, 7.5). Ration and nutrient composition affect passage rates (eq. 7.1, 7.2, 7.3, 7.4, 7.5), rumen load index (eq. 7.15), efficiency of microbial protein synthesis in the rumen (eq. 7.28), and ration fill (eq. 6.10, 6.11).

Therefore, in a non-additive system it is important to consider and decide which 'level' to use for feed value calculations when reporting experimental production results. Examples of grouping on animal level could be per individual animal, per parity, per breed. Examples on grouping over time could be per day, per week, or for the whole exp. period. An alternative could be to use planned feed values from feed plan, or standard values (e.g. NEL20, AAT20 and PBV20). Use of standard values for feeds in the ration (NEL20, AAT20, PBV20) would be equal to a classical additive system and would therefore be easy. Use of standard values means that the procedure can be documented (by date of access to the NorFor feed table and/or the NorFor Feed Ration Optimizer). Also, use of planned ration energy values from the feed plan would be an easy solution, but difficult to document and not very scientific, as planned feed intake probably would not be identical to observed feed intake when animals are fed ad libitum. Scientific sound solutions would be to use observed feed intake and analysed feed/ration composition to calculate more 'correct' NorFor feed values. However, this requires a severe workload calculating the energy values on the desired level (individual or

group, and eventually on day or week basis). In the examples in the present study, grouping was for the whole exp. (Table 1) or the whole exp. period (Table 2), and in both cases per parity.

Whether live weight changes should be included in calculations is questionable. Live weight changes during exp. period is not expected to affect the frame of the cow and thereby 'physical gastrointestinal' size which could affect e.g. passage rate as modelled in Norfor. Therefore, live weight changes during the experiment should not be included in feed value calculations. However, for growing animals including primiparous cows, inclusion of live weight changes per experimental period could be relevant for studies running for a time span long enough for the animal to increase gastrointestinal size. Furthermore, if the Danish version (DMS) of NorFor Feed Ration Optimizer is used, live weight cannot be included at present, whereas it can be included in the international NorFor Feed Ration Optimizer and in Development Tool. In the examples presented in the present study, live weight changes during the study has not been included.

For fill, standard values can differ considerable from ration values, and therefore fill values for feed intake prediction might present an even bigger challenge than energy and protein values. However, this has not been evaluated in the present study.

A further consideration is, whether calculations at individual animal level is appropriate for a model like NorFor parameterised generally using group data (treatment means). Jensen et al. (2012) showed that use of individual animal data for estimation of response functions resulted in considerably different response functions compared to use of group data.

The examples from Table 1 and 2 shows that feed value (NEL, AAT, PBV) calculations as treatments averages seems robust to whether input data is on an 'individual' or 'group' basis. Therefore, average treatment values will probably only to a minor and practically insignificant level be affected by method used for feed value calculation (individual or group). However, the much higher variation means that statistics (variation and statistical significance) will be very dependent on method. Therefore, when reporting results from experiments, calculations and statistical evaluations of treatment effects on e.g. energy, AAT and PBV intake and efficiency estimates could be heavily affected by method applied.

Conclusions

Average feed values seem robust to whether input data are on individual or group basis, but variation and statistical significance would be affected by method used. The challenges of reporting feed values from non-additive systems should be examined using more experimental data and performing statistical analyses of treatment effects on obtained feed values. Beside energy and protein feed values, also effects on e.g. energy efficiencies (milk energy per feed energy) and on feed intake estimations (Fill) should be examined for effects of evaluation 'approach' on the statistical outcome.

Furthermore, parameterisation of the NorFor model is based on group data, this further requires a discussion on whether it is appropriate to subsequently calculate feed values on individual animal levels.

References

Alstrup, L., Søegaard, K. & Weisbjerg, M.R., 2016. Effect of maturity and harvest season of grass-clover silage and of forage-to-concentrate ratio on milk production of dairy cows: doi:10.3168/jds.2015-9802. J. Dairy Sci. 99, 328-340.

Jensen, C., Weisbjerg, M.R. & Østergaard, S., 2012. Evaluation of individual versus group level observations and different feed ration evaluation systems for estimating milk yield responses. Summaries of communications. Proc. 2012 Meeting of the Animal Science Modelling Group. Can. J. Anim. Sci. 92, 562-563.
Jensen, C., Østergaard, S., Schei, I., Bertilsson, J. & Weisbjerg, M.R., 2015. A meta-analysis of milk production responses to increased net energy intake in Scandinavian dairy cows. http://dx.doi.org/10.1016/j.livsci.2015.02.009. Livst. Sci. 175, 59-69.
INRA. 2018. INRA feeding system for ruminants. Institut National de la Recherche Agronomique (INRA). Wageningen Academic Publishers, Wageningen, The Netherlands, 640 pp.
Kristensen, V.F., Weisbjerg, M.R., Børsting, C.F., Aaes, O. & Nørgaard, P., 2003. Malkekoens energiforsyning og produktion. I: Kvægets ernæring og fysiologi. Bind 2 – Fodring og produktion. Ed.: F. Strudsholm & K. Sejrsen. DJF rapport nr. 54. 73-112.
Volden, H., 2011. NorFor – The Nordic feed evaluation system. EAAP publication No. 130. Wageningen Acedemic Publishers, 180 pp.
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. In: Udén, P., Eriksson, T., Müller & C.E., Liljeholm, M. (Eds.), Proc. 1st Nordic Feed Science Conference, Uppsala, Sweden, 22-23 June 2010. Rapport 274, SLU, p. 51-64.

Evaluation of NorFor's prediction of neutral detergent fibre digestibility in dairy cows

M. Åkerlind[1] & N. I. Nielsen[2]
[1]Växa Sverige, Box 288, 751 05 Uppsala, Sweden. [2] SEGES, Landbrug & Fødevarer F.m.b.A., Agro Food Park 15, DK 8200 Aarhus N, Denmark.
Correspondence: maria.akerlind@vxa.se

Introduction

Neutral detergent fibre (NDF) often comprises 30 to 40% of dry matter (DM) in Nordic diets to dairy cows and digested NDF is an important part of energy supply. Both NDF digestibility and its contribution to energy are predicted in the NorFor system (Volden, 2011a). Rumen degraded NDF is one of the most important contributors to energy supply and substrate to rumen microbial protein synthesis. It is the nutrient fraction that shows the largest variation in digestibility and, therefore, NDF digestibility is difficult to predict compared to e.g. starch and crude protein. Since NDF digestibility varies, it is important that feed evaluation systems can predict this variation.

The digestion model of NorFor is based on the AAT model by Volden (2001) and is therefore primarily based on older Norwegian digestion trials. The aim was to evaluate the NorFor digestion model of NDF on a broader basis of digestion trials and identify areas for improvements.

Material and methods

The dataset comprised 212 treatment means from 29 studies where total diet digestibility of NDF (NDFD) had been measured using different markers (see Table 1). The studies had been performed in Denmark (n=5), Finland (n=13), Norway (n=1), Sweden (n=8) and UK (n=1) More information of the studies is in Table 1.

Study criterion beyond NDFD were also determination of organic matter digestibility (OMD) of the forages, which is a central input to the NorFor model. The OMD had been determined by four different methods: OMD *in vivo* by sheep used in trials from Norway, UK and Finland or estimated from either enzymatic and or *in vitro* methods, where D-value (digestible OM in the DM) was used in Finland (Nousiainen *et al*., 2003), IVOS (in vitro OMD) in Denmark (Møller *et al*., 1989) or VOS (in vitro OMD) in Sweden (Lindgren, 1979). For further details on these methods, see Table 1 footnotes.

Forage indigestible NDF (iNDF) was estimated according to Equation 1 and 2 below, and potentially degradable NDF was calculated according to Equation 3. Degradation rate of potentially degradable NDF (kdNDF) was estimated from OMD according to equation 6.9 in the NorFor book (Volden, 2011b), where Equation 3 was used instead of equation 6.7. The non-linear rumen passage rate for forage NDF (rkpNDFr) in the NorFor model (Equation 7.5; Volden & Larsen, 2011) is shown below as it is an important equation for the estimated NDFD in NorFor.

These equations were used for the evaluation:

iNDF was calculated for forages including more than 50% legume plants as:

$$iNDF = \frac{940 - 10.6 \cdot OMD - 0.517 \cdot Ash}{NDF} \cdot 1000 \qquad \text{Equation 1}$$

iNDF was calculated for forages including less than 50% legume plants as:

$$iNDF = \frac{506 - 5.60 \cdot OMD - 0.519 \cdot Ash}{NDF} \cdot 1000 \qquad \text{Equation 2}$$

$$pdNDF = 1000 - iNDF \qquad \text{Equation 3}$$

$$rkpNDFr = 0.480339 + \frac{0.78 \cdot 1.93668}{1+\left(\frac{NDFI}{BW \cdot 7.48383}\right)^{-3.19822}}$$ Equation 7.5

, where iNDF is indigestible NDF (g/kg NDF), OMD is organic matter digestibility *in vivo* (% of OM) from Equations 5.12 to 5.18 (Åkerlind *et al.*, 2011), Ash is ash content (g/kg DM) and NDF is NDF content (g/kg DM), pdNDF is the potentially degraded NDF (g/kg NDF); rkpNDFr is the rumen passage coefficient of potentially degraded NDF in roughage (%/h); NDFI is the NDF intake (g/day) and BW is the animal's body weight (kg)

Input of feed characteristics, in addition to data presented in the articles, such as rumen degradation rate of crude protein, indigestible crude protein, indigestible starch, concentrate iNDF and kdNDF were taken from the NorFor feedstuff table for corresponding feeds used in the studies (http://feedstuffs.norfor.info). For calculations NorFor's prediction of NDFD, we used the NorFor Development tool (NorFor 1.24.0.655, FST revision 1.99, FRC revision 1.92. NorFor Amba, Denmark).

Data were analysed using the MIXED procedure in SAS with experiment as a random effect to evaluate measured (observed) tdNDF against NorFor predictions. Differences between predicted and observed tdNDF were also analysed and the full model included: OMD-method (D-value, *in vivo*, IVOS, VOS), forage type (grass silage, maize silage or whole crop silage), fatty acids (g/kg DM), protein balance in rumen (PBV; g/kg DM) and rumen load index (RLI).

Forage type was classified as grass silage (GS), whole crop (WCS) or maize silage (MS). GS consisted of grass-clover silages with 50 to 100% of grass of total forage (n=136). WCS consisted whole crop small grain cereal-grass silages, where whole crop small grain cereals were included at a level of 30 to 100% (n=29, of which 100% whole crop n=4). MS consisted of maize-grass silages, where maize was included from 30 to 100% (n=19 of which 100% maize n=7).

The equation of rkpNDFr was further developed by the SOLVER function in Microsoft Excel 2010. Coefficients for a linear equation of rkpNDFr were chosen by Excel to minimize the sum of squared differences between observed and predicted tdNDF. Accuracy and precision (R^2) and mean prediction error (MPE) of the equations of predicted tdNDF were calculated according to Bibby and Toutenberg (1977).

Results and Discussion

The evaluated diets had a wide range of characteristics both in forage share, forage type and NDF content (Table 2). Approximately, half of the diets (n=113) were outside the range of NorFor recommendation for diet formulation, where recommendation for e.g. RLI is less than 0.6 and for PBV is between 10 and 40 g per kg DM). NorFor's prediction of NDFD and total amount of NDF were on average good, where mean prediction error (MPE) was 10% for NDFD and 9.3% for tdNDF (Figure 1 and 2). The intercept and slope differed significantly from 0 and 1, respectively. For diets within recommendations for RLI and PBV (n=113) MPE was lower (8.2% and 8.3%, respectively).

Table 1 Description of methods and forages in the studies used in the evaluation

Reference	NDFD method[1]	OMD method[2]	Forage type
Danish trials			
Alstrup *et al.*, 2016	TiO	IVOS	Mixture of maize silage and ryegrass and red clover silage
Brask *et al.*, 2013	Cr	IVOS	Silage of ryegrass and red clover mixture and maize silage
Hymøller *et al*., 2005	iNDF	IVOS	Maize silage and mixture of maize and grass silage
Johansen *et al*., 2017	TiO	IVOS	Silage of pure ryegrass, meadow fescue, tall fescue, red clover, white clover and mixtures of ryegrass and red clover and ryegrass and white clover
Lund *et al.*, 2007	Cr	IVOS	Silages of grass, whole crop pea, whole crop barley and maize
Finnish trials			
Heikkilä *et al.*, 1998	AIA	D-value	Silage of timothy and meadow fescue mixture
Huhtanen, 1991	AIA	D-value	Silage of timothy and meadow fescue mixture
Jaakkola, 2006	AIA	D-value	Silage of timothy and meadow fescue mixture
Jaakkola *et al.*, 2009	AIA	D-value	Silages of timothy and meadow fescue mixture and whole crop barley and whole crop wheat and mixtures of timothy meadow fescue and whole crop barley and timothy meadow fescue and whole crop wheat
Khalili *et al.*, 1999	AIA	D-value	Silage of timothy, meadow fescue and red clover mixture
Khalili *et al.*, 2001	AIA	D-value	Silage of timothy and meadow fescue mixture
Khalili *et al.*, 2005	AIA	D-value	Silage of timothy and meadow fescue mixture
Kokkonen *et al.*, 2000	AIA	D-value	Silage of timothy and fescue mixture
Kokkonen *et al.*, 2002	AIA	D-value	Silage of grass
Puhakka *et al.*, 2016	AIA	D-value	Silage of timothy and meadow fescue mixture
Rinne *et al.*, 1999	AIA	*In vivo*	Silage of timothy and meadow fescue mixture
Rinne *et al.*, 2002	AIA	*In vivo*	Silage of timothy and meadow fescue mixture
Shingfield *et al.*, 2001	AIA	D-value	Silage of timothy and fescue mixture
Shingfield *et al.*, 2002a; 2002b	AIA	D-value	Silage of timothy and meadow fescue mixture
Norwegian trial			
Prestløkken *et al*., 2008a; 2008b; Garmo *et al.*, 2008	Total	*In vivo*	Silage of timothy, meadow fescue and red clover mixture
Swedish trials			
Bertilsson and Murphy, 2003	AIA	VOS	Silages of pure red clover, white clover, and rye grass and mixtures of rye grass and red clover silage, and white clover and ryegrass silage
Bertilsson *et al.*, 2017	AIA	VOS	Silage of ryegrass and red clover mixture
Eriksson *et al.*, 2004	Total	VOS	Mixture of timothy and lucerne silages
Eriksson, 2010	AIA	VOS	Silage of timothy, meadow fescue and red clover mixture
Eriksson *et al.*, 2012	AIA	VOS	Silage mixtures of birdsfoot trefoil and ryegrass, and white clover and ryegrass
Eriksson and Rustas, 2014	AIA	VOS	Silage of timothy and red clover mixture
Rondahl *et al*., 2007	AIA	VOS	Silages of whole crop pea and oat, mixture of whole crop pea and oat and timothy, meadow fescue and red clover
Wallsten and Martinsson, 2009	AIA	VOS	Silage of timothy, meadow fescue, red clover and whole crop barley mixture
UK trial			
Ferris *et al.*, 2000	Total	*In vivo*	Silage of ryegrass, and mixture of ryegrass silage and straw, fresh grass, mixture of fresh grass and straw

[1]Method for measuring NDF digestibility: AIA = acid insoluble ash; Cr = chromic oxide; iNDF = indigestible NDF, 504 hours incubation; TiO = titanium oxide; Total = total collection of faeces. [2]Method for determining organic matter digestibility (OMD) of forages: sheep = OMD determined *in vivo* in sheep; D-value= enzymatic method using cellulase and pepsin; IVOS = *in vitro* method using rumen liquid 48 h and enzymes 48 h; VOS = *in vitro* incubation for 96 h using rumen liquid.

Table 2 Diet and animal characteristics in the studies used in the evaluation. (n=212)

	Unit	Average	Minimum	Maximum	Standard deviation
Diet					
Total dry matter intake	kg DM/day	20.0	12.9	23.9	1.9
Forage intake	kg DM/day	12.2	7.2	18.9	2.1
Concentrate intake	kg DM/day	7.8	0.9	13.4	2.0
Forage share	% of DM	61	37	95	9
Forage dry matter	g/kg	300	148	860	107
Crude protein	g/kg DM	167	113	249	24
Crude fat	g/kg DM	39	17	83	10
Fatty acid	g/kg DM	24	10	54	8
NDF	g/kg DM	385	186	516	60
Starch	g/kg DM	147	36	329	53
Starch +WCS	g/kg DM	202	69	345	52
Rumen load index	g/g fibre	0.35	0.01	0.88	0.14
Net energy (NE)	MJ/kg DM	6.36	4.77	7.21	0.37
Protein balance in rumen (PBV)	g/kg DM	26	-15	111	22
Available amino acid absorbed in small intestine (AAT)	g/MJ NE	15.1	9.0	20.8	2.1
Cow performance					
Cows in each trial	number	22	4	64	
Days in milk	days	129	61	295	49
Energy corrected milk yield	kg/cow/day	29.5	18.6	41.2	3.6
Milk yield	kg/cow/day	28.4	15.6	40.8	4.1
Milk fat	g/kg	43.8	33.8	54.1	4.1
Milk protein	g/kg	33.4	30.1	39.4	1.9
Body weight (BW)	kg	592	516	814	36
NDF intake	g/kg BW	13.0	5.0	17.0	2.3
Observed NDF digestibility	% of NDF	63.1	37.0	78.9	7.7
Predicted NDF digestibility	% of NDF	64.9	47.2	86.4	6.6
Observed total digested NDF	kg/day	4.86	1.48	7.12	0.98
Observed total digested NDF	kg/day	4.99	1.85	7.11	0.98

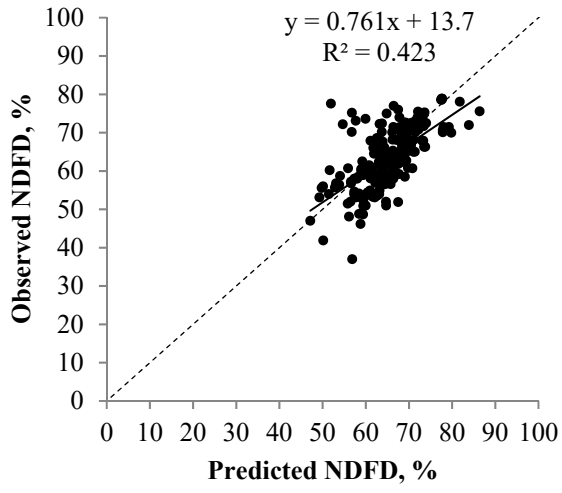


Figure 1 NorFor predicted NDF digestibility (NDFD) against observed NDFD (n=212). Mean prediction error (MPE) was 10.0% and root means square prediction error (RMSPE) was 6.3 % units. General bias was 7.7 %, line bias 6.2 % and random bias 86.0 %.

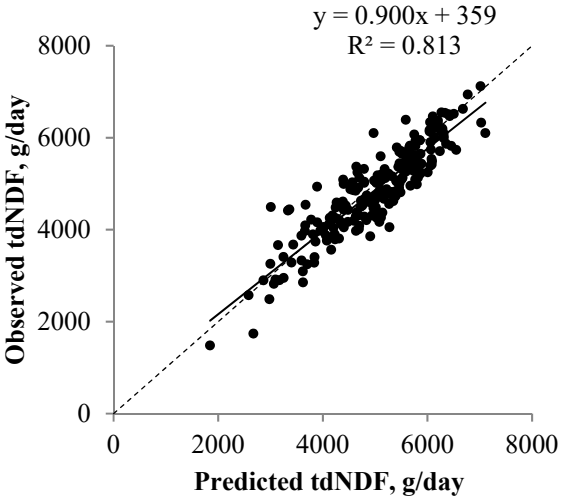


Figure 2 NorFor predicted total digested NDF (tdNDF) against observed tdNDF (n=212). The dotted line represents X = Y. Mean prediction error (MPE) was 9.3% and root means square prediction error (RMSPE) was 453 g. General bias was 9.3 %, line bias 4.6 % and random bias 86.1%.

Applying the energy constant of 14.5 MJ metabolizable energy per kg digested carbohydrate from the NorFor system and assuming utilisation of 60% for net energy, the prediction error of 453 g NDF corresponds approximately to 3.9 MJ NEL which again corresponds to 1.3 kg ECM yield or approximately 0.7 kg DM in a typical ration for lactating dairy cows.

This dataset showed that neither OMD method (D-value, *in vivo*, IVOS, VOS) nor forage type could explain the variation of the residual differences between observed and predicted tdNDF.

Starch and water-soluble carbohydrate (WSC) in the diets and, hence, RLI had a strong effect (p=0.0013) on the variation of the differences between observed and predicted tdNDF (Figure 3). It could be further investigated how the RLI equation could be modified to improve NorFor prediction of NDF digestibility. Also, PBV had an effect on the residuals (Figure 4) (p=0.019).

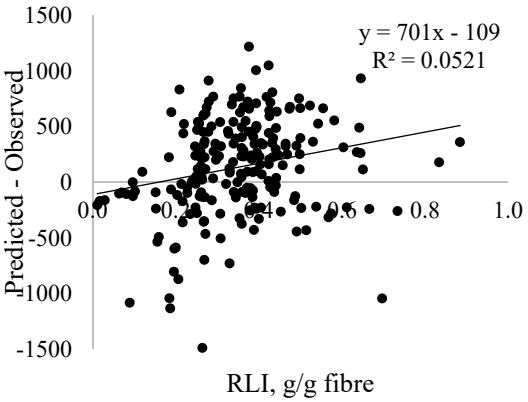


Figure 3 Differences between predicted and observed total digested NDF plotted against rumen load index (RLI; g starch and WSC per g fibre).

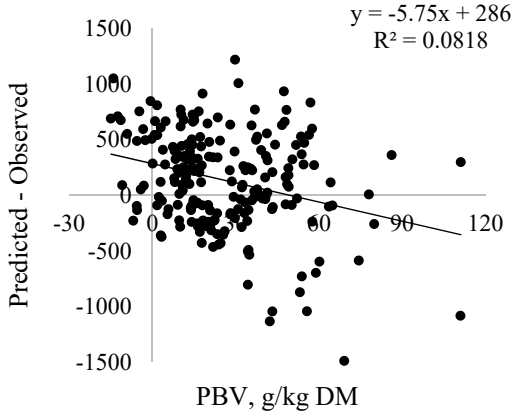


Figure 4 Differences between predicted and observed total digested NDF plotted against protein balance in rumen (PBV).

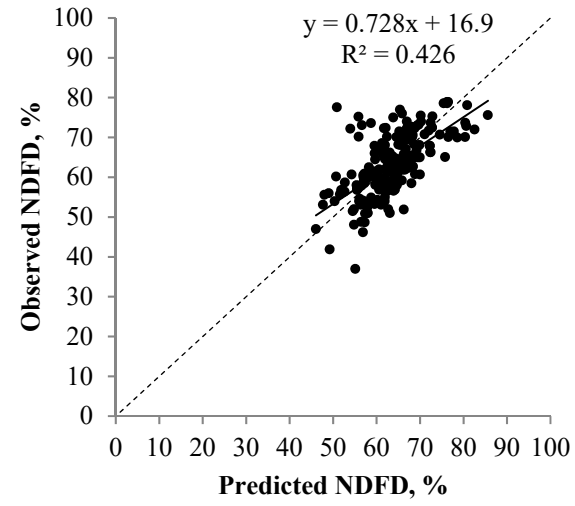


Figure 5 Equation 4 predicted NDF digestibility (NDFD; %) plotted against observed NDFD (n=212). The dotted line represents X = Y. Mean prediction error (MPE) was 9.7% and root means square prediction error (RMSPE) was 6.1 % unit. General bias was 0.4 %, line bias 9.4 % and random bias 90.2 %.

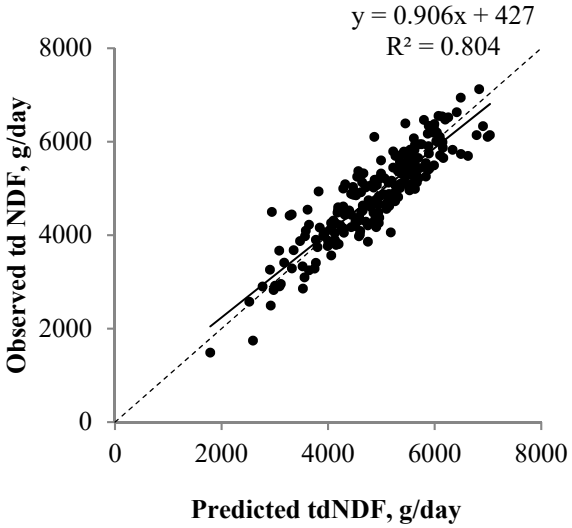


Figure 6 Equation 4 predicted total digested NDF (tdNDF, g/day) plotted against observed tdNDF (n=212). The dotted line represents where X is equal to Y. Mean prediction error (MPE) was 9.1 % and root means square prediction error (RMSPE) was 442 g. General bias was 0.5 %, line bias 4.2 % and random bias 95.3%.

An Excel Solver solution for linear passage rate of potentially degradable NDF in forage (rkpNDFr) resulted in Equation 4.

$$rkpNDFr = 0.7792 + 0.09296 \cdot \frac{\text{NDFI}}{\text{BW}} \qquad \text{Equation 4}$$

, where, rkpNDFr is the passage coefficient for potentially degradable NDF in forage (%/h), NDFI is the total daily intake of NDF (g/day) and BW is the animal's body weight (kg).

When using Equation 4 instead of 7.5 for rkpNDFr, prediction of NDFD and tdNDF resulted in slightly lower MPE (Figure 5 and 6). This change would have a slight effect on net energy supply of approximately -1.5 MJ per day and a predicted milk protein production of -12 g per day.

Conclusions

The prediction of digested NDF in the NorFor model performed fairly well with a MPE of 9.1 % and a RMSPE of 442 g per cow and day. A linear passage rate for forage pdNDF performed slightly better than the present non-linear function in the NorFor model. Rumen load index should be further investigated for improving the prediction of NDFD and tdNDF.

References

Alstrup, L., Søegaard, K. & Weisbjerg M. R., 2016. Effects of maturity and harvest season of grass-clover silage. J. Dairy Sci. 99, 328-340.
Bertilsson J. Åkerlind, M & Eriksson T., 2017. The effects of high-sugar ryegrass/red clover silage diets on intake, production, digestibility, and N utilization in dairy cows, as measured in vivo and predicted by the NorFor model. J Dairy Sci. 100, 7990-8003.
Bertilsson J. & Murphy M., 2003. Effects of feeding clover silages on feed intake, milk production and digestion in dairy cows. Grass Forage Sci 58, 309-322.
Bibby, J. & Toutenberg, H., 1977. Prediction and Improved Estimation in Linear Models, Ch. 1. John Wiley & Sons, New York, NY, USA.
Brask, M., Lund P, Hellwing A.L.F, Poulsen M. & Weisbjerg, M.R., 2013 Enteric methane production, digestibility and rumen fermentation in dairy cows fed different forages with and without rapeseed fat supplementation. Anim. Feed Sci Tech. 184, 67-79.
Eriksson T., Murphy, M., Ciszuk, P. & Burstedt E., 2004. Nitrogen balance, microbial protein production, and milk production in dairy cows fed fodder beets and potatoes, or barley. J Dairy Sci 87, 1057-1070.
Eriksson T., 2010. Nitrogen metabolism in dairy cows fed restricted amounts of grass–clover silage supplemented with seeds from narrow-leafed lupin or pea. Livestock Prod. Sci. 131, 39-44.
Eriksson, T., L. Norell & N. Nilsdotter-Linde, 2012. Nitrogen metabolism and milk production in dairy cows fed semi-restricted amounts of ryegrass-legume silage with birdsfoot trefoil (Lotus corniculatus L.) or white clover (Trifolium repens L.) Grass Forage Sci 67, 546-558.
Eriksson, T. & Rustas, B.-O., 2014. Effects on milk urea concentration, urine output, and drinking water intake from incremental doses of potassium bicarbonate fed to mid-lactation dairy cows. J. Dairy Sci. 97, 4471-4484.
Ferris, C.P., Patterson D.C., Gordon F. J. & Kilpatrick D. J., 2000. The effects of incorporating small quantities of straw in grass/grass silage-based diets for dairy cows. Grass and Forage Sci 55, 146-158.

Heikkilä T., Toivonen, V. & Huhtanen P., 1998. Effects of and interactions between the extent of silage fermentation and protein supplementation in dairy cows. Agric. Food Sci. Finland 7, 329-343.
Huhtanen, 1991. The response to replacement of barley with wheat bran and treatment of rapeseed meal in the diets of dairy cows given grass silage *ad libitum*. Acta Agric. Scand. Sect. A. 41, 415-426.
Hymøller L., Weisbjerg M.R., Nørgaard P., Børsting C.F. & Kristiansen N.B., 2005 Majsensilage til malkekøer. Report no 65. Danmarks jordbrugsforskning Foulum, Denmark (in Danish).
Jaakkola S., 2006. Effects of restriction of silage fermentation with formic acid on milk production. Agric. Food Sci. 15, 200-218.
Jaakkola S, Saarisalo, E. & Heikkilä 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. Agric. Food Sci. 18, 234-256.
Johansen M., Søegaard K., P. Lund P. & Weisbjerg M.R., 2017. Digestibility and clover proportion determine milk production when silages of different grass and clover species are fed to dairy cows. J. Dairy Sci. 100, 8861-8880.
Khalili H., Kuusela E., Saarisalo, E. & Suvitie M., 1999. Use of rapeseed and pea grain protein supplements for organic production. Agric. Feed Sci. in Finland 8, 239-252.
Khalili H., Sairanen A., Hissa K. & Huhtanen P., 2001. Effects of type and treatment of grain and protein source on dairy cow performance. Anim. Sci. 72, 573-584.
Khalili H., Sairanen A., Nousiainen J. & Huhtanen P., 2005. Effects of silage made from primary or regrowth grass and protein supplementation on dairy cow performance. Livest. Prod. Sci. 96, 269-278.
Kokkonen T., Tuori M., Leivonen V. & Syrjälä-Qvist L.,2000. Effect of silage dry matter content and rapeseed meal supplementation on dairy cows. 1. Milk production and feed utilisation. Anim. Feed Sci. Tech. 84, 213-228.
Kokkonen T., Tsehai Tesfa A., Tuori M., Yrjänen S. & Syrjälä-Qvist L., 2002. Effect of concentrate crude protein level on grass silage intake, milk yield and nutrient utilisation by dairy cows in early lactation. Arch. Anim. Nutr. 56, 213-227.
Lindgren, E., 1979. Vallfodrets näringsvärde bestämt in vivo och med olika laboratoriemetoder. Rapport 45. Dept of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Sweden (in Swedish).
Lund P., Weisbjerg M.R., Hvelplund T. & Bach Knudsen K.E., 2007. Determination of digestibility of different forages in dairy cows using indigestible NDF as marker. Acta Agric. Scand. Sect. A 57, 16-29.
Møller, E., Andersen P.E. & Witt N., 1989. En sammanligning af in vitro opløslighed in vivo fordøjlighedaf organisk stof i grovfoder. Beretning fra Faellesudvalget for Statens Planteavls- og Husdyrbrugsforøg, Denmark (in Danish)
Nousiainen, J., Rinne, M. Hellämäki, M. & Huhtanen P., 2003. Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsin-cellulase solubility. Anim. Feed Sci. Tech. 103, 97-111.
Prestløkken, E., Randby, Å.T., Eknæs, M. & Garmo, T.H., 2008a. Effect of harvesting time and wilting on feed intake and milk production. Grassl. Sci. Europe 13.
Prestløkken E., Randby Å.T., Eknæs M. & Garmo T.H., 2008b, Effect of harvesting time and wilting of silage on digestibility in cows and sheep. Grassl. Sci. Europe 13.

Garmo T.H., Randby Å.T., Eknæs M., Prestløkken E. & Nørgaard P., Effect of grass silage chop length on chewing activity and digestibility. Grassl. Sci. Europe 13.
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. J. Dairy Sci. 99, 7993-8006.
Rinne M., Jaakkola S., Kaustell K., Heikkilä T. & Huhtanen P., 1999. Silage harvested at different stages of grass growth versus concentrate foods as energy and protein sources in milk production. Anim. Sci. 69, 251-263.
Rinne M., Huhtanen P. & Jaakkola S., 2002. Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. J. Anim. Sci. 80, 1986-1998.
Rondahl T., Bertilsson J & Martinsson K., 2007. Mixing whole-crop pea–oat silage and grass–clover silage: positive effects on intake and milk production of dairy cows. Grass Forage Sci. 62, 459-469
Shingfield K.J., Jaakkola S. & Huhtanen P., 2001. Effects of level of nitrogen fertilizer application and various nitrogenous supplements on milk production and nitrogen utilization of dairy cows given grass silage-based diets. Anim. Sci. 73, 541-554.
Shingfield K.J., Jaakkola S. & Huhtanen P., 2002a. Effect of forage conservation method, concentrate level and propylene glycol on diet digestibility, rumen fermentation, blood metabolite concentrations and nutrient utilisation of dairy cows. Anim. Feed Sci. Tech. 97, 1-21.
Shingfield K.J., Jaakkola S. & Huhtanen P., 2002b. Effect of forage conservation method, concentrate level and propylene glycol on intake, feeding behaviour and milk production of dairy cows. Anim. Sci. 74, 383-397.
Volden H., 2001. Utvikling av et mekanistisk system for vurdering av fôr til drøvtyggere, AAT-modellen. In: Fôropptak og Fôrmiddelvurdering hos Drøvtyggere. Fagseminar 18.-19. september 2001. Quality Hotell Halvorsbøle, Jevnaker (in Norwegian).
Volden, H., 2011a. In: NorFor - The Nordic Feed Evaluation System. EAAP publication No 130. Wageningen Academic Publishers, The Netherlands, 180 pp.
Volden, H., 2011b. Feed calculations in NorFor. Chapter 6. In: NorFor - The Nordic Feed Evaluation System. EAAP publication No 130. Wageningen Academic Publishers, The Netherlands.
Volden, H. & Larsen, M., 2011. Digestion and metabolism in the gastrointestinal tract. Chapter 7. In: NorFor - The Nordic Feed Evaluation System. EAAP publication No 130. Wageningen Academic Publishers, The Netherlands.
Wallsten J. & Martinsson K., 2009. Effects of maturity stage and feeding strategy of whole crop barley silage on intake, digestibility and milk production in dairy cows. Livest. Sci,121, 155-162
Åkerlind, M. Weisbjerg, M., Eriksson, T., Tøgersen, R., Udén, P. Olafssen B.L., Harstad, O.M. & Volden H., 2011. Feed Analyses and digestion methods. Chapter 5. In: NorFor - The Nordic Feed Evaluation System. EAAP publication No 130. Wageningen Academic Publishers, The Netherlands.

Replacing timothy silage by whole crop barley silage improved intake and growth performance of beef bulls

A. Huuskonen[1] & K. Manni[2]
[1]Natural Resources Institute Finland (Luke), Production Systems, Halolantie 31A, FI-71750 Maaninka, Finland; [2]Natural Resources Institute Finland (Luke), Production Systems, Tietotie 2C, FI-31600 Jokioinen, Finland.
Correspondence: katariina.manni@luke.fi

Introduction

Small grain cereal based whole crop silages provide an opportunity to improve efficiency of forage production for ruminants under Nordic conditions (Rustas, 2009). Whole crop silages have a potential to lower costs, which have increased the interest to use them in feeding. Whole crop silage can be used as sole forage or simultaneously with grass silage in diets of growing cattle (Huuskonen, 2013). The objective of the present study was to determine the effects of whole crop barley silage (BS) on feed intake, growth and carcass traits of growing and finishing beef bulls when timothy silage (TS) was partially or completely replaced by BS.

Materials and Methods

A feeding experiment was carried out in the experimental cattle unit of Natural Resources Institute Finland (Luke) in Ruukki. The experiment was conducted using 60 beef bulls [30 Hereford (HF) and 30 Charolais (CH)] with an initial live weight (LW) of 440 (±50.8) kg. At start of the experiment, the bulls were on average 311 (±15.2) days old. During the experiment, the bulls were housed in an uninsulated barn in pens (10.0 × 5.0 m; 5 bulls in each pen), providing 10.0 m^2/bull. A GrowSafe feed intake system (model 4000E; GrowSafe Systems Ltd., Airdrie, AB, Canada) was used to record individual daily feed intakes so that each pen contained two GrowSafe feeder nodes.

At the beginning of the experiment, both HF and CH bulls were randomly allotted to pens and then randomly allotted to three feeding treatments so that each treatment included two HF pens and two CH pens (20 bulls per treatment). Two bulls were excluded from the experiment due to reasons unrelated to the treatments. The bulls were fed total mixed rations (TMR) *ad libitum* (proportionate refusals of 5%). Rations were mixed in a mixer wagon (Trioliet, BW Oldenzaal, the Netherlands) once a day.

The three experimental diets were:

1) TSB [(g kg^{-1} dry matter (DM)] = TS (600), rolled barley (335), rapeseed meal (50) and mineral-vitamin mixture (15)

2) TSBSB (g kg^{-1} DM) = TS (300), BS (300), rolled barley (335), rapeseed meal (50) and mineral-vitamin mixture (15)

3) BSB (g kg^{-1} DM) = BS (600), rolled barley (335), rapeseed meal (50) and mineral-vitamin mixture (15).

Both TS and BS were produced at the experimental farm of Luke in Ruukki (64°44'N, 25°15'E). The GS was harvested from a primary growth stand which was cut by a mower conditioner and harvested using a precision-chop forage harvester approximately 24 hours after cutting. The BS was harvested at the soft dough stage (growth stage Z85; Zadoks et al., 1974) of the cereal using a direct-cut flail harvester and at a stubble height of about 10 cm.

Both silages were treated with a formic acid-based additive (GrasAAT SX, Addcon Group GmbH) applied at a rate of 5 L/t of fresh forage and stored in bunker silos.

During the feeding experiment, silage sub-samples were taken twice a week, pooled over periods of approximately four weeks and stored at –20 ºC prior to analyses. Thawed samples were analysed for DM, ash, crude protein (CP), neutral detergent fibre (NDFom) exclusive of residual ash, crude fat (analysed as ether extract), starch, silage fermentation quality: pH, water soluble carbohydrates, lactic and formic acids, volatile fatty acids and ammonia N, and also for digestible organic matter (DOM) in DM (D-value) as described by Pesonen et al. (2013). Concentrate sub-samples were collected weekly, pooled over periods of eight weeks and analysed for DM, ash, CP, NDFom, crude fat and starch. The metabolisable energy (ME) concentration of TS was calculated from the concentration of DOM using the equation ME (MJ/kg DM) = 16.0 (MJ/kg DM) × DOM (kg/kg DM) (MAFF, 1984). For BS, a coefficient of 15.5 instead of 16.0 was used (MAFF, 1984). The ME concentrations of the concentrate feeds were calculated based on concentrations of digestible crude fibre, CP, crude fat and nitrogen-free extract described by MAFF (1984). The digestibility coefficients of the concentrates were taken from the Finnish Feed Tables (Luke, 2019). Amino acids absorbed from the small intestine (AAT) and protein balance in the rumen (PBV) were calculated according to the Finnish Feed Tables (Luke 2019). The relative intake potential of silage DM (SDMI index) was calculated as described by Huhtanen et al. (2007).

The bulls were weighed on two consecutive days at the beginning of the experiment and thereafter approximately once every 28 days. Before slaughter, the bulls were weighed on two consecutive days. The target for the average carcass weight was 380 kg and 400 kg for HF and CH bulls, respectively. The bulls were selected for slaughter based on LW and slaughtered in the Atria Ltd. commercial slaughterhouse in Kauhajoki, Finland in three batches. All three feeding treatments were represented in all batches. After slaughter, the carcasses were weighed hot. The cold carcass weight was estimated as 0.98 of the hot carcass weight. The carcasses were classified for conformation and fat using the EUROP classification (EC, 2006).

Results are shown as least squares means. The data were subjected to analysis of variance using the SAS GLM procedure. The statistical model used was $y_{ijklm} = \mu + \delta_k + \alpha_i + \gamma_j + \theta_{ijm} + \beta x_{ijkl} + e_{ijklm}$, where μ is the intercept and e_{ijklm} is the residual error term associated with l^{th} animal. α_i, γ_j and δ_k are the effects of i^{th} diet (TSB, TSBSB, BSB), j^{th} breed (HF, CH) and k^{th} slaughtering batch (1,2,3), respectively, while θ_{ijm} is the effect of pen. The effect of pen was used as an error term when differences between feeding treatments were compared because treatments were allocated to animals penned together. Initial LW was used as a covariate (βx_{ijkl}) in the model. The effect of BS inclusion was further divided into linear and quadratic effects using orthogonal polynomial contrasts.

Results and Discussion

Chemical composition and feeding values of the experimental feeds and TMRs used are in Table 1. Due to the weather conditions during harvesting, the DM concentration of TS was 41% lower compared to BS. According to the feed analyses, CP and NDF concentrations were 49% and 39% higher, respectively, and ME content 8% higher in TS compared to BS. However, BS had 26% higher SDMI index compared to TS. The fermentation characteristics of both silages were good, as indicated by low concentrations of ammonia N in total N and low total fermentation acids (Table 1). The barley grain had typical chemical composition

and feeding values, corresponding to the average values in the Finnish Feed Tables (Luke, 2019).

Table 1 Chemical composition and feeding values of the experimental feeds and total mixed rations (calculated)

	Feeds				Total mixed rations		
	TS	BS	Barley	RSM	TSB	TSBSB	BSB
Number of feed samples	6	6	3	2			
Dry matter (DM), g/kg feed	205	350	872	876	295	360	460
Organic matter (OM), g/kg DM	931	946	977	924	932	937	941
Crude protein, g/kg DM	148	99	105	377	143	128	113
Neutral detergent fibre, g/kg DM	559	402	213	284	421	374	327
Starch, g/kg DM	6	308	569	13	195	285	376
Ether extract, g /kg DM	38	22	17	29	30	25	20
Metabolisable energy, MJ/kg DM	11.0	10.2	13.2	11.4	11.6	11.3	11.1
AAT, g/kg DM	88	82	95	169	93	91	89
Protein balance in the rumen, g/kg DM	19	-22	-38	154	6	-6	-18
Digestible OM in DM, g/kg DM	685	659					
Silage DM intake index	98	123					
Fermentation quality of the experimental silages							
pH	3.72	3.90					
Volatile fatty acids, g/kg DM	17	10					
Lactic + formic acid, g/kg DM	68	39					
Water soluble carbohydrates, g/kg DM	53	60					
NH_4N in total N, g/kg	50	26					

TS = timothy silage, BS = whole crop barley silage, RSM = rapeseed meal, TSB (g/kg DM) = TS (600), rolled barley (335), RSM (50) and mineral-vitamin mixture (15), TSBSB (g/kg DM) = TS (300), BS (300), rolled barley (335), RSM (50) and mineral-vitamin mixture (15), BSB (g/kg DM) = BS (600), rolled barley (335), RSM (50) and mineral vitamin mixture (15), AAT = Amino acids absorbed from small intestine

Due to the differences in chemical composition and feeding values of the feeds, TMR composition differed among the treatments (Table 1). Compared to TSB, BSB had 56% higher DM content and 93% higher starch concentration. The CP and NDF concentrations were 21% and 22% lower, respectively, in BSB compared to TSB. The ME content differed slightly and was 4% lower in BSB compared to TSB. The Finnish recommendation for growing cattle above 200 kg LW is that diet PBV should be above -10 g/kg DM (Luke, 2019) and this was fulfilled in TSB and TSBSB rations. In BSB ration, the PBV value was slightly lower than recommended. However, based on the meta-analysis of the feeding experiments, Huuskonen et al. (2014) concluded that recommended PBV could be even lower than the current -10 g/kg DM without harmful effects on live weight gain (LWG).

Digestibility and ME content of whole-crop silages are generally lower than those of good quality grass silage, as was also observed in the present and previous experiments (Keady et al., 2007; Huuskonen, 2013). However, in the present experiment, a lower digestibility and ME content were compensated by higher DM intake (DMI) (Table 2). When BS was

included in the diet, DMI increased 10% and 13% in TSBSB and BSB bulls, respectively, when compared to TSB bulls (linear effect $P<0.001$). As a result of increased DMI, ME intake increased 7% and 8% in TSBSB and BSB, respectively, compared to TSB (linear effect $P<0.01$).

Table 2 Intake, growth performance and carcass characteristics of the bulls

	Diets				*P*-values	
	TSB	TSBSB	BSB	SEM	L	Q
Number of bulls	18	20	20			
Duration of the experiment, d	167	159	149	3.2	<0.001	0.716
Intake						
Dry matter (DM), kg/d	9.44	10.36	10.65	0.219	<0.001	0.209
DM, g/kg metabolic live weight	80.2	88.6	90.7	1.69	<0.001	0.105
Metabolisable energy (ME), MJ/d	111	119	120	2.5	0.007	0.221
Crude protein (CP), g/d	1356	1345	1209	26.8	<0.001	0.050
Neutral detergent fibre, g/d	3994	3974	3530	85.8	<0.001	0.037
Starch, g/d	1928	2988	4132	66.7	<0.001	0.592
Live weight gain (LWG), g/d	1641	1666	1841	45.7	0.002	0.165
Carcass gain, g/d	989	993	1083	30.1	0.027	0.221
Feed conversion rate						
kg DM/kg carcass gain	9.70	10.67	10.06	0.241	0.279	0.007
MJ/kg carcass gain	114.1	122.7	113.8	2.73	0.932	0.007
g CP/kg carcass gain	1395	1384	1145	30.3	<0.001	0.002
Live weight, kg						
Initial	435	440	445	12.3	0.564	0.989
Final	710	702	713	10.5	0.812	0.419
Slaughter age, d	479	468	462	4.9	0.011	0.690
Carcass characteristics						
Carcass weight, kg	391	384	390	6.9	0.875	0.392
Dressing proportion, g/kg	551	546	545	3.3	0.213	0.550
Conformation, EUROP	9.6	9.2	9.6	0.26	0.999	0.143
Fat score, EUROP	2.5	3.2	3.0	0.14	0.007	0.009

SEM = standard error of the mean, TSB (g/kg DM) = timothy silage (600), rolled barley (335), rapeseed meal (50) and mineral-vitamin mixture (15), TSBSB (g/kg DM) = timothy silage (300), barley silage (300), rolled barley (335), rapeseed meal (50) and mineral-vitamin mixture (15), BSB (g/kg DM) = barley silage (600), rolled barley (335), rapeseed meal (50) and mineral vitamin mixture (15), L = linear effect of barley silage inclusion, Q = quadratic effect of barley silage inclusion, Conformation: (1=poorest, 15=excellent), Fat score: (1=leanest, 5=fattest)

The SDMI index can be affected by various silage characteristics including DM content, fermentation quality, D-value and NDF concentration (Huhtanen et al., 2007). The SDMI index quantitatively estimates the effects of these factors on silage intake (Huhtanen et al., 2007). In the present experiment, higher DM content, lower concentration of NDF and lower amount of total fermentation acids in BS may have compensated for the negative effects of lower D-value on DMI. The SDMI index was consistent with increased DMI.

In previous experiments, the effects of the whole crop silage on DMI are not consistent. Similarly as in the present study, Keady et al. (2007) observed that DMI increased when whole crop silage was included in the diet but there also are experiments where whole crop inclusion has not affected DMI (e.g. Huuskonen & Joki-Tokola, 2010; Huuskonen, 2013). According to Huhtanen et al. (2007), the highest DMI in dairy cows was achieved when the proportion of whole crop silage was 0.48 of total silage DM, but above this level, it started to decrease quadraticaly.

Replacing TS by BS increased starch intake linearly ($P<0.001$) while CP and NDF intakes decreased ($P<0.001$). High starch intake may lead to low rumen pH and increase the risk of diseases such as rumen acidosis developing (Krause & Oetzel, 2006). In the present experiment, starch concentrations in the diets were 195, 285 and 376 g/kg DM in TSB, TSBSB and BSB, respectively, and were below harmful levels. According to Huuskonen et al. (2014), a starch level of 400 g/kg DM is not too high for growing bulls when the diet contains forage NDF of at least 180 g/kg DM. In the present experiment forage NDF levels were 335, 288 and 241 g/kg DM in TSB, TSBSB and BSB, respectively.

Live weight gain ($P<0.01$) and carcass gain ($P<0.05$) of the bulls improved linearly when TS was replaced by BS. A likely explanation for this was probably differences in ME intake. Based on the meta-analysis, Huuskonen & Huhtanen (2015) found that energy intake was clearly the most important variable affecting LWG of growing cattle whereas the results showed only marginal effects of protein supply on growth. The results of the present experiment support this because the highest LWG was achieved at the highest ME intake level while the CP intake was the lowest.

Inclusion of the BS into the diet had a quadratic effect on feed conversion rate ($P<0.01$) (FCR; kg DM or MJ/kg carcass gain) with the poorest FCR observed in TSBSB bulls. Whole crop barley inclusion decreased CP intake and improved protein conversion rate (g CP/kg carcass gain, linear effect $P<0.001$, quadratic effect $P<0.01$).

Replacing TS by BS did not affect carcass weight, dressing proportion or carcass conformation of the bulls. When TS was replaced by BS, carcass fat score increased 28% and 20% in TSBSB and BSB bulls, respectively (linear and quadratic effects $P<0.01$). It is well established that increased energy intake of growing cattle increases carcass fatness (Pesonen et al., 2013; Huuskonen & Huhtanen, 2015; Manni et al., 2016), which is supported by the results of the present experiment. Based on the meta-analysis of data from growing cattle, Huuskonen & Huhtanen (2015) found that increased ME intake improved carcass conformation. However, this was not found in the present experiment.

Conclusions

Our study demonstrated that BS is a potential forage for feeding growing beef bulls. Both daily DM and energy intakes, as well as growth rates of the bulls, increased when TS was replaced by BS. However, inclusion of BS into the diet did not improve DM or ME conversion rate but improved CP conversion rate. Replacing TS by BS increased carcass fatness but other treatment responses in carcass characteristics were not found.

References

EC, 2006. Council Regulation (EC) No 1183/2006 of 24 July 2006 concerning the Community scale for the classification of carcasses of adult bovine animals. Off. J. Europ. Union L, 214, 1-6.
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.
Huuskonen, A., 2013. Performance of growing and finishing dairy bulls offered diets based on whole-crop barley silage with or without protein supplementation relative to grass silage-based diet. Agric. Food Sci. 22, 424-434.
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.
Huuskonen, A. & Joki-Tokola, E., 2010. Performance of growing dairy bulls offered diets based on silages made of whole-crop barley, whole-crop wheat, hairy vetch and grass. Agric. Food Sci. 19, 116-126.
Huuskonen, A., Huhtanen, P. & Joki-Tokola, E., 2014. Evaluation of protein supplementation for growing cattle fed grass silage-based diets: a meta-analysis. Animal 8, 1653-1662.
Keady, T.W.J., Lively, F.O., Kilpatrick, D.J. & Moss, B.W., 2007. Effects of replacing grass silage with either maize or whole-crop wheat silages on the performance and meat quality of beef cattle offered two levels of concentrates. Animal 1, 613-623.
Krause, K.M. & Oetzel, G.R., 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: a review. Anim. Feed Sci. Technol. 126, 215- 236.
Luke, 2019. Feed Tables and Nutrient Requirements. [cited 6.3.2019]. Available at: http://www.luke.fi/feedtables
Manni, K., Rinne, M. & Huuskonen, A., 2016. Effects of barley intake and allocation regime on performance of growing dairy bulls offered highly digestible grass silage. Livest. Sci. 191, 72-79.
MAFF (Ministry of Agriculture, Fisheries and Food), 1984. Energy Allowances and Feeding Systems for Ruminants. ADAS Reference book 433. London, United Kingdom.
Pesonen, M., Honkavaara, M., Kämäräinen, H., Tolonen, T., Jaakkola, M., Virtanen, V. & Huuskonen, A., 2013. Effects of concentrate level and rapeseed meal supplementation on performance, carcass characteristics, meat quality and valuable cuts of Hereford and Charolais bulls offered grass silage-barley-based rations. Agric. Food Sci. 22, 151-167.
Rustas, B.-O., 2009. Whole-crop cereals for growing cattle. Effects of maturity stage and chopping on intake and utilisation. Doctoral Thesis. Swedish University of Agricultural Sciences, Skara, Sweden.
Zadoks, J.C., Chang, T.T. & Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Res. 14, 415-421.

Estimates for rumen dry matter degradation of concentrates are higher, but not consistently, when evaluated based on *in sacco* as compared to *in vitro* methods

L.E. Sembach[1], H.H. Hansen[1], R. Dhakal[1], T. Eriksson[2], N.I. Nielsen[3] & M.O. Nielsen[1]
[1]*University of Copenhagen, Grønnegårdsvej 3, 1870 Frederiksberg C, Denmark*
[2]*Swedish university of agricultural sciences,* Ulls väg 26, 750 07 *Uppsala, Sweden*
[3]*NorFor & SEGES, Agro Food Park 15, 8200 Aarhus N, Denmark*
Correspondence: lasse.emil.sembach@sund.ku.dk

Introduction

NorFor – the Nordic feed evaluation system - currently bases estimates of ruminal crude protein and NDF degradation on data from the *in sacco* nylon bag technique. These degradation profiles are fitted to an exponential model with an intercept as proposed by Ørskov and McDonald (1979), where the intercept represents the soluble part of the substrate, while the asymptote is the potentially degradable part (Åkerlind *et al.,* 2011). The *in vitro* gas production technique (IVGPT) is a method used to estimate fermentation kinetics of feeds, by incubating the feeds in buffered rumen fluid and measuring the gas produced over time (Theodorou *et al*., 1994). Cumulative gas production profiles have been related to dry matter (DM) degradation, by assuming a potentially degradable pool equal to the amount of substrate degraded at the end-point of the fermentation and a constant yield factor of gas to degraded substrate (Dhanoa *et al.,* 2000). The aim of this study was to examine whether DM degradation estimated from mathematical models fitted to degradation profiles of concentrates obtained through the *in sacco* technique correspond to estimates by the rapid and less costly IVGPT.

Materials and Methods

IVGPT

Nine concentrates were tested in two *in vitro* gas production trials. The concentrates were ground, using a cyclone mill (Cyclotec 1093 sample mill, Foss Analytical A/S, Hillerød, Denmark), to pass a 2-mm screen and run in quadruplicates in each trial. Samples of 500 ± 1 mg were weighed into 100-ml glass jars. Rumen fluid with particulate matter was collected from two rumen cannulated, overnight fasted Jersey heifers on the morning of the experiment and transported for approximately 45 minutes in four pre-heated thermo bottles. The rumen fluid was then filtered through 2 layers of cheesecloth and added to a pre-heated (39°C) buffered solution (1:2 ratio) (Mencke & Steingass, 1988) that was kept anaerobic by continual CO_2 flushing. Ninety ml of the buffered rumen fluid solution was dosed into each of the 100 ml jars, and three jars without substrate were included for blank correction. The jars were placed in a preheated (39°C) thermoshaker with 40 rotations pr. minute. The ANKOM^{RF} Gas Production System (Ankom Technology, Macedon NY, US) was used to register gas production every 10 minutes for each sample during the fermentation period, with gas released at a pressure of 0.0517 bars. After 9 hours of incubation, half of the samples were removed, and the medium filtered through pre-weighed 25 µm F57 filter bags (ANKOM Technology). The remaining samples were removed and filtered through F57 bags after 48 hours of fermentation. Samples removed after 9 hours had an average of 54 gas readings, while samples removed after 48 hours had an average of 288 gas readings. One sample of palm expeller incubated for 48 hours was excluded due to the filter bag breaking during filtering. After drying, the undegraded residue was determined after correcting for microbial weight gain in the blank bottles.

In sacco nylon bag technique

The same nine concentrates were ground to pass a 1.5-mm screen using a cutter mill with four stationary and three rotating knives (Model 880803, Brabender OHG, Duisburg, Germany). Samples of 1700 ± 5 mg were weighed into 35-µm polyester bags (Saatifil PES 28/31, Saatitech S.p.A., 22070 Veniano, Italy). The bags were soaked in 39°C tap water for 20 minutes prior to incubation. The samples were incubated in the rumen of three non-lactating dairy cows fed at maintenance level, for 0, 2, 4, 8, 16, 24 and 48 hours, respectively. Two bags of each sample for each timepoint were incubated in each cow giving a total of six replicates for each feedstuff at each timepoint. After incubation, the bags were washed in a domestic washing machine, dried at 45° C and weighed. The bags were then emptied, vacuum cleaned and weighed to calculate the weight of air-dried sample residue.

C*omputational analysis*

Gas production profiles of the samples from the IVGPT were converted into ml gas (g DM)$^{-1}$ at Standard Pressure and Temperature (STP). A mean cumulative gas production profile for each feed was estimated from replicates incubated *in vitro* for 48 hours and mean dry matter degradation was calculated based on undegraded feed residue after 9 and 48 hours of incubation. The program R (R Core Team, 2019) with the "drc-package" (Ritz *et al.*, 2015) was used to fit eight mathematical models to the gas production and feed degradation profiles obtained *in vitro* and *in sacco*, respectively.

After fitting the observed curves, the in vitro results were converted from ml gas produced to percentage of DM degraded. This was done by assuming a potential DM degradation equal to what was degraded after 48 hours of incubation and a constant yield factor (ml gas (g DM)$^{-1}$). The estimated gas production at time 't' was then divided by the yield factor and reported as a proportion of the original sample DM.

The eight models initially fitted to each degradation profile are in Table 1. The best fit was selected based on AIC (Akaike's Information Criterion) and used to calculate total rumen dry matter degraded for both the *in vitro* and the *in sacco* degradation profiles of each concentrate, assuming a mean retention time in the rumen of 16 hours.

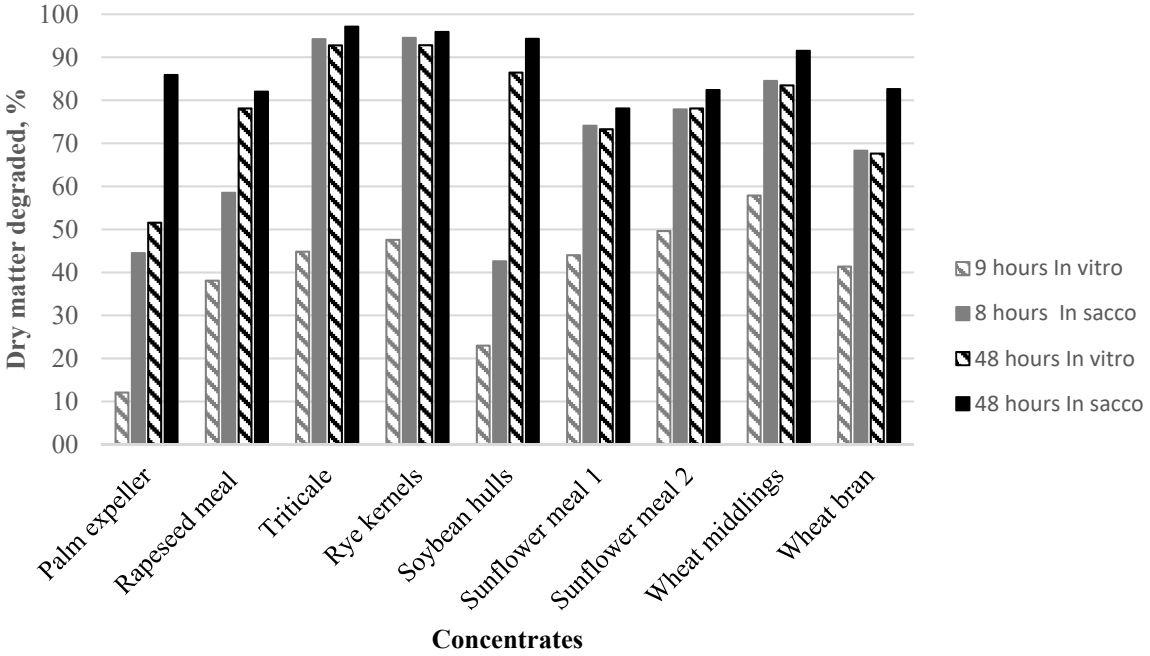
This estimated rumen retention timed was derived from the equation used in NorFor to calculate passage rate of potential degradable starch and crude protein from concentrate (Åkerlind *et al.,* 2011), assuming a dairy cow with a body weight of 640 kg, a daily DM intake of 22 kg and a concentrate share of 50% of the feed ration.

Table 1 Mathematical models fitted to degradation profiles of nine concentrates

Abbreviation	Model type	Equation
EXP	An exponential model	$Y_t = B*(1-e^{-ct})$
EXP.L	An exponential model with a lag phase	$Y_t = B*(1-e^{-c(t-L)})$
Ø & M	An exponential model with an intercept (Ørskov & McDonald, 1979)	$Y_t = A+B*(1-e^{-ct})$
EXP.IL	An exponential model with an intercept and a lag phase	$Y_t = A+B*(1-e^{-c(t-L)})$
Groot	A sigmoidal model (Groot *et al.*, 1996)	$Y_t = A/(1+(b^c/t^c))$
Weibull	A continuous probability distribution	$Y_t = A+B*\exp^{-\exp(b*(\log(t)-\log(e)))}$
Gompertz	A sigmoid curve with slow growth at the start and end of the curve	$Y_t = A+B*\exp^{-\exp(b*(t-e))}$
M-M	A Michealis-Menten curve	$Y_t = A+B/(1+(e/t))$

Results and discussion

Across feeds, DM degradation was significantly higher ($P<0.05$) for the samples incubated *in sacco* at eight and 48 hours compared to the samples incubated *in vitro* for nine and 48 hours, respectively (Figure 1). Mean DM degradation across feeds after 8 hours of incubation *in sacco* was 71.0 ± 19.4%, while it was 39.8 ± 14.1% after nine hours of incubation *in vitro.* Mean dry matter degradation across feeds after 48 hours of incubation *in sacco* was 87.8 ± 7.0%, while it was 78.2 ± 13.1% after 48 hours of incubation *in vitro.*



percentage of dry matter degraded after 9 and 48 hours of incubation, with the lowest correlation being for wheat middlings (r=0.94, $P<0.005$). Feed degradation profiles were best fitted to different mathematical models, depending on whether feeds had been incubated *in sacco* (Weibull, Ø & M or M-M) or *in vitro* (Groot, Gompertz and Ø & M), and in no case was the best fit model for a feed identical for the two methods (Table 2).

Table 2 Best fit mathematical models used to describe feed degradation profiles based on AIC, depending on concentrate and technique

Concentrate	In vitro	In sacco
Palm expeller	Ø & M	Weibull
Rapeseed meal	Groot	Weibull
Triticale	Groot	Weibull
Rye kernels	Gompertz	Ø & M
Soy bean hulls	Ø & M	Weibull
Sunflower meal 1	Gompertz	Ø & M
Sunflower meal 2	Gompertz	Ø & M
Wheat middlings	Groot	Weibull
Wheat bran	Groot	M-M

For rye seeds, sunflower meal and wheat middlings, DM degradation was very different in the early compared to the final stage of *in vitro* fermentation, which was not the case when incubated *in sacco* (Figure 1).

Estimated dry matter degradation, assuming 16 hours retention in the rumen and using the predicted degradation curves, was significantly greater ($P<0.05$) for *in sacco* compared to *in vitro* estimates for all concentrates (Table 3). There was a non-significant ($P=0.66$) smaller

mean standard deviation for estimated DM degradations based on degradation profiles obtained *in sacco* (SD =1.38 unit) compared to *in vitro* (SD =1.64). Use of the best fit model resulted in only minimal differences in degradation estimates at 16 hours rumen retention when compared to the other tested models (data not shown).

Table 3 Estimated rumen dry matter degradation (%) derived from *in vitro* and *in sacco* degradation profiles for 9 concentrates, according to the best fit model and assuming a mean rumen retention time of 16 hours

Concentrate	In vitro	In sacco
Palm expeller	21.6[a]	76.8[d]
Rapeseed meal	57.0[b]	72.9[d]
Triticale	73.3[b]	95.6[d]
Rye kernels	72.8[c]	95.5[a]
Soy bean hulls	44.3[a]	67.8[d]
Sunflower meal 1	59.7[c]	77.6[a]
Sunflower meal 2	63.0[c]	81.7[a]
Wheat middlings	69.9[b]	87.4[d]
Wheat bran	52.5[b]	73.0[e]

a, b, c, d, e: = Estimated using the Ø & M, Groot, Gomperts, Weibull and M-M models, respectively.

The reasons for the observed discrepancies between the two techniques are not known. Estimates for early degradation by the *in sacco* method are affected by amount of substrate escaping (but not necessarily degraded) at time zero (intercept) from the bags placed in the rumen (Huntington & Givens, 1995). Such an escape is not possible from bottles used *in vitro*. However, any initial loss of material from *in sacco* bags should not affect estimates of degradation rates for remaining feed in the bag, since several of the applied mathematical models included an intercept. Particle size of feed samples is another issue to consider, since feed samples used in the *in sacco* trial were ground through a smaller sieve size (1.5 mm) than those used *in vitro* (2 mm). However, the cyclone mill used for *in vitro* samples has been shown to create a smaller mean particles size compared to other mill types (Hoffman *et al*., 2012). A linear relationship has been shown between bag pore size and estimates of undegraded particle loss (Michalet-Doreau & Ould-Bah, 1992). There was a difference in pore size between the filter bags used *in vitro* (25 µm) and *in sacco* (35 µm). It is possible to use filter bags with smaller pore sizes in the *in vitro* system compared to the *in sacco* method, since the *in vitro* samples are incubated directly in the fluid and bags are only used to retain undegraded feed material at the end of the incubation. *In sacco* degradation requires bags with pore sizes large enough to allow for passage of microorganisms. This raises the question whether differences in degradation between the two methods to a large extent could be explained by loss of undegraded particles in the *in sacco* method, and subsequent inflated increase in estimates for degraded particles. Another factor that may contribute to uncertainties related to the IVGPT, is the microbial concentration and microbial activity in the rumen fluid. The activity and concentration are known to be affected by time of rumen fluid sampling, relative to the time when the cow was fed (Mould *et al*., 2005). The rumen fluid used in the *in vitro* system was collected from heifers that have been fasted overnight to obtain standardized results from run to run. A greater gas production in the early stages of fermentation could perhaps be expected, if the cows were recently fed. Less gas is produced from degradation of protein than degradation of carbohydrates (Cone & van Gelder, 1999). This means that gas production relative to unit of DM degraded is not constant. DM degradation in the *in vitro* system is estimated from amount of gas produced and DM degraded at the end of incubation (48 hours). Since soluble protein will mostly be degraded in the early fermentation (Cone & van Gelder, 1999), the deviation between estimated DM

degraded to actual DM degraded is expected to be larger at that stage. In the *in vitro* system DM degraded, and gas produced had a correlation ≥0.94 for all samples, considering both 9 and 48 hours of incubation. As the estimated rumen retention time was 16 hours, the effect of protein is assumed to be minimal.

Conclusions

In sacco estimates of DM degradation (assuming a 16 hour rumen retention time for all feeds) were significantly greater across feeds compared to *in vitro* estimates. These differences cannot readily be explained by the characteristics of the type of concentrate. There were no major differences in estimates for DM degradation depending on the mathematical model applied, when assuming a mean rumen retention time of 16 hours. Further studies are needed to determine which method most accurately describes differences between feeds with respect to dry matter degradation in the rumen, and whether the rapid and cheap *in vitro* method could complement normal feed analyses to refine description of degradation kinetics.

References

Cone, J.W. & van Gelder, A.H., 1990. Influence of protein fermentation on gas production profiles. Anim. Feed Sci. Technol. 76, 251-264.

Dhanoa, M.S., Lopez, S., Dijkstra, J., Davies, D.R., Sanderson, R., Williams, B.A., Sileshi, Z., & France, J., 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: comparison of models. Br. J. Nutr. 83, 131–142.

Groot, J.C.J., Cone, J.W., Williams, B.A., Debersaques, F.M.A., & Latinga, E.A., 1996. Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. Anim. Feed Sci. Technol. 64, 77–89.

Hoffman, P.C., Mertens, D.R., Larson, J., Coblentz, W.K., & Shaver, R.D., 2012. A query for effective mean particle size in dry and high-moisture corns. J. Dairy. Sci. 95, 3467-3477.

Huntington, J.A. & Givens, D.I., 1995. The in situ technique for studying rumen degradation of feeds: a review of the procedure. Nutr. Abstr. Rev. 65b, 63-93.

Menke, K.H. & Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Anim. Res. Dev. 28, 7–55.

Michalet-Doreau, B. & Ould-Bah, M.Y., 1992. In vitro and in sacco methods for the estimation of dietary nitrogen degradability in the rumen: a review. Anim. Feed Sci. Technol. 40, 57-86.

Mould, F.L., Kliem, K.E., Morgan, R., & Mauricio, R.M., 2005. In vitro microbial inoculum: A review of its function and properties. Anim. Feed Sci. Technol. 123-124, 31–50.

R Core Team., 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ritz, C., Baty, F., Streibig, J.C., & Gerhard, D., 2015. Dose-Response Analysis Using R. PLoS ONE 10(12): e0146021. https://doi.org/10.1371/journal.pone.0146021.

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B. & France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feedstuffs. Anim. Feed Sci. Technol. 48, 185–197.

Ørskov, E.R. & I. McDonald., 1979. The estimation of protein degradability in the rumen from measurements adjusted for rate of passage. J. Agric. Sci. 92, 499-503.

Åkerlind, M., Weisbjerg, M., Eriksson, T., Tøgersen, R., Udén, P., Ólafsson, B.L., Harstad, O.M., & Volden, H., 2011. Feed analyses and digestion methods, in: Volden, H. (Ed.), NorFor - The Nordic feed evaluation system, pp. 41 – 54. EAAP – European Federation of Animal Science, vol 30. Wageningen Academic Publishers, The Netherlands.

Prediction of intake, digestibility and weight gain of sheep fed urea-wood ash treated maize cobs from *in vitro* degraded substrate

A. Abdulazeez[1], C.M. Tsopito[2] & O.R Madibela[2]
[1]*Federal College of Education (Tech) Gusau, Zamfara State, Nigeria.* [2]*Botswana University of Agriculture and Natural Resources.*
Correspondence: azeezrazaq70@gmail.com

Introduction

Many workers have observed that *in vivo* studies are very expensive, laborious, requires large quantity of feed, time-consuming and not suitable for large scale feed analysis (Carro et al., 1994; Chenost et al., 1997; Fernandez - Rivera, 1997; Macheboeuf et al., 1997; Romney et al., 1997; Blümmel et al., 1997). The major constraint in the utilization of roughages by ruminants is voluntary intake, which is dependent on how digestible the roughage is (Minson, 1990; Ørskov& Ryle, 1990). Data generated from *in vitro* gas production studies could be used to predict *in vivo* observations using regression equations to overcome the shortcomings associated with *in vivo* studies. The objective of this study was to predict nutrient intake, digestibility, weight gain and feed intake of sheep fed urea/wood ash treated maize cob-based diet from *in vitro* truly degraded substrate (IVTDS) parameters.

Materials and Methods

The study was conducted at Botswana University of Agriculture and Natural Resources, Content Farm, located 10 km north of Gaborone in SE Botswana and consisted of two gas in vitro experiments (Experiment I and II) and an in vivo experiment (Experiment III) with urea and wood ash treated maize cobs.

In vitro studies

Maize cobs were acquired from Molepolole village, 70 km from Gaborone after harvest and ground (4 mm sieve used) in preparation for treatments in Experiment I. Two hundred g of maize cobs were treated with a total of 100 ml of two water solutions containing either urea (5 g/100 ml) or wood ash (30 g/100 ml) in the following proportions: 100% urea+0% wood ash, 75% urea+25% wood ash, 50% urea+50% wood ash, 25% urea+75% wood ash and 0% urea+100% wood ash.
After treatments, samples were stored in airtight plastic bags for 7 days, which was adequate for urease activity under an ambient temperature of 37°C (Sundstøl et al., 1978). This was followed by laboratory analysis in triplicates to determine their chemical composition. The samples were then milled through a 1-mm sieve and incubated in rumen fluid in calibrated glass syringes following the procedure of Menke and Steingass (1988). Rumen fluid was obtained from fistulated steers. The steers were fed a commercial concentrate combined with crushed maize cobs. The liquor was collected in a flask flushed with carbon dioxide to maintain anaerobic environment. Five hundred milligrams of sample were weighed in triplicate for each treatment into calibrated glass syringes of 100ml. The syringes were pre warmed at 39°C followed by injection of 30ml rumen fluid-buffer (1:2 v/v) mixture into each syringe under continuous flushing with CO_2, then incubated in a water bath at 39°C. The buffer was made up of (A) $MgSO_4.H_2O$+ NaCl + KH_2SO_4 + $CaCl_2.H_2O$ + Urea and (B) $NaSO_4.9H_2O$ + $NaCO_3$. Gas production was recorded at 6, 12, 24, 48 and 72 hours after incubation. Three runs were carried out and means for each run were used as replicates. In vitro truly degraded substrate (IVTDS) was determined according to the procedure outlined

by Makkar (2010). Residues from *in vitro* gas production were treated with neutral detergent solution (NDF) solution (in order to detach microbes from the residues) in a beaker for one hour then filtered into a crucible, washed with hot water and finally oven dried at 100°C over night. The undegraded residue was then transferred to the muffle furnace and ashed at 550°C. IVTDS parameters were then determined according to the procedure outlined by Makkar (2010).

At the end of Experiment I, cobs treated with 25% urea + 75% wood ash gave better results in terms of the parameters investigated and was used to replace maize grain in Experiment II. The IVTDS parameters were: degraded substrate in milligrams (TDS mg), degraded substrate in percentage (TDS%), gas production in millilitre (GP ml), microbial mass production in milligrams (MMP mg), efficiency of microbial mass production (EMMP) and partitioning factor (PF).

In Experiment II, effects of substituting maize grain in a complete diet with graded levels of maize cobs treated with 25% urea plus 75% wood ash on IVTDS parameters was determined. The treatments were: 100% maize grain, 66% maize grain plus 34% treated maize cobs, 34% maize grain plus 66% treated maize cobs and 100% treated maize cobs (Table 1). Their IVTDS were determined according to the procedures of Menke and Steingass (1988) and Makkar (2010) as described in Experiment I (Table 2).

In Vivo Study

In Experiment III, four Tswana male sheep sourced from the school farm (18 months old, 32±1 kg) were fed the experimental diets used in Experiment II in a 4×4 Latin Square Design (Table 3). The sheep were housed in metabolism cages for collection of urine and faeces separately. Prior to commencement of the trial, the sheep were weighed, vaccinated against Enterotoxaemia Pasteurella followed by deworming with Evomectin 14 days later. The trial lasted for forty-eight days with 7 days of adaptation and 5 days for collection. Each period was restricted to 12 days due to insufficiency of maize cobs to substitute maize grain because of the drought experienced in Botswana during the experimental period.

Data Collection and Chemical Analysis

Feed offered and refused were recorded daily throughout the experimental period. Feed were offered twice daily (500g at 8am and 500g at 4pm) while samples of the experimental diets including refusals and faeces (5% sub samples) were collected daily in the morning before feeding during the 5-d collection period. Samples of concentrate mixtures and faeces for each treatment group were then composited and stored at -20°C for later chemical analysis. The samples were divided into two parts, first part for dry matter (DM) analysis, while the second part was kept and pooled for chemical analysis. Feed, refusal and faecal DM for all samples were determined by drying in forced air oven at 60°C for 24 hr (AOAC, 1999; ID number 930.15). Organic matter (OM) and ash were obtained by difference in weight after ignition at 550°C in a muffle furnace (Muffle Furnace Size 3, Gallenkamp, UK) according to AOAC (1999), ID number 942.05. The neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined with an ANKOM fibre analyser (Ankom Technology Corporation, Fairport, NY, USA) according to the procedure of Van Soest et al. (1991). In the analysis of NDF, sodium sulphite and alpha amylase were also added. Nitrogen was determined by a Kjeldahl method (AOAC, 1999; ID number 955.04) and crude protein (CP) was calculated as N*6.25 (AOAC, 1999; ID number 954.01). Daily collections of urine

for each treatment before feeding were acidified with 10ml50% H_2SO_4/L to prevent ammonia volatilization, stored and then pooled at the end of the trial. The urine samples were then analysed for N according to AOAC (1999).

Table 1 Ingredients and chemical composition of experimental diets

	Treatments			
Ingredients (%)	100M	66M34C	34M66C	100C
Maize cob	0	15	30	45
Maize grain	45	30	15	0
Lucerne	39	36	32.5	29.5
Wheat bran	10	10	10	10
Sun Flower Cake	5	8	11.5	14.5
Salt	0.5	0.5	0.5	0.5
DCP	0.5	0.5	0.5	0.5
Chemical composition (g/kg)				
DM	945	950	950	955
ASH	82	100	107.9	175.4
OM	918	900	892.1	824.6
CP	134.3	131	133.5	133.5
NDF	470	470	480	570
ADF	160	220	250	360
ADL	53.2	63.2	85.2	114.4
HC	310	250	230	210
NDS	530	530	520	430
ME MJ/kg	18.94	19.19	19.28	17.24

DM= dry matter, OM=organic matter, CP=crude protein, NDF=neutral detergent fibre, ADF=acid detergent fibre, ADL=acid detergent lignin, HC=hemicellulose, NDS=neutral detergent soluble, ME=metabolizable energy, DCP= Di calcium phosphate. 100M= 100% maize, 66M34C=66% maize 34% treated maize cobs, 34M66C=34% maize 66% treated maize cobs, 100= 100% treated maize cobs.

Table 2 IVTDS parameters of experimental diets

	Treatments					
IVTDS parameters	100M	66M34C	34M66C	100C	SE	P value
TDS (mg)	353.7^{a}	352.5^{a}	363.7^{a}	288.7^{b}	7.84	<0.0001
TDS (%)	81.6^{b}	82.5^{b}	85.8^{a}	73.3^{c}	1.22	<0.0001
GP (ml)	117.5^{a}	119.5^{a}	120.0^{a}	105.0^{b}	1.66	<0.0001
MMP (mg)	95.3^{ab}	89.6^{b}	99.8^{a}	57.8^{c}	4.41	<0.0001
EMMP	26.9^{ab}	25.40^{b}	27.4^{a}	20.0^{c}	0.81	<0.0001
PF	3.01^{ab}	2.94^{b}	3.03^{a}	2.75^{c}	0.03	<0.0001

100M= 100% maize, 66M34C=66%maize 34% treated maize cobs, 34M66C=34% maize 66% treated maize cobs, 100C= 100% treated maize cobs, TDS= truly degraded substrate, GP= gas production at 24 hours, MMP= microbial mass.

Regression equations for predicting DMD, OMD and NDFD from TDS (mg), TDS (%) GP, MMP, EMMP and PF are in Table 5. TDS (mg), TDS (%) GP, MMP, EMMP and PF explained 33 – 66% of the variability in DMD. Combinations of TDS (mg), TDS (%) GP, MMP, EMMP and PF in a multiple regression improved the accuracy of prediction from 66 to 90%. TDS (mg), TDS (%) GP, MMP, EMMP and PF explained 45 – 88% variability in OMD. Their combinations in a multiple regression failed to increase accuracy of predictions above 88%. TDS (mg), TDS (%) GP, MMP, EMMP and PF explained 40 – 66% of the variability in NDFD but the multiple regression improved the accuracy of prediction to 91%.

Regression equations for predicting FI and WG from TDS (mg), TDS (%) GP, MMP, EMMP and PF are in Table 6. Gas production and TDS (%) explained 26 – 33% variability in FI.

Table 3 Nutrient intake and digestibility of Tswana sheep fed experimental diet

	Treatments					
Parameters	100M	66M34C	34M66C	100C	SE	P value
			Nutrient intake (kg/day)			
CPI	0.08^{c}	0.13^{a}	0.13^{a}	0.09^{b}	0.01	<0.0001
DMI	0.67^{b}	0.95^{a}	0.95^{a}	0.56^{c}	0.04	<0.0001
OMI	0.55^{c}	0.90^{a}	0.89^{a}	0.58^{b}	0.04	<0.0001
NDFI	0.37^{d}	0.53^{a}	0.48^{b}	0.41^{c}	0.02	<0.0001
			Nutrient digestibility (%)			
CPD	94.1^{a}	81.3^{b}	79.6^{b}	81.4^{b}	1.63	0.0011
DMD	59.1^{c}	65.5^{b}	68.0^{b}	56.2^{d}	1.26	<0.0001
OMD	59.8^{b}	66.3^{a}	67.2^{a}	50.6^{c}	1.8	0.0003
NDFD	56.8^{a}	56.0^{a}	57.8^{a}	50.1^{b}	0.9	0.0199

100M= 100% maize, 66M34C=66%maize 34% treated maize cobs, 34M66C=34% maize 66% treated maize cobs, 100C= 100% treated maize cobs, CPI= crude protein intake, DMI= dry matter intake, OMI=organic matter intake, NDFI= neutral detergent fibre intake, CPD=crude protein digestibility, DMD= dry matter digestibility, OMI= organic matter digestibility, NDFD= neutral detergent fibre digestibility.

Combinations of TDS (mg), TDS (%) GP, MMP, EMMP and PF in a multiple regression improved the accuracy of predicting FI from 33 to 73%. TDS (mg), TDS (%), GP, MMP, EMMP and PF explained 52 – 83% variability in WG while their combinations in a multiple regression improved the accuracy of prediction slightly from 83 to 91%.

Table 4 Prediction of *in vivo* CPI, DMI and OMI from IVTDS parameters

Equations	R^2	RSD	P value
CPI= -0.1266 +0.0029TDS (%)	0.35	18.3	0.0155
CPI= -0.1095 +0.0019GP	0.28	19.3	0.0368
CPI= 66.17 + 0.0556TDS (mg) + 0.0939TDS (%) – 0.1878GP+ 0.9263EMMP – 29.83PF	0.83	11.2	0.0015
DMI=-0.8596 +0.0203TDS (%)	0.32	19.1	0.0226
DMI= -0.7397+0.0132GP	0.25	20.1	0.0486
DMI= 398.7+0.3432TDS (mg) +0.0939TDS (%) – 1.1506GP +5.5372EMMP-179.6PF	0.74	14.1	0.0101
OMI=-0.3245+0.0031TDS (mg)	0.32	20.1	0.0221
OMI=-1.1956+0.0238TDS (%)	0.46	18.0	0.0040
OMI=-1.1339+0.0161GP	0.39	19.0	0.0097
OMI=348+0.3043TDS(mg)+0.0780TDS(%) +1.0123GP+4.8345EMMP-152.2PF	0.75	14.5	0.0083

TDS= truly degraded substrate, GP= gas production at 24 hours, MMP= microbial mass production, EMMP= efficiency of microbial mass production, PF= partitioning factor, CPI= crude protein intake, DMI= dry matter intake, OMI=organic matter intake. Y= response variable. X = predictor variable.

Ørskov and Ryle (1990) reported that intake could be predicted from chemical composition of forages. Khazaal et al. (1995) reported an improvement in the accuracy of predicting DMI from chemical composition when CP, NDF, ADF and ADL were combined in a multiple regression analysis. Feeds with higher PF improves microbial production, which is digested post-ruminally, resulting in higher intake and weight gain (Preston and Leng, 1987; Blümmel et al., 1997). It has also been reported that feeds with higher PF have higher intakes, lower methane production and higher excretions of purine derivatives (Blümmel et al., 1997). The positive relationship that was established between TDS, intake, digestibility, feed intake and weight gain implied that the higher the TDS parameters, the higher the intake, digestibility, feed intake and weight gain. Several authors have reported relationships between chemical composition, *in vitro* gas production parameters, *in sacco* degradation parameters, intake, digestibility and growth (Ørskov and Ryle, 1990; Khazaal et al., 1995; Chermiti et al., 1996;

Karsli &Russell, 2002; Moujahed et al., 2011). These authors have also reported improvements in accuracy of predictions when two or more dependent variables are combined in a regression equation.

Table 5 Prediction of *in vivo* DMD, OMI and NDFD from IVTDS parameters

Equations	R^2	RSD	P value
DMD=23.01+0.1155TDS (mg)	0.51	5.8	0.0017
DMD=-5.6937+0.8408TDS (%)	0.66	4.9	0.0001
DMD=-3.6400+0.5703GP	0.57	5.5	0.0001
DMD=46.28+0.1864MMP	0.43	6.3	0.0060
DMD=39.43+0.9148EMMP	0.35	6.8	0.0160
DMD=-6.9356+23.56PF	0.33	6.8	0.0188
DMD=4505+7.555TDS(mg)+0.8457TDS(%)-22.28GP+39.82EMMP-18.73PF	0.90	3.1	<0.0001
OMD=-2.3119+0.1863TDS (mg)	0.66	7.1	0.0001
OMD=-39.06+1.2381TDS (%)	0.70	6.6	<0.0001
OMD=-45.49+0.9217GP	0.73	6.4	<0.0001
OMD=35.28+0.3001MMP	0.54	8.3	0.0011
OMD=23.78+1.492EMMP	0.45	9.0	0.0042
OMD=-51.59+38.34PF	0.88	3.3	<0.0001
OMD=1874+7.858TDS(mg)-1.0711TDS(%)-20.44GP-11.40EMMP-596.5PF	0.88	5.0	<0.0001
NDFD=25.29+0.0880TDS (mg)	0.60	4.2	0.0004
NDFD=10.78+0.5497TDS (%)	0.57	4.4	0.0008
NDFD=4.960+0.4349GP	0.66	3.9	0.0001
NDFD=43.04+0.1420MMP	0.49	4.8	0.0025
NDFD=37.51+0.7089EMMP	0.42	5.1	0.0068
NDFD=1.679+18.23PF	0.40	5.2	0.0085
NDFD=-8194-3.501TDS(mg)-1.307TDS(%)+14.84GP-136.2MMP+3824PF	0.91	2.4	<0.0001

TDS= truly degraded substrate, GP= gas production at 24 hours, MMP= microbial mass production, EMMP= efficiency of microbial mass production, PF= partitioning factor, DMD= dry matter digestibility, OMD=organic matter digestibility, NDFD=neutral detergent fibre digestibility. Y= response variable. X = predictor variable.

Table 6 Prediction of feed intake (FI) and weight gain (WG) from *in vitro* truly degraded substrate parameters

Equations	R^2	RSD	P value
FI=-0.9324+0.0218TDS (%)	0.33	18.9	0.0198
FI=-0.8101+0.0142GP	0.26	19.9	0.0432
FI=403.1+0.3489TDS(mg)+0.0971TDS-1.1685GP+5.588EMMP-181.6PF	0.73	14.1	0.0107
WG=-0.3422+0.0053TDS (mg)	0.70	7.8	<0.0001
WG=-1.549+0.0374TDS (%)	0.83	5.9	<0.0001
WG=-1.459+0.0254GP	0.71	7.6	<0.0001
WG=0.7067+0.0089MMP	0.62	8.7	0.0003
WG=0.3399+0.0453EMMP	0.54	9.6	0.0012
WG=-1.973+1.173PF	0.52	9.8	0.0015
WG=124.6+0.1546TDS(mg)+0.0586TDS(%)-0.4833GP+1.4587EMMP-54.82PF	0.91	5.1	<0.0001

TDS= truly degraded substrate, GP= gas production at 24 hours, MMP= microbial mass production, EMMP= efficiency of microbial mass production, PF= partitioning factor. Y= response variable. X = predictor variable.

In the present study, combinations of IVTDS parameters greatly improved accuracy of predicting nutrient intake, digestibility, feed intake and weight gain.

Conclusions

Nutrient intake, digestibility, feed intake and weight gain of Tswana sheep fed urea-wood ash treated maize cob based diets could be predicted from individual TDS (mg), TDS (%), GP, MMP, EMMP, PF or their combinations.

Acknowledgement

Tertiary Education Trust Fund (TETFUND) Abuja, Nigeria is acknowledged for funding the research.

References

AOAC, 1999. Official Methods of Analysis, 16th ed. Official Methods of Analysis of AOAC International, Gaithersburg, MD, USA

Blümmel, M., Makkar, H.P.S., Chisanga, G., Mtimuni, J. & Becker, K., 1997. The prediction of dry matter intake of temperate and tropical roughages from *in vitro* digestibility/gas production data, and the dry matter intake and *in vitro* digestibility of African roughages in relation to ruminant live weight. Anim. Feed Sci. Technol. 69, 131-141.

Carro, M.D., Lopez, S., Gonzalez, J.S. & Ovejero, F.J., 1994. Comparison of laboratory methods for predicting digestibility of hay in sheep. Small Rumin. Res. 14, 9–17.

Chenost, M., Deverre, F., Aufrere, J. & Demarquilly, C., 1997. The use of gas-test technique for predicting the feeding value forage plants. In: In Vitro Techniques for Measuring Nutrient Supply to Ruminants. Proc. Occ. Meet. Br. Soc. Anim. Sci., 8–10 July 1997, Univ. Reading, UK.

Chermiti, A., Nefzaoui, A, Teller, E., Vanbelle, M., Ferchichi, H. & Rokbani, N., 1996. Prediction of the voluntary intake of low quality roughages by sheep from chemical composition and ruminal degradation characteristics Anim. Sci. 62, 57-62.

Fernandez-Rivera, S., 1997. Relationships between gas release *in vitro* and *in vivo* quality measures of tropical forages. In: *In vitro* techniques for measuring nutrient supply to ruminants, Proc. Occ. Meet. Br. Soc. Anim. Sci., 8–10 July 1997, Univ. Reading, UK.

Karsli, M.A.& Russell, J.R., 2002. Prediction of the voluntary intake and digestibility of forge-based diets from chemical composition and ruminal degradation characteristics. Turk J Vet. Anim. Sci.26, 249-255.

Khazaal, K., Dentinho, M.T., Robeiro, J.M. & Ørskov, E.R., 1995. Prediction of apparent digestibility and voluntary intake of hays fed to sheep: comparison between using fibre components, *in vitro* digestibility or characteristics of gas production or nylon bag degradation.Anim.Sci.61, 527-538.

Macheboeuf, M., Jestin, M., Andrieu, J. & Martin-Rosset, W., 1997. Prediction of the organic matter digestibility of forages in horses by the gas test method. In: *In vitro* techniques for measuring nutrient supply to ruminants. Proc. Occ. Meet. Br. Soc. Anim. Sci., 8–10 July 1997, Univ. Reading, UK.

Makkar, H.P.S., 2010. *In vitro* Screening of Feed Resources for Efficiency of Microbial Protein Synthesis. Retrieved on 18thNovember, 2016 DOI: 10.1007/978-90-481-3297-3_7.

Menke, K.H., Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev. 28, 7-55.

Minson, D.J., 1990. Forage in Ruminant Nutrition. New York, NY,Acad.Press.

Moujahed, N., Khelfa, A., Bouaziz, Y., Ben Mustapha, C. & Kayouli, C., 2011. Prediction of feeding value of some Tunisian hays using chemical composition and *in vitro* fermentation characteristics. In: Ranilla, M.J., Carro, M.D., BenSalim, H. & Morand-Fehr, P., (Eds.), Challenging Strategies to Promote the Sheep and Goat Sector in the Current Global Context, Zaragoza: CIHEAMCSISUniversidadDeLeon, FAO, p. 239-243.
Nolte, M.E., Cline, J.H., Dehority, B.A., Loerch, S.C. & Parker, C.F., 1987. Treatment of wheat straw with alkaline solutions prepared from wood ashes to improve fibre utilization by ruminants. J. Anim. Sci. 64, 669-677.
Ørskov, E.R.&Ryle, M., 1990. Energy Nutrition in Ruminants. Applied Sci., Oxford 149 pp.
Preston, T.R.& Leng, R.A., 1987. Supplementation of diets based on fibrous residues and by-products. In: Straw and Other Fibrous By-Products as Feeds. Sundstøl, F., Owen, E., (Eds.) Elsevier press, Amsterdam, Netherlands, p 373-413.
Romney, D.L., Cadario, F.C., Owen, E. & Murray, A.H., 1997. Comparison of parameters from the Theodorou gas production technique using nitrogen-free and nitrogen-rich media as predictors of DM intake and digestibility. In: *In Vitro* Techniques For Measuring Nutrient Supply to Ruminants. Proc. Occ. Meet. Br. Soc. Anim. Sci., 8–10 July 1997, Univ. Reading, UK.
SAS, 2002. Statistical Analysis System. SAS user's guide: Statistics, SAS Inst. Inc., Carry, NC.
Sundstøl, F., Coxworth, E. & Mowat, D.N., 1978.Improving the nutritive value of wheat straw &other low quality roughages by treatment with ammonia. World Anim. Rev, 26, 13-21.
Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods of dietary fibre, NDF and non-starch polysaccharides in relation to animal material. J. Dairy Sci. 74, 3583-3595.

Aerobic stability of fresh and ensiled potato by-product treated with preservatives and yield fractions from a biorefinery process

M. Franco, T. Stefanski, T. Jalava, M. Lehto, M. Kahala, E. Järvenpää & M. Rinne
Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland.
Correspondence: marcia.franco@luke.fi

Introduction

Incremental demands by consumers and growth of population have resulted in large quantities of vegetable by-products worldwide (Karak *et al*., 2012). Large amounts of by-products from vegetable industry are generated and could potentially be incorporated into the animal feed chain, which could represent a feasible way to efficiently utilize them. The use of potato by-products as animal feed would have a great positive impact on economy and sustainability of both vegetable industry and animal production. Generally, local farmers are willing to use by-products to feed fattening beef cattle, but very little is still known about chemical composition, feed value for ruminants and microbial quality of various types of by-products. A further challenge would be how to preserve and store such residues as they are typically wet and prone to fast deterioration. Additives can be used to preserve these materials and/or enhance quality, such as aerobic stability (AS) (Rinne *et al*., 2017; Franco *et al*., 2018a).

Separating feeds into liquid and solid fractions is typically the first step of a biorefinery process and it may make feeds easier to handle and suitable for different animal groups. In a green biomass production, the concept of the biorefinery is based on a multiproduct system, which utilises green biomass for manufacturing industrial products (Xiu & Shahbazi, 2015). This concept can be expanded to a range of versatile and abundant raw materials, such as vegetable by-products. Biorefining can create innovative feeds with different purposes for different animal species, a source for biogas production and fertilizers, improving profitability and sustainability of the agricultural production system. The objective of the current study was to evaluate the effect of silage additives on AS of fresh or ensiled potato by-product as such or mixed with straw and to evaluate efficiency of the biorefinery process on yield and AS of the solid and liquid fractions.

Materials and Methods

A potato by-product was collected from a Finnish company in September 2018 and comprised end-product waste with peels. It was collected in plastic bags, closed air-tight and transported to Luke's laboratory. The experiment was performed on the same day of sampling. A representative sample of raw material was taken for laboratory analyses. The raw material was divided into three portions, according to the following purposes: 1. aerobic stability of fresh sample, 2. ensiling of potato by-product with preservatives and 3. biorefining the potato by-product and AS of liquid and solid fractions separately.

Aerobic Stability of Fresh Sample

Aerobic stability was evaluated through visual inspection, since rise in temperature seems not work for this type of material (Franco *et al*., 2018a). Half a kg of raw material was weighed into a plastic containers and five preservatives were carefully mixed with it according to the commercial instructions (Table 1): control treatment without preservative (C), formic acid based preservative (FA; AIV Ässä Na, Eastman Chemical Company, Oulu, Finland at 5 l/t), propionic acid based preservative (STAB; Xtrasil Stabilizer, Konsil Scandinavia AB,

Tvååker, Sweden at 3 l/t), salt based preservative (HD; Xtrasil Excel HD, Konsil Scandinavia AB, Tvååker, Sweden at 1.5 l/t) and a lactic acid bacteria additive (LAB; KOFASIL® LAC; ADDCON Bitterfeld-Wolfen, Germany at 1 g/t).

Table 1 Description of the preservatives used in the experiment

Abbr.	Company	Composition	Name	Dosage
FA	Eastman Chemical Company	Formic acid Propionic acid Potassium sorbate Sodium formate	AIV Ässä Na	5 l/t
STAB	Konsil Scandinavia AB	Propionic acid Ammonium propionate Sodium benzoate Potassium sorbate	Xtrasil Stabilizer	3 l/t
HD	Konsil Scandinavia AB	Potassium sorbate Sodium benzoate Ammonium propionate	Xtrasil Excel HD	1.5 l/t
LAB	ADDCON	*Lactobacillus plantarum* DSM 3676 *Lactobacillus plantarum* DSM 3677 (min. 1×10^{11} CFU/g)	KOFASIL® LAC	1 g/t

Preservatives were pipetted into the raw material, but since dosages were different for each treatment, additional water was added to those preservatives used in smaller quantities. The potato by-product was divided into 4 plastic containers (3 cm layer, ≈100 g), which were covered with perforated plastic film and kept at +20°C. Aerobic stability was evaluated once daily by visually observing growth of yeasts and moulds on the surface of the raw material using a scale: 0 = no mould, 1 = slight mouldiness, 2 = moderate mouldiness, 3 = severe mouldiness. Samples were discarded when severely moulded.

Ensiling of Potato By-Product with Preservatives

Three batches of seven kg of raw material per treatment were weighed into plastic containers and carefully mixed with additives using same treatments previously described (Table 1). The potato by-product was ensiled in glass jars (ca. 1.6 l) at a density of 1,087 kg/m^3, which were filled as full as possible to minimize headspace and were covered with a plastic foil. Each batch was divided into three replicates per treatment that were opened at three different occasions: after 2 days, after 7 days and after 28 days of ensiling (3 replicates × 3 opening times × 5 treatments; altogether 45 jars).

In addition to these 45 jars, control and formic acid based treatments were also tested when ensiled with straw in a fresh matter proportion of 85:15 of potato by-product and straw, comprising 18 jars (3 replicates × 3 opening times × 2 treatments). The density of potato by-product and straw was 751 kg/m^3. When opening the jars, samples were taken to evaluate AS by visual inspection as previously described. Furthermore, potato by-product ensiled with straw was also evaluated for AS through increase in temperature. Aerobic stability was carried out according to Luke's standard method, where thermocouple wires in polystyrene boxes were connected to a data logger with temperature automatically recorded at 10-minute intervals. Aerobic stability was defined as the time taken to increase temperature of the sample 2°C or 3°C above ambient temperature. Samples were weighed before and after the aerobic incubations for estimation of losses.

Biorefining the Potato By-Product and AS of Liquid and Solid Fractions Separately

The potato by-product was biorefined using a laboratory scale pneumatic press (in-house built equipment; Luke, Jokioinen, Finland) for liquid-solid separation. About 200 g sample was put into a bag made from cheese cloth and squeezed between two piston plates during 2 minutes at 6 bars (× 100 kPa) of pressure. Liquid was quantitatively collected and weighed to estimate liquid yield.

Several replicates were fractionated to obtain enough material to apply the preservative treatments (Table 1) of the liquid and solid fractions and for sampling of fractions for analysis of chemical composition. Aerobic stability was evaluated through visual inspection of liquid and solid fractions.

All samples were stored in a -20°C and analysed using routine methods at Luke's laboratory as described by Franco *et al*. (2018a). The data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability. The sums of squares were further partitioned into contrasts, where preservative effects were tested. Replicates were taken as a random effect in the model.

Results and Discussion

The chemical composition, feed values and microbial quality of potato by-product, biorefined fractions as well as silage (potato by-product + straw) are in Table 2. The potato by-product had lower DM concentration than the reference value for whole potatoes in the Finnish tables (220 g/kg; Luke, 2019), because water was used during peeling. Due to low DM of the potato by-product, it was ensiled with straw. The potato by-product had low concentration of CP, but on the other hand, it was high in starch.

Aerobic Stability of Fresh Sample

Preservative treatments resulted in greater ($P<0.05$) AS of the potato by-product (Fig. 1). Formic acid based preservative improved ($P<0.05$) AS for up to 1.5 months in comparison to control treatment with only 5 days of stability. Similar results were found by Franco *et al*. (2018b) with carrot by-products. Treatment with STAB and HD resulted in greater AS than control, but LAB was not effective in improving stability. According to McDonald *et al*. (1991), preservatives can be divided into fermentation inhibitors (e.g. organic acids), which were more effective in controlling fermentation of the potato by-product and fermentation stimulators (e.g. strains of lactobacilli). The latter stimulated fermentation, resulting in a more rapid deterioration. Thus, LAB was used erroneously, since the operating principle is not suitable for this type of raw material. Addition of straw resulted in lower ($P<0.05$) AS and the reason could have been introduction of microorganism by the straw, which on average had higher concentrations of enterobacteria, moulds and total bacteria (Table 2) than the potato by-product raw material.

Ensiling of Potato By-Product with Preservatives

Additives were efficient in improving ($P<0.05$) AS during the aerobic phase of silages opened after 2 and 7 days (Table 3), but no difference ($P>0.05$) was identified for silages after 28 days. Ensiling resulted in greater ($P<0.05$) AS than fresh raw material for all treatments except for FA. This treatment had better AS than all other treatments and no difference was observed throughout the period. According to Rinne *et al*. (2017), FA also

restricted fermentation during ensiling process of carrot by-product and only minor effects could be detected from using different LAB strains.

Table 2 Composition and microbial quality of potato by-products, straw and fractions used in the experiment

	Potato by-product	Straw	Fractions		Silage (potato + straw)
			Solid	Liquid	
Proportion, g/kg			353	647	850:150
Dry matter (DM), g/kg	179	847	460	106	279
pH	5.97	8.86			
In DM, g/kg					
Ash	57	99	23	100	63
Crude protein	76	65	25	123	74
Starch	654	24			560
Sugar	22	19			22
Neutral detergent fibre	58	705			155
Feed values for ruminants					
IVOMD, g/g	0.930	0.521			
Metabolizable energy, MJ/kg DM	14.0	7.5			
MP, g/kg DM	96	55			
PBV, g/kg DM	-70	-18			
Microbial quality					
Enterobacteria, cfu/g	1.0×10^5	2.0×10^6	1.3×10^5	6.9×10^4	
Lactic acid bacteria, cfu/g	7.5×10^6	5.4×10^3	7.4×10^7	1.4×10^7	
Yeast, cfu/g	6.1×10^4	3.7×10^6	3.2×10^4	1.7×10^4	
Mould, cfu/g	1.6×10^4	1.5×10^7	3.8×10^4	1.5×10^4	
Total bacteria, cfu/g	1.3×10^7	8.8×10^7	1.1×10^7	8.9×10^6	
Clostridia	23	<3.0	23	0.92	
Total coliforms			2.3×10^5	2.0×10^5	
Escherichia coli			$<1.0\times10^1$	$<1.0\times10^1$	

IVOMD: In vitro organic matter digestibility - pepsin-cellulase method calculated with the general equation of Huhtanen *et al*. (2006); MP: metabolizable protein; and PBV: protein balance in the rumen (Luke, 2019).

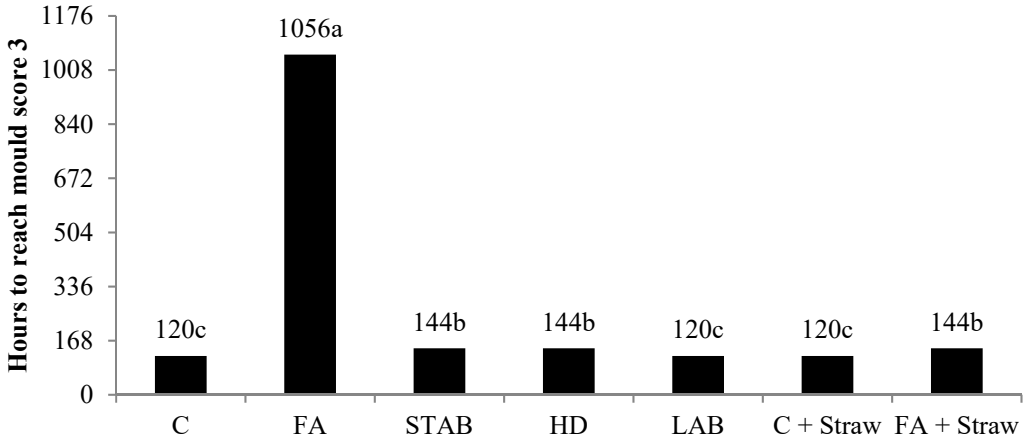


Figure 1 Hours to reach mould score 3 of potato by-product treated with preservatives. For treatment descriptions see Table 1. C vs others P<0.01; Straw P<0.01. Means without same letter differ significantly (P<0.05). SEM 3.7 h.

Table 3 Aerobic stability (visual inspection; hours to reach mould score 3) of potato by-product fresh and after 2, 7 and 28 days fermentation period treated with preservatives

	C	FA	STAB	HD	LAB	C + Straw	FA + Straw	SEM	Add	Straw
Fresh	120^{cC}	1056^{aA}	144^{bC}	144^{bD}	120^{cC}	120^{cD}	144^{bC}	3.7	<0.01	<0.01
2 days	72^{cD}	1064^{aA}	144^{cC}	168^{cC}	96^{cD}	360^{bB}	376^{bB}	22.5	<0.01	<0.01
7 days	144^{bB}	1040^{aA}	216^{bB}	216^{bB}	144^{bB}	320^{bC}	392^{bAB}	75.0	0.01	0.01
28 days	336^{cA}	704^{abA}	336^{cA}	336^{cA}	336^{cA}	816^{aA}	440^{bcA}	58.0	0.18	0.09
SEM	0.0	156.5	0	0	0	8.9	13.9			
Fresh vs silage	<0.01	0.49	<0.01	<0.01	<0.01	<0.01	<0.01			
Linear	<0.01	0.14	<0.01	<0.01	<0.01	<0.01	<0.01			
Quad	<0.01	0.27	<0.01	<0.01	<0.01	<0.01	0.01			

For treatment descriptions see Table 1. Means without same lower case letter in a row and upper case letter in a column differ significantly (P<0.05). SEM: standard error of the mean. Add: additive effect, control vs others; Straw: effect of straw in ensiling; Fresh vs silage: effect of ensiling; Linear: linear effect of ensiling period; Quad: quadratic effect of ensiling period.

There was a quadratic effect (P<0.05) of ensiling period on AS for both threshold temperatures and a longer AS was obtained for silages opened after the 28-d fermentation period (Table 4). Greater losses of potato by-product + straw silages were observed (P<0.05) for samples ensiled during the longer fermentation period.

Table 4 Aerobic stability (AS; time taken to increase the temperature for 2 °C or 3 °C above the ambient temperature, hours) and losses during aerobic phase of potato by-product ensiled with or without formic acid based additive and straw opened at different times

	2 days		7 days		28 days		SEM	Significance	
	C + Straw	FA + Straw	C + Straw	FA + Straw	C + Straw	FA + Straw		Linear	Quad
AS, 2 °C	79^{b}	60^{b}	68^{b}	77^{b}	501^{a*}	448^{a}	15.1	<0.01	<0.01
AS, 3 °C	87^{b}	63^{b}	76^{b}	89^{b}	501^{a*}	475^{a}	10.7	<0.01	<0.01
Losses, g/kg FM	15^{c}	17^{c}	22^{b}	23^{b}	23^{b}	29^{a}	0.59	<0.01	0.01

Means without same letter differ significantly (P<0.05). SEM: standard error of the mean. Linear: linear effect of ensiling period; Quad: quadratic effect of ensiling period. FM: fresh matter. *Treatment did not reach the threshold during the evaluation period.

Biorefining the Potato By-Product and AS of Liquid and Solid Fractions Separately

Biorefining the potato by-product resulted in a 65% liquid yield with greater proportion of ash (0.67) and CP (0.62) retained in the liquid fraction (Table 2). Preservatives improved (P<0.05) AS of both fractions (Table 5), FA being the most effective, followed by STAB, HD and LAB, with no difference (P>0.05) among them. Both fractions presented greater (P<0.05) AS than fresh raw material in all treatments, except for FA. The solid fraction had a better AS than the liquid fraction, proving that moisture reduction can extend shelf life and improve logistics.

Part of the of the preservative responses may have been due to the amounts of product recommended by the manufacturer, suggesting a need for future optimizing of preservative levels and taking into account the cost of using additives. Due to the extensive fermentation of potato the by-product during ensiling, a practical farm scale option would be the use of chemical preservative to improve the shelf life.

Table 5 Aerobic stability by visual inspection (h to reach mould score 3) of biorefined fractions of potato by-product treated with silage preservatives

	C	FA	STAB	HD	LAB	SEM	Preservative
Fresh	120^{cC}	1056^{aB}	144^{bC}	144^{bC}	120^{cC}	3.7	<0.01
Solid	496^{bA}	1336^{aA}	552^{bA}	512^{bA}	496^{bA}	24.3	<0.01
Liquid	168^{cB}	1384^{aA}	296^{bB}	216^{bcB}	296^{bB}	24.5	<0.01
SEM	5.2	60.9	5.2	10.3	6.3		
Fresh vs fractions	<0.01	0.01	<0.01	<0.01	<0.01		
Solid vs liquid	<0.01	0.57	<0.01	<0.01	<0.01		

For treatment descriptions see Table 1. Means without same lower case letter in a row and upper case letter in a column differ significantly (P<0.05). SEM: standard error of the mean. Preservative: control vs others.

Conclusions

Chemical preservatives typically used as silage additives were efficient in extending aerobic stability of the fresh or fermented potato by-product. Formic acid was superior compared to the other types of additives, which was at least partly due to the high dosage level. Biorefining has a great potential in improving AS of fractions creating better opportunities in terms of logistics and targeted nutritional value of the products.

Acknowledgements

This project was conducted as part of the Circwaste project (http://www.circwaste.fi).

References

Franco, M., Jalava, T., Kahala, M., Järvenpää, E., Lehto M. & Rinne, M., 2018a. Preservatives can improve aerobic stability of potato by-products. Proc. 9th Nordic Feed Sci. Conf., 12-13 June, Uppsala, Sweden.

Franco, M., Jalava, T., Järvenpää, E., Kahala, M. & Rinne, M., 2018b. Carrot by-product fermentation quality and aerobic stability could be modified with silage additives. XVIII Internat. Silage Conf., 24-26 July, Bonn, Germany.

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

Karak, T., Bhagat, R.M. & Bhattacharyya, P., 2012. Municipal solid waste generation, composition, and management: the world scenario. Crit. Rev. Env. Sci. Tech. 42, 1509-1630.

Luke, 2019. Feed Tables and Nutrient Requirements. Available at: www.luke.fi/feedtables

McDonald, P., Henderson, A.R. & Heron, S.J.E., 1991. The Biochemistry of Silage, 2nd edn. Marlow, UK, Chalcombe Publ., 340 pp.

Rinne, M., Jalava, T., Siljander-Rasi, H., Kuoppala, K., Blasco, L., Kahala, M. & Järvenpää, E., 2017. Improving the usability of carrot by-products as animal feeds by ensiling. Proc. 8th Nordic Feed Sci. Conf., 13-14 June 2017, Uppsala, Sweden.

Xiu, S. & Shahbazi, A., 2015. Development of green biorefinery for biomass utilization: a review. Trends Renew. Energy 1, 4-15.

Manipulation of mixed red clover and grass silage quality through compaction, soil contamination and use of additives

M. Franco, T. Stefański, T. Jalava, A. Huuskonen & M. Rinne
Natural Resources Institute Finland (Luke), Finland.
Correspondence: marcia.franco@luke.fi

Introduction

In Finland, red clover is used successfully as a silage crop, largely because of its winter hardiness and good nutritional quality (Järvenranta *et al.*, 2016). In Northern areas, due to climatic conditions with a short grazing period, most forage is provided to ruminants as silage. Ensiling of forages has become a global practice for forage preservation and is particularly prevalent in wet climates, where the conservation of dried forage is difficult (Wilkinson and Rinne, 2018).

The improvement of silage quality in practice is still a concern and work to achieve it is continuously needed. Poorly packed silage and soil contamination are common reasons for spoilage and aerobic deterioration of silage before feeding. General good management practices in silage making include avoiding soil contamination to prevent inoculation with spoilage microbes and tight compaction to ensure anaerobic conditions and accumulation of lactic acid that results in a low pH, inhibits microbial metabolism and preserves nutrients. Thus, improving fermentation quality of silages could provide a substantial advantage to farmers. Many approaches have been used to improve fermentation quality of silages such as use of additives (Muck and Kung, 1997). The objective of this experiment was to evaluate how different types of silage additives are able to manipulate the ensiling process of a mixed red clover and timothy grass under varying management conditions represented by two levels of compaction and soil contamination.

Materials and Methods

Organically grown mixed red clover (*Trifolium pratense*) and timothy (*Phleum pratense*) grass with a botanical proportion of 0.765 and 0.235, respectively, was harvested from a second cut on August 1st 2018 at Häme University of Applied Sciences in Mustiala, Finland (60°83'N, 23°77'E). One day later, the grass was precision chopped using farm scale machinery and transported to the laboratory.

Silages were prepared using two compaction levels that was achieved by manually dropping an 8--kg lead plummet (80 cm height) ten and two times on a handful of grass in a cylindrical silo, for tight and loose compactions, respectively. In order to challenge the effect of additives on fermentation quality of a low-hygienic quality raw material, contamination with soil from a slurry-treated area was conducted for the tightly compacted silos.

Four additive treatments were used according to commercial instructions (Table 1):

1. Control: negative treatment without additive;
2. Formic acid (FA) based additive (AIV Ässä Na, Eastman Chemical Company, Oulu, Finland at 5 l/t);
3. Homofermentative strains of the lactic acid bacteria (LAB) *Lactobacillus plantarum* (KOFASIL® LAC, ADDCON, Bitterfeld-Wolfen, Germany at 1 g/t); and
4. Salt based additive (Safesil Challenge, Salinity AB, Göteborg, Sweden at 2 l/t).

The grass was ensiled in cylindrical pilot scale silos with 12-L capacity using three replicates per treatment. Silos were stored at room temperature with protection from light and opened after an ensiling period of about 3 months. Deteriorated parts from top of the silos were discarded and silage was carefully mixed and samples taken and analysed for chemical composition, fermentation quality, aerobic stability and microbial quality at the laboratory of Luke using standard methods.

Ensiling losses were calculated according to Knicky and Spörndly (2015) by assuming weight loss to be CO_2 leaving the silo during fermentation. It was assumed that for each mole of CO_2, 1 mol of H_2O was produced. It means that for each gram of weight decrease due to CO_2 loss, 0.44 g of dry matter (DM) in the silo became water, which represents loss even if it is still inside the silo. Therefore, the DM loss was calculated as the decrease in weight of the silo multiplied by a factor of 1.44, expressed as g/kg initial DM.

Table 1 Description of the additives used in the experiment

Abbr.	Company	Composition	Name	Dosage
FA	Eastman Chemical Company	Formic acid Propionic acid Potassium sorbate Sodium formate	AIV Ässä Na	5 l/t
LAB	ADDCON	*Lactobacillus plantarum* DSM 3676 *Lactobacillus plantarum* DSM 3677 (min. 1×10^{11} CFU/g)	KOFASIL® LAC	1 g/t
Salt	Salinity AB	Sodium nitrite, E 250 Sodium benzoate Potassium sorbate Active substance: 35 % Water: 65 %	Safesil Challenge	2 l/t

Aerobic stability testing was carried out according to Luke's standard method, where thermocouple wires in polystyrene boxes was connected to a data logger and temperature was automatically recorded at 10-minute intervals. Aerobic stability was defined as the time taken to increase the temperature of the sample 2°C above ambient temperature. Samples were weighed before and after aerobic incubation for estimation of losses.

All samples were stored in -20°C prior to analysis according to standard laboratory methods. The DM concentration was determined by drying at 105°C for 16 h. Ash (method 942.05) and crude protein (CP; method 968.06 using Leco FP 428 nitrogen analyser - Leco Corp., USA and the correction factor 6.25×N) were determined according to AOAC (1990). Concentration of neutral detergent fibre (NDF) was determined in an ANKOM 220 Fiber Analyzer (ANKOM Technology, USA) according to Van Soest *et al*. (1991) using sodium-sulphite and α-amylase and expressed as ash free. Volatile fatty acids were determined according to Huhtanen *et al*. (1998), lactic acid according to Haacker *et al*. (1983), sugars according to Somogyi (1945) and ammonia according to McCullough (1967). The N content of the herbage before ensiling was used to express ammonia-N proportions in total N after fermentation. A spectrophotometric method was used for the determination of ethanol from water-silage extracts (commercial kit cat. No. 10 176 290 035, Boehringer Mannheim GmbH, Mannheim, Germany) according to application instructions. In vitro organic matter digestibility was measured according to Nousiainen *et al.* (2003). Results were calculated with correction equations to convert pepsin-cellulase solubility values into *in vivo* digestibility by equations based on a data set comprising of Finnish *in vivo* digestibility trials

according to Huhtanen *et al*. (2006). The concentration of propionic acid was corrected for the amount added in the additive. Yeasts and moulds were determined on Dichloran Rose Bengal Chloramphenicol Agar medium (Lab M Ltd, LAB217) which was supplemented with 50 µg/ml of oxytetracycline hydrochloride (AppliChem BioChemica A5257). The Petri dishes were incubated at 25°C. The colonies were counted after 3 and 5 d. The aerobic plate count was determined on Plate Count Agar (Lab M Ltd, Lab010) dishes incubated at 30°C during 72 hours.

Data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability with additive, compaction and soil contamination as fixed effects and replicates as random effect.

Results and Discussion

Grass DM (Table 2) was slightly lower than typical grasses used in Northern Europe according to reference value (321 g/kg DM; Salo *et al*., 2014). However, the content of ash, CP and NDF was representative for a standard raw material used for ensiling. Water soluble carbohydrates and in vitro organic matter digestibility were lower than typical grasses in Finland. The densities reached by the silages were 500 and 665 kg/m^3 for tight and loose compactions, respectively.

Table 2 Composition and microbial quality of mixed red clover and timothy grass

	Grass	Soil contamination solution	Contaminated raw material
Dry matter (DM), g/kg	261		
Buffering capacity, g lactic acid/100 g	7.4		
Chemical composition, g/kg DM			
Ash	100		
Crude protein	162		
Water soluble carbohydrates	37		
Neutral detergent fibre	460		
Feed values for ruminants			
IVOMD, g/kg OM	670		
Metabolizable energy, MJ/kg DM	9.6		
MP, g/kg DM	82		
PBV, g/kg DM	43		
Microbial quality			
Yeasts, cfu/g	1.1×10^6	1.1×10^3	7.6×10^5
Moulds, cfu/g	1.3×10^6	1.3×10^3	9.6×10^5
Total bacteria, cfu/g	1.8×10^9	7.0×10^6	4.4×10^8
Clostridia, spore/g	3.6	2 800	3.6

IVOMD: In vitro organic matter digestibility - pepsin-cellulase method calculated with the general equation of Huhtanen *et al*. (2006). OM: organic matter. MP: metabolizable protein and PBV: protein balance in the rumen (Luke, 2019). cfu: colony-forming units.

Silages treated with FA resulted in lower ($P<0.05$) concentration of ammonia N and also the lowest ($P<0.05$) pH values (Table 3). There was no effect ($P>0.05$) of compaction and soil contamination on these parameters, except for pH, which was higher ($P<0.05$) for contaminated silages. Proportion of ammonia N in total N is generally considered as a good indicator of silage fermentation quality (McDonald *et al*., 1991). According to Wilkinson (1990), grass silage with ammonia N in a range of 50 – 100 g/kg total N is regarded as well fermented, showing that even if ammonia N in control, LAB and Salt was higher than in FA, they were still well preserved, while grass silage having ammonia N below 50 g/kg total N is very well fermented.

Table 3 Chemical composition, fermentation quality, aerobic stability, ensiling losses and microbial quality of mixed red clover and timothy grass silages treated with additives under different compaction (Comp) and soil contamination (Cont) levels

Contamination	Non-contaminated								Soil contaminated				SEM[1]	P-value[2]	
Compaction	Loose				Tight				Tight						
Additive	Control	FA	LAB	Salt	Control	FA	LAB	Salt	Control	FA	LAB	Salt		Comp	Cont
Dry matter (DM), g/kg	274	264	272	274	271	271	268	269	259	274	269	268	4.6	0.77	0.31
pH	4.55^{abc}	4.37^{de}	4.54^{abcd}	4.48^{bcd}	4.56^{ab}	4.40^{cde}	4.59^{ab}	4.49^{bcd}	4.70^{a}	4.31^{e}	4.64^{ab}	4.53^{abcd}	0.034	0.28	0.02
Ammonia N, g/kg N	61^{a}	38^{b}	60^{a}	61^{a}	59^{a}	37^{b}	58^{a}	58^{a}	64^{a}	40^{b}	62^{a}	56^{a}	1.6	0.18	0.06
Chemical composition, g/kg DM															
Ash	108	105	74	108	107	104	108	106	110	106	109	109	10.1	0.31	0.19
Crude protein	182	175	181	172	182	173	183	181	181	173	178	183	2.5	0.22	0.59
Ethanol	5.9^{b}	1.9^{e}	5.1^{bc}	2.8^{de}	5.8^{b}	1.9^{e}	5.5^{b}	3.1^{de}	7.5^{a}	2.4^{de}	5.6^{b}	3.7^{cd}	0.31	0.48	<0.01
Sugars	3.3^{c}	34.8^{a}	2.7^{c}	4.4^{c}	3.9^{c}	40.4^{a}	2.9^{c}	5.7^{c}	2.4^{c}	22.4^{b}	2.3^{c}	2.9^{c}	1.30	0.04	<0.01
Acids, g/kg DM															
Formic	0^{b}	13.8^{a}	0^{b}	0^{b}	0^{b}	13.3^{a}	0^{b}	0^{b}	0^{b}	13.4^{a}	0^{b}	0^{b}	0.16	0.28	0.30
Lactic (LA)	76.7^{a}	39.5^{de}	71.5^{ab}	69.9^{ab}	72.2^{ab}	30.4^{e}	72.2^{ab}	64.6^{ab}	62.3^{abc}	45.4^{cde}	55.4^{bcd}	64.8^{ab}	3.55	0.08	<0.01
Acetic	33.8^{a}	15.0^{b}	36.9^{a}	34.2^{a}	34.0^{a}	14.2^{b}	37.3^{a}	37.5^{a}	42.3^{a}	20.0^{b}	44.1^{a}	38.3^{a}	2.26	0.64	<0.01
Propionic[3]	0.23^{b}	0.42^{ab}	0.23^{b}	0.12^{b}	0.25^{b}	0.55^{ab}	0.22^{b}	0.14^{b}	0.93^{a}	0.39^{b}	0.53^{ab}	0.40^{b}	0.100	0.58	<0.01
Butyric	0.38	0.05	0.40	0.04	0.24	0.04	0.09	0.04	0.74	0.04	0.21	0.04	0.223	0.48	0.79
Total volatile fatty acids	34.5^{a}	15.5^{b}	37.7^{a}	34.5^{a}	34.6^{a}	14.8^{b}	37.7^{a}	37.8^{a}	44.2^{a}	20.5^{b}	45.0^{a}	39.0^{a}	2.28	0.67	<0.01
Total fermentation acids	111.2^{a}	58.5^{bc}	109.2^{a}	104.4^{a}	106.8^{a}	48.6^{c}	109.9^{a}	102.4^{a}	106.5^{a}	69.2^{b}	100.4^{a}	103.7^{a}	2.52	0.03	0.64
LA/total fermentation acids	0.69^{a}	0.67^{ab}	0.65^{ab}	0.67^{ab}	0.68^{ab}	0.63^{ab}	0.66^{ab}	0.63^{ab}	0.58^{ab}	0.66^{ab}	0.55^{b}	0.62^{ab}	0.026	0.18	<0.01
Total fermentation products	117^{a}	60b^{c}	114^{a}	107^{a}	113^{a}	50^{c}	115^{a}	106^{a}	114^{a}	72^{b}	106^{a}	107^{a}	2.7	0.06	0.98
Aerobic stability[4], hours	150^{c}	185^{c}	194^{c}	246^{c}	135^{c}	178^{c}	176^{c}	445^{ab}	202^{c}	477^{a}	265^{bc}	551^{a5}	38.7	0.16	<0.01
Ensiling losses, g/kg of initial DM	20^{abc}	11^{c}	22^{abc}	14^{bc}	28^{ab}	12^{c}	33^{a}	18^{bc}	28^{ab}	15^{bc}	29^{ab}	22^{abc}	2.9	<0.01	0.76
Losses during aerobic phase, g/kg initial DM	40	38	33	22	44	43	34	32	51	45	41	44	7.0	0.36	0.18
Clostridia, spore/g	-	-	-	-	5.7	3.2	9.9	5.1	4.7	3.2	16.5	<3			
Yeasts, cfu/g	1.0×10^{4}	$<1.0\times10^{2}$	$<1.0\times10^{2}$	$<1.0\times10^{2}$	$<1.0\times10^{2}$	$<1.0\times10^{2}$	$<1.0\times10^{2}$	$<1.0\times10^{2}$	$<1.0\times10^{2}$	$<1.0\times10^{2}$	$<1.0\times10^{2}$	$<1.0\times10^{2}$			
Moulds, cfu/g	5.3×10^{2}	2.8×10^{3}	3.5×10^{2}	$<1.0\times10^{2}$	1.7×10^{4}	5.3×10^{2}	$<3.0\times10^{2}$	$<2.0\times10^{2}$	1.0×10^{3}	4.3×10^{4}	2.7×10^{3}	$<3.0\times10^{2}$			

Values with same letter in a row are not significantly different at 5% Tukey test.
[1]Standard error of the mean. [2]Effect of compactions and soil contamination. [3]Corrected for its amount in the FA based additive. [4]Time taken to increase the temperature of samples by 2 °C above the ambient temperature (22 °C). Data collection lasted for 551 h. [5]Treatment did not reach the threshold during the evaluation period.

There was no effect (P>0.05) of compaction on ethanol production in the silages, but an effect (P<0.05) was found for contamination with higher concentrations for silages contaminated with soil (Table 3). Lower concentrations (P<0.05) of ethanol was found for FA treated silages.

Among non-contaminated silages, the only effect of additive treatments on aerobic stability was the greater (P<0.05) aerobic stability of Salt compared to the other treatments (Figure 1). Soil contaminated silages resulted in higher (P<0.05) aerobic stability than the uncontaminated ones probably due to "wild-type" fermentation (Table 3) with greater concentration of acetic and propionic acids and lower concentration of lactic acid. Although this type of fermentation provides stability to the silages it is not recommended and may lead to reduced feed intake, decreased milk quality and unexpected losses. Acetic and propionic acids are good antifungal acids (Woolford, 1975), but according to Kung (2010), production of acetic acid through "wild-type" fermentation pathways may be less desirable than if produced via "controlled" pathways.

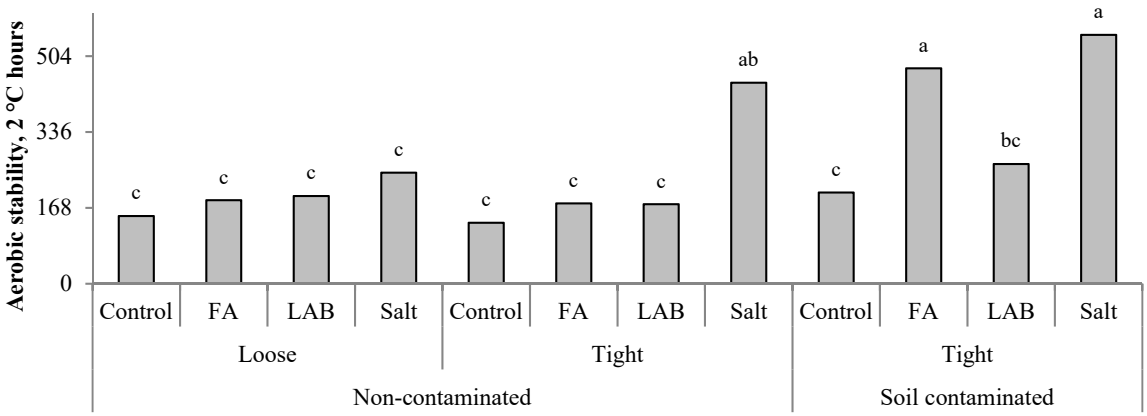


Figure 1 Aerobic stability (time taken to increase the temperature of samples by 2 °C above the ambient temperature [22 °C]) of mixed red clover and timothy grass silages treated with additives under different compaction (Comp) and soil contamination (Cont) levels. Comp P=0.16; Cont P<0.01; SEM: 38.7h.

There was no effect (P>0.05) of additive, compaction and soil contamination on silage composition regarding DM, ash and crude protein, but there was a positive effect (P<0.05) of compaction and soil contamination on sugar levels with higher concentrations for non-contaminated and tight compacted silages (Table 3). Fermentation during the ensiling process reduced proportions of yeasts and moulds in comparison to the raw material in all treatments. According to Pahlow *et al*. (2003), yeasts are generally initiators of aerobic deterioration, consuming sugars and fermentation acids and raising silage temperature and pH. The FA addition effectively restricted fermentation as consistently observed by Franco *et al*. (2018). For LAB and Salt, only very few differences were observed compared to control.

Conclusions

The results of the current experiment confirmed the practical guidelines of good silage production management including use of additives, careful compaction and avoiding soil contamination. Use of additives, such as formic acid, lactic acid bacteria strains and salt based additives improved fermentation quality of the mixed red clover and timothy grass ensiled under different management conditions. Silages were slightly better preserved under tight compaction. Silage soil contamination stimulated a non-desired type of fermentation

with greater amount of ethanol, acetic and propionic acid and higher pH, thus it should be avoided in farm scale.

References

AOAC, 1990. Official methods of analysis. Arlington, VA: Association of Official Analytical Chemists, Inc., 1298 p. ISBN 0-935584-42-0.
Franco, M., Jalava, T., Stefański, T., Kuoppala, K., Timonen, P., Winquist, E., Siika-aho, M. & Rinne, M., 2018. Grass silage for biorefinery - effect of additives on silage quality and liquid-solid separation in timothy and red clover silages. Proc. 9th Nordic Feed Sci. Conf., 12-13 June, Uppsala, Sweden.
Haacker, K., Block, H.J. & Weissbach, F., 1983. Zur kolorimetrischen Milchsäurebestimmung in Silagen mit p-Hydroxydiphenyl. [On the colorimetric determination of lactic acid in silages with p-hydroxydiphenyl]. Arch. Tierernähr. 33, 505-512.
Huhtanen, P., Nousiainen, J. & Rinne, M., 2006. Recent developments in forage evaluation with special reference to practical applications. Agric. Food Sci. 15, 293-323.
Huhtanen, P.J., Blauwiekel, R. & Saastamoinen, I., 1998. Effects of intraruminal infusions of propionate and butyrate with two different protein supplements on milk production and blood metabolites in dairy cows receiving grass silage based diet. J. Sci. Food Agr. 77, 213-222.
Järvenranta, K., Kuoppala, K., Rinne, M. & Virkajärvi, P., 2016. Legumes in ruminant production systems in European cold climates - Limitations and opportunities. Legume Perspectives, Issue 12, p. 34-35.
Knicky, M. & Spörndly, R., 2015. Short communication: Use of a mixture of sodium nitrite, sodium benzoate, and potassium sorbate in aerobically challenged silages. J. Dairy Sci. 98, 5729-5734.
Kung, L. Jr., 2010. Aerobic stability of silage. Proc. 2010 California Alfalfa & Forage Symp.. Visalia, CA, UC Davis. Available at: https://alfalfa.ucdavis.edu/+symposium/proceedings/2010/10-89.pdf
Luke, 2019. Feed Tables and Nutrient Requirements. Available at: www.luke.fi/feedtables
McCullough, H., 1967. The determination of ammonia in whole blood by direct colorimetric method. Clin. Chim. Acta 17, 297-304.
McDonald, P., Henderson, A.R. & Heron, S.J.E., 1991. The Biochemistry of Silage, 2nd edn. Marlow, UK, Chalcombe Publ. 340 pp.
Muck, R. & Kung, L., 1997. Effects of silage additives on ensiling. Proc. Silage: Field to Feedbunk N. Americ. Conf., Hershey, PA, Ithaca: Northeast Regional Agricultural Engineering Service.
Nousiainen, J., Rinne, M., Hellämäki, M. & Huhtanen, P., 2003. Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsin-cellulase solubility. Anim. Feed Sci. Tech. 103, 97-111.
Pahlow, G., Muck, R.E., Driehuis, F., Elferink, S.J.W.H.O. & Spoelstra, S.F., 2003. Microbiology of Ensiling, in: Silage Science and Technology, p 31–93.
Buxton, D.R., Muck, R.E. & Harrison, J.H.(Ed.). Monograph No. 42. American Society of Agronomy, Madison, WI, USA.

Salo, T., Eurola, M., Rinne, M., Seppälä, A., Kaseva, J. & Kousa, T., 2014. The effect of nitrogen and phosphorus concentrations on nutrient balances of cereals and grass silage. MTT Report 147, Jokioinen, Finland, 37 pp. Available at: http://jukuri.luke.fi/bitstream/handle/10024/482918/mttraportti147.pdf
Somogyi, M., 1945. A new reagent for the determination of sugars. J. Biol. Chem. 160, 61-68.
Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.
Wilkinson, J.M., 1990. Silage. 6th edn., Marlow, UK: Chalcombe Publ., 186 pp.
Wilkinson, J.M. & Rinne, M., 2018. Highlights of progress in silage conservation and future perspectives. Grass Forage Sci. 73, 40-52.
Woolford, M.K., 1975. Microbiological screening of the straight chain fatty acids (C1-C12) as potential silage additives. J. Sci. Food Agric. 26, 219-228.

Digestibility of grass silage treated with a feruloyl esterase producing inoculant

K. Kelkay Haile & K. Mogodiniyai Kasmaei
Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Box 7024, 750 07 Uppsala
Correspondence: Kamyar.mogodiniyai.kasmaei@slu.se

Introduction

Lignocellulosic biomass is the main ingredient of dairy cattle rations and is an important renewable carbon resource for biorefineries. This type of biomass has a relatively low digestibility in most cases and intensive research is carried out in several places to improve its digestibility.

The lignocellulosic biomass comprises mainly cellulose, hemicellulose and lignin, collectively referred to as fibre. Under anaerobic conditions (e.g. in the rumen or biogas reactors), only cellulose and hemicellulose can be utilized. In plant cell walls of monocots (e.g. cereals and grasses), lignin and hemicellulose binds together via linkages mediated mainly by ferulic acid. The lignin-hemicellulose matrix encrusts the cellulose, forming a complex structure believed to be the main cause of recalcitrance of fibres to utilization (Rubin, 2008; Pu et al., 2013). These linkages can be cleaved by feruloyl esterases (FAE) (EC 3.1.1.73), members of carboxylic esterases (EC 3.1.1), to enhance bioavailability of cellulose and hemicellulose.

Ensiling is the main form of forage preservation in temperate regions. In this study, we aimed at breaking lignin-hemicellulose linkages in silage by the combined effects of mechanical treatment and inoculation with a feruloyl esterase producing inoculant.

Materials and Methods

One 2nd cut ryegrass with fully developed heads and one 2nd cut meadow fescue sample with no heads were obtained from fields within Uppsala, Sweden during autumn 2018. Samples were chopped with a stationary chopper to ~5 cm and stored at -20°C. *Lactobacillus buchneri* LN4017 (ATCC no. PTA-6138) was used as a FAE producing inoculant. FAE activity of this strain had been confirmed in a previous experiment (Nsereko et al., 2008). For inoculum preparation, the bacterium was cultivated at 37°C for 48 h in MRS broth (Merck KGaA, Darmstadt, Germany) before centrifugation at 4000 × *g* for 5 min. The bacterial pellet was suspended in a Ringer solution (Merck KGaA, Darmstadt, Germany), divided into batches of 1 mL and stored at -80°C. For the ensiling trials, forage samples were thawed and wilted at room temperature to reach ~35% dry matter (DM). Two mechanical treatments were applied: beating a 200-g sample with a metal rod (4.8 kg) 100 times from a 55-cm distance (‘Mild’) or grinding a 200-g sample in a meat grinder (12.8 mm die) (‘Harsh’). An amount of 100 g of the individually treated samples was sprayed with 5 mL inoculum to reach 10^5 CFU/g forage. Treatment combinations, in triplicate, were: (i) untreated (‘Control’), (ii) inoculation only (‘Inoculation’), (iii) Mild, (iv) Harsh, (v) Mild × Inoculation and (vi) Harsh × Inoculation. Samples were then ensiled in 100-mL glass tubes fitted with top mounted water-locks for 48-49 d. Forage epiphytic lactic acid bacteria (LAB) and *L. buchneri* added to forage were enumerated by the spread-plate method on MRS agar (Merck KGaA, Darmstadt, Germany) after a 48-h anaerobic incubation at 37°C.

Upon silo opening, 15 g samples were mixed with 15 g distilled water and stored overnight at 4°C. Silage juice was thereafter extracted and pH was measured by a pH meter. The

remaining silage samples were dried at 60°C for 18 h and milled in a hammer mill to pass a 1-mm sieve. In an attempt to partially extract fibre, an amount of 5 g of dried and ground silage sample was incubated with 50 mL distilled water at 85°C for 2 h. The extracted residue was dried at 60°C for 18 h. The effect of treatment on digestibility of silage fibre was estimated by an *in vitro* rumen incubation assay. An amount of 0.5 g DM of water extracted fibre was incubated with 50 mL buffered rumen liquid for 96 h at 38°C according to the 'VOS' method (Lindgren, 1983), as described by Åkerlind et al. (2011). The rumen fluid was collected from a fistulated dry cow, fed at maintenance level, two hours after morning feeding. From each treatment, two replicates were connected to each gas measurement unit of the 'GAS ENDEAVOUR' (Bioprocess Control, Lund, Sweden) and a third one was fitted with a water-lock. Upon terminating the incubations, pH of the fermentation medium was measure by a pH meter. The content of neutral detergent fibre (NDF) before and after *in vitro* incubation was estimated by incubation with neutral detergent solution at 85°C for 20 h (Chai and Udén, 1998).

Statistical analysis was done by GLM in Minitab (Minitab®18.1, Minitab, Ltd., Coventry, UK) with treatment as main factor. Pairwise comparison was made by the Tukey method. Significant level was declared at $P < 0.05$.

Results and Discussion

The counts of epiphytic LAB of ryegrass and meadow fescue were 10^7 and 10^6 CFU/g forage, respectively and count of *L. buchneri* added to forages prior to ensiling was 10^6 CFU/g forage. *In vitro* NDF digestibility of ryegrass and meadow fescue silages is in Table 1. For ryegrass silage, one of the replicates of Harsh × Inoculation treatment was lost. For meadow fescue silage, estimated NDF digestibilities of one replicate of Mild × Inoculation and one replicate of Harsh × inoculation were only, on average, 11 and 17% of the other two respective replicates, respectively and were excluded. In both forages, inoculation resulted in the highest silage pH (Table 1). Silage pH was generally low in all treatments, indicating a successful fermentation.

Table 1 Silage pH and NDF digestibility (%) of silage fibres estimated by *in vitro* rumen incubation (n=3). Values are least square means±SEM

	Treatments						SEM	*P* value
	Control	Mild	Harsh	Inoculation	Mild × Inoculation	Harsh × Inoculation		
Ryegrass silage								
pH	4.41^{b}	4.26^{bc}	4.23^{c}	4.72^{a}	4.62^{a}	4.39^{bc}	0.03	<0.001
NDF digestib.	73	75	74	73	70	70[1]±2	1	0.296
Meadow fescue silage								
pH	4.52^{bc}	4.37^{cd}	4.30^{d}	4.73^{a}	4.62^{ab}	4.43^{cd}	0.03	<0.001
NDF digestib.	55	51	53	47	49[1]±5	53[1]±5	4	0.774

Values with different superscript within a row differ at $P < 0.05$. [1]n=2. Mild=manually pounded forage, Harsh=minced forage, Inoculation=inoculation with a feruloyl esterase producing inoculant.

There was no effect of treatment on NDF digestibility. The effects of this inoculant on digestibility of silage fibre have been inconsistent. Kang et al. (2009) inoculated two maize hybrids, planted in adjacent fields, with this inoculant. Despite a similar chemical composition of forages, digestibility of silage fibre was improved only in one of these whole-crop silages. One possible explanation for this mixed effect of *L. buchneri* is that the bacterium does not always dominate fermentation, something dependent on the levels of epiphytic LAB of forages. This can for instance be the case in our experiment or in the

experiment of Lynch et al. (2015), in which, the counts of epiphytic LAB were similar or higher than the inoculation level. No information is available about the levels of epiphytic LAB in the study of Kang et al. (2009). Our theory can be evaluated by studying expression of FAE gene during ensiling by means of real-time quantitative PCR. The counts of epiphytic LAB on fresh grasses is generally low, something $<10^4$ CFU/g forage (Mogodiniyai Kasmaei et al., 2013). In this experiment, we used frozen grasses and it seems that LAB grew during thawing and wilting. The mechanical treatment was to open fibre structure and thereby to enhance access of FAE to target linkages between lignin and hemicelluloses.

Table 2 Cumulative gas production (mL) from water extracted fibre of ryegrass and meadow fescue silages during 96-h incubations in buffered rumen fluid and final pH. Gas values (mean±sd, n=2) are corrected for gas produced from buffered rumen fluid (n=2) as baseline. pH values are mean±sd (n=3)

		Treatments					
		Control	Mild	Harsh	Inoculation	Mild × Inoculation	Harsh × Inoculation
Ryegrass silage							
Hour	0	0±0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	24	1.6±4.9	6.6±0.1	-1.7±6.7	8.1±12.4	4.3±5.3	0.7±2.4
	48	13.5±8.1	16.4±3.5	3.5±11.0	20.2±18.4	7.5±5.4	2.8±1.3
	72	20.9±10.7	20.6±7.8	5.6±10.5	24.9±22.6	15.7±9.3	8.8±1.3
	96	20.9±10.7	21.2±8.7	5.6±10.5	25.5±23.5	15.7±9.3	12.0±1.3
pH		7.3±0.0	7.3±0.1	7.3±0.1	7.3±0.1	7.2±0.0	7.3±0.1
Meadow fescue silage							
Hour	0	0±0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	24	-8.9±3.6	5.1±5.8	-1.5±1.3	4.8±2.8	-2.9±2.6	-2.5±0.3
	48	-8.5±3.1	10.0±12.7	-0.8±1.3	5.2±2.3	-2.9±2.6	-2.2±0.8
	72	-8.2±2.6	15.5±20.5	-0.1±1.3	5.5±1.8	-2.9±2.6	-1.9±1.2
	96	-8.2±2.6	18.0±24.0	4.6±2.5	7.4±0.8	-2.9±2.6	-1.9±1.2
pH		7.2±0.0	7.2±0.0	7.3±0.0	7.3±0.0	7.4±0.3	7.4±0.3

Mild=manually pounded forage, Harsh=minced forage, Inoculation=inoculation with a feruloyl esterase producing inoculant.

Data of gas production during the 96-h *in vitro* incubation are in Table 2. Unfortunately, within replicate variation was large, not allowing comparing degradation rates of treatments. Possible explanations for this large variation are that the gas measurement unit used is not sensitive enough for low quantities of gas and/or VOS setup is not suitable for real-time measurements of gas production. In the meadow fescue trial, the gas productions of the Control, Mild × Inoculation and Harsh × Inoculation treatments were less than that of the buffered rumen fluid without substrate as baseline, resulting in negative net gas productions.

Conclusions

Inoculation with *Lactobacillus buchneri* LN4017 alone or coupled with mechanical treatment did not improve fibre digestibility of ryegrass and meadow fescue silages. It is likely that the bacterium did not dominate fermentation owing to high counts of epiphytic forage LAB.

References

Chai, W. & Udén, P., 1998. An alternative oven method combined with different detergent strengths in the analysis of neutral detergent fibre. Anim. Feed Sci. Technol. 74, 281-288.
Kang, T.W., Adesogan, A.T., Kim, S.C. & Lee, S.S., 2009. Effects of an esterase-producing inoculant on fermentation, aerobic stability, and neutral detergent fiber digestibility of corn silage. J. Dairy Sci. 92, 732-738.

Lindgren, E., 1983. Nykalibrering av VOS-metoden för bestämning av energivärde hos vallfoder. Working paper. Department of Animal Nutrition and Management, Swedish Universitiy of Agricultural Sciences, 4 pp. (in Swedish).
Lynch, J.P., Baah, J. & Beauchemin, K.A., 2015. Conservation, fiber digestibility, and nutritive value of corn harvested at 2 cutting heights and ensiled with fibrolytic enzymes, either alone or with a ferulic acid esterase-producing inoculant. J. Dairy Sci. 98, 1214–1224.
Mogodiniyai Kasmaei, K., Spörndly, R. & Udén, P., 2013. A sterilization technique with applications to silage research and inoculant evaluation. Grass Forage Sci. 69, 724-728.
Nsereko, V.L., Smiley, B.K., Rutherford, W.M., Spielbauer, A., Forrester, K.J., Hettinger, G.H., Harman, E.K. & Harman, B.R., 2008. Influence of inoculating forage with lactic acid bacterial strains that produce ferulate esterase on ensilage and ruminal degradation of fiber. Anim. Feed Sci. Technol. 145, 122-135.
Pu, Y., Hu, F., Huang, F., Davison, B.H. & Ragauskas, A.J., 2013. Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments. Biotechnol. Biofuels 6, 15.
Rubin, E.M., 2008. Genomics of cellulosic biofuels. Nature 454, 841-845.
Åkerlind, M., Weisbjerg, M., Eriksson, T., Tøgersen, R., Udén, P., Ólafsson, B.L., Harstad, O.M. & Volden, H., 2011. Feed analyses and digestion methods, in: Volden, H. (Ed.), NorFor – the Nordic feed evaluation system. Wageningen Academic Publishers, Wageningen, The Netherlands, p. 41-54.

Estimation of VOC emission from silages in Sweden

M. Knicky, R. Spörndly & H. Gonda
Department of Animal Nutrition & Management, Swedish University of Agricultural Sciences, Box 7024, 750 07 Uppsala, Sweden
Correspondence: martin.knicky@slu.se

Introduction

Emissions of volatile organic compounds (VOCs) from silage, the dominant feed for cattle, have been identified as a cause of poor air quality (Howard et al., 2010). Silage is produced by storing of wet forage (in Sweden usually grass, clover, lucerne and whole crop grain) under anaerobic conditions to promote bacterial fermentation. The result of bacterial activity is the formation of fermentation products, mainly lactic acid (McDonald et al., 1993) which preserves the feed. Besides lactic acid, the fermentation process results in formation of other substances that are emitted as gas and are considered as VOC. The major substances are acids (acetic and propionic), alcohols (methanol, ethanol, 1-propanol, 1-butanol), aldehydes (acetaldehyde, valeraldehyde, hexanal, 3-methylbutanal), and esters (methyl acetate, ethyl acetate, propyl acetate), together with smaller amounts of ketones (acetone, 2-butanone, 3-hydroxy-2-butanone). According to Hafner et al (2013), alcohols, mainly ethanol and occasionally 1-propanol, contribute most to VOC emissions. This is due to the combination of their large amounts and high volatility. Organic acids are produced in even larger extents than alcohols in silage, but their lower volatility reduce their importance for VOC emission. Emission of VOC from silages occurs primarily during feed-out, when silage is exposed to air. Prediction of VOC emission is difficult due to a variety factors such as substance volatility, airflow, and temperature. As a possible measure of VOC emission the model of Hafner et al (2012) can be used, which predicts an average of 2.79 grams of evaporated VOC per kg of corn silage at 35% DM content at 25ºC. However, maize silage is not a common feed in Sweden and also the temperature used in his model is high for Sweden. Therefore, the aim of the study was to provide basic data to estimate amounts of VOC emitted from silages in Sweden.

Materials and Methods

A typical clover-grass mixture was used. The crop represented a mixture of perennial ryegrass, meadow fescue and timothy (56%, in the vegetative stage), and red clover (vegetative stage, 44%). It was fertilized with a manure slurry and harvested as a fourth cut on 17th of October 2018. The forage was wilted to DM contents of 35% and 55%. After wilting, the forage mass was chopped using a stationary chopper to a nominal chop length of 2 cm and ensiled in laboratory silos (1.7 L volume) according to the DLG guideline for testing efficiency of silage additives WR1 (DLG, 2009). Both DM variants were ensiled in three replicates (six silos in total) for 30 days at 20°C. After 30 days, the silos were placed in a room at 5°C where silo contents were emptied into separate plastic bags and mixed thoroughly. Extracted silages were divided into 3 sub-samples: chemical sample, aerobic test sample and reserve sample. Sample was were analysed for DM, pH, and fermentation products such as acetic acid, butyric acid, lactic acid, ethanol, butanediol, propionic acid, butanol, propanol, methanol, acetaldehyde, butyraldehyde, propionaldehyde, methyl acetate, propyl acetate, ethyl acetate and ethyl butyrate. Fermentation products and pH were analysed from silage juice extracted at 5°C. HPLC (Ericson & André, 2010.) and gas chromatography/mass spectrometry at the Eurofins laboratory was used to identify

fermentation products in the silage samples. To simulate losses of VOC from an open silo, samples (300 g of fresh silage) of each DM variant were loosely placed on aluminium trays, stored in a ventilated refrigerator for another 24 hours at 10°C and then analysed for DM, pH and VOC using the same methods.

Results and Discussion

The DM content of the forages was 32.2% and 53.1%. Chemical composition of silage after 30 days of fermentation and after another 24 hours of air exposure at 10°C are in Tables 1 and 2. A dominance of lactic acid and an absence of butyric acid among the fermentation products indicate a good fermentation process in all silage (Pahlow et al., 2003). The pH of the silage is consistent with the degree of fermentation that can be expected from the particular DM levels and crop composition.

Table1 Chemical composition of silages at 35% dry matter (DM) variant before and after 24 h air exposure at 10 °C (n=3)

Item	Unit	Air exposure		LSD	P value
		Before	After		
DM	%	32.2	37.7		
pH		4.2	4.2	0.1	0.9
Lactic acid	g/kg FM	23.9	28.9	5.4	0.06
Acetic acid	g/kg FM	6	6.3	0.6	0.1
Propionic acid	g/kg FM	0.51	0.49	0.04	0.2
Butyric acid	g/kg FM	<0.17	<0.16	0.01	0.2
Ethanol	g/kg FM	2.45	0.97	0.43	0.005
2.3 butanediol	g/kg FM	0.84	0.81	0.06	0.2
1.2 propandiol	g/kg FM	<0.17	<0.16	0.01	0.2
1.3 propandiol	g/kg FM	<0.17	<0.16	0.01	0.2
Methylacetate	g/kg FM	0.0085	0.0003	0.003	0.01
Dimethylsulfide	g/kg FM	0.0099	0.0074	0.012	0.5
Butanol	g/kg FM	0.0092	0.0089	0.0009	0.2
3-Methylbuthanal	g/kg FM	0.021	0.022	0.011	0.7

Table2 Chemical composition of silages at 55% dry matter (DM) variant before and after 24 h air exposure at 10 °C (n=3)

Item	Unit	Air exposure		LSD	P value
		Before	After		
DM	%	53.1	56.9		
pH		4.9	4.8	0.2	0.7
Lactic acid	g/kg FM	23.9	28.9	5.4	0.06
Acetic acid	g/kg FM	4.1	4.4	1.15	0.3
Propionic acid	g/kg FM	0.45	0.44	0.02	0.1
Butyric acid	g/kg FM	<0.15	<0.15	0.01	0.1
Ethanol	g/kg FM	1.73	0.58	0.26	0.003
2.3 butanediol	g/kg FM	0.75	0.73	0.03	0.1
1.2 propandiol	g/kg FM	<0.15	<0.15	0.01	0.1
1.3 propandiol	g/kg FM	<0.15	<0.15	0.01	0.1
Methylacetate	g/kg FM	0.0051	0.0002	0.011	0.03
Dimethylsulfide	g/kg FM	0.0069	0.0068	0.0024	0.9
Butanol	g/kg FM	0.0082	0.008	0.0002	0.04
3-Methylbuthanal	g/kg FM	0.0125	0.0125	0.005	1.0

The amount of fermentation products and, hence, VOC formed in silage is mainly determined by the DM content of a forage (Pahlow et al. 2003). The wetter the forage is the higher production of volatile substances can be expected in silage.

The majority of VOC emissions occurs during the time when silage is fed. At this time, anaerobic conditions in silage is terminated and silage is exposed to the air. The extent of VOC emission also greatly depends on the time silage is exposed to the air before it is consumed. It is not uncommon that it can take up 24 h between silage removal from the silo and actual consumption. The present experiment displayed the variation in volatility between different fermentation products, where ethanol had the greatest volatility. The concentration of ethanol at both DM variants decreased significantly ($P<0.01$) after 24 hours of air exposure. In silage with 32% DM content, the ethanol lost was equivalent to 1.45 g per kg of fresh silage, while 1.15 g of ethanol disappeared from one kg of fresh silage at 53% DM content. These results are in accordance with Hafner et al. (2013) that alcohols, and primarily ethanol, has the highest VOC emission at the beginning of silage exposure to air. Besides ethanol, methylacetate displayed a reduction ($P<0.05$) in concentration before and after air exposure in both DM variants as well as for butanol at 53% DM. Other volatile fermentation compounds were not detected or found to differ between before and after air exposure measurements. On the other hand, lactic and acetic acid showed a tendency to increase concentrations after air exposure. This is thought to be a consequence of water losses increasing DM concentration over time in the silage, resulting in higher acid concentrations.

To estimate VOC emission for Sweden from this study, it was assumed that about 4.5 million tons of DM forage is conserved (SCB, 2016), of which approximately 4 million tons of DM as silage. Of this silage amount, 2.2 million tons of DM silage is preserved with an average DM content of 35%, corresponding to 6.3 million tons of fresh weight silage, which gives 9166 tons of VOC emissions in the form of ethanol and methylacetate per year. The rest, 1.8 million tons of DM silage in Sweden, is preserved in round bales with an average 50% DM content, which gives 3.6 million tons of fresh weight silage emitting 4150 tons of ethanol, methylacetate and butanol. Total estimated amount of VOC emissions during silage 24 hours air exposure can correspond to about 13316 tons per year. This value is lower compared to the Hafner et al. (2012) model with an average of 2.79 grams of evaporated VOC per kg of corn silage. Using Hafner's model would result in an estimation of 27621 tons of total annual VOC emission in Sweden.

Conclusions

The study confirmed earlier findings that alcohols and primarily ethanol contribute mostly to VOC emission from silages at an early phase of exposure. Based on the results of the study, it was estimated that the extend of annual VOC emission in Sweden can be up to 13316 tons.

References

DLG. 2009. DLG-Richtlinien für die Prüfung von Siliermitteln auf DLG-Gütezeichen--Fähigkeit (DLG guidelines for the test of silage additives for approval of DLG quality labels). DLG (www.dlg.org/home-en.html).
Ericson. B. & André. J., 2010. HPLC – applications for agricultural and animal science. Proc. 1st Nordic Feed Sci. Conf., 22-23 June. Uppsala. Sweden.
Hafner, S.D., Montes, F. & Rotz, C.A., 2012. A mass transfer model for VOC emission from silage. Atmosph. Environm. 54, 134-140.

Hafner, S., Howard, C., Muck, R., Franco, R., Montes, F., Green, P., Mitloehner, F., Traube, S & Rotz, C., 2013. Emission of volatile organic compounds from silage: Compounds, sources and implications. Atmosph. Environm., 77, 827-839
Pahlow, G., Muck, R., Driehuis, F., Oude Elferink & S., Spoelstra, S., 2003. Microbiology of ensiling. In: Buxton, D., Muck, R., Harrison, J. (Eds.), Silage Science and Technology. Am. Soc. Agron., Madison, WI, p. 31-93.
SCB, 2016. Jordbruksstatistisk sammanställning 2016. Statistiska Centralbyrån och Jordbruksverket. Statistics Sweden, Örebro, Sweden

The effect of silage additives onto fermentation of late autumn cut grass

A. Milimonka[1], A. Zeyner[2] & G. Glenz[1]
[1]ADDCON Europe GmbH, Parsevalstr. 6, 06749 Bitterfeld, [2]Martin Luther Universität, Institut für Agrar- und Ernährungswissenschaften, Professur Tierernährung, Theodor-Lieser-Str. 11, 06120 Halle (Saale)
Correspondence: Andreas.Milimonka@addcon.com

Introduction

Grass cuts are in a DM range which need to be wilted to become suitable for ensiling (Weißbach et al., 1974). But, wilting conditions vary widely within and among years. Temperature in Europe is continuously increasing and the vegetation period is extended (Chmielewski et al., 2004). A warming climate may lead to more intensive summer rainfall or, the rains will fail to appear. A longer vegetation period gives farmers a chance to cut later in the year or take an additional cut. Droughts, such as in 2018, forced farmers to cut late autumn crops to fill up the stocks. However, in late autumn wilting conditions get worse. A lower temperature and shorter day length in the autumn results in a reduced formation of new leafs and tillers (Jones & Lazenby, 1988). Cloudier weather may lower photosynthesis with little effect on N uptake, which may lead to higher crude protein contents. With increasing soil moisture in the autumn, there is an increased risk of deeper tire tracks in the fields, resulting in higher crude ash contents in the material to be ensiled and thus, higher number of clostridia may be expected. The probability of an increasing number of soil born clostridia is higher if the land is grazed or slurry fertilized (Driehuis et al., 2016). Less chances for intensive wilting, higher risk of crude ash and higher crude protein will result in a reduced fermentation coefficient, less pH reduction and higher DM losses in silage (Kalzendorf & Milimonka, 2018). Different additive concepts may have different effects on fermentation and clostridia growth (Polip, 2002). Therefore, this experiment examines the effect of different additive concepts on fermentation of a wet autumn cut grass.

Material and Methods

A late cut was taken 18th of October 2017 from a *Lolium* dominated multi species natural grassland in the district of Wittenberg, Germany. The crop traits for the grass were (g/kg DM): dry matter (DM) 238, crude protein (CP) 195, crude ash (CA) 103, water soluble carbohydrates (WSC) 99, nitrate 0.7, buffer capacity (BC) 4.1 g lactic acid/100 g DM, S/BC 2.4 and the fermentation coefficient was 43. Thus, the conditions for fermentation were rather good.
The crop material was ensiled in 1.5 litre jars. Each treatment was prepared from 5 kg fresh crop and treated with: 1) KL2, nitrite + HMTA with 2 litres/t fresh matter (FM), KOFASIL liquid, ADDCON Europe GmbH, 2) KL3 (3 litres/t FM), 3) KL2z10, KL2 plus 100 g sugar/kg additive (2 litres/t FM), 4) KL3z10 (3 litres/t FM), 5) NDF4, formic acid, sodium format (4 kg/t FM), ADDCON Nordic, 6) NDF6 (6 kg/t FM), 7) SS2, sodium nitrite, sodium benzoate, potassium sorbate (2 litres/t FM), Safesil, Salinity, 8) SS3 (3 litres/t FM), 9) Control (no additive). The additives were applied according to the dosages mentioned above but for better distribution, the liquid additives were filled up to 50 ml with water prior to dosing. The grass of the NDF treatments and Control was sprayed with 50 ml of water. The jars were filled with 0.97 kg chopped grass on average within a three-hour period.
The silages were opened after 90 days. Crop parameters from fresh crop as well as for the silage were evaluated based on the VDLUFA protocol. Silage DM was measured and

corrected for loss of volatiles during drying (Weißbach & Kuhla, 1995). The following parameters of the silage were analysed: pH (VDLUFA, 2011), lactic acid, acetic acid, butyric acid (HPLC; VDLUFA, 2011), NH_3 (auto-analyser; VDLUFA, 2011). Fermentation loss was calculated after Weißbach (2005). The setup of the experiment was a random block design 3 times replicated. Statistical analyses were done using the ANOVA procedure, program "R". When the overall P-value was significant at the 5% level, pair wise comparisons between LSMEANS of treatments were done using Tukey´s test.

Results and Discussion

After 7 days, all silage additives resulted in reduced fermentation losses compared to Control. The high dosage of the formic acid based NDF additive gave the lowest losses (Figure 1). This was reported by Wen et al. (2017) also. Until the end of the fermentation period, DM losses increased in all treatments but was lowest in KL3. This long lasting effect of KL was often described for grasses by Weißbach (1989) and in field bean silages by Kalzendorf et al. (2016). After 90 days, the benzoate based additive had the weakest effect on DM loss. A similar combination was less effective in field beans also (Kalzendorf et al. 2016).

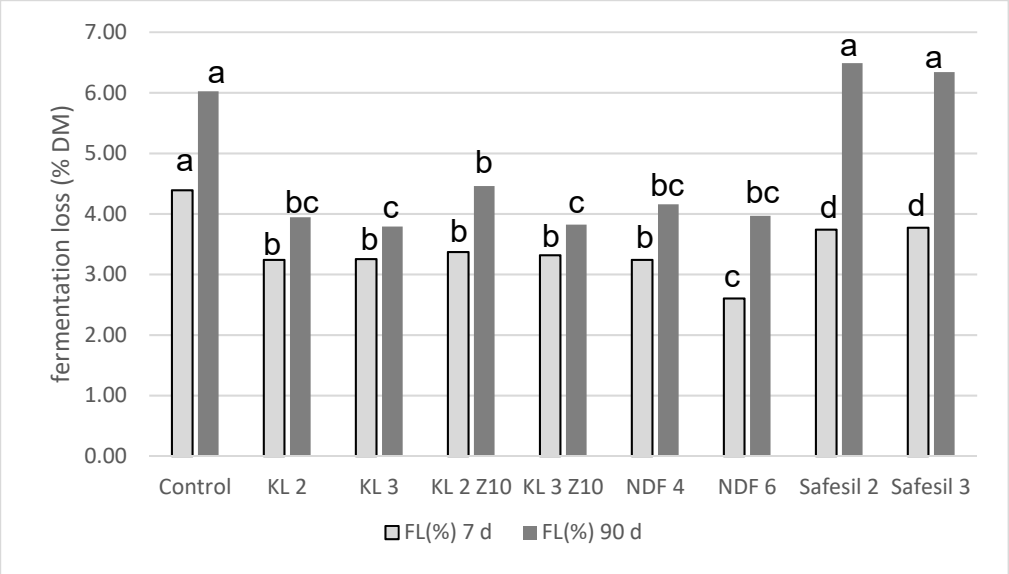


Figure 1 Fermentation loss (% DM) after 7/90 days of a grass silage (25% DM) treated with different additives KL2: nitrite, HMTA, 2 litres/t FM, KL3: 3 litres/t FM, NDF4: formic acid, sodium format, 4 kg/t FM, NDF6: 6 kg/t FM, SS2: sodium nitrite, sodium benzoate, potassium sorbate, 2 litres/t FM, SS3: 3 litres/t FM
Different letters show significant differences between treatments, $P<0.05$.

Fermentation conditions during the experiment were not optimal. The quite high CP and CA contents were challenging in combination with a FC of 43. After 90 days of fermentation, pH was not lower than 4.3 (Control) and in the treated silages higher than in control (Table 1). Also, the additive treated silages had not been different from Control for the lactic acid content, except SS3. The addition of sugar had a minimal effect on lactic acid fermentation and pH. For acetic acid, there was a tendency for a response from the KL3 and NDF treatments (Table 1). The KL2 dosage was less than recommended. The low-dose KL2 and the SS2/SS3 treatment showed the highest levels of acetic acid. That may have been the result of a longer period of enterobacteria activity caused by a delayed and insufficient lactic acid formation and, thus, a delayed pH drop (Pobednov, 1997). However, in all treatments and the control, only low levels of butyric acid were observed.

Table 1 Fermentation acids (% DM), NH3-N in % of total N and pH of a grass silage (25% DM) treated with different additives

	Control	KL2	KL3	KL2 z10	KL3 z10	NDF4	NDF6	SS2	SS3
pH	4,3a	4,6adc	4,7c	4,4d	4,6dc	4,3af	4,7bc	4,6bc	4,6bc
LA	7,8a	7,7a	6,5ab	8,1a	6,9ab	7,6a	5,1ab	5,2ab	4,2b
AA	2,1ac	1,9ac	1,1c	2,5ac	1,3c	0,8c	0,9c	4,2a	5,4b
BA	0,20a	0,20a	0,20a	0,21ac	0,19b	0,19d	0,20d	0,21f	0,20a
NH3-N (%) N_t*	10,16a	6,50b	6,44b	6,95b	6,50b	8,46c	8,64c	11,23d	10,69a

*NH3-N corrected for ammonia coming from the additive
KL2: nitrite, HMTA, 2 litres/t FM, KL3: 3 litres/t FM, KL2z10: 2 litres/t FM+sugar, KL3z10: 3 litres/t FM+sugar, NDF4: formic acid, sodium format, 4 kg/t FM, NDF6: 6 kg/t FM, SS2: sodium nitrite, sodium benzoate, potassium sorbate, 2 litres/t FM, SS3: 3 litres/t FM; LA=lactic acid, AA=acetic acid, BA=butyric acid
Different letters show significant differences between treatments, P<0.05.

In the KL treatments, some ammonia-N was coming from HMTA decomposition. Therefore, ammonia-N levels were corrected for this contribution. In the Control and the silages treated with benzoic acid based additives (SS2 and SS3), the ammonia-N proportion of total N (NH_3-N of N_t) was higher than in the KL and NDF treatments (Table 1). A slow pH-drop for this treatments and the control and, thus, a prolonged protein degradation might have been the reason for this. Obviously, benzoic and sorbic acid based mixtures were less suited to improve fermentation in the silages, characterized by a FC<45. On the other hand, Knicky & Spörndly (2009) reported different results according to the combination of nitrite, benzoic and sorbic acid.

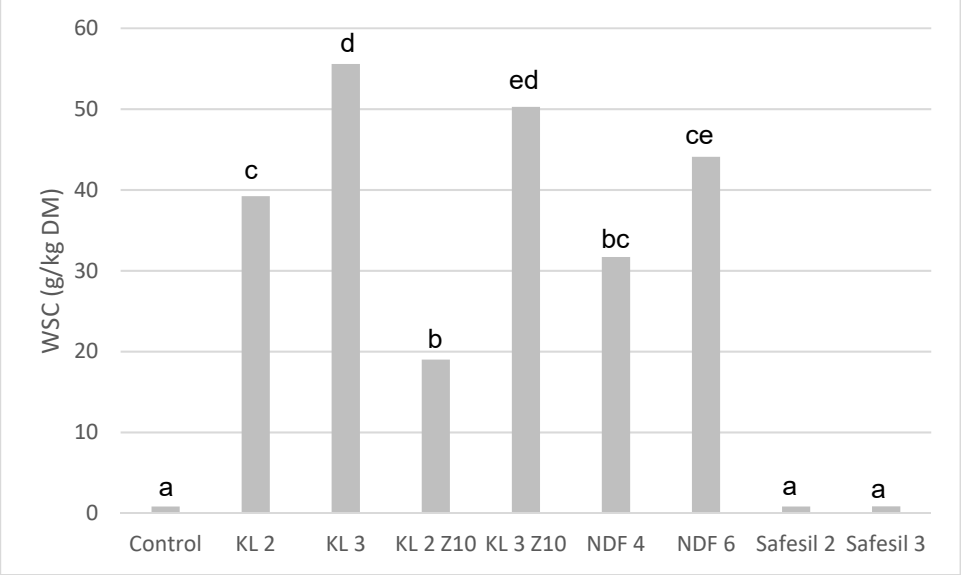


Figure 2 Water soluble carbohydrates (g/kg DM) after a 90-d fermentation of a grass silage (25% DM) treated with different additives.
KL2: nitrite, HMTA, 2 litres/t FM, KL3: 3 litres/t FM, NDF4: formic acid, sodium format, 4 kg/t FM, NDF6: 6 kg/t FM, SS2: sodium nitrite, sodium benzoate, potassium sorbate, 2 litres/t FM, SS3: 3 litres/t FM
Different letters show significant differences between treatments, P<0.05.

The reduction of water soluble carbohydrates differed strongly among treatments (Figure 2). Untreated and SS-additive treated silages resulted in a high consumption of sugar. But, in the lower dosed KL2 and NDF4 treatments, the sugar consumption was higher also. All the

treatments, which showed high sugar losses, also had higher total amounts of fermentation acids (lactic + acetic). For wet grass silages, Hoedke & Zeyner (2010) reported a more intensive fermentation characterized by higher lactic and acetic acid contents compared to wilted one. As acetic acid levels tended to be higher in the low-sugar containing treatments SS2 and SS3 as well as in the Control, it might be assume that the sugars were mainly used by the enterobacteria.

In case of the KL and NDF treatments, the higher application rates tended to result in higher residual sugar contents, higher pH and less lactic acid compared to Control. Obviously, fermentation was reduced by the higher dosages. As a result, less amounts of easy fermentable substances were used by the microbes and thus, the fibre content in the treated silages was less compared to Control and SS-additives (Table 2). That may have caused the slightly higher energy contents in the KL and NDF silages.

Table 2 Acid detergent fiber content (ADFom) and net energy of lactation (NEL) in a grass silage (25% DM) treated with different additives

	Control	KL2	KL3	KL2 z10	KL3 z10	NDF4	NDF6	SS2	SS3
ADFom (g/kg DM)	248a	230b	229b	237b	228b	234b	232b	253a	261a
NEL (MJ/kg DM)	5,9ac	6,1ab	6,1ab	6ab	6,1ab	6,1b	6,2b	5,8c	5,8c

KL2: nitrite, HMTA, 2 liters/t FM, KL3: 3 liters/t FM, NDF4: formic acid, sodium format, 4 kg/t FM, NDF6: 6 kg/t FM, SS2: sodium nitrite, sodium benzoate, potassium sorbate, 2 liters/t FM, SS3: 3 liters/t FM
Different letters show significant differences between treatments, $P<0.05$.

Conclusions

A grass cut in the late autumn season and characterized by a low a DM content tended to ferment with high DM losses. This may have been the result of the poor fermentation characteristics of the crop with a slow pH drop and, thus, high amounts of acetic acid. Silage additives can control DM losses and fermentation if appropriately dosed. Most effective appear to be nitrite and HMTA or formic acid/format based additives. They showed the lowest DM losses, better fermentation profiles as well as less fibre and higher energy contents of the silages. The addition of sugar did not have any significant improvement on the fermentation pattern.

References

Chmielewski, F., Müller, A., & Bruns, E., 2004. Climate changes and trends in phenology of fruit trees and field crops in Germany, 1961-2000. Agric. Forest Meteorol., DOI: 10.1016/S0168-1923(03)00161-8.

Driehuis, F., Hoolwerf, J. & Rademaker, J.L.W., 2016. Concurrence of spores of Clostridium tyrobutyricum, Clostridium beijerinckii and Paenibacillus polymyxa in silage, dairy cow faeces and raw milk. Intern. Dairy J., 63, 70-77.

Hoedke, S. & Zeyner, A., 2010. Comparative evaluation of laboratory-scale silages using standard glass jar silages or vacuum-packed model silages. J. Food Sci. and Agric., 91, 841-849.

Jones, M.B. & Lazenby, A., 1988. The Grass Crop. London: Chapman and Hall.

Kalzendorf, C., Milimonka, A. & Richardt, W. 2016. Ensiling of whole crop field beans (Vicia faba) as affected by silage additives. 7th Nordic Feed Science Conference, Uppsala, 35-40.

Kalzendorf, C. & Milimonka, A., 2018. Effect of chemical additives, lactic acid bacteria and their combinations on the fermentation of low dry matter crops. Proceedings of the XVIIIth International Silage Conference, Bonn, 236-237.
Knicky, M. & Spörndly, R., 2009. Sodium benzoate, potassium sorbate and sodium nitrite as silage additives. J. Food Sci. and Agric., 89, 2659–2667.
Polip, I., 2002. Untersuchungen zur Unterbindung von Buttersäuregärung und Clostridienaktivität in Silagen aus nitratarmem Grünfutter. Diss. Berlin.
Popednov, J., Weißbach, F. & Pahlow, G., 1997. Über den Effekt von Milchsäurepräparaten auf die Säuerungsgeschwindigkeit und die Gärqualität von Welksilage. Landbauforschung Völkenrode, Heft 3, 97-102.
VDLUFA, 2011. VDLUFA Methodology, Vol. III., 1997-2011, VDLUFA-Verlag, Darmstadt.
Weißbach, F., Schmidt, L. & Hein, E., 1974. Method of anticipation of the run of fermentation in silage making, based on the chemical composition of green fodder. Proceedings XII International Grassland Congress, Moskov, 663-673.
Weißbach, F., Zwierz, P., Reuter, B., Köller, S., Weißbach, H. & Baronius, W. 1989. Control of the silage fermentation by chemical preserving agents. Chimica*oggi*, October, 57-60.
Weißbach, F. & Kuhla, S., 1995. Substance losses in determining the dry matter content of silage and green fodder: arising errors and possibilities of correction. Übersichten Tierernährung, 23, 189-214.
Weißbach , F., 2005. A simple method for the correction of fermentation losses measured in laboratory silos. Proc. XIVth Int. Silage Conf., Belfast, UK, 278.
Wen, A., Wena, A., Yuana, X., J., Wanga, J., Destaa, S., T. & Shao, T., 2017. Effects of four short-chain fatty acids or salts on dynamics of fermentation and microbial characteristics of alfalfa silage. Animal Feed Sci. Techn., 223, 141–148.

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

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

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

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

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

bioprocess CONTROL

www.bioprocesscontrol.com

Senast publicerade titlar i denna serie:
Latest published in this series:

Nr	År	Titel och författare
Nr 283	2013	Phosphorus to horses and cows. Ögren, G. *Licentiate thesis*
Nr 284	2013	Microbial characterization of wet pig feed. Borling. J. *Licentiate thesis*
Nr 285	2013	Renhjord i kollaps – produktivitet, kondition och renförluster i Njaarke sameby. Åhman, B.
Nr 286	2013	Mjölk på bara vall och spannmål. Spörndly, R. och Spörndly, E.
Nr 287	2013	Proceedings of the 4th Nordic Feed Science Conference, Uppsala, Sweden. Editors: Udén, P., Spörndly, R., Rustas, B-O., Eriksson, T., Müller, C. and Liljeholm, M.
Nr 288	2013	Performance of dairy cows and calves in agro-pastoral production systems. Johansson, C. *Licentiate thesis*
Nr 289	2013	Utfodringsrekommendationer för häst. Redaktör: Jansson, A.
Nr 290	2014	Proceedings of the 5th Nordic Feed Science Conference, Uppsala, Sweden,. Editor: Udén, P.
Nr 291	2015	Proceedings of the 6th Nordic Feed Science Conference, Uppsala, Sweden. Editor: Udén, P.
Nr 292	2016	Updating Swedish emission factors for cattle to be used for calculations of greenhouse gases. Bertilsson, J.
Nr 293	2016	Proceedings of the 7th Nordic Feed Science Conference, Uppsala, Sweden. Editors: Udén, P., Eriksson, T., Rustas, B-O. and Danielsson, R.
Nr 294	2016	Renar och vindkraft II – Vindkraft i drift och effekter på renar och renskötsel. Skarin, A., Sandström, P., Moudud, A., Byhot, Y. och Nellemann C.
Nr 295	2016	Single cell protein in fish feed: Effects on gut microbiota. Nyman, A. *Licentiate thesis*
Nr 296	2017	Proceedings of the 8th Nordic Feed Science Conference, Uppsala, Sweden. Editors: Udén, P., Eriksson, T., Spörndly, R., Rustas, B-O., Mogodiniyai Kasmaei, K. and Liljeholm, M.
Nr 297	2018	Betesdjur och betestryck i naturbetesmarker. Spörndly, E. och Glimskär, A.
Nr 298	2018	Proceedings of the 9th Nordic Feed Science Conference, Uppsala, Sweden. Editors: Udén, P., Eriksson, T., Spörndly, R., Rustas, B-O. and Liljeholm, M.
Nr 299	2019	Tekniklösningar för egenproducerat kraftfoder i besättningar med mjölkkor - en exempelsamling. Carlsson, M.P. och Gustafsson, A.H.
Nr 300	2019	Grundläggande foderhygien – med fokus på mikrobiologiska faror i lokalproducerat foder till mjölkkor. Elving, J.
Nr 301	2019	Ersättningsfoder till nötkreatur vid grovfoderbrist. Spörndly. R., Bergkvist, G., Nilsdotter-Linde, N. och Eriksson, T.
Nr 302	2019	Proceedings of the 10th Nordic Feed Science Conference, Uppsala, Sweden. Editors: Udén, P., Eriksson, T., Spörndly, R., Rustas, B-O. and Karlsson, J.

DISTRIBUTION:
Sveriges Lantbruksuniversitet
Institutionen för husdjurens utfodring och vård
Box 7024
750 07 UPPSALA
Tel. 018-67 20 26
Marianne.Lovgren@slu.se