Proceedings of the 6th Nordic Feed Science Conference, Uppsala, Sweden

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

Rapport 291
Report

Uppsala 2015
ISSN 0347-9838
ISRN SLU-HUV-R-291-SE

Proceedings of the 6th Nordic Feed Science Conference, Uppsala, Sweden

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

Rapport 291
Report

Uppsala 2015

ISSN 0347-9838
ISRN SLU-HUV-R-291-SE

Published by:
Organising committee of the 6th Nordic Feed Science Conference
Department of Animal Nutrition and Management
Swedish University of Agricultural Sciences (SLU)
SE- 753 23 Uppsala, Sweden

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/husdjur-utfodring-vard

QR code reader for accessing conference posters can be downloaded at:
http://app.scanlife.com/appdownload/dl

Proceedings from the Nordic Feed Science conferences are indexed and archived in CAB Abstracts since 2010

Foreword

This year we hold the 6th annual Nordic Feed Science Conference in Uppsala. The aim of the conference is still to create an arena for Nordic feed scientists to meet and discuss ruminant and horse feeds and feeding. We are particularly happy this year to have received some funding from the Swedish Research Council Formas. This has enabled us to have three distinguished keynote speakers – Professor Peter Robinson, UC Davis, Dr Padraig O'Kiely, Teagasc, Ireland and Professor Peiqiang Yu, University of Saskatchewan, Canada. We wish to welcome these eminent scientists for coming all the way to join us in this conference.

Topics this year include ensiling, grain processing, biogas from crops, modeling and ration formulation as well as beef and dairy animal nutrition. In the evening session, we will discuss amino acid delivery to dairy cows in connection with two presentations.

You are all most welcome to the conference! For downloading proceedings of earlier conferences, please go to the first of our homepage where you also find a list of all titles.

Uppsala 2015-05-21

Peter Udén

Contents

Methods and modelling

Feed conservation and processing

Ruminant nutrition

Bio-Synchrotron Based Feed Research Program:
Synchrotron-Based Technology as a New Approach for Plant-Based Feed Protein Structure Research at Ultra-Spatial Resolutions

Peiqiang Yu, PhD.
Professor and Ministry of Agriculture Strategic Research Chair
Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, S7N 5A8, Canada
Correspondence: Tel: +1 306 966 4132; E-mail: peiqiang.yu@usask.ca

Introduction

Novel research ideas and/or novel research tools play a significant role in advances in feed science and animal nutrition research (Yu, 2004). In feed analysis, wet analytical methods are often suggested to be used (eg. NRC-2001 dairy model). However this type of analysis often result in destruction or alteration of feed intrinsic structures during processing for analysis (Budevska, 2002; Marinkovic *et al*., 2002; Yu, 2004), because the wet chemical analyses rely heavily on the use of harsh chemicals, which altering the native feed structures and possibly generating artifacts (Budevska, 2002; Yu, 2004).

Advanced synchrotron radiation powered infrared microspectroscopy has been developed as a rapid, direct, non-destructive and bioanalytical technique (Wetzel *et al*., 1998; Marinkovic & Chance, 2006; Miller & Dumas, 2006; Yu *et al*., 2008). In contrast to traditional wet chemical analytical methods which during processing for analysis often result in destruction or alteration of the intrinsic structures (eg. protein) (Budevska, 2002; Yu, 2004), this cutting-edge synchrotron-based analytical technique, taking advantages of synchrotron light brightness and small effective source size (Marinkovic & Chance, 2006; Miller & Dumas, 2006), is capable of exploring the molecular chemistry or structure of a biological tissue without the destruction of inherent structures at ultra-spatial resolutions (Wetzel *et al*., 1998, 2003; Doiron *et al*., 2009a,b; Yu *et al*. 2008b; Zhang & Yu, 2014). With synchrotron based analytical technique, several information can be obtained simultaneously, such as feed tissue composition, tissue make-up, tissue environment, and tissue structure (Budevska, 2002; Yu, 2011; Yu *et al*., 2013).

To date there has been little application of synchrotron radiation-based infrared microspectroscopy to the study of feed inherent structures (eg. protein) in relation to nutrient availability in animal science community (Yu, 2010; Yu & Nuez-Ortín, 2010). Protein inherent structure, among other factors such as protein matrix, affects nutritive quality, bio-function, fermentation, and degradation behavior in animals (Zhang & Yu, 2012; Yu, 2012). Protein structure profile influences protein value (eg. absorbed protein in small intestine) and functionality (eg. solubility) and affects the access of gastrointestinal digestive enzymes to the protein (Abeysekara *et al*., 2013; Yu *et al*., 2014). Reduced accessibility results in poor digestibility and as a result, low protein nutritive value (Becker & Yu, 2013; Yang *et al*., 2013; Thedoridou *et al*., 2014).

The objective of this presentation is to introduce the potential of using the advanced synchrotron-based bioanalytical technology as a new approach to study feed molecular structure and detect feed treatment or feed processing-induced structural changes in relation to nutrient availability. The outline of my presentation include: I. what is Synchrotron? a) short definition; b) major components of synchrotron; II. synchrotron molecular spectroscopy

techniques; a) principle and advantage of synchrotron radiation; b) advantages of synchrotron radiation infrared microspectroscopy; III: applications: synchrotron-based research programs; a) feed structure in relation to nutrient availability; b) feed molecular-chemical make-up; c) feed molecular chemistry imaging; d) effect of gene transformation on feed structure and nutrient availability; e) heat-induced changes in structure and relation to nutrient availability; f) effect of bioethanol processing on feed structure and quality.

Unique Bio-Synchrotron Based Feed Programs

First of all, what is a synchrotron? The short definition is that a synchrotron, a giant particle accelerator that turns electrons into light, is composed of six major components: electron gun, linear accelerator, booster ring, storage ring, beamlines, and end experimental station (Wetzel *et al*., 1998; Marinkovic & Chance, 2006; Miller & Dumas, 2006). The size of a synchrotron is just like a soccer field, for example, Canadian Light Source (CLS) on our campus of the University of Saskatchewan. But some synchrotrons are very large, for example, Advanced Photo Sources (APS) in Chicago, USA. So the size is partially dependent on synchrotron target energy level (0.8 to 8.0 Gev). When high speed and high energy electrons are accelerated, it will produce extremely brilliant, full spectrum photon beam known as synchrotron light (CLS, 2015). At the end experimental stations, researchers collect synchrotron-based data to determine molecular structure of a sample. The extremely bright synchrotron light makes it possible to detect biomaterial structure (chemical make-up) at both molecular and cellular levels (Dumas, 2003; Yu, 2004; Marinkovic & Chance, 2006; Miller & Dumas, 2006; Yu *et al*., 2007).

Plant protein has unique molecular structures; therefore it has its own infrared spectrum. Plant protein infrared spectrum has two primary features, the amide I (ca. 1600-1700 cm^{-1}) and amide II (ca. 1500-1560 cm^{-1}) both of which arise from specific stretching and bending vibrations within the protein backbone (Kemp, 1991; Jackson & Mantsch, 1995, 1996; Kneipp *et al*., 2003). The amide I, not amide II, is usually used for protein 2^{nd} structure analysis, such as alpha-helix structure, beta-sheet structure, random coil and beta turn (Seguchi *et al*., 2004; Yu 2005).

Two multivariate molecular spectral analyses can be used to discriminate and classify plant protein structures and protein structure changes. The first method is agglomerative hierarchical molecular spectral cluster analysis. The second multivariate analysis used to determine major sources of variation is principle component analysis, a statistical data reduction method that transforms the original set of variables to a new set of uncorrelated variables called principle components. The advantage of multivariate analyses is that we do not need to know what the spectral assignments are, just want to qualitatively separate one group from another (Yu 2005, 2006).

Exampled Studies of Using Synchrotron-Based Analytical Methods for Feed Research

Area I: Using Synchrotron-Based Analytical Methods for Feed Molecular Chemistry Imaging (Yu, 2005). For example, imaging molecular chemistry of wheat (Wetzle *et al*., 1998), Pioneer corn (Yu *et al*., 2004) and sorghum (Yu, 2011) and effect of heat treatment on cotyledon tissues in yellow-type of Canola (Brassica) Seeds (Yu *et al*., 2013). One example of imaging application: from the molecular chemistry imaging, we can see difference between frost-damaged wheat and normal wheat.

Area II: Using Synchrotron-Based Analytical Methods to Detect Foreign gene-transformation induced protein structure change on a molecular basis. This exampled study was to use advanced synchrotron technique to compare protein molecular structure of transgenic alfalfa plant tissues transformed with the maize *Lc* regulatory gene with non-transgenic alfalfa protein within cellular and subcellular dimensions and to quantify protein inherent structure profiles using Gaussian and Lorentzian methods of multi-component peak modeling (Yu *et al*., 2009; Jonker *et al*., 2010; 2011; 2012). Currently we are using this technique to detect double-gene and two foreign gene inserted alfalfa (Heendeniya *et al*., 2014a,b) and gene-knock down effect on alfalfa structure change in relation to nutrient value (Li *et al*., 2015). Our results showed that Lc-gene-transformation is expected to increase dairy cow milk yield from a 650 kg dairy cow with 2 kg/d if grazing on pasture with a dry matter intake of 17 kg/d.

Area III: Using Synchrotron-Based Analytical Methods to Detect Heat-induced protein structure and subfractions in relation to protein degradation kinetics and intestinal availability (Doiron *et al*., 2009a,b; Yu *et al*., 2010, 2014; Pend *et al*., 2015). These exampled studies were to reveal protein structures of feed tissues affected by heat processing (Autoclaving, roasting, etc) at a cellular level, using the advanced synchrotron technology as a novel approach, and quantify protein structure in relation to protein digestive kinetics and nutritive value. The parameters assessed included protein structure α- helix to β-sheet ratio, protein subfractions profiles (using CNCPS system, protein degradation kinetics and effective degradability, intestinal digestion of protein, predicted nutrient supply in terms of the intestinally absorbed protein supply (DVE), or metabolizable protein (MP) and degraded protein balance (OEB) (Doiron *et al*., 2009a, b; Yu *et al*., 2010, 2014; Pend *et al*., 2015). This method can also be used to check how carbohydrate structure change affect nutrient availability in dairy cattle (Yu, 2012). In our recent study, we found that with molecular structure spectral profile data (eg. TCP1A and TCP2H), we can predict both metabolizable protein and degraded protein balance with $R^2 > 0.9$ without expensive and time consuming dairy cow trial.

Area IV: Using Synchrotron-Based Analytical Methods to Detect Bio-ethanol processing induced structure changes on a molecular basis: relationship of protein structures to metabolic characteristics. This exampled study (Yu & Nuez-Ortín, 2010) aimed to reveal protein molecular structures of the new co-products of bioethanol production affected by bio-ethanol processing, identify the differences in protein molecular structure between grains and new co-products and between different types of the bioethanol co-products and quantify protein molecular structures in relation to protein nutritive values. In this study, proteins were from wheat, corn, wheat DDGS, corn DDGS and blend DDGS (wheat:corn=70:30) from bioethanol production (Yu & Nuez-Ortín, 2010). Currently this technique is used to study interactive association between the molecular structure and nutrient utilization of the new co-products (Carinata Meal) from bio-fuel processing as a new alternative feed for dairy cattle (Ban *et al*., 2015).

Summary

In conclusion, the above exampled studies demonstrate the potential of ultra-spatially resolved advanced synchrotron radiation based technique to reveal feed inherent structural change induced by various feed treatment and processing and can be used to reveal relationship between feed molecular structure changes and nutritive availability.

Acknowledgments

The National Synchrotron Light Source in Brookhaven National Laboratory (NSLS-BNL, New York, USA) is supported by the U.S. Department of Energy. Canadian Light Source Inc. at University of Saskatchewan (Saskatoon, Canada) is supported by, various Canadian federal and provincial funds. The author is grateful to Drs. Lisa Miller, Chithra Karunakaran, Tim May etc for synchrotron beamtime support, discussion and/or collaborations, and Randy Smith at NSLS-BNL (New York) for helpful synchrotron data collection and Ferenc Borondics, Xia Liu, Tor Pederson nad Luca Quaroni for helpful data collection at 01B1-1 station, Canadian Light Sources. Feed Research Chair Programs have been supported by the Natural Sciences and Engineering Research Council of Canada (NSERC- Individual Discovery Grant and CRD Grant), the Ministry of Agriculture Strategic Feed Research Chair Program, the Saskatchewan Agricultural Development Fund, Thousand-Talent-People Program (Tianjin) and University Innovation Team "Veterinary Biotechnology" in Tianjin (TD12-5019), Saskatchewan Canola Development Commission, Western Grain Foundation, and Saskatchewan Forage Council etc.

References

Abeysekara, S. D. A. Christensen, Z. Niu, K, Theodoridou & P. Yu, 2013. Molecular Structure, Chemical and Nutrient Profiles and Metabolic Characteristics of the Proteins and Energy in New Cool-Season Corn Cultivars Harvested as Fresh Forage for Dairy Cattle. J. Dairy Sci., 96, 6631–6643

Ban, Y., D. A. Christensen, J. J. McKinnon & P. Yu, 2015. Chemical Profiles, Energy Values, Protein and Carbohydrate Fractions of New Co-products (Carinata Meal) from Bio-fuel Processing as a New Alternative Feed for Dairy Cattle in Comparison with Canola Meal. Advances in Dairy Technology, 27, 359

Becker, P.M. & P. Yu, 2013. What Makes Protein Indigestible from Tissue, Cellular and Molecular Structure Aspects? Molecular Nutrition & Food Research, 57, 1695–1707.

Budevska, B. O., 2002. Applications of Vibrational Spectroscopy in Life, Pharmaceutical and Natural Sciences. In: Chalmers, J. M., Griffiths, P. R. (Eds). Handbook of Vibrational Spectroscopy, Vol. 5. John Wiley and Sons, Inc., New York, NY, USA, pp 3720-3732.

Doiron, K. J., Yu, P., McKinnon, J. J. & Christensen, D. A., 2009a. Heat-induced protein structures and protein subfractions in relation to protein degradation kinetics and intestinal availability in dairy cattle. J. Dairy Sci., 92, 3319-3330.

Doiron, K. J., Yu, P., Christensen, C. R., Christensen, D. A. & McKinnon, J. J., 2009b. Detecting molecular changes in Vimy flaxseed protein structure using synchrotron FTIRM and DRIFT spectroscopic techniques: Structural and biochemical characterization. Spectroscopy. 23, 307–322

Dumas, P., 2003. Synchrotron IR Microspectroscopy: A Multidisciplinary Analytical Technique. The 6th Annual Synchrotron CLS Users' Meeting and Associated Synchrotron Workshops University of Saskatchewan, Canada. Nov 13-15, 2003.

Heendeniya Vidanaral, R. G., M. Y. Gruber,Y. Wang, D. A. Christensen, J. J. McKinnon, B. Coulman & P. Yu., 2014a. Effect of co-expression of Lc and C1 flavanoid regulatory genes in alfalfa on nutritive value and ruminal methane production. J. Anim. Sci. Vol. 92, E-Suppl. 2/J. Dairy Sci. Vol. 97, E-Suppl. 1: 490.

Heendeniya Vidanaral, R. G., M. Y. Gruber,Y. Wang, D. A. Christensen, J. J. McKinnon, B. Coulman, & P. Yu., 2014b. Nutrient composition and degradation characteristics of anthocyanidin containing alfalfa transformed with Lc, C1, and Lc × C1 regulatory genes.. J. Anim. Sci. Vol. 92, E-Suppl. 2/J. Dairy Sci. Vol. 97, E-Suppl. 1: 105.

Huang, X., C. Christensen & P. Yu, 2015. Effects of Conditioning Temperature and Time during the Pelleting Process on Feed Molecular Structure, Pellet Durability Index, Metabolic Features of Co-Products from Bio-Oil Processing in Dairy Cows. J. Dairy Sci. In Press (JDS-14-9290)

Jackson, M. & Mantsch, H. H., 1995. The use and misuse of FTIR spectroscopy in the determination of protein structure. Biochemistry and Molecular Biology, 30, 95-120.

Jackson, M. & Mantsch, H. H., 1996. Biomedical Infrared Spectroscopy, In: Mantsch, H. H., Chapman, D. (Eds), Infrared Spectroscopy of Biomolecules, Wiley-Liss, New York, pp 311-340.

Jonker, A., M. Y. Gruber, M. McCaslin, Y. Wang, B. Coulman, J. J. McKinnon, D. A. Christensen & P. Yu, 2010. Nutrient Composition and Degradation Profiles of Anthocyanidin-Accumulating Lc-Alfalfa Populations. Can. J. Anim. Sci., 90, 401-412

Jonker, A., M. Y. Gruber, Y. Wang, B. Coulman, J. J. McKinnon, D. A. Christensen & P. Yu, 2012. Foam Stability of Leaves from Anthocyanidin-Accumulating Lc-Alfalfa and Relation to Molecular Structures Detected by FTIR Vibration Spectroscopy. Grass Forage Sci., 67, 369-381

Jonker, A., M. Y. Gruber , Y. Wang , B. Coulman , A. Azarfar , J. J. McKinnon , D. A. Christensen & P. Yu, 2011. Modeling Degradation Ratios and Nutrient Availability of Anthocyanidin-Accumulating Lc-Alfalfa Populations in Dairy Cows. J. Dairy Sci., 94, 1430–1444.

Katerina Thedoridou, Sally Vail & Peiqiang Yu, 2014. Explore Protein Molecular Structure in Endosperm Tissues in Newly Developed Black and Yellow Type Canola Seeds by Using Synchrotron-based Fourier Transform Infrared Microspectroscopy. Spectrochimica Acta Part A - Molecular and Biomolecular Spectroscopy, 120, 421–427

Kemp, W., 1991. Organic Spectroscopy. 3rd ed. W.H. Freeman and Company, New York, USA.

Kneipp, J., Miller, L. M., Joncic, M., Kittel, M., Lasch, P., Beekes, M. & Naumann, D., 2003. In situ identification of protein structural changes in prion-infected tissue. Biochim. Biophys. Acta., 1639, 152-58.

Li, X., A. Hannoufa, M. Y. Gruber, Y. Zhang & P. Yu, 2015. Effect of gene modification on protein and energy values in new alfalfa for dairy cattle. Advances in Dairy Technology, 27, 379 (Proceedings of the 33rd Western Canadian Dairy Seminar, Lorraine Doepel, Editor, Published by University of Alberta, Edmonton, Alberta, Canada.

Marinkovic, N. S. & Chance, M. R., 2006. Synchrotron Infrared Microspectroscopy. In: Meyers, R. (Ed), Encyclopedia of Molecular Cell Biology and Molecular Medicine, 2nd ed., Vol 13, Wiley Inc., pp 671-708.

Marinkovic, N. S., Huang, R., Bromberg, P., Sullivan, M., Toomey, J., Miller, L. M., Sperber, E., Moshe, S., Jones, K. W., Chouparova, E., Lappi, S., Franzen, S. & Chance, M. R., 2002. Center for Synchrotron Biosciences' U2B beamline: an international resource for biological infrared spectroscopy. J. Synchrotron Rad., 9, 189-197.

Miller, L. M. & Dumas, P., 2006. Chemical imaging of biological tissue with synchrotron infrared light. Biochim. Biophys. Acta., 1758, 846-857.

Peng, Q., N. A. Khan, Z. Wang, X. Zhang & P. Yu, 2014. Effect of Thermal Processing on Estimated Metabolizable Protein Supply to Dairy Cattle from Camelina Seeds: Relationship with Protein Molecular Structural Changes. J. Agric. Food Chem., 62, 8263−8273

Seguchi, M., Takemoto, M., Mizutani, U., Ozawa, M., Nakamura, C. & Matsumura, Y., 2004. Effects of secondary structures of heated egg white protein on the binding between prime starch and tailings fractions in fresh wheat flour. Cereal. Chem., 81, 633-636.

Wetzel, D. L., Eilert, A. J., Pietrzak, L. N., Miller, S. S. & Sweat, J. A., 1998. Ultraspatially resolved synchrotron infrared microspectroscopy of plant tissue in situ. Cell. Mol. Bio., 44, 145-167.

Wetzel, D. L., Srivarin, P. & Finney, J. R., 2003. Revealing protein infrared spectral detail in a heterogeneous matrix dominated by starch. Vibrational Spectroscopy. 31, 109-114.

Yang, L., D. A. Christensen, J. J. McKinnon, A. D. Beattie, H. Xin & P. Yu, 2013. Investigating the Molecular Structure Features of Hulless Barley (*Hordeum vulgare* L.) in Relation to Metabolic Characteristics Using Synchrotron-based Fourier Transform Infrared Microspectroscopy . J. Agric. Food Chem., 61, 11250−11260.

Yu, P., 2004. Application of advanced synchrotron-based Fourier transform infrared microspectroscopy (SR-FTIR) to animal nutrition and feed science: a novel approach. British J. Nutri., 92, 869-885.

Yu, P, McKinnon, J. J., Christensen, C. R. & Christensen, D. A., 2004. Imaging Molecular Chemistry of Pioneer Corn. J. Agric. Food Chem., 52, 7345-7352.

Yu, P., 2005. Multi-component peak modeling of protein secondary structures: comparison of Gaussian with Lorentzian analytical method for plant feed and seed molecular biology and chemistry research. Applied Spectroscopy, 59, 1372-1380.

Yu, P., 2005. Molecular chemistry imaging to reveal structural features of various plant feed tissues. J. Structural Biol., 150, 81-89.

Yu, P., 2005. Protein secondary structures (α-helix and β-sheet) at a cellular level and protein fractions in relation to rumen degradation behaviors of protein: A novel approach. British J. Nutri., 94, 655-665.

Yu, P., 2006. Synchrotron IR Microspectroscopy for Protein Structure Analysis: Potential and Questions: A Review. Spectroscopy: An International Journal. 20, 229-251.

Yu, P., Block, H., Niu, Z. & Doiron, K., 2007. Rapid characterization of molecular chemistry and nutrient make-up and microlocalization of internal seed tissue. J. Synchrotron Rad. 14, 382-390.

Yu, P., 2008. Synchrotron-Based Microspectroscopic Analysis of Molecular and Biopolymer Structures Using Multivariate Techniques and Advanced Multi-Components Modeling. Can. J. Analytical Sci. Spectroscopy, 53, 220-231.

Yu, P., Doiron, K. & Liu, D., 2008. Shining light on the difference in molecular structural chemical make-up and the cause of distinct biodegradation behavior between malting- and feed-type barley: A novel approach. J. Agric. Food Chem. (Molecular Nutrition Section). 56, 3417-3426.

Yu, P., Jonker, A. & Gruber, M., 2009. Molecular basis of protein structure and nutritive value in proanthocyanidin-enhanced Lc-transgenic alfalfa using synchrotron-radiation FTIR microspectroscopy. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 73, 846–853.

Yu, P., 2010. Plant-Based Food and Feed Protein Structure Changes Induced by Gene-Transformation, Heating and Bio-Ethanol Processing: A Novel Synchrotron-Based Molecular Structure and Nutrition Research Program. Molecular Nutrition and Food Research, 54, 1535–1545.

Yu, P. & W.G. Nuez-Ortín, 2010. Relationship between Protein Molecular Structures and Metabolisable Proteins in Different Types of Dried Distillers Grains with Solubles: a novel approach. Br. J. Nutr., 104, 1429–1437.

Yu, P., Niu, Z. & Damiran, D., 2010. Protein molecular structures and protein fraction profiles of new co-products of bioethanol production: A novel approach. J. Agric. Food Chem., 58, 3460–3464.

Yu, P., 2011. Microprobing Molecular Spatial Distribution and Structural Architecture of Sorghum Seed Tissue (*Sorghum Bicolor* L.) with SR-IMS Technique. Journal of Synchrotron Radiation, 18, 790-801.

Yu, P., 2012. Relationship of Carbohydrate Molecular Spectroscopic Features to Carbohydrate Nutrient Profiles in Co-Products from Bioethanol Production. J. Dairy Sci., 95, 2091-2096.

Yu, P., K. Theodoridou, H. Xin, P-Y. Huang, Y-C. Lee & B. R. Woods, 2013. Synchrotron-Based Microspectroscopic Study on the Effect of Heat Treatment on Cotyledon Tissues in Yellow-Type of Canola (*Brassica*) Seeds. J. Agric. Food Chem., 61, 7234–7241.

Yu, P., I.H. Gamage & X. Zhang, 2014. New Approaches and Recent Advances on Characterization of Chemical Functional Groups and Structures, Physiochemical Property and Nutritional Values in Feedstocks and By-Products: Advanced Spectroanalytical and Modeling Investigations. Applied Spectroscopy Reviews 49, 585–602.

Zhang, X. & P. Yu, 2012. Molecular basis of protein structure in combined feeds (hulless barley with bioethanol coproduct of wheat dried distillers grains with solubles) in relation to protein rumen degradation kinetics and intestinal availability in dairy cattle. J. Dairy Sci., 95, 3363–3379.

Zhang, X. & P. Yu, 2014. Using Non-Invasive Technique in Nutrition: SR-IMS Spectroscopic Characterization of Oil Seeds Treated with Different Processing Conditions on Molecular Spectral Factors Influencing Nutrient Delivery. J. Agric. Food Chem., 62, 6199–6205.

Karoline in Excel

P. Huhtanen
Swedish University of Agricultural Sciences (SLU), Department of Agriculture for Northern Sweden, 90 183 Umeå, Sweden
Correspondence: pekka.huhtanen@slu.se

Introduction

Karoline is a mechanistic and dynamic dairy model that was published in detailed book chapters (Sveinbjörsson *et al*., 2006; Danfær *et al*., 2006a; 2006b). The model was developed in a collaborative Nordic project and was partially based on the Doctor of Science thesis of Allan Danfær (1990). Original model (Danfær, 1990) was written in Fortran that makes using and understanding the model difficult for "amateurs". Graphic modelling software can make it much easier to learn modelling and to understand and use by researchers. This type of software also facilitates close collaboration between modelling and experimental research, of which both should benefit.

Karoline was built using the graphic Powersim® software. Input data (intake, diet composition, body weight, etc.) are formulated in an Excel spreadsheet which communicates with the Powersim model via the dynamic data exchange feature in Powersim. Basal calculations, for example diet composition from intake and composition of dietary ingredients and aggregated digestion kinetic parameters, are first made in Excel. Model output data is also returned to Excel so that further calculations can be made. Powersim is also an excellent tool in teaching, because dynamic processes can easily be demonstrated by simple models.

In the original model evaluation (Danfær *et al*.,2006b), Karoline predicted ruminal and total digestibility of nutrients and flow of microbial and feed protein to the small intestine accurately and precisely. Karoline also showed potential to predict methane emissions accurately (Huhtanen & Ramin, 2012). Thereafter, some revisions were made on the digestion model, especially on the methane sub-model, and local sensitivity analysis was performed (Huhtanen *et al*., 2015). Compared to other mechanistic models, performance of Karoline was good in terms of small root means squared prediction errors of 10 and 6% of observed mean with fixed and mixed model regression analysis, respectively (Ramin & Huhtanen, 2015).

Using Powersim version 2.5 has become difficult, since the software does not work in modern computers with 64 bit system. One possibility is to install Windows 7 with a 32 bit system. Another alternative is to divide the computer into another virtual computer with 32 bit system and Powersim in virtual mode. Especially the latter system has encountered problems. Because of the development of computers and systems, it is evident that we may not be able to use the Powersim 2.5 version in the near future. Therefore, other alternatives must be sought to be able to run and develop Karoline and other models developed in Powersim. More recent versions of Powersim are not able to read and run models made with the version 2.5. In addition, Powersim is not commonly used among animal scientists. One alternative is to rewrite Karoline in Excel. Preliminary testing using small NDF digestion models suggested that mechanistic dynamic models can also be built in an Excel spreadsheet. Numerical solutions for compartmental sizes and fluxes were found to be identical in Excel and Powersim applications. The objectives of this paper is to present principles how to build

mechanistic dynamic models using Excel spreadsheet and present comparisons of model solutions of Karoline digestion model by Powersim and Excel softwares.

Principles

In Powersim compartments, auxiliaries, constants and fluxes have different graphic symbols. A simple one-compartment model describing digestion of the dietary component potentially degradable neutral detergent fibre (pdNDF) is described in Figure 1. Time graphs can easily be made to illustrate changes in pool sizes, fluxes and auxiliaries during simulation. The changes in 'Pool' over time are described by the differential equation:

Pool dt/dx = -dt*Passage – dt*Digestion + dt*Intake

An advantage of Powersim is that when the fluxes are built manually by a graphical tool, the differential equations are automatically developed.

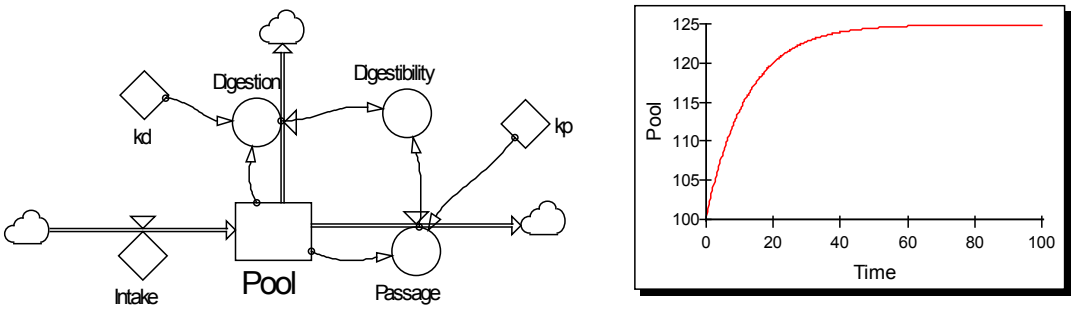


Figure 1. Graphic presentation of a simple dynamic model in Powersim.

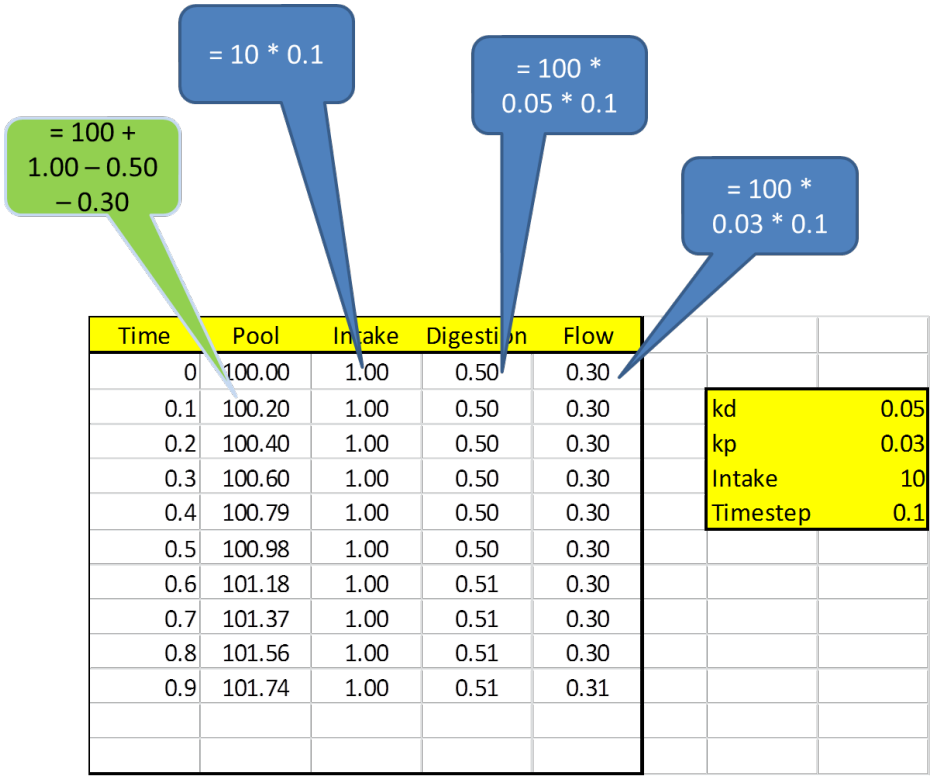
When the same model is constructed in Excel, we need to write the explicit equations for pools (compartments), fluxes and auxiliaries from Powersim (Figure 2) on different columns with each time step on a different row.

```
init    Pool = 100
flow    Pool = -dt*Passage
        -dt*Digestion
        +dt*Intake
aux     Digestion = Pool*kd
aux     Passage = Pool*kp
aux     Digestibility = Digestion/(Digestion+Passage)
const   Intake = 10
const   kd = 0.05
const   kp = 0.03
```

Figure 2. Equations of the graphic model presented in Figure 1.

The graphic model presented in Figure 1 can be written in Excel as described in Figure 3. The model requires 5 lines and 1 000 rows for a 100-h simulation time with a time step = 0.1 h. In practise, simulations times should often be longer than 100 h to ensure reaching a steady-state solution. The last hour simulation is shown in Figure 4. In this example digestibility

coefficients were calculated from the last hour data, but in practise it is recommended to present fluxes based on the last 24 h data. It is worth noting that the model did not reach absolute steady-state during this 100-h simulation.



Time	Pool	Intake	Digestion	Flow
0	100.00	1.00	0.50	0.30
0.1	100.20	1.00	0.50	0.30
0.2	100.40	1.00	0.50	0.30
0.3	100.60	1.00	0.50	0.30
0.4	100.79	1.00	0.50	0.30
0.5	100.98	1.00	0.50	0.30
0.6	101.18	1.00	0.51	0.30
0.7	101.37	1.00	0.51	0.30
0.8	101.56	1.00	0.51	0.30
0.9	101.74	1.00	0.51	0.31

kd	0.05
kp	0.03
Intake	10
Timestep	0.1

Figure 3. Excel application of the first hour simulation of the graphic model presented in Figure 1.

Time	Intake	Pool	Digestion	Flow
99.1	1.000	124.991	0.625	0.375
99.2	1.000	124.991	0.625	0.375
99.3	1.000	124.991	0.625	0.375
99.4	1.000	124.991	0.625	0.375
99.5	1.000	124.992	0.625	0.375
99.6	1.000	124.992	0.625	0.375
99.7	1.000	124.992	0.625	0.375
99.8	1.000	124.992	0.625	0.375
99.9	1.000	124.992	0.625	0.375
100	1.000	124.992	0.625	0.375
Sum	**10.000**		**6.250**	**3.750**

Digestistibity = 6.25 / 10.00 = 0.625
Digestibility = (10.00 – 3.75) / 10.00 = 0.625

Figure 4. Excel application of the last hour simulation of the graphic model presented in Figure 1.

Karoline in Excel

Karoline includes both digestion and metabolism of nutrients and is a very large model that is described graphically in 54 pages (nutrient digestion 24 pages) including 102 compartments, 292 flows, 197 constants and 528 auxiliaries. In Excel format, the nutrient digestion model required 1 075 200 cells (224 columns and 4 800 lines; time step = 0.1). The same Excel spreadsheet for fermentation equations, ration formulation and computing diet composition and aggregated digestion kinetic parameters is used as for the Powersim application. The

input data is then transferred by a macro to another Excel file (Karoline Excel), in which it takes approximately 4 s to execute the model. In Powersim, a run takes about 22 s when graphic is turned off. Further calculations of results are made and presented in 13 Tables in another spreadsheet in the same file. The results section is more comprehensive than the previous version, including e.g. amounts of nutrients absorbed in moles, grams and MJ per day and calculated potential for gluconeogenesis (AA not included). The results (125 variables) are also collected in the 'LOG' sheet in the same file with one simulation per line from the tables by use of a separate macro.

One potential problem in writing large models like Karoline in Excel could be using Euler's algorithm, where first initial conditions for the compartments (pools) and flows are calculated, as described earlier. The flows are used to update the compartments and the new values are then used to recalculate the flows. It only uses derivative information at the beginning of each time step. Making time steps shorter can reduce the error imposed by the Euler method. When time step approaches zero, the Euler approximation approaches an exact solution. Fourth order of Runge-Kutta (RK4) algorithm is a more advanced method that uses four flow calculations within each time step, one initial, two at mid-points and one at the end-point. From these, a weighted average value is calculated. Using the RK4 algorithm in Excel would make the model considerably larger since 4 extra columns for each flow would be required. In evaluations of simple models, the two algorithms deviated at the beginning of the simulations when the rate constants were high and time steps long, but using a 0.1 h time step, the same steady state was reached approximately at a time was much less than 480 h in the current application.

Table 1. Selected pool sizes of nutrients in digestive tract simulated by Powersim using RK4 and Euler algorithms or by Excel spreadsheet

	Powersim		Excel
	RK4	Euler	
Rumen NDF pools (g)			
Forage pdNDF-NEP[1]	1602.46	1602.46	1602.42
Forage iNDF-EP[2]	1004.52	1004.52	1004.49
Concentrate-pdNDF-EP	424.93	424.93	424.93
Rumen N pools (g)			
Rumen ISN (g)			
Forage ISN-EP	17.37	17.37	17.38
Concentrate ISN-EP	30.05	30.05	30.05
Peptide N	11.269	11.269	11.266
Free AAN	3.196	3.196	3.196
Ammonia N	7.762	7.735	7.807
Hind-gut (g)			
iNDF	324.00	324.00	324.00
pdNDF	346.93	346.93	346.90
Ammonia N	2.2388	2.2372	2.2660

[1]NEP = Rumen non-escapable pool; [2]EP = Rumen escapable pool

The differences between RK4 and Euler algorithms in pools sizes of various nutrients were negligible, if any, in Powersim simulations (Table 1). Similarly, only minor numerical differences in pool sizes of different nutrients were observed between Powersim and Excel simulations. Actually, the values estimated by Excel can be more exact, since Excel uses 15 decimals compared to only 5 decimals in Powersim. Calculated diet composition, passage kinetic parameters and aggregated digestion rates of pdNDF, starch and insoluble protein usually have more than 5 decimals that can result in minor differences in fluxes and pool sizes compared with input data presented only with 5 decimals. Also in fluxes the differences between the systems were negligible. For example, digestibility of organic matter was 74.25 vs. 74.23% and microbial N flow 273.08 and 273.30 g/d with Excel and Powersim simulations, respectively.

Table 2. Predicted values from Karoline simulations when the same diet (grass silage – barley – rapeseed meal 60:30:10; CP 168 g/kg DM, NDF 439 g/kg DM) was fed to sheep, growing cattle and dairy cows.

	Sheep	Growing cattle	Dairy cow
Body weight, kg	60	350	600
DM intake, kg	1.0	8.0	20.0
Rumen pool, kg			
NDF	0.35	2.72	6.40
iNDF	0.13	0.97	2.13
Organic matter	0.62	4.71	11.05
Total VFA, mmol/L	89.3	102.3	119.0
Digestibility			
Organic matter	0.762	0.751	0.733
NDF	0.678	0.662	0.635
pdNDF	0.817	0.797	0.765
CP	0.745	0.735	0.724
MFOM[1], g/kg DM intake	86.4	90.1	95.9
N flow, g/d			
Microbial N	13.6	114.9	310.3
Feed N	5.3	47.1	136.5
Non-ammonia N	18.9	162.0	446.8
CP degradability	0.801	0.778	0.743
Microbial N, g/kg TDOM[2]	18.5	19.9	22.1
Methane			
g/kg DM intake	26.7	24.8	22.1
kJ/MJ GE intake	83.1	77.1	68.6

[1] MFOM = Metabolic faecal output (OM – NDF); [2] TDOM = Truly digested organic matter in the rumen.

The performance of the model was evaluated using the same diet (grass silage-barley rapeseed meal: 60-30-10 on DM basis) for sheep (body weight 60 kg), growing cattle (350 kg) and lactating dairy cows (600 kg). Respective DM intakes were 1.0, 8.0 and 20.0 kg/d. Initial pool sizes were typical for dairy cows fed approximately 20 kg DM/d. Some selected simulation results are shown in Table 2. Although the Karoline model was developed for

dairy cows, the simulated values for sheep fed slightly above maintenance requirement (feeding level 1.3) were within the range of expected values. This suggests that the mechanisms of transactions in the digestive tract are described with a reasonable accuracy in the Karoline model. For example, predicted differences in OM digestibility (OMD) between sheep and dairy cows at a production level intake were close to the difference between observed OMD in dairy cows and OMD predicted at maintenance level from *in vivo* or in *vitro* OMD of forages and tabulated digestibility coefficients for concentrate ingredients (Huhtanen *et al*., 2009).

Conclusions

The digestion sub-model of the Nordic dairy cow model Karoline was transferred to the Excel format. Comparisons of the Excel model with different algorithms in Powersim confirmed that with a 0.1 h time step and 480 h simulation time, both softwares gave identical solutions. The Excel version has two advantages: the model is easily available to possible users and it runs faster. On the other hand, demonstrating transactions and mechanisms in nutrient digestion is more illustrative with graphic softwares than with the Excel spreadsheet. Model development would also be easier with Powersim compared with Excel. Based on my own experience, graphic modelling softwares like Powersim make both learning and teaching mechanistic modelling easier. Graphic modelling software also can improve communication and mutual understanding between modellers and experimentalist.

References

Danfær, A., 1990. A Dynamic Model of Nutrient Digestion and Metabolism in Lactating Dairy Cows. 671 Berething fra Statens Husdyrforsøg. National Institute of Animal Science, Denmark. 511p.

Danfær, A., Huhtanen, P., Udén, P., Sveinbjörnsson, J. & Volden, H., 2006. The Nordic Dairy Cow Model, Karoline - Evaluation. In: Kebreab, E. *et al*. (eds) Nutrient Digestion and Utilization in Farm Animals: Modelling approaches. CAB International, pp. 407-415.

Danfær, A., Huhtanen, P., Udén, P., Sveinbjörnsson, J. & Volden, H., 2006. The Nordic Dairy Cow Model, Karoline - Description. In: Kebreab, E. *et al*. (eds) Nutrient Digestion and Utilization in Farm Animals: Modelling approaches. CAB International, pp. 383-406.

Huhtanen, P. & Ramin, M., 2012. Evaluation of the Nordic Dairy cow model Karoline in predicting predicting methane production. Acta Agric Scand., Sect. A Anim. Sci. 62, 295-299.

Huhtanen, P., Ramin, M. & Udén, P., 2015. Nordic dairy cow model Karoline in predicting methane emissions: 1. Model description and sensitivity analysis. Livest. Sci. (accepted).

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

Ramin, M. & Huhtanen, P., 2015. Nordic dairy cow model Karoline in predicting methane emissions: 2. Model evaluation. (submitted).

Sveinbjörnsson, J., Huhtanen, P. & Udén, P., 2006. The Nordic dairy cow model, Karoline – Develpment of volatile fatty acid sub-model. In: Kebreab, E. *et al*. (eds) Nutrient Digestion and Utilization in Farm Animals: Modelling approaches. CAB International. pp 1-14.

The effect of sodium sulfite on fiber contents of red clover when using the ANKOM® detergent fiber fractionation system

H. H. Hansen[a], H. M. Ebeid[a,b], K. Manolopoulos[a], M. Toldbod Nielsen[a] & E. Nadeau[c]
[a]*Department of Large Animal Sciences, University of Copenhagen, Grønnegårdsvej 2, 1870 Frederiksberg, Denmark.*
[b]*Department of Dairy Science, National Research Centre, Dokki, Giza, 12311, Egypt.*
[c]*Department of Animal Environment and Health, The Swedish University of Agricultural Sciences, Box 234, SE-532 23 Skara, Sweden.*
Correspondence: hhh@sund.ku.dk

Introduction

Several different modifications of cell wall analysis (neutral detergent fiber (NDF), acid detergent fiber (ADF) and sulphuric acid lignin (lignin (sa)) have been described and continue to be developed for forages, in particular for ruminant feeds. However, a large variation between laboratory results for fiber fractions of red clover grown in Sweden created the need to investigate the effect of specific modifications of the technique on the estimates of cell wall structure. An editorial in Animal Feed Science and Technology (2005) reviews the evolution of some overall modifications from the 60's and was used as a start for the research. In 2002 the American Organisation of Analytical Chemists approved the NDF methodology (Mertens, 2002). This article concludes that "Sixty min of refluxing at boiling temperatures achieves asymptotic extraction" and "the proposed method uses 2 additions of heat-stable amylase: one in neutral detergent (ND) solution after initiation of boiling, and one in the first residue washing step" in order to remove starch. Finally, this article states that "research by the Study Director indicated that sodium sulfite is critical for removal of proteinaceous matter in heated or cooked feeds, and it was reintroduced in the method that is being evaluated". Hintz *et al*. (1996) recommend 1:1 grams/gram of sulfite to sample, based on a series of trials. Hunt *et al*. (1995) investigated 4 neutral detergent extraction methods to determine where changes due to sodium sulfite occurred. They concluded that use of sodium sulfite in the rinse was a viable method to decrease sample analysis time without compromising NDF results. However, use of sodium sulfite is still controversial. Already in 1991, in a review of the methodology, Van Soest *et al*. (1991) stated that "Sulfite also attacks lignin and therefore should not be used in sequential analyses leading to lignin determination…"

The ANKOM^{200} Fiber Analyzer® is a machine used to determine NDF and ADF residues in 24 filter bags at a time. It has an inner chamber that can heat (100 ± 0.5°C) 2 liters of fluid and a bag suspender with trays that move vertically and rotate while maintaining a pressure of 10-25 psi. This instrument is made to be capable of creating a similar flow of solution around each sample to ensure uniformity of extraction (ANKOM^{2000} with 65 rpm agitation, ANKOM Technology). These filter bags are nitrogen and ash free, can withstand 72% sulfuric acid, and have a porosity of 25 microns. The residual material in the fiber bags is weighed after washing with ANKOM® ND solution, ANKOM® Acid Detergent Solution (AD solution) or 72% sulfuric acid and the residual expressed as % of DM or g/kg of the original DM.

On the basis of the uncertainty of 1) reflux time for NDF using the ANKOM® Fiber Analyzer, 2) the number of necessary alpha amylase additions and 3) the potential effects of sulfite on fiber fractionation, a series of trials were conducted to test the following hypotheses:

1) NDF concentration in red clover achieves an asymptote after 3 sequential washes
2) NDF and ADF concentrations in red clover are reduced with increasing doses of sodium sulfite and increasing exposure time.
3) Lignin (sa) concentration in red clover, determined sequentially after NDF and ADF determination, is reduced with increasing doses of sodium sulfite in the ND solution wash.

Materials and Methods

Five varieties of red clover (*Trifolium pratensis*: Ares, Nancy, Roseta, Taifun, and Vicky), grown at the Rådde Experimental Station, the Rural Economy and Agricultural Society, in southwest Sweden were used to test the hypotheses. The varieties were harvested June 11, 2012 from a clover variety experiment designed as a randomized block with three field blocks. The crude protein concentrations ranged from 160 to 180 g/kg DM (Nadeau *et al*., 2014). The samples were dried and ground to pass through a 1 mm screen. Approximately 0.5 g of this material was placed in an ANKOM® "F57" filter bag. Four batches of fiber fractionation with a different treatment for each batch were analysed (first four treatments in Table 1). The same five red clover varieties were analyzed in each treatment with either 2 (SS0*3, SS20W, SS11W, SS11R) or 3 (SS0*2) replicated bags with the plant sample from each block. All samples were analyzed for NDF, ADF and lignin (sa). The results from the fifth treatment (SS02*A) were from another research project using the same samples (5 clover varieties) but including more harvest times. Only samples from same harvest and blocks were included for comparison. The SS02*A was considered the "reference" treatment as it adheres to the AOAC methodology (Mertens, 2002).

The aNDF and ADF concentrations were determined in differing batches of ANKOM®Fiber Analyzer A200 runs. The treatments used were based on the variations in methodology reported above, and were as described in Table 1.

Table 1 Abbreviations and descriptions of NDF methodology modification

Treatment name	Treatment description
SS0*3	75 minutes washing in ND solution with no addition of sulfite. Three (3) subsequent rinses with deionized water for 5 minutes each. Addition of 4 ml heat stable amylase to the wash and to the subsequent first two rinses. The bags were dried and weighed and the treatment repeated twice. The bags were thereafter analyzed for ADF and lignin (sa) sequentially.
SS20W	75 minutes washing in ND solution with addition of 20 g of sulfite in 2L ND solution. Three (3) subsequent rinses with deionized water for 5 minutes each. Addition of 4 ml heat stable amylase to the wash and to the subsequent first two rinses. This is the protocol recommended by ANKOM® (ANKOM Technology, Macedon, NY) The residual material was analyzed for ADF and lignin (sa) sequentially.
SS11W:	As S20W but with only 11 g sulfite in the ND solution. The residual material was analyzed for ADF and lignin (sa) sequentially.
SS11R:	75 minutes washing in ND solution with three subsequent rinses with deionized water of 5 min. Eleven (11) g sodium sulfite added to the first rinse. Addition of 4 ml heat stable amylase to the wash and to the subsequent first two rinses. The residual material was analyzed for ADF and lignin (sa) sequentially.
SS02*A:	75 minutes washing in ND solution with no sodium sulfite, three subsequent rinses with deionized water for 5 minutes each. Addition of 4 ml heat stable amylase to the wash and to the subsequent first rinse. The residual material was analyzed for ADF and lignin (sa) sequentially. This was considered the reference treatment.

The NDF and ADF residues were determined using the ANKOM® Fiber Analyzer A200 (ANKOM Technology, 2010). The ANKOM® DaisyII Incubator was used for lignin (sa) determination. Empty bags were included in all runs to correct for empty bag weight changes. All calculations are reported on a g/kg DM basis inclusive of residual ash (aNDF, ADF).

All statistical analyses were conducted with the R statistical software (R Development Core Team, 2013) using a linear mixed-effects models the (LMER package in R) (Bates *et al*., 2014) with treatment as a fixed variable and feed as a random variable. The number of observations for Vicky, Nancy and Taifun in the first 4 treatments (SS0*3, SS20W, SS11W, SS11R) was: 3(varieties) × 4(treatments) ×2(blocks) × 2(bags=reps) = 48. No differences were found between blocks and effect of block was not included in the final model. The number of observations for Ares and Roseta in the first 4 treatments was: 2(varieties) × 4(treatments) ×1(block) ×2(reps) =16. The number of observations for Ares, Nancy, Taifun, and Vicky in the fifth treatment (SS0*2A) were 4(varieties) × 1(treatment) × 2(blocks) × 3(reps) =24: and for Roseta: 1(variety) × 1(treatment) × 1(block) × 3 (reps) =3. All 91 samples were included in the treatment analyses model. No differences were found between blocks and effect of block was not included in the final model. Only the aNDF concentration of the final SS0*3 treatment (third wash) was included in the statistical model for differences in treatments. Due to differences between the varieties, but the use of the same variety in each treatment, clover variety was included as a random variable in the model. Each treatment was tested against the reference treatment (SS02*A) alone and thereafter against all other treatments.

Results and Discussion

The average decrease of the aNDF concentrations from the first to the second ND solution wash (SS0*3) ranged from 11 to 13%, while the decrease from the second to the third ND solution wash ranged from 3 to 5%. The aNDF concentration did not reach an asymptote after 3 sequential washes, as can be seen in Figure 1. The reduction between second and third wash was different from zero (asymptote; $P < 0.05$). However, it should be noted that this research was undertaken by subjecting the same filter bags to repeated washes. Despite the "gentleness" of the washing and rinsing procedures, a certain continued loss might be expected through the filter bag pores, primarily due to heterogeneous particle size. More research is needed with prolonged single (non-sequential) wash times.

The sequential ND solution wash treatment (SS0*3) decreased ($P < 0.05$) NDF, ADF and lignin (sa) compared to the reference (SS02*A) treatment (Figure 2). Furthermore, the sequential ND solution treatment (SS0*3) reduced the numerical concentration of NDF, ADF and lignin (sa) more than any of the sulfite treatments (SS20W, SS11W, SS11R). Use of 11 g of sulfite in the rinse resulted in more NDF, ADF and lignin (sa) compared to the reference treatment (SS02*A) ($P<0.05$). This result was surprising and no reasonable explanation found.

The NDF concentration after SS11W was not s different ($P>0.05$) from the reference treatment (SS02*A) or 20 g sulfite in the wash (SS20W). However, a numerical decrease of NDF and lignin (sa) concentrations was seen when using 11 g of sodium sulfite in the wash, compared to both the reference treatment (SS02*A) and 20 gram in the wash treatment (SS20W). This suggests that lignin is not solubilized and lost from red clover when using

sodium sulfite in sequential NDF, ADF and lignin (sa) analyses. However, the variation between sample results (magnitude of SEM) was greater when using 20 g in the wash compared to 11 g in the wash and therefore the addition of 11 g in the ND solution wash is recommended.

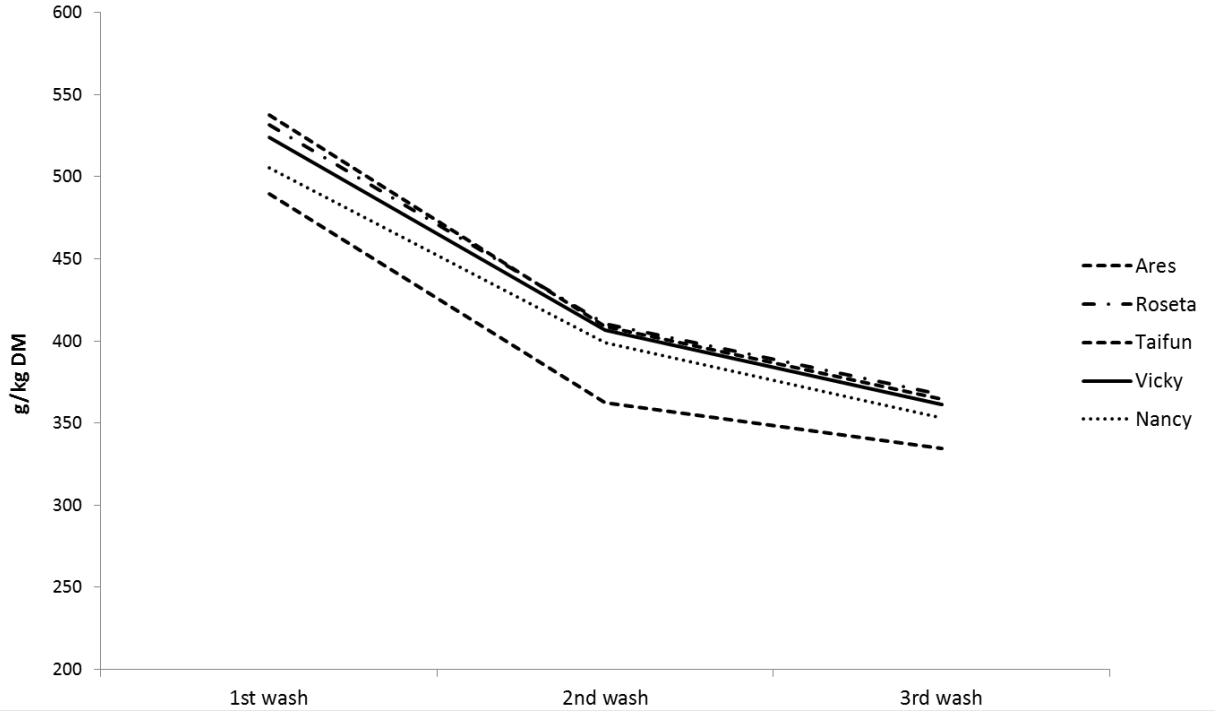


Figure 1 NDF (g/kg DM) after 3 sequential washes with neutral detergent solution.

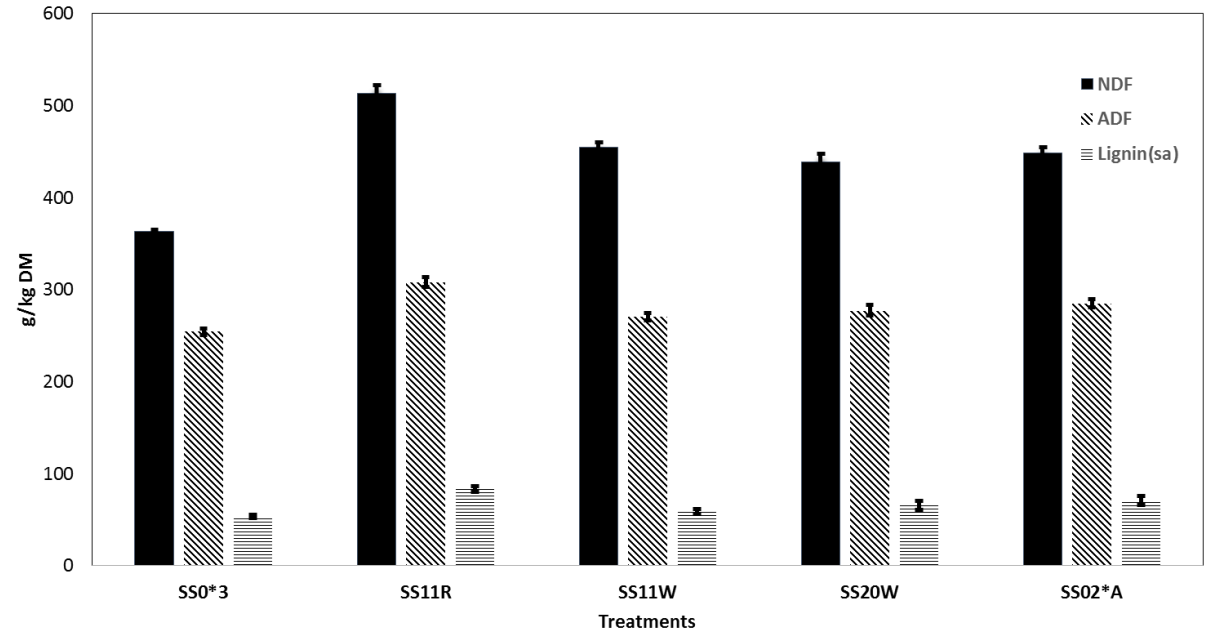


Figure 2 Comparison of NDF, ADF and lignin (sa) concentrations between different treatments. See text for treatment details and labels (SEM shown by error bars).

Conclusions

aNDF values of red clover did not reach an asymptote after three repeated analyses in the ANKOM® Fiber Analyzer. More research is needed to ascertain the correct reflux time. Repeated NDF washing affected aNDF, ADF and lignin (sa) concentrations more than addition of sodium sulfite. Use of 20 g sulfite in the wash does not produce statistically different results compared to the use of 11 g sodium sulfite for NDF, ADF and lignin (sa) values. Use of 11 g of sodium sulfite in the wash produced less variation in NDF, ADF and lignin (sa) results and is therefore recommended for red clover varieties. The ANKOM® fiber fractionation system is an extremely easy method to measure fiber. However, the differing results from varying ND solution reflux times as well as varying sodium sulfite doses and exposure times have underlined the need for more research to standardize the method in order to ensure the integrity of the plant structural components.

References

ANKOM Technology, 2010. Method 6, 4/13/11: Neutral Detergent Fiber in Feeds Filter Bag Technique (For A200, A200I). Ankom Technology, Macedon, NY, Accessed April 10, 2015 from https://ankom.com/neutral-detergent-fiber.aspx

Bates, D., Maechler, M., Bolker, B. & Walker, S., 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7, Accessed April 10, 2015 from http://CRAN.R-project.org/package=lme4.

Hintz, R. W., Mertens, D. R. & Albrecht, K. A., 1996. Effects of sodium sulfite on recovery and composition of detergent fiber and lignin. J. AOAC Int. 79, 16-22.

Hunt, J., Pinchak, W. & Hutcheson, D., 1995. Effects of incubation time and sodium sulfite upon in-vitro digestibility estimates and sample filtering time. J. Range Manage. 48, 451-454.

Nadeau, E., Hallin, O., Richardt, W., Hansen, H., Arnesson, A. & Jansson, J. 2014. Protein och fiber i rödklöver. Sid 85-88. Vallkonferens 2014. Rapport nr 18, Institutionen för växtproduktionsekologi, Sveriges lantbruksuniversitet. Konferensrapport 5-6 februari, Uppsala, Sverige, Nilsdotter-Linde, N., Bernes, G., Liljeholm, M. & Spörndly, R. (ed).

R Development Core Team, 2013. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Accessed April 10, 2015 from http://www.r-project.org

Reed, J.D., 2001. Effects of proanthocyanidins on digestion of fiber in forages. J. Range Manage. 54, 466-473.

Udén, P., Robinson, P. & Wiseman, J., 2005. Use of detergent system terminology and criteria for submission of manuscripts on new, or revised analytical methods as well as descriptive in formation on feed analysis and/or variability - Editorial. Anim. Feed Sci Technol. 118, 181-186.

Van Soest, P. J., Robertson, J. B. & Lewis, B. A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

Van Soest, P. J. & Wine, R. H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. J AOAC 50, 50-55.

Biogas from grass silage

P. O'Kiely[1], P. Nolan[1,2], D.M. Wall[3,1], H. Himanshu[1,3], J.J. Lenehan[1], E.M. Doyle[2] & J.D. Murphy[3]

[1]Teagasc, Animal & Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland. [2]School of Biology and Environmental Science and Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland. [3]Science Foundation Ireland (SFI) Marine Renewable Energy (MaREI) Centre, Environmental Research Institute, University College Cork, Cork, Ireland.

Correspondence: padraig.okiely@teagasc.ie

Broad perspective

Considerations regarding the economic role of grass silage as a ruminant feed or as a feedstock for mesophilic anaerobic digestion (AD) have much in common. Furthermore, the factors affecting grass silage digestibility by ruminants have much in common with those influencing methane production potential during mesophilic AD.

Biomass produced by grassland is a reservoir of solar-derived chemically-stored energy. This energy is most often utilised by herbivores and the major commercial emphasis is on exploiting it with ruminants. The availability of this energy to ruminants is influenced by numerous crop, animal and management factors, but particularly by the amount (including relative proportions) and form of the various chemical compounds in the herbage. These influence the content of energy (a) directly available to the animal, (b) indirectly provided by the activity of bacteria, archaea, protists and fungi in the animal's reticulo-rumen and large intestine, or (c) not available to the animal. Crucial to the evolution of ruminants being able to exploit fibrous diets is the symbiosis with a complex ecosystem comprised of the vast, diverse, interdependent and collaborative population of micro-organisms living in their digestive tract. These micro-organisms permit the ruminants to access the energy stored within the fibre fraction of feeds and, with many forages, this represents the majority of the energy potentially available from the plant. In return, the animal provides the micro-organisms with an anaerobic environment where, in a liquid medium maintained within a narrow temperature range, a complex and balanced nutrient supply is matched by the continuous removal of the products of microbial digestion. A side-effect of how this fibre-digesting process has evolved is the production of methane as a 'waste' by-product. Because of the loss of energy that methane represents to the animal, but particularly because of its greenhouse gas effect, much research in recent years has focussed on how to curtail this methanogenesis process.

Biogas is produced by the AD of feeds and its commercial value resides within its content of methane (biogas is approximately 55% methane). This methane is valuable as a fuel (its energy is used for electricity generation, heat provision, powering transport vehicles or other functions also provided by natural gas) and as a chemical feedstock (for manufacturing diverse products). The biological processes that take place during the mesophilic AD of feeds to produce biogas have much in common with ruminant microbial digestion, but the commercial emphasis with biogas production is on greatly increasing methane output. Most of the chemical and some of the physical characteristics of feeds that determine the extent of their digestion by ruminants similarly affect biogas production from an AD reactor. However, whereas the ruminant removes and utilises some of the soluble nutrients contained within the feed together with much of the initial breakdown products from the microbial digestion of the

same feeds, the vast, diverse, interdependent and collaborative population of microbes in an AD reactor combine to continue the digestion of available organic substrate (including the initial products of microbial digestion) to a much more advanced state of degradation, thereby greatly increasing methane production. The other major difference between the feed-related digestion processes in ruminants and mesophilic AD reactors is that ruminants up- or down-regulate their intake of feed depending on an array of feed (and animal, management and environmental) characteristics whereas the operator of an AD reactor largely ignores these traits and pre-programmes the daily input of feed.

The technical and economic efficiency of transferring the chemically-stored energy from a standing crop through to ingestion by ruminants impact greatly on the sustainability of the process. Thus, when forages are ensiled as a strategy to efficiently provide a secure future supply and desired quality of feed to ruminants, considerable effort is invested in constraining losses at the field, silo and feedout phases of this feed provision process. This technical efficiency is similarly of considerable economic importance when conserving forages as feeds for biogas production. Also of great importance in defining the economic sustainability of farm-based ruminant and biogas production systems is the yield of crop biomass per ha since, in general, the greater the amount of available crop energy over which to apportion biomass provision costs the lower the cost per unit biomass energy provided to the animal or AD reactor.

The practice of using whole-crop large- (e.g. maize) and small- (e.g. wheat, barley, triticale) grain cereal silages for biogas production has been in place for more than two decades in many European countries. Their use reflects their suitability to local farming circumstances. Many of the now relatively mature technologies associated with the design and operation of AD reactors on European farms were developed with these cereal silages as their major feedstock and consequently some of the features of these facilities reflect optimal adaptation to the particular physical and chemical traits of these feedstocks. A considerable repository of published research underpinned and responded to this use of whole-crop (above ground) cereal silages for biogas production (Lebuhn *et al.*, 2008; Brunia *et al.*, 2010; Hutňan *et al.*, 2010; Herrmann *et al.*, 2012a; Rincón *et al.*, 2012; Colussia *et al.*, 2013; Tóthné Zsubori *et al.*, 2013).

In some European countries biomass from permanent grassland is the predominant energy-rich feedstock grown on farms and this, together with slurry produced by housed livestock, would be the obvious feedstocks to be considered for sustainable biogas production on these farms. This paper will provide an interpretation of the published literature on mesophilic biogas production from temperate (C3) grass silages.

Energy yield

Although the plant species present in temperate permanent grassland normally convert less than 1% of incident solar energy into biomass, highly productive meadows have the potential to capture over 280 GJ gross energy/ha annually in above-ground biomass (15 t dry matter (DM)/ha at 18.7 MJ/kg DM). The extent to which grassland biomass will support a high methane output/ha will vary widely, reflecting ranges in the biomass yield/harvest, the number of harvests within a year and the methane production potential of the biomass. In farm practice the biomass output/ha grassland is often low, but McEniry *et al.* (2013a) identified the potential to greatly increase annual grassland biomass output per ha by optimally applying available technologies while still adhering to environmental constraints.

Grass and grass silage can be excellent feedstocks for biogas production, however a wide range in values (198-467 L CH_4/kg organic matter (aka volatile solids)) for specific methane yield has been reported (Nizami *et al.*, 2012; Murphy *et al.*, 2011; Prochnow *et al.*, 2009). Thus, a wide range in methane outputs/ha can occur, with Amon *et al.* (2005) describing scenarios where methane yields of 500 to over 3000 Nm^3/ha/a would be produced and Wall *et al.* (2013) indicating yields of over 4000 Nm^3 methane/ha/a.

Chemical composition of biomass

The impact of sward botanical composition, growth stage at harvest, conservation efficiency, etc., on the range in gross energy (i.e. calorific) values per unit silage organic matter is quite modest. Where the range is wide, however, is in the extent to which the anaerobic microbial community in the rumen or a mesophilic AD reactor can access this gross energy, and particularly so for the proportion within plant fibre. This, in turn, has a major influence on the yield of biogas or methane that can be produced from grassland biomass by AD and on how the above and other factors can impact on the methane potential of such biomass.

Among the temperate grass species typically found in grassland associated with commercial ruminant production the main effects of grass species on methane output during AD depend on grass phenological growth stage more than harvest date. Similar harvest date dependent differences in methane output would likely also occur among varieties within a species. However, when methane outputs were standardised to common phenological growth stages across various grass species [perennial ryegrass (*Lolium perenne* L.var. Gandalf), Italian ryegrass (*Lolium multiflorum* Lam. var. Prospect), tall fescue (*Festuca arundinacea* Schreb. var. Fuego), cocksfoot (*Dactylis glomerata* L. var. Pizza) and timothy (*Phleum pratense* L. var. Erecta)], rather than to harvest dates, then species did not have a significant effect on methane output/kg organic matter incubated (McEniry et al., 2013b). A similar absence of effect would be expected among varieties within species if they were compared at common growth stages. Pre- and post-ensilage biomass harvested from landscape management and high nature-value grassland can be quite variable in composition, and grassland rich in moor grasses, some non-legume forbs and, in particular, sedges may not be suited to methane production by mesophilic AD (Hermann *et al*., 2013) if they are of low digestibility. In addition to the methane output/kg organic matter incubated associated with different grassland species, grassland botanical composition can indirectly affect biomass suitability for methane production by the extent to which it can generate economically sustainable yields of biomass and the extent to which it naturally undergoes an efficient conservation process with low losses.

Grass phenological growth stage has a considerable impact on the proportion of fibre and on the latter's refractory nature when subjected to microbial digestion and, as a consequence, it impacts on the amount of methane produced from biomass. McEniry *et al.* (2013b) demonstrated this with monocultures of each of five temperate grass species where mean neutral detergent fibre (NDF) values increased from 519 to 644 g/kg dry matter (DM; aka total solids) and DM digestibility declined from 782 to 583 g/kg between the harvesting of immature (12 May) and mature (7 July) phases of the primary growth of these grasses. The corresponding reduction in specific methane output was from 253 to 225 NL CH_4/kg organic matter when grass samples were anaerobically digested under standardised test conditions.

Even though growth stage (or harvest date) significantly alters grass digestibility (King *et al.*, 2012), this relationship can be modified by other factors altering the lignification of stem

(e.g. solar radiation, temperature, soil moisture deficit) or the accumulation of dead vegetation at the base of a silage sward. These factors impact on herbage nutritive value for ruminants (O'Kiely *et al.*, 1995; Bertrand *et al.*, 2008; Virkajarvi *et al.*, 2012; Jing *et al.*, 2013) and would likely similarly impact on the potential methane yield from a mesophilic AD reactor.

The efficiency of the conservation process between mowing a standing crop and inputting silage to an AD reactor will impact on methane output/ha due to both quantitative and qualitative losses throughout the process. Quantitative losses of crop DM can range from <150 to >300 g/kg and the resultant different amounts of silage biomass available for AD result in corresponding greater or lesser outputs of methane/ha. Qualitative losses also reduce methane output/ha even though in cases where ensilage losses of DM are greater than the losses of energy (Kreuger *et al.*, 2011) the methane output/kg organic matter incubated may be increased. This latter outcome has been demonstrated by Pakarinen *et al.* (2008), Herrmann *et al.* (2011) and Herrmann *et al.* (2012b), although the effect has not always been consistent (Kreuger *et al.*, 2011; McEniry & O'Kiely, 2014; Nolan *et al.*, 2014). The actual type of primary fermentation and the extent of any secondary fermentation will impact on both the efficiency of conserving DM and energy (Kreuger *et al.*, 2011) and methane output/kg organic matter incubated. Thus, for example, Herrmann *et al.* (2011) have shown that silages with fermentations that produced elevated concentrations of ethanol or butyric acid can produce more methane/kg organic matter incubated. This results from their considerably higher carbon and less oxidised molecular state than lactic acid, acetic acid, glucose or fructose. It is important to remember that the apparent benefit of higher methane output/kg organic matter incubated for silages of elevated ethanol or butyric acid concentration is an outcome of the greater loss of organic matter that occurred during the conservation of such silages, so there is at best no advantage to methane output/ha from such fermentations. In fact, the results of McEniry *et al.* (2014a) indicate that the ensilage losses far outweigh any modest gain in specific methane yield (i.e. methane output/kg organic matter incubated) where a silage had undergone a clostridial fermentation, resulting in a major reduction in methane output/ha.

Once the ensilage process has stabilised, and if a lactic acid fermentation dominates, it would be expected that the methane production potential of the silage will remain stable over an extended duration of anaerobic storage. In contrast, the methane production potential can continue to change over time when a secondary fermentation ensues over the extended storage duration (Pakarinen *et al.*, 2008; Herrmann *et al.,* 2011).

Qualitative conservation losses that result in a decline in herbage digestibility (e.g. respiration, leaf shatter, rain leach, effluent) would be expected to lead to a reduction in methane output/kg organic matter incubated. Even though silage effluent can be a sizeable quantitative and qualitative loss, it can be collected and fed into the AD reactor, with Abu-Dahrieh *et al.* (2011) reporting methane outputs of 0.385 m^3/kg chemical oxygen demand. Thus, effluent need not constitute a loss from the overall system, and McEniry *et al.* (2011) demonstrated the economic benefit such conversion of silage effluent energy to methane energy can contribute in an AD system. Losses due to respiration can be quite considerable in some silages and in most cases represent a loss of digestible substrate that would have been particularly efficient for methane production during AD. McEniry *et al.* (2014a) confirmed this loss by demonstrating reduced methane output/kg organic matter incubated due to

exposing a range of silages to air for eight days. It is also possible that some mycotoxins produced by moulds would have detrimental effects on the AD process.

Considerable caution is needed with 'effects of ensilage' assessments because losses of volatile organic constituents during oven drying can result in (a) underestimation of silage DM content and thus an overestimation of methane output/kg DM incubated, and (b) underestimation of methane output potential where dried rather than fresh samples are incubated in laboratory-scale incubators. The more extensive the silage fermentation and the greater the proportion of ethanol, volatile fatty acids and ammonia within the fermentation products, the more severe the extent that these errors can be. In principle, comparable errors are a risk if estimating the DM content of other feedstocks that contain compounds more volatile than water during oven drying, including cattle and pig slurry.

The feedstock input to an AD reactor must meet the collective biochemical, mineral and physical needs of its complex microbial ecosystem so that it efficiently produces high and stable outputs of methane over an extended duration. This requires the input of nutrients to avoid excess, deficient or imbalanced contents of essential nutrients, and avoids inappropriate inputs of phytotoxins, mycotoxins, etc., via the feedstock. Chen *et al.* (2008) indicated that a wide range of inhibitory substances need to be avoided including ammonia, sulphide, light metal ions, heavy metals, and some organic compounds. It is also likely that nitrates could inhibit methanogenesis (Navarro-Villa *et al.*, 2011), although they are more likely to be present in pre- rather than post-ensiled grass. Hansen *et al.* (1998) confirmed the inhibitory effect of ammonia on methane production during AD of swine manure, and this risk with ammonia needs to be reviewed when using silages made from leafy grass (particularly vegetative grass in autumn) (O'Kiely, 1993), heavily nitrogen-fertilised grass (Keady *et al.*, 1996) or grass with a high clover content (Winters *et al.*, 2004), as these contain high amounts of nitrogen-based compounds.

Grass can be physically, chemically or biologically treated pre- or post-ensilage to improve its suitability for methane production. Thus, for example, additives are sometimes applied to grass at harvesting to potentially improve conservation efficiency by promoting a more lactic acid dominant fermentation (e.g. efficient lactic acid bacteria, additional fermentable substrate, acid, fibrolytic enzyme, inhibitors of undesirable bacteria such as Clostridia), reducing or eliminating effluent outflow (e.g. absorbents) or improving aerobic stability at feedout (e.g. chemical or biological agents). They may also be applied to potentially increase the extent or pattern of methane output/kg organic matter incubated (e.g. added energy, protein or trace elements, fibrolytic enzymes). It can reasonably be expected that additives that reduce quantitative and/or qualitative conservation losses will improve organic matter yield conserved and/or methane output/kg organic matter incubated, and thus methane output/ha harvested. However, the scale of these effects, if they occur, may not always be economically rewarding. Technical benefits from bacterial silage additives have not been consistent (Pakarinen *et al.*, 2008; Plochl *et al.*, 2009b; Herrmann *et al.*, 2013; McEniry *et al.*, 2014a). Parawira (2012) outlined the mechanisms by which fibrolytic enzymes could facilitate the anaerobic digestion of lignocellulosic substrate, and the extent to which these could increase the extent and/or rate of methane production during AD has been demonstrated by Suarez Quinones *et al.* (2012). Such enzymes could also impact on methane output via their influence on silage fermentation, aerobic stability or effluent output, and Plochl *et al.* (2009a) have shown their potential to alter the fluidity characteristics of the biomass, and this could be advantageous in some practical circumstances.

Parawira (2012) also outlined a range of other pretreatments that could be used to increase the biodegradability of lignocellulosic biomass, including milling, irradiation, steam explosion, ammonia fibre explosion, supercritical carbon dioxide explosion, alkaline hydrolysis, liquid hot-water pretreatment, organosolvent processes, wet oxidation, ozonolysis and acid hydrolysis. Thus, for example, Xie *et al.* (2011a) demonstrated the ability of thermo-chemical pre-treatment of grass silage to increase methane output/kg organic matter incubated.

Physical characteristics of biomass

Field wilting of grass between mowing and harvesting is undertaken to evaporate water from biomass and thus reduce or eliminate effluent production, restrict silage fermentation or direct it towards greater dominance by lactic acid bacteria, and produce a feedstock that is easier to mechanically transport and handle. However, successfully wilted silages may be more susceptible to aerobic spoilage during feedout. The value obtained from wilting depends on the efficiency with which unwilted grass would have conserved, on the speed and extent of drying of grass during field wilting, on the extent of physical losses during wilting and harvesting, and on aerobic losses incurred during feedout. Thus, a range of contrasting methane output responses to wilting have been recorded (Pakarinen *et al.*, 2008; McEniry *et al.,* 2014).

Biomass from grassland can be comminuted pre- (Herrmann *et al.*, 2012b) or post- (Lindmark *et al.*, 2012) ensilage. In farm practice, silages can be successfully ensiled without chopping as is evidenced by efficient baled silage systems where grass often receives no chopping. The chop length ranges investigated by Herrmann *et al.* (2012b) were all shorter than the 19 mm nominal chop length often targeted if chopping grass when producing silage as a feed for ruminants. Under these circumstances still finer chopping increased silage lactic acid content, reduced in-silo losses, increased silage density (which could have beneficial effects on silage aerobic stability at feedout but could be problematic regarding effluent outflow if wet biomass were ensiled) and increased methane output/kg organic matter incubated. Thus, the effects on methane output/kg organic matter ensiled were even larger. Very fine post-ensilage chopping and physical disruption can also lead to potentially large increases in methane output/kg organic matter incubated (Lindmark *et al.*, 2012). However, all of these comminution processes require considerable energy inputs which in turn have obvious net energy gain and economic consequences.

Co-digestion with cattle slurry

In general, laboratory-scale anaerobic digestion tests show higher methane outputs/kg organic matter incubated for grass silage compared to cattle (Lehtomaki *et al.*, 2007; Wall *et al.*, 2014b) or pig (Asam *et al*., 2011) slurries, although this relativity can disappear for sufficiently low digestibility grass silage (Xie *et al.*, 2011b; Himanshu *et al.*, 2015). Similar benefits of grass silage over cattle slurry have been shown in farm-scale mesophilic AD reactors (Luostarinen *et al.*, 2013). In longer term continuous digestion tests, benefits to the stability of an efficient AD process can accrue from inclusion of slurry, as the latter can help stabilise pH, counteract ammonia inhibition and provide a more optimum C:N ratio for the process (Murphy *et al.*, 2013). The operational conditions for specific grass silage and cattle slurry feedstocks and for an AD reactor type have been provided by Wall *et al.* (2013 & 2014b), and the importance of overcoming a deficiency of specific essential trace elements in

order to ensure a prolonged duration of efficient methane production have been highlighted (Wall *et al.*, 2014a).

AD reactors for grass silage

Many combinations of digester types for grass silage can be configured, and the benefits and weaknesses of these have been discussed by Nizami *et al.* (2010). The suitability of the digester configuration is linked to the specific feedstock characteristics. Wet-based systems such as the continuously stirred tank reactors (CSTRs) are suitable for grass silage with higher DM digestibility values. This is a traditional one-phase system, operating within a DM range of 20-120 g/kg, which benefits from a liquid co-substrate (such as slurry or recycled digestate liquor) to operate. Previous work by Wall *et al.* (2014) has shown mesophilic mono-digestion of grass silage performs comfortably up to an organic loading rate (OLR) of 3.5 kg organic matter per m^3 reactor per day in a CSTR. This gave the optimum methane yield with the highest feedstock throughput. With the addition of slurry, a higher OLR of 4 kg organic matter per m^3 reactor per day was achieved due to longer retention times and added availability of micronutrients in the slurry. In such systems it is recommended that the hydraulic retention time (HRT) exceeds 20 days to avoid washout of methane-producing bacteria. Difficulties associated with grass silage-fed CSTRs include fibrous materials floating on the reactor surface. A sufficient mixing system (that breaks the digestate surface) is crucial in a CSTR to prevent floating accumulations of grass silage and to ensure the micro-organisms interact effectively with the grass silage (Thamsiriroj *et al.*, 2010).

Nizami *et al.* (2011) investigated the use of a two-phase digestion process suitable for grass silages with higher NDF values. In this system the AD process is broken into two phases: 1) hydrolysis/acidogenesis and 2) acetogenesis/methanogenesis. Leach-bed reactors (LBRs) are sequentially fed and run in tandem with a high rate methanogenic reactor known as an upflow anaerobic sludge blanket (UASB). A high strength liquor leachate is produced from the LBRs (similar to silage effluent), which is subsequently fed to the UASB to produce a very high methane-content biogas (approximately 70%). Such systems cope well with feedstocks of high DM contents (200-400 g/kg) and have a lower parasitic energy demand than a CSTR as no mixing is required.

Concluding perspective

Grass silage can be used to efficiently produce high outputs of methane, both per kg organic matter incubated and per ha, over extended durations. The knowledge, technologies and skills required to provide this silage are largely similar to those already employed by commercial farmers operating efficient grass silage-dependent ruminant production systems. In fact, indices of forage digestibility developed to estimate the nutritive value of a range of silages for ruminants correlate well with the methane output that will be obtained from standard mesophilic AD batch tests (McEniry *et al*, 2013b; Seppälä et al., 2013).

Considerable potential exists to further improve the sustainability of these systems by increasing biomass output/ha for crops at the appropriate phenological growth stage, and by reducing conservations losses and costs – the latter includes incorporating silage effluent into the feedstock stream and judiciously landspreading digestate to reduce inorganic N fertiliser purchases (McEniry *et al.*, 2011). Considerable potential must also reside in developing pre- and/or post-ensilage technologies to improve the extent, rate and/or stable duration of methane output/kg organic matter incubated. These could involve enzymatically 'upgrading'

silage lignocellulosic substrate, supplementation or co-digestion with complementary ingredients or feedstocks, etc. It will also be important to provide guidelines on AD reactor design specifications and operation procedures that are tailored to optimising the efficient (e.g. energy, labour) and safe production of biogas from a solely or predominantly grass silage feedstock. Finally, broader issues regarding the integration of AD for biogas and methane production into the fabric of individual farms or other businesses, or into groups of farms or businesses within a locality, or regionally/nationally, need to be considered. Aspects of these have been provided by Gunnarsson *et al.* (2008 & 2009), O'Keeffe *et al.* (2011a & b) and Smyth *et al.* (2010 & 2011).

Acknowledgements

Funding for this work has been provided by (a) Teagasc [RMIS 6254], (b) Department Agriculture Food & the Marine [RSF 07 557], (c) European Union's 7th Framework Programme for research, technological development and demonstration [Marie-Curie Initial Training Network] under grant agreement n. 316838 (ATBEST) and (d) Science Foundation Ireland (Grant No. 12/RC/2302).

References

Abu-Dahrieh, J., Orozco, A., Groom, E. & Rooney, D., 2011. Batch and continuous production from grass silage liquor. Bioresource Technol. 102, 10922-10928.

Amon, T., Kryvoruchko, V., Amon, B., Bodiroza, V., Zollitsch, W., Boxberger, J. & Pötsch, E., 2005. Biogas Production from grassland biomass in the Alpine region. Landtechnik 60, 336-337.

Asam, ZUZ., Poulsen, T.G., Tjalfe, G., Nizami, A.S., Rafique, R., Kiely, G. & Murphy, J.D., 2011. How can we improve biomethane production per unit of feedstock in biogas plants? Applied Energy 88, 2013-2018.

Bertrand, A., Tremblay, G.F.,.Pelletier, S., Castonguay, Y. & Belanger, G., 2008. Yield and nutritive value of timothy as affected by temperature, photoperiod and time of harvest. Grass Forage Sci. 63, 421-432.

Brunia, E., Jensenb, A.P., Pedersen, E.S. & Angelidaki, I., 2010. Anaerobic digestion of maize focusing on variety, harvest time and pretreatment. Appl. Energ. 87, 2212–2217.

Chen, Y., Cheng, J.J. & Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A review. Bioresource Technol. 99, 4044-4064.

Colussia, I., Cortesia, A., Del Piccolob, C., Galloa, V., Rubesa Fernandeza, A.S. & Vitanzaa, R., 2013. Improvement of methane yield from maize silage by a two-stage anaerobic process. Chem. Eng. Trans. 32, 151-156.

Gunnarsson, C., Sporndly, R., Rosenqvist, H., de Toro, A. & Hansson, P-A., 2009. A method of estimating timeliness costs in forage harvesting illustrated using harvesting systems in Sweden. Grass Forage Sci. 64, 276–291.

Gunnarsson, C., Vagstrom, L. & Hansson, P-A., 2008. Logistics for forage harvest to biogas production – timeliness, capacities and costs in a Swedish case study. Biomass Bioenerg. 32, 1263-1273.

Hansen, K.H., Angelidaki, I. & Ahring, B.K., 1998. Anaerobic digestion of swine manure: inhibition by ammonia. Water Res. 32, 5-12.

Herrmann, A. & Rath, J., 2012a. Biogas production from maize: current state, challenges, and prospects. 1. Methane yield potential. BioEnergy Research 5, 1027-1042.

Herrmann, C., Heiermann, M. & Idler, C., 2011. Effects of ensiling, silage additives and storage period on methane formation of biogas crops. Bioresource Technol. 102, 5153-5161.

Herrmann, C., Heiermann, M., Idler, C. & Prochnow, A., 2012b. Particle size reduction during harvesting of crop feedstock for biogas production. 1: Effects on ensiling process and methane yields. Bioenerg. Res. 5, 926-936.

Herrmann, C., Prochnow, A., Heiermann, M. & Idler, C., 2013. Biomass from landscape management of grassland used for biogas production: effects of harvest date and silage additives on feedstock quality and methane yield. Grass Forage Sci. 69, 549-566.

Himanshu, H., Murphy, J.D. & O'Kiely, P., 2015. Biomethane production from co-digestion of forage silage and cattle slurry. Proceedings of 14th World Congress on Anaerobic Digestion, 14-18 November, Viña del Mar, Chile (in press).

Hutňan, M., Špalková, V. & Bodík, I., Kolesárová, N. & Lazor, M., 2010. Biogas production from maize grains and maize silage. Polish J. of Environ. Stud. 19, 323-329.

Jing, Q., Bélanger, G., Qian, B. & Baron, V., 2013. Timothy yield and nutritive value under climate change in Canada. Agron. J. 105, 1683-1694.

Keady, T.W.J. & O'Kiely, P., 1996. An evaluation of the effects of nitrogen fertilisation of grassland on silage fermentation, in-silo losses, effluent production and aerobic stability. Grass Forage Sci. 51, 350-362.

King, C., McEniry, J., Richardson, M. & O'Kiely, P., 2012. Yield and chemical composition of five common grassland species in response to nitrogen fertiliser application and phenological growth stage. Acta Agr. Scand. B-S P 62, 644-658.

Kreuger, E., Achu Nges, I. & Bjornsson, L., 2011. Ensiling of crops for biogas production: effects on methane yield and total solids determination. Biotechnology for Biofuels 4, 1-8.

Lebuhn, M., Liu. F., Heuwinkel, H. & Gronauer, A., 2008. Biogas production from mono-digestion of maize silage-long-term process stability and requirements. Water Sci. Technol. 58, 1645-51.

Lehtomaki, A., Huttunen, S. & Rintala, J.A., 2007. Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio. Resour. Conserv. Recy. 51, 591-609.

Lindmark, J., Leksell, N., Schnurer, A. & Thorin, E., 2012. Effects of mechanical pre-treatment on the biogas yield from ley crop silage. Appl. Energ. 97, 498-502.

Luostarinen, S., Pyykkönen, V., Hyrkäs, M., Räty, M. & Virkajärvi, P., 2013. Farm-scale co-digestion of manure and grass silage for improved energy yield and nutrient recycling. In: (eds. Á. Helgadóttir & A. Hopkins) Proceedings of 17th EGF (European Grassland Federation) Symposium, 23-26 June, Akureyri, Iceland. Grassland Sci. Eur. 18, 539-541.

McEniry, J., Allen, E., Murphy, J.D. & O'Kiely, P., 2014a. Grass for biogas production: the impact of silage fermentation characteristics on methane yield in two contrasting biomethane potential test systems. Renew. Energ. 63, 524-530.

McEniry, J., Crosson, P., Finneran, E., McGee, M., Keady, T.W.J. & O'Kiely, P., 2013a. How much grassland biomass, in excess of livestock requirements, is available in Ireland? Irish J. Agr. Food Res. 52: 67-80.

McEniry, J. & O'Kiely, P., 2013b. Anaerobic methane production from five common grassland species at sequential stages of maturity. Bioresource Technol. 127, 143-150.

McEniry, J. & O'Kiely, P., 2014b. Methane production by anaerobic digestion of tall fescue samples pre- and post-ensiling, prepared by thermal or freeze drying. Agric. Eng. Int. CIGR Journal, 16, 133-142.

McEniry, J., O'Kiely, P., Crosson, P., Groom, E. & Murphy, J.D., 2011. The effect of feedstock cost on biofuel cost as exemplified by biomethane production from grass silage. Biofuel., Bioprod. Bior. 5, 670-682.

Murphy, J. D., Braun, R., Weiland, P. & Wellinger, A., 2011. Biogas from crop digestion, IEA Bioenergy. Available from: www.iea-biogas.net/_download/publi-task37/Update_Energy_crop_2011.pdf .

Murphy, J.D., Wall, D. & O'Kiely, P., 2013. Second generation biofuel: Biomethane from co-digestion of grass and slurry. In: (eds. Á. Helgadóttir & A. Hopkins) Proceedings of 17th EGF (European Grassland Federation) Symposium, 23-26 June, Akureyri, Iceland. Grassland Sci. Eur. 18, 505-513.

Navarro-Villa, A., O'Brien, M., Lopez, S., Boland, T.M. & O'Kiely, P., 2011. In vitro rumen methane output of red clover and perennial ryegrass assayed using the gas production technique (GPT). Anim. Feed Sci. Tech. 168: 152-164.

Nizami, A-S. & Murphy, J.D., 2010. What type of digester configurations should be employed to produce biomethane from grass silage? Renew. Sust. Energ. Rev. 14, 1558-1568.

Nizami, A. S., Orozco, A., Groom, E., Dieterich, B. & Murphy, J. D., 2012. 'How much gas can we get from grass?', Appl Energ, 92, 783–790.

Nolan, P., McEniry, J., Doyle, E.M. & O'Kiely, P., 2014. Comparison of specific methane yield of perennial ryegrass prepared by thermal drying versus non-thermal drying in small-scale batch digestion tests. Bioresource Technol. 169, 484-489.

O'Keeffe, S., Schulte, R.P.O., Lalor, S.T.J., O'Kiely, P. & Struik, P.C., 2011a. Green biorefinery (GBR) scenarios for a two-cut silage system: Investigating the impacts of sward botanical composition, N fertilisation rate and biomass availability on GBR profitability and price offered to farmers. Biomass Bioenerg. 35, 4699-4711.

O'Keeffe, S., Schulte, R.P.O., Sanders, J.P.M. & Struik, P.C., 2011b. I. Technical assessment for first generation green biorefinery (GBR) using mass and energy balances: Scenarios for an Irish GBR blueprint. Biomass Bioenerg. 35, 4712-4723.

O'Kiely, P., 1993. Influence of a partially neutralised blend of aliphatic organic acids on fermentation, effluent production and aerobic stability of autumn grass silage. Irish J. Agr. Food Res. 32, 13-27.

O'Kiely, P. & O'Riordan, E.G., 1995. Effects of defoliating grass swards in winter and spring on subsequent silage yield and quality. Irish J. Agr. Food Res. 34, 81.

Pakarinen, O., Lehtomaki, A., Rissanen, S. & Rintala, J., 2008. Storing energy crops for methane production: Effects of solids content and biological additive. Bioresource Technol. 99, 7074-7082.

Parawira, W., 2012. Enzyme research and applications in biotechnological intensification of biogas production. Crit. Rev. Biotechnol. 32, 172-186.

Plochl, M., Hilse, A., Heiermann, M., Suarez Quinones, T., Budde, J. & Prochnow, A., 2009a. Application of hydrolytic enzymes for improving biogas feedstock fluidity. Agricultural Engineering International: the CIGR e-Journal. Manuscript 1529. 9, 1-16.

Plochl, M., Zacharias, H., Herrmann, C., Heiermann, M. & Prochnow, A., 2009b. Influence of silage additives on methane yield and economic performance of selected feedstock. Agricultural Engineering International: the CIGR e-Journal. Manuscript 1123, 11, 1-16.

Prochnow, A., Heiermann, M., Plöchl, M., Linke, B., Idler, C., Amon, T. & Hobbs, P. J., 2009. 'Bioenergy from permanent grassland – a review: 1. Biogas', Bioresource Technol. 100, 4931–4944.

Rincón, B., Heaven, S., Banks, C.J. & Zhang, Y., 2012. Anaerobic digestion of whole-crop winter wheat silage for renewable energy production. Energy Fuels 26, 2357–2364.

Seppälä A., Kässi P., Ervasti S., Kuoppala K., Sormunen-Cristian R., Särkijärvi S., Heikkilä T., Luostarinen S. & Rinne M., 2013. Biochemical methane potential of timothy, tall fescue and red clover silages harvested at different stages of maturity. In: (eds. Á. Helgadóttir & A. Hopkins) Proceedings of 17th EGF (European Grassland Federation) Symposium, 23-26 June, Akureyri, Iceland. Grassland Sci. Eur. 18, 525-527.

Smyth, B.M., Smyth, H. & Murphy, J.D., 2010. Can grass biomethane be an economically viable biofuel for the farmer and the consumer? Biofuel., Bioprod. Bior. 4, 519-537.

Smyth, B.M., Smyth, H. & Murphy, J.D., 2011. Determining the regional potential for a grass biomethane industry. Appl. Energ. 88, 2037-2049.

Suarez Quinones, T., Plochl, M., Budde, J. & Heiermann, M., 2012. Results of batch anaerobic digestion test – effect of enzyme addition. Agricultural Engineering International: CIGR e-Journal 14, 1-19.

Thamsiriroj, T. & Murphy, J. D., 2010. Difficulties associated with monodigestion of grass as exemplified by commissioning a pilot-scale digester. Energy & Fuels 24, 4459-4469.

Tóthné Zsubori, Z., Pintér, J., Spitkó, T., Hegyi, Z., Csaba, L. & Marton, A., 2013. Yield and chemical composition of plant parts of silage maize (*Zea mays* L.) hybrids and their interest for biogas production. Maydica 58, 34-41.

Virkajarvi, P., Hyrkas, M., Pakarinen, K. & Suomela, R., 2012. Importance of senescence and dead material on nutritive value of grass silage. Proceedings of the XVI International Silage Conference, Hameenlinna, Finland, 2-4 July 2012. (Edited by: Kuoppala, K.; Rinne, M.; Vanhatalo, A.). p188-189.

Wall, D.M., Allen, E., Straccialini, B., O'Kiely, P. & Murphy, J.D., 2014a. The effect of trace element addition to mono-digestion of grass silage at high organic loading rates. Bioresource Technol. 172, 349–355.

Wall, D.M., Allen, E., Straccialini, B., O'Kiely, P. & Murphy, J.D., 2014b. Optimisation of digester performance with increasing organic loading rate for mono- and co-digestion of grass silage and dairy slurry. Bioresource Technol. 173, 422-428.

Wall, D.M., O'Kiely, P. & Murphy, J.D., 2013. The potential for biomethane from grass and slurry to satisfy renewable energy targets. Bioresource Technol. 149: 425-431.

Winters, A.L., Minchin, F.R., Davies, Z., Kingston-Smith, A.H., Theodorou, M.K., Griffith, G.W. & Merry, R.J., 2004. Effects of manipulating the protein content of white clover on silage quality. Anim. Feed Sci. Tech. 116, 319-331.

Xie, S., Frost, J.P., Lawlor, P.G., Wu, G. & Zhan, X., 2011a. Effects of thermo-chemical pre-treatment on grass silage on methane production by anaerobic digestion. Bioresource Technol. 102, 8748-8755.

Xie, S., Lawlor, P.G., Frost, J.P., Hu, Z. & Zhan, X., 2011b. Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion off concentrated pig manure and grass silage. Bioresource Technol. 102, 5728-5733.

What are the real 'shrink' losses in maize silage piles?

P.H. Robinson[1], N. Swanepoel[1], J. Heguy[2] & D. Meyer[1]
[1]Department of Animal Science; [2]UCCE Stanislaus, San Joaquin & Merced Counties
University of California, Davis, CA, USA 95616
Correspondence: phrobinson@ucdavis.edu

Introduction

Maize silage has been an important silage crop for a very long time. And in the world of today, of over 6 billion people and a high requirement for dairy products leading to large commercial dairy enterprises, maize silage is far and away the most important ensiled crop in most developed dairy areas. As in most aspects of life, where we want to receive all of whatever it is that we pay for, losses of maize silage post-harvest during ensiling are an economic loss to the dairy industry. Generally referred to as 'shrink', although seldom clearly defined, it is the proportion of fresh crop weight that is not recovered from the pile as feedable, or sometimes expressed as total, silage. Shrink can refer to wet weight (WW) recovery of silage or as oven dry weight (oDW) recovery. But, however you express it, shrink can be costly. For example, 10% WW shrink on a 15,000 tonne maize silage pile represents a loss of US$90,000 if WW maize silage is valued at $60/tonne WW. In addition to an economic loss to dairy farmers, shrink represents a loss of carbon compounds to waterways as weepage from the pile, or to aquifers as leachate, or to the atmosphere as gases. As such, silage shrink losses have attracted the attention of regulatory agencies, especially water and air districts in California which are tasked with reducing environmental impacts of farming as a way to create cleaner water and air. These regulatory efforts have in some cases resulted in semi-mandatory mitigations to dairy farmers to reduce silage shrink (e.g. Rule 4570 of the: San Joaquin Valley Unified Air Pollution Control District, 2010); mitigations (based upon limited data of questionable relevance to large commercial silage piles), which may or may not actually reduce silage shrink which itself may or may not be a problem of a magnitude equal to that assumed by the regulatory agencies. As with many governmentally regulated areas in our societies, nothing is simple.

Nevertheless, reducing shrink is important. Shrink numbers in the commercial literature are commonly in the 5 to 20+% range, and numerous management strategies have been suggested to reduce it (e.g. Wilkinson & Davies, 2012). These include use of an inoculant at chopping, building silos on a concrete base, creating high pack density at silage pile building, use of a plastic cover, rapid covering of the mass with that plastic cover, use of an inner plastic film, use of an inner film with enhanced oxygen barrier characteristics, sealing the pile periphery with dirt or weights, minimizing exposed face at feed out, removing maximum depth of silage at feed out, maintaining a 'smooth' silage face, using moveable weight lines along the cut surface of the plastic, only removing as much silage as is immediately needed, use of mechanical defacers, use of block cutter defacers, and leaving no overnight piles of loose silage. Quite an extensive list – some simple and some not so simple. The common feature of all of them is that they will increase silage costs, while the telling feature is that it would take a team of 20 scientists about 10 years to investigate the efficacy of each individually, and about forever to investigate them concurrently. So lots of costly suggested mitigations of silage shrink with no guarantee that any of them are cost effective – in fact little evidence for many that they reduce shrink at all. Thus, in general, common silage-sense

and experience are the bulk of what dairy farmers have to go on. Not a great situation, especially when the actual extent of the base 'problem' that the mitigations are designed to address - that silage shrink is substantive economically and environmentally – has little or no supporting data in real world maize silage piles.

Defining 'shrink'

Shrink losses of maize silage can be defined in many ways. However the most common definition is the proportion of the WW fresh crop packed into a silo structure (including a pile) which is later placed into a TMR mixer as silage. Under this definition, spoilage which is removed by hand (in most cases) and disposed of by land application or feeding to heifers counts as shrink. However shrink as defined by air and water boards typically includes wastage since this material is actually recovered (and not 'lost'). The interpretive limitation of WW shrink is that much of it will be water, which has no substantive economic or environmental impact. Thus some dairy producers and regulatory boards also measure shrink on an oven dry weight (oDW) basis.

To convert WW shrink to oDW shrink it is necessary to collect many samples of fresh cut crop at ensiling, as well as collect many samples of the silage that is put into the TMR mixer. This is a time consuming chore which involves collecting and pooling many samples over the period of silo structure building, as well as many samples over the often long feed out period, in order to create pooled samples representative of the crop ensiled and of the silage fed out. Both of these tasks are prone to poor practices and creation of samples which are not representative of the fresh cut crop ensiled and/or the silage placed into the TMR mixer. While these issues can be dealt with by using defined sample collection protocols, a serious structural issue is that oDW shrink will always overestimate real DW shrink by adding volatile carbon compounds lost during oven drying to the shrink estimate, because drying fresh chopped maize crop in an oven will almost exclusively drive off water, since very few volatile carbon compounds are in a fresh chop maize crop, but drying maize silage in an oven will drive off volatile carbon compounds, most of which will actually be fed to the cows, as well as water. Volatile carbon compounds commonly found in maize silage include the volatile fatty acids (VFA) acetic, propionic and butyric, the alcohols ethanol, 1,2 propanediol and 2,3 butanediol, as well as a host of minor volatile carbon compounds (Weissbach & Strubelt, 2008). Even lactic acid, always found in maize silage, will be lost to some extent during oven drying – and the 'oven volatility' of all of these compounds differs, and also differs within compound in the range of normal oven drying temperatures (Porter & Murray, 2001). As a group, potentially volatile compounds in maize silage typically make up 2 to 5% of the fresh weight and, depending upon their proportions in the silage, up to 60% of them could be lost during oven drying. Thus 'oDW shrink', where the DW is determined by oven drying, could overestimate actual DW shrink by up to 5 % units. In other words, an oDW shrink of 10% might only be ~5% when corrected for the volatiles lost during oven drying.

To correct oDW shrink for volatiles lost in the drying oven (i.e. volatiles corrected oDW; vcoDW) it is necessary to measure concentrations of volatiles in fresh and oven dried silage, and then arithmetically add those volatiles lost in the oven back to oDW to create vcoDW, similar to a technique used by Vahlberg *et al*., (2013). Thus vcoDW is the most accurate measure of real shrink in a maize silage pile.

Silage sources (areas) of 'shrink'

Maize silage shrink losses originate from many facets of the ensiling process such as during pile building after the fresh chop material is weighed but prior to plastic covering, from the mass while it is ensiled, from the silo 'face' at (or near) exposure to air, during defacing, after silage has been defaced but before it is moved to the TMR mixer and, finally, during transport to the TMR mixer – which is typically where fed out silage is measured.

Losses during pile building

Once the fresh chop maize crop is weighed in the trucks, small quantities of it could be lost on the way to the pile due to wind or by falling off the trucks. However this is unlikely to be substantial during the life of a silo building operation. A more likely loss of weight is evaporation of water) from the fresh chop material once it is placed on the pile since, typically, piles are built on pleasant sunny days when solar radiation levels are high. Such losses will impact WW shrink to a greater extent than oDW shrink since there is little opportunity for non-water compounds to evaporate because their levels in a freshly chopped maize crop are very low. Another possible source of oDW shrink is from plant respiration because the plants are not really dead when they are delivered to the pile. Until the plants are fully dead, due to creation of an acidic environment and/or heat and/or they consume the sugars in their biomass, they will continue to be metabolically active and, once no longer in the sun and photosynthesizing, they will utilize stored sugars to meet their energy requirements which will result in creation of CO_2 which will largely be released to the atmosphere. Such CO_2 losses would be measured as WW, oDW and vcoDW shrink since the carbon atoms are coming from metabolized sugars. Unlike water losses, carbon losses as CO_2 impact the total nutritional value of the silage pile, but would have no air quality impact in most regulated air districts at this time (i.e. CO_2 is a greenhouse gas (GHG), but not a volatile carbon compound which impacts air quality).

Losses from the silage mass

Once fresh chop maize crop is packed and covered, it goes through fermentation starting with aerobic bacteria (which create heat) and finishes with anaerobic bacteria, which create the alcohols and acids, primarily lactic, acetic and propionic, which drives down the pH to create a 'stable' silage mass. This silage mass, if protected from oxygen penetration, should be unchanging for prolonged periods of time. However, it is likely that due to the long period of ensiling, >12 mo in some cases, and low level penetration of oxygen through and around the plastic cover (as well as into the face once it is exposed) that some losses of gases, and vaporized water, could occur. Indeed, as most silage piles have the plastic peeled back up to 2 m from the exposed face, evaporative losses of water and volatilization of carbon based compounds (i.e. the surface dries out) could occur from exposed silage. If this exposed silage is rained on, losses could turn into weight gains, but only as WW. A silage weight loss which could be negligible or substantive is weepage and leaching of low DW fluid from the silage mass. The extent of this loss will be impacted by the moisture content of the fresh cut crop as well as its pack density which are positively correlated (i.e. it is hard to obtain high pack density of a low moisture crop no matter how much packing pressure is applied).

Losses from the silage 'face' at (or near) its exposure to air

This is an area of interest from regulatory agencies as it seems intuitive that silage weight losses occur from silo faces once exposed to air. Such losses would be water, but there must

be losses of alcohols and VFA since they are easily detected by simply smelling a freshly exposed maize silage face. Losses would likely be impacted by the orientation of the exposed face (south faces in the northern hemisphere having higher losses than north faces due to sun exposure), temperature and humidity during face exposure (higher temperatures associated with higher losses), smoothness of exposed faces ('rough' surfaces creating more surface area to emit volatiles than smooth), wind (higher wind speeds leading to higher losses) and exposure time of the face (emissions/unit area declining with time of exposure).

Losses from silage during defacing

In all silage face removal systems, there are likely to be losses of water and volatiles as the silage collapses into a pile after defacing. Such losses would likely be impacted by the violence of the defacing process. For example, mechanical rotating defacers are relatively violent, front end loader buckets intermediate and block cutters relatively benignly violent removal methods. A greater extent of silage disturbance during defacing could increase immediate losses of volatiles and water, as well as create the potential for higher losses of volatiles and water while it is in the 'drop down pile' awaiting transport to the TMR mixer.

Losses from silage after defacing

In all systems where silage is left on the ground for a period of time between defacing and placement in a TMR mixer (i.e. in a 'drop-down pile'), there are likely to be losses of water and volatiles as the silage waits for removal and loading into a TMR mixer. Such losses would likely be impacted by the length of the delay between defacing and placement in the TMR mixer (for example overnight delays might be expected to maximize losses), the size of the drop-down pile (larger piles emitting less per unit weight) as well as the environmental conditions during that wait, as were discussed earlier for losses from the face.

Losses of silage during transport to the TMR mixer

Such losses include silage which is never picked up from the ground or falls off the load while it is being transported to the TMR mixer. However it could also result in a weight gain for silage piles on dirt bases if some of that dirt is picked up with the silage. In total, these losses are unlikely to be substantial.

Overall, there are a number or areas of the ensiling process where silage weight, as fresh or dry material, can be lost (or gained in a few cases) between when fresh crop is weighed into the pile until silage is weighed into a TMR mixer. But we had questions. The first addressed the issue of extent of maize silage shrink as WW, oDW and vcoDW, because that quantities the extent of the silage shrink 'problem' from the perspective of dairy farm economics and potential impacts on air and water quality. The second questions addressed the issue of where in the entire process (as outlined above) shrink is occurring because that suggests where mitigations should be focused to reduce it. Finally, the third questions addressed the issue of which ensiling practices and characteristics exacerbate or mitigate shrink, and where that mitigation occurs in the ensiling process, because that suggests which mitigations would likely be most efficacious in terms of reducing shrink.

First questions: measuring total silage 'shrink' losses

Silage piles can be very large – 15,000 tonne piles are not uncommon – and can be fed out over periods > 12 mo. This makes measuring shrink a challenging task, and identification of

where shrink occurs even more challenging. While it is not difficult to measure shrink in mini- or model silos of a few kg to a few hundred kg, it is unlikely that such models can be expected to fully represent a 1,000+ tonne silage pile. Total shrink losses in commercial silage piles can be measured by recording the total WW of fresh cut maize crop delivered to a silage pile at building relative to the amount of WW maize silage measured as placed into the TMR mixer at feed out. We used 7 maize silage piles (2 wedge/rollover, 1 bunker, 4 wedge) ranging in size from 950 to 12,204 tonnes (as built), on concrete (4), dirt (2) and a combination base (1), on 4 dairy farms, in 2 areas of the San Joaquin Valley of California (USA), all covered within 48 h by professional crews with an oxygen barrier inner film and black/white outer plastic weighted with tire chains and fed out by professional crews using a silage tracking system, and all from the 2013 crop year. On these 7 piles, average WW shrink losses (i.e. where silage recovered, but not fed, is not classified as shrink) were 9.0 ± 1.69%, a number within the range suggested by many persons working on silage issues.

Conversion of WW to oDW losses occurs by creating pooled samples of the incoming fresh cut maize crop and fed out maize silage, which are both oven dried at 55°C. These pooled samples are then assayed in both their 'as sampled' and 'oven dried' forms, and then arithmetically adding back to recovered oDW the amount of volatile compounds lost during oven drying. Using this approach, oDW losses were 6.8 ± 1.82% (similar to values of Kohler *et al*., measured in German maize bunker silos) and vcoDW losses were 2.8 ± 2.08%, confirming that a lot of measured WW shrink is really water, and some of what is measured as oDW shrink is actually volatile compounds driven off in the drying oven.

Second questions: measuring where silage 'shrink' losses occur

While it is critical to know shrink losses for silage piles, as this effects environmental impacts and farm economics, it is as interesting to know where in the silage creation and feed out process, those losses occur since this suggests where mitigation efforts should be directed.

Losses from the silage mass

If protected from oxygen, the silage mass should be relatively unchanging for prolonged periods of time. However losses could occur as gases or liquids during this period. The extent of this loss was measured by burying Nylon mesh bags of fresh crop in the pile at filling and recovering them from the face at silage removal (Figure 1). We utilized a grid of 14 bags (Figure 2) in each of 4 of the maize silage piles. Data from the bags suggests that the WW, oDW and vcoDW losses from the mass were 3.9 ± 2.40, 7.2 ± 1.12 and 3.5 ± 1.27%, respectively. As with total pile shrink losses, as noted above, a lot of what is measured as oDW shrink actually contains a lot of volatile compounds driven off in the drying oven, and not actually lost from the pile.

Losses from the silage 'face' at (or near) its exposure to air

This is an area of interest to regulatory agencies as it seems intuitive that losses of silage will occur as volatile compounds and water are lost from silo faces once they are exposed to air. The extent of this loss was measured by coring each silage pile on two occasions in a 4 core grid (Figure 3), to 50.8 cm of depth from a freshly exposed face (new face) and from a face exposed for ~20 h (old face) at ~1.5 m above grade. WW, oDW and vcoDW losses from the face were 1.3 ± 1.16, -0.6 ± 1.55 and 0.1 ± 1.40% respectively. Although these values are low, they confirm suggestions that most weight loss from the face is water.

Figure 1 Buried bags prior to burying and after recovery.

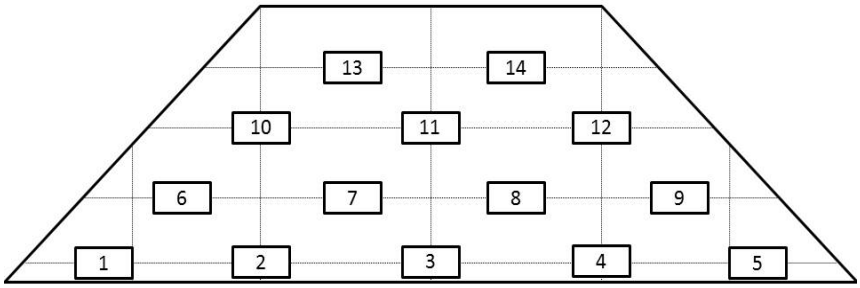


Figure 2 The 14-buried bag grid.

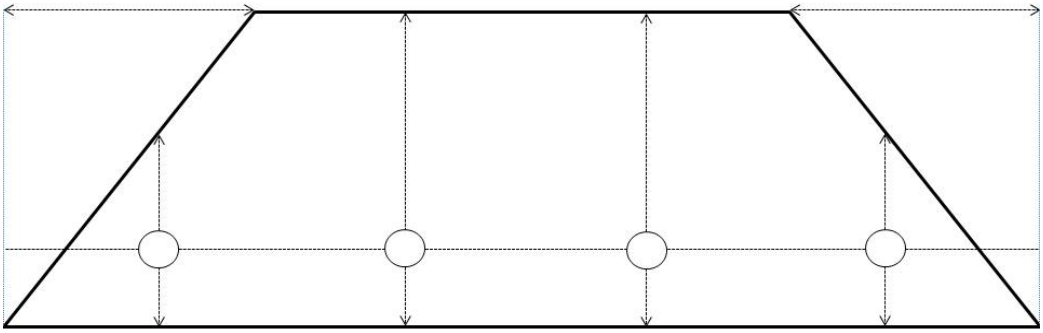


Figure 3 The 4-location lace coring grid (samples cored to 50 cm depth 1.5 m above grade).

Losses from Silage During and After Defacing

In all silage face removal systems there are likely to be losses of water and volatiles as the silage collapses into a pile after defacing. The extent of this loss was estimated by comparing the composition of the silage in the 'old face' with the composition of the silage in the drop down pile that was loaded into the TMR mixer. These WW, oDW and vcoDW losses from the drop down piles were 0.9 ± 0.54, -0.6 ± 2.27 and -1.5 ± 2.17% respectively.

Other Losses

Such losses include fresh chop crop which is weighed but never makes it to the pile, evaporative losses from the pile surface during building, continued plant respiration in the pile as CO_2, weepage and seepage, and silage which is never picked up from the ground or falls off the loader in transport to the TMR mixer.

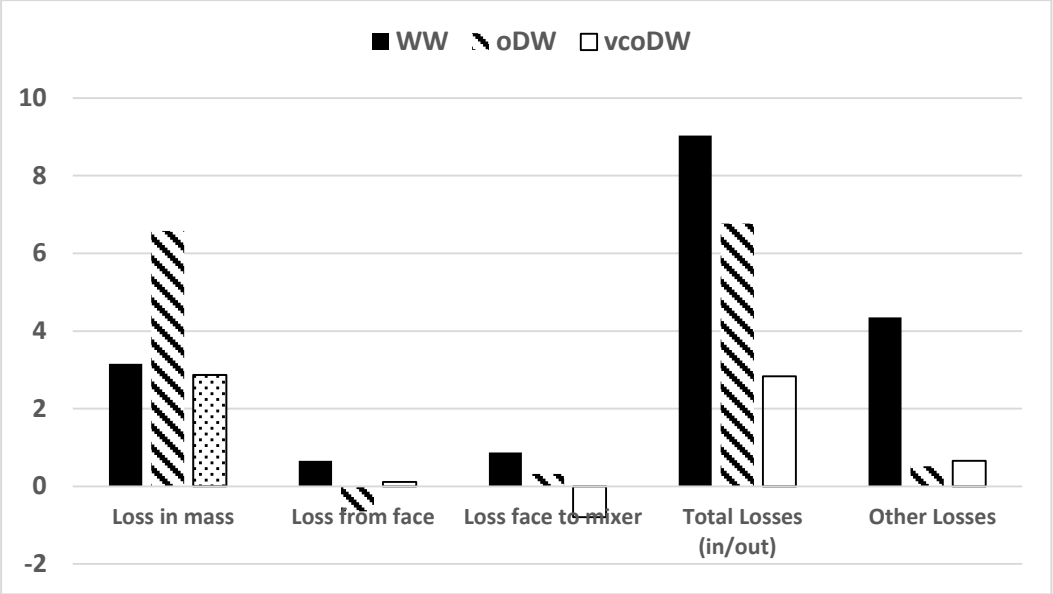


Figure 4 Losses of wet weight (WW), oven dry weight (oDW) and volatile corrected dry weight (vcoDW) from the seven maize silage piles.

In general, shrink losses were highest when measured as WW, intermediate as oDW and lowest as vcoDW losses, were measureable from most phases of ensiling, and occurred at relatively low levels. However percentage losses from the face are quantitatively far from the most important shrink losses, which are summarized in Figure 4 above.

Third questions: factors impacting silage 'shrink' losses

As already discussed, there are numerous factors which could impact total shrink losses from maize silage piles. In fact many of these factors can be controlled (i.e. they are chosen) by the farmer, at least to some degree. For example, pile orientation (S, N, E, W) and its base (dirt or concrete), use of a thin underlay film with or without enhanced oxygen barrier characteristics, chop length of the crop, use rate (i.e. cm/d) of the pile and face management can be virtually fully controlled. However factors such as the moisture level of the crop, pack density and environmental conditions during feedout can only be partially controlled (or anticipated) by the operator.

With only 7 silage piles, where each pile differed from all other piles in many ways, while being the same in many ways, it was difficult to assess the efficacy of individual mitigations. For example, as all piles had an oxygen barrier underlay, were harvested and packed by professional crews, were rapidly covered (within 48 h) with an inner oxygen barrier film and black/white outer plastic, were weighted with tire chains, and were opened and fed out by professional crews, none of these ensiling practices can be evaluated. However, some practices can, although the data requires care in interpretation.

Shrink losses as affected by silage density

This is an area of interest to regulatory agencies as it seems intuitive that losses of silage weight would be reduced if the silage was packed more densely. However in our maize silage piles there was no apparent relationship of bulk density and shrink.

Shrink losses as affected by speed of face use, 'smoothness' of the face and face orientation

Speed of face use is an area of interest from regulatory agencies as it also seems intuitive that losses of silage weight would be reduced if the silage was fed out more quickly. However in our piles there was no apparent relationship between the speed of feed out and shrink, possibly because speed of feed out was relatively fast and face losses were very low overall.

'Smoothness ' of the exposed face is also an area of interest to regulatory agencies as it seems sensible that losses of silage would be lower if silage faces were 'smooth' at the end of the day. To assess this possibility, faces were scored subjectively on a scale of 1 (really rough) to 5 (really smooth). In our piles, there was no relationship of face 'smoothness' and shrink.

Face orientation is a practice which could, at least theoretically, be changed on-farm – certainly long term. However we found no relationship of face orientation and shrink.

Shrink losses and pile base and pile size

Pile base (i.e. concrete vs. dirt) is a practice which could be changed on-farm. Although it seems sensible that a concrete base would reduce leaching losses, there was no relationship of pile base and shrink in our piles.

Pile size is also a practice which could easily be changed on-farm. However there was no clear relationship of pile size and shrink.

Shrink Losses and Age of the pile at Feedout

The age of a silage structure at feedout is not entirely within the control of dairy producers, but it is clear that pile age during feedout increased shrink losses (Table 1), although this relationship was much stronger, and quantitatively most important, for WW losses, seemingly suggesting, again, that the bulk of WW shrink losses are water.

Table 1 Relationships between wet weight (WW), oven dry weight (oDW) and volatile corrected oDW (vcoDW) shrink and age of the pile at feedout

	Intercept (% loss)	Slope (% loss/month)	R^2
WW	-0.28	1.09	0.78
oDW	1.54	0.61	0.21
vcoDW	-3.82	0.78	0.25

Shrink losses and chemical composition of the fresh chop maize crop

The fresh chop maize was analyzed for its moisture content (i.e. oDW) as well as the level of neutral and acid detergent fiber, ash, fat and crude protein in the oDW. There were no meaningful relationships (i.e., r^2 <0.15) of these components to any shrink measure.

A failure to identify mitigations or practices associated with reduced silage shrink is discouraging as it could be interpreted to suggest that silage shrink is random. This is unlikely to be the case. The more likely explanation is that only 7 piles is too small a data set to examine practices associated with shrink, especially when the number of defined practices that differed among piles, and might be expected to impact shrink losses, is more than the number of piles examined. Thus the possibility of inter-correlations is high, which could lead to concluding that a mitigation is efficacious when it is not because it is related to a mitigation that is effective.

Another reason for the failure to identify practices that reduce silage shrink may simply be a combination of the variability in the methods which were deployed to examine shrink in large commercial silage structures combined with the relatively small shrink values, especially vcoDW shrink, compared to expectations at the start of the study. With total shrink in the 3 to 8% range, it would likely have required at least 20 piles to create meaningful relationships of silage shrink and practices/mitigations that may have impacted it. There are clearly limits to what can be done in a study such as this where the piles, albeit carefully selected to be representative and well managed, exhibit a host of differences in the factors that may impact silage shrink and, perhaps critically, very low levels of shrink no matter how it is expressed.

Conclusions

The extent of silage shrink has been overestimated in large well managed commercial maize silage piles, likely due to incorrect assumptions and inappropriate research models to measure it. However, the most important reason may have been due to the failure to measure real shrink (i.e. vcoDW) in favour of WW shrink (which is exaggerated due to losses of water) or oDW shrink (which is exaggerated by losses of volatile compounds during oven drying). When the correct measure of shrink is used (i.e. vcoDW), it was <3% in our commercial maize silage piles. Within the context of these low overall vcoDW losses, losses from the face and after defacing were trivial contributors to shrink in contrast to losses while the silage was in the mass prior to face exposure. While the number of silage piles used were too small (relative to the number of definable differences between them) to allow examination of many practices commonly used to minimize shrink (and because many piles had similar characteristics by design), the commonly suggested mitigations of increasing bulk density, increasing face feedout rate and maintaining a 'smooth' face had no discernable impact on total shrink losses, probably because these mitigations are all designed to reduce losses from the exposed face which was a trivial contributor to overall shrink. Only the average silage pile age at feedout impacted shrink, with older silos having higher shrink, but mainly in the form of WW.

While maize silage shrink exists, and can be costly to dairy producers and impactful to air and water quality, its extent in large well managed maize silage piles can be low and the ability to mitigate it seems, unfortunately, to be very low due to our inability to find support for several commonly accepted mitigations. Nevertheless, dairy producers should continue to use good silage practices (i.e. common silage sense) in creating maize silage structures, but recognize that shrink is only likely to become excessive under extreme conditions.

Acknowledgements

This project would not have been possible without inputs of time and effort of Trish Price, Grace Cun and Henco Leicester, as well as the four co-operating dairy owners who prefer to remain in the shadows. This research was funded in part by grant Agreement 13-0099 SA from the California Department of Food and Agriculture.

References

Köhler, B., Diepolder, M., Ostertag, J., Thurner, S. & Spiekers, H., 2013. Dry matter losses of grass, Lucerne and maize silages in bunker silos. Agric.Food Sci. 22, 145-150.

Porter, M.G. & Murray, R.S., 2001. The volatility of components of grass silage on oven drying and the inter-relationship between dry matter content estimated by different analytical methods. Grass Forage Sci. 56, 405-411.

San Joaquin Valley Unified Air Pollution Control District, 2010. Rule 4570. Confined Animal Facilities. Adopted June 15, 2006. Amended October 21, 2010. San Joaquin Valley Unified Air Pollution Control District. http://www.valleyair.org/rules/currentrules/R4570_1010.pdf (accessed 8 April 2015).

Vahlberg, C., Nordell, E., Wiberg, L. & Schnürer, A., 2013. Method for correction of VFA loss in determination of dry matter in biomass. Swedish Gas Technology Centre Report C SGC2013-273, Malmö, Sweden, 51 pp.

Weissbach, F & Strubelt, P., 2008. Correcting the dry matter content of maize silage as a substrate for biogas production. Landtechnik 63, 82-83.

Wilkinson, J.M. & Davies, D.R., 2012. The aerobic stability of silage: key findings and recent developments. Grass Forage Sci. 68, 1-19.

Monitoring the bacterial dynamics during ensiling and aerobic stress with molecular methods

K. Graf[1], A. Ulrich[2], C. Idler[1] & M. Klocke[1]
[1] *Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB), Department Bioengineering, D-14469 Potsdam, Germany*
[2] *Leibniz Centre for Agricultural Landscape Research (ZALF), Institute for Landscape Biogeochemistry, D-15374 Müncheberg, Germany*
Correspondence: mklocke@atb-potsdam.de

Introduction

Grass silage is an important ruminant feedstuff in dairy farms. Many factors, for example, the herbage dry matter concentration, the ensiling system and the herbage compaction are known to influence the ensiling process. One of the main factors that affects the silage quality, is the rapid removal of air from the forage mass and preventing the infiltration of air during silage storage and subsequent application (Woolford, 1990). At anaerobic conditions, mainly lactic acid bacteria ferment the water soluble carbohydrates to lactic acid and in minor amounts also to acetic acid. In response to ongoing fermentation, pH of the ensiled material decreases and aerobic spoilage organisms are inhibited. As silage is exposed to air, aerobic spoilage occurs, heating the silage, due to degradation of the lactic acid. An increasing pH allows opportunistic bacteria and molds to grow which reduces the silage quality (McDonald *et al*., 1991).

Aerobic spoilage is a known problem, however, the microbial assemblages, supporting aerobic spoilage, remains uncertain. Hence, the aim of the study was to unravel the microbial community dynamics during the silage fermentation using a cultivation-independent molecular fingerprint technique, the terminal restriction fragment length polymorphism (TRFLP). In particular the effect of herbage compaction on the bacterial community in the silage and resulting aerobic stability was investigated.

Materials and Methods

Perennial ryegrass was harvested and wilted in the field for 24 h. After wilting, the crop was chopped by a field chopper to pieces of approximately 12 mm average length. Prior to ensiling, replicated samples of the plant material were analysed for chemical and molecular characterization and dry matter (DM) content of the crop was determined.

Silages were made at two compaction levels in 1.5-L laboratory scale jars (DLG, 2000). The compaction level was achieved with a compression of 0.14 kg DM L^{-1} (high density) and simulated ideal herbage compression with low amounts of residual air. Insufficient compaction was achieved by filling the jars at two-thirds with herbage (low density). The low density variants were ensiled in glass jars with a small hole (6 mm diameter) in the lid and in the bottom of the jar, enabling access of air. After filling, all laboratory silos were sealed airtight and stored at a constant temperature of 21°C prior to sampling. To intensify the effect of air in the low density variants, the rubber stoppers in the jar and lid were removed after 28 and 42 days and replaced after 24 hours with new sterile ones.

The grass was ensiled in three replicates (*i.e.* jars) for each ensiling time (3, 10 and 49 days; only data of 49 days were shown) and in two compaction variants. After 49 days the silages were used for the aerobic stability test (ASTA) about 2, 4 and 7 days. A total of 36 samples were examined. Silages were analyzed after 49 days, to determine storage losses, chemical

composition and for molecular characterization of the bacterial community. To determine aerobic stability, an aerobic stability test (ASTA) was conducted according to the method published by Honig (1990). After 49 days ensilage time, the material was placed in permeable plastic buckets and kept in a closed climate chamber at 21°C ambient temperature. The temperature of each silage was measured every hour by a data logger placed in the center of the sample. Three replicates of silages were taken after 2, 4 and 7 days of aerobic storage for molecular and chemical analyses. Aerobic stability was defined as the number of hours, until silage temperature increased 3ºC above ambient temperature.

The dry matter content of forage and silages were determined by drying the material at 105°C to constant weight. DM values of silages were corrected, regarding the content of volatile components, as described by Weißbach & Kuhla (1995). The pH, water soluble carbohydrates (WSC), crude protein and fermentation products (acetic acid, butyric acid, lactic acid, propionic acid, ethanol, 1,2 propanol, 2,3 butanediol) were determined. Analyses were carried out primarily according to VDLUFA (1997). Determination of fermentation products were performed by modified methods according to Block and Weißbach (1982).

To investigate the structure and dynamic of the bacterial community, three replicate samples of the forage crop, prior to ensiling (day 0), after 49 days ensiling time and during aerobic storage about 2, 4 and 7 days were analyzed using the terminal restriction fragment polymorphism (TRFLP) and complementary clone libraries of the 16S rRNA gene.

Accordingly, total DNA was extracted from grass and silages using a commercial DNA isolation kit (PowerSoil® DNA Isolation Kit, MO BIO Laboratories, Inc.). The bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) and the amplicons were digested with two restriction enzymes (*Hin*6I, *Rsa*I). After electrophoretic separation, fluorescence detection and analysis of the terminal restriction fragments (TRFs) a profile of the bacterial community was obtained. For identification and taxonomic assignment of TRFs, corresponding 16S rRNA gene libraries were constructed and analyzed as published by Rademacher *et al.* (2012).

Mean values and standard deviations were calculated from three replicate samples of high density and low density variants, at five time points (0, 49 days ensilage time and 2, 4, 7 days ASTA) for DM, WSC, pH and fermentation products. Only fermentation products with significant changes are presented below. Bacterial community profiles were analyzed as described above. The similarity of TRFLP profiles for each silage compaction and time point was examined with the Bray-Curtis index as measure of similarity, using the software Past (version 3.04, Hammer *et al.*, 2001). For the stacked column chart, the mean overall abundance of each TRF was calculated and TRFs were ranked according to abundance. The ranking was used to include only TRFs with relative abundance about 1% for visualization in the column chart (Figure 1b).

Results and Discussion

The chemical composition of the wilted grass, indicate a grass material that is difficult to ensile. A low dry matter content of 21%, buffering capacity (144.6 g lactic acid kg^{-1} DM), water soluble carbohydrate concentration (67.2 g kg^{-1} DM) and ash (187 g kg^{-1} DM) were determined in the starting material. Nonetheless, a good silage quality and aerobic stability up to two days after ensiling was reached in all laboratory silages, also in the low density variant.

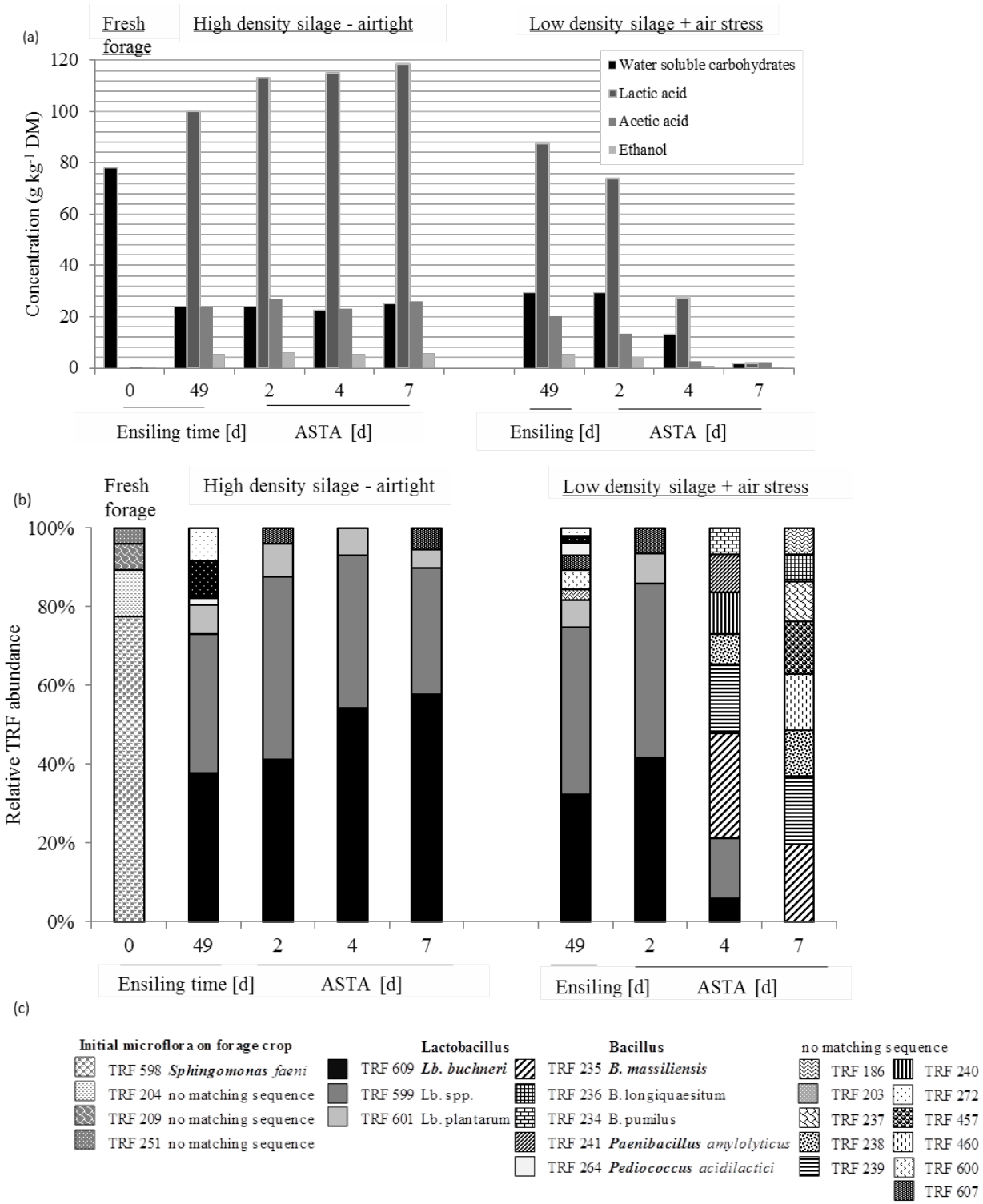


Figure 1 (a) Effects of herbage compaction on fermentation products and water soluble carbohydrate concentration. (b) Bacterial community profiles of grass silage during ensiling and subsequent aerobic stability test (ASTA) determined by TRFLP analysis. (c) Detected TRFs phylogenetically assigned based on 16S rRNA gene sequence libraries.

TRFLP analysis of the 16S rRNA gene reveals the dynamics of the microbial community structure during the fermentation process in relation to herbage compaction and under aerobic stress conditions. The bacterial community in the grass prior to ensiling (Day 0, Figure 1), was dominated by eight TRFs with highest abundances of TRF 598 (65.7%), TRF 204 (10.1%), TRF 209 (5.6%) and TRF 251 (3.4%). After 49 days of ensiling, this initial community was completely replaced by a pure *Lactobacillus* community dominated by TRF 609, TRF 599 and TRF 601.

The aerobic stability test was started with 49-d old silages containing very similar community structures in both compaction variants (as indicated by a Bray-Curtis similarity of 78%). Also after two days of aerobic storage, the community structure of the two compaction variants were nearly similar (Bray-Curtis index = 79%). In contrast, seven days after opening, only traces of the anaerobic community were determined in the insufficiently compacted silages (Bray-Curtis index = 2%).

An increase in temperature was solely observed in the low density variant and, for the first time, after two and a half days of aerobic exposure. This was associated with an intensive community shift, detected after four days of aerobic exposure. The *Lactobacillus* community was completely displaced by TRF 234, TRF 235, TRF 236, TRF 241 and TRF 264 that were determined as community with species of the genus *Bacillus, Paenibacillus* and *Pediococcus*. The fast spoilage process of the insufficiently compacted variant was also characterized by decreased lactic acid and acetic acid concentrations, high losses of water soluble carbohydrates and increased pH values (from pH 4.1 to pH 8.8 after seven days aerobic storage).

Surprisingly, the *Lactobacillus* community in the high density compaction silages remained stable at aerobic storage conditions up to seven days. The Bray-Curtis index showed a high similarity of 76% of the microbial community after seven days aerobic exposure in comparison to the 49-day silages. The *Lactobacillus* community seemed to proliferate under aerobic stress, as indicated by an increase of TRF 609 (*Lactobacillus buchneri*) from 28.1% relative abundance after 49 days to 40.3% after seven days of ASTA. At the same time, the lactic acid content increased (Figure 1a). In detail, TRF 599 increased within the first two days but decreases with continued aerobic exposure. Similar development was also observed for TRF 601. Presence of TRF 263 (7.2%), TRF 272 (6.2%) and TRF 264 (2.7%) completely disappeared after two days of aerobic storage.

The proliferating species *Lactobacillus buchneri* (TRF 609) is known as a microbial additive to improve aerobic stability in silages, as reviewed by Holzer *et al.*, (2003). Furthermore, the findings reveal that no TRFs of the wilted grass material or at early stages of ensiling were present during the aerobic storage phase.

Conclusions

The naturally occurring microorganisms on the crop material underwent intensive structural changes during ensiling. In this study, an insufficient compaction and air infiltration resulted in a silage microorganism community with a lower stability under aerobic conditions. Furthermore, this study revealed that the bacterial community of the grass before ensiling differs completely from the community in the silage and in the aerobic spoilage process. Further work is necessary to elucidate the origin of the aerobic spoilage microorganisms and to clarify the relevance of the initial microbial population of the crop on the ensiling process.

References

Block, H.J. & Weißbach, F., 1982. Zur gaschromatographischen Bestimmung flüchtiger Fettsäuren in Silagen mit innerem Standard. Archiv. Tierernähr. 32, 693-702.

DLG [Hrsg.], 2000. DLG-Richtlinien für die Prüfung von Siliermitteln auf DLG-Gütezeichen-Fähigkeit. DLG-Verlag.

Hammer, Ø., Harper, D.A.T. & Ryan, P. D., 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontol. Electron. 4(1): 9pp.

Holzer, M., Mayrhuber, E., Danner, H. & Braun, R., 2003. The role of *Lactobacillus buchneri* in forage preservation. Trends Biotechnol. 21, 282-287.

Honig, H., 1990. Evaluation of aerobic stability. In: Lindgren, S.; Lundén, P. (ed.): Proc. EUROBAC Conf., Uppsala, 1986, Grass and Forage Reports 3, 76-82.

McDonald, P., Henderson, A.R. & Heron, S.J.E., 1991. The Biochemistry of Silage. 2nd edition, Marlow, U.K. Chalcombe Publ.

Rademacher, A., Nolte, C., Schönberg, M. & Klocke, M., 2012. Temperature increases from 55 to 75 degrees C in a two-phase biogas reactor result in fundamental alterations within the bacterial and archaeal community structure. Appl. Microbiol. Biotechnol. 96, 565-576.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 1997. VDLUFA-Methodenbuch Band III „Die chemische Untersuchung von Futtermitteln“, 3. Auflage 1997.

Weißbach, F.& Kuhla, S., 1995. Stoffverluste bei der Bestimmung des Trockenmassegehaltes von Silagen und Grünfutter. Entstehende Fehler und Möglichkeiten der Korrektur. Übersicht Tierernähr. 23, 189-214.

Woolford, M. K., 1990. The detrimental effects of air on silage. J. Appl. Bacteriol. 68, 101-116.

Effect of hammer milling, roller milling and pelleting on technical characteristics of barley for ruminants

G. Q. Khan[1] & E. Prestløkken[1]
[1]*Norwegian University of Life Sciences (NMBU), Department of Animal and Aquacultural Sciences, 1432 Ås, Norway*
Correspondence: ghulam.khan@nmbu.no

Introduction

Barley is rich in starch and is an efficient dietary energy source in dairy and beef farming. It also has considerable amount of proteins and when compared to wheat and corn, it is usually cheaper, hence cost efficient. However, the whole barley kernel has a thick fibrous outer covering (hull) which offers resistance to digestion in the rumen and making it largely indigestible (Beauchemin *et al*., 1994). Also, 80 to 90% of barley starch is degraded in rumen (Nocek & Tamminga, 1991) increasing the risk of sub acute ruminal acidosis (SARA) (Krause & Oetzel, 2006) which often reduces feed intake in cattle (Allen, 2000). Therefore, there is a need of optimum processing of barley grains to achieve maximum benefit of its nutritive qualities. Particle size reduction of grains is an important process with respect to nutritional and physical quality of feed. Grinding and dry rolling are the two most commonly used processing methods to reduce particle size. Either type of processing method can produce a satisfactory particle size for ruminants. However, extent of reducing particle size is usually higher for grinding than dry rolling giving different patterns of particle size distribution along with differences in energy consumption (Koch, 1996). Pelleting, a more intensive method of processing, involves addition of steam and particle size reduction by grinding ability in the press. The heat added during the process of pelleting, also affects the digestibility of the different nutrients and the pellet quality. The aim of current study was to figure out how grinding and pelleting influence particle size distribution, energy consumption and pellet quality when processing barley to ruminants

Materials and Methodology

Processing of diets

In total, 12 pure barley feed samples were produced at ForTek (NMBU) with hammer milling (HM) and roller milling (RM) followed by pelleting. The HM samples were produced using screen sizes of 2, 4 and 6 mm with a hammer mill (Muench E-22115 TF, Wuppertal, Germany). Samples of the mash were taken for analysis and then the HM material was pelleted to 5 mm diameter in a pellet press (RPM 350.100, Munch-Edelsthal,Wuppertal, Germany). The RM samples were produced on a DT900-12, CPM roller mill (Roskamp, USA) using roller gap distances of 0.25, 0.75 and 1.5 mm. Samples of the mash were taken and then the material was pelleted as for the HM material. In both milling treatments, pelleting was done at as similar conditions as possible. Conditioning temperature was 75°C and the capacity 1000 kg per hour. Specific energy consumption, both at milling and pelleting, was recorded as kWh consumption per ton of feed processed.

Analysis of particle size distribution and pellet quality

Particle size distribution of mash was measured by both dry and wet sieving on a Retsch AS200 sieving machine (Retsch GmbH & Co., Haan, Germany) while particle size distribution of the pelleted feeds were determined by the wet sieving method only. In both dry and wet sieving, screen sizes were, 2.8, 2.0, 1.6, 1.0, 0.6 and 0.2 mm. In dry sieving,

100 g material was placed at the top screen and sieved for 3 min using a frequency of 50 Hz and amplitude of 1.5 mm. Particles, smaller than 0.2 mm, were collected in a bottom pan. In the wet sieving method, 100 g feed samples (both mash and pellets) were soaked in water for 2 hours prior to sieving so that all pellets were completely dissociated. Thereafter, the dissolved material was transferred to the top sieve. Then, the material was sieved with flowing water (about 3 l/min) applied to the top sieve for 10 minutes. As for dry sieving, the frequency was 50 Hz and the amplitude 1.5 mm. Instead of a bottom pan, excess water was discharged through a outlet. All sieves with remaining feed material were placed in an oven at 103°C overnight. The particle size distribution was then calculated by measuring the material left on each sieve. In wet sieving, particles smaller than 0.2 mm is calculated as the difference between applied dry matter of material and accumulated material on the six screens. The mean particle size distribution was determined by ASAE Method S319.4 (ASAE, 2008). The pellet quality was determined by Holmen Tester, NHP 200 (TekPro Ltd., UK). In this method, 100 g pellet, free of fines, were circulated by air flow for 60 seconds. The pellet quality was then calculated as the proportion of pellet remaining on screen No. 5 and was presented as pellet durability index (PDI).

Results

There was an increase in particle sizes with increasing hammer mill screen size and gap between the rolls in the mill as shown in Fig 1. Mean particle sizes for the hammer mill with screen sizes 2, 4, 6 mm were 504, 820, 985 µm respectively and for the roller mill with gap distance between rolls 0.25, 0.75, 1.5 mm, they were 1069, 1552, 2199 µm, respectively. The hammer mill produced more fine particles then roller mill. Wet sieving analysis yielded more fine particles in case of both hammer and roller mill, especially with small screen sizes in the hammer mill and smaller gap distance between rolls in the roller mill (Fig 2).

Wet sieving showed more fine particles for RM 0.25 mm as compared to HM 2 mm although in case of dry sieving analysis, proportions of fines were considerably higher for HM 2 mm than for RM 0.25 mm. Also, the curves for hammer mill were steeper than roller mill in case of dry sieving (Fig 1) where as wet sieving analysis showed less steep curves (Fig 2).

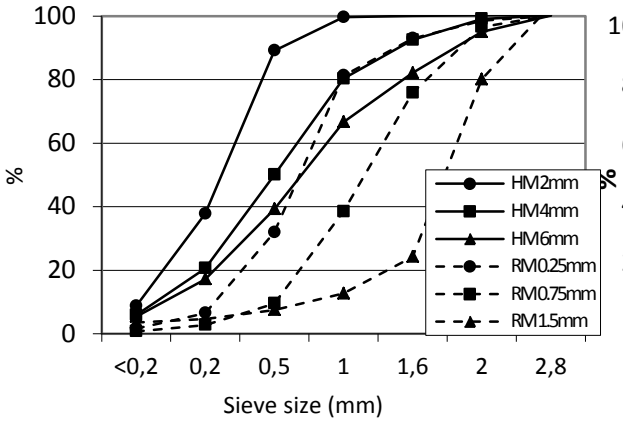


Fig 1. Cumulative particle size distribution in hammer mill and roller mill by dry sieving analysis. HM= hammer mill, RM= roller mill

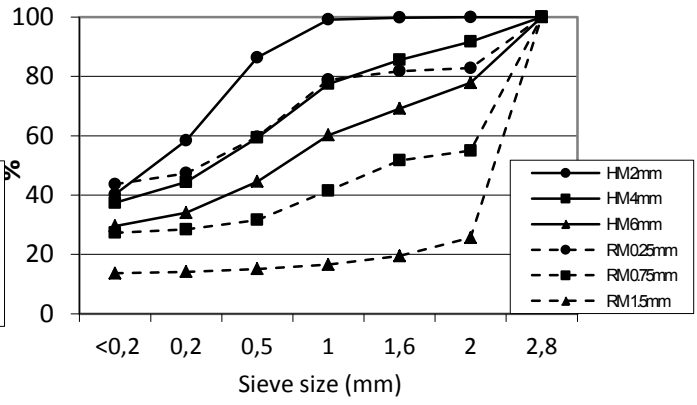


Fig 2. Cumulative particle size distribution in hammer mill and roller mill by wet sieving analysis. HM= hammer mill, RM= roller mill

Pelleting reduced mean particle size to 247, 411, 568, 364, 540 and 827 µm for HM 2, 4 and 6 mm and RM 0.25, 0.75 and 1.5 mm ground feeds, respectively (Fig. 3). It also decreased the differences in particles size between hammer and roller milling, making the curves less steep.

The processing data for pelleting along with energy consumption milling are shown in Table, 1. Specific energy consumption for milling decreased with increasing screen size in the hammer mill and with gap distance in the roller mill. However, overall energy consumption was higher for the roller mill, as compared to the hammer mill.

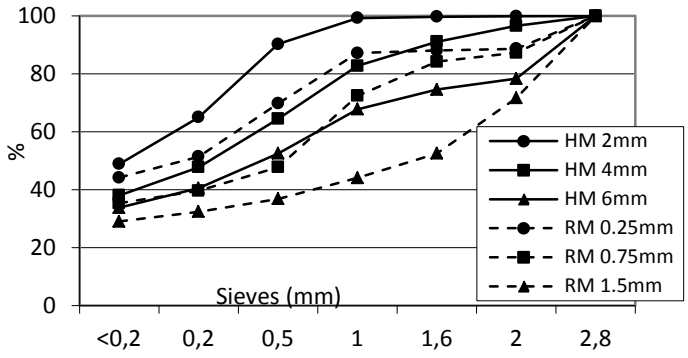


Fig 3. Cumulative particle size distribution after pelleting by hammer mill and roller mill (wet sieving analysis). HM= hammer mill, RM= roller mill

Table, 1. Processing data for pelleting and energy consumed in the milling process

	Hammer Mill			Roller Mill		
Processing data	2 mm	4 mm	6 mm	0.25 mm	0.75 mm	1.5 mm
Bulk density kg/l	0.56	0.52	0.48	0.41	0.39	0.44
Capacity, kg/h	1000	1000	1000	1000	1000	1000
Conditioning temperature, ºC	75	75	75	75	75	75
Specific energy consumption (kWh/t) for milling	15.7	15.3	14.6	16.6	15.7	15.1
Specific energy consumption (kWh/t) for pelleting	19.9	19	18.7	19.6	20.8	21.1
Total specific energy consumption (kWh/t)	35.6	34.3	33.3	36.2	36.5	36.2
PDI[a] (%)	94.05	86.98	81.26	90.27	77.99	60.97

[a] Pellet durability index

Similarly, pelleting of hammer mill feeds consumed less energy than roller mill feeds. Feeds ground on the roller mill with 0.75 and 1.5 mm gap distance between the rolls, consumed more energy during pelleting than the rest. Overall pellet durability values were higher for hammer milled feeds as compared to roller milled and the roller mill 1.5 mm ground feed gave the lowest PDI value.

Discussion

In the present study, lower values of mean particle size were found for the hammer mill as compared to the roller mill (Fig. 1), which is in accordance with previous studies (Koch,

1996; Waldroup, 1997; Boyles *et al*., 2001). Impact is the primary force used in hammer mills to reduce particle size, while in roller mills, particle size reduction is accomplished by a combinations of forces (compression and shearing) and design features of the rolls (Koch, 1996). Designing of rolls affect particle size distribution as well as the geometry of the particles, however, this was fixed in the current experiment. In hammer milling, fine particle are produced by the transfer of energy from high-speed rotation hammer tips (high kinetic energy) to slow moving grains (low kinetic energy). The process is aided by a fluidized bed of material swept along the face of the screen by the hammers (Koch 1996; Anderson, 1994). Thus, the speed of the hammers and the screen size determine particle size in a hammer mill. In roller mills, particle size is determined by distance between the rolls and number of pairs of rolls, together with uniform and constant supply of material. The roller mill produced a more uniform particle size distribution, gave fewer fines than hammer mill (Fig. 1) which is in agreement with the previous studies (Koch, 1996; Waldroup, 1997; Boyles *et al*., 2001). But, with respect to mean particle sizes, the roller mill consumed relatively more energy than the hammer mill (Table 1). This contradiction might be caused by the properties of the material being ground, as the roller mill has little effect on the fiber fraction (Koch, 1996).

A reduced particle size by pelleting (Fig. 3) is in accordance with previous studies (Svihus *et al*., 2004b). The reduction in particle size is due to the grinding action of the rolls in the pellet press. The effect of grinding is higher on coarse than on fine particles (Svihus *et al*., 2004b) and this can be seen as a difference in mean particle sizes before and after pelleting. Thus, in practice, pelleting evens out differences in particle distribution both between and within hammer and roller milling, resulting in an increased homogeneity (Fig. 3). As expected, the pellet durability index (PDI) values were higher for finer grinding (Table 1) and hammer milling gave more durable pellet than roller milling. A probable explanation is that fine grinding increases water absorption capacity of feed particles (Hemmingsen *et al*., 2008), aiding gelatinization and making starch stickier. In addition, compression of feed material becomes easier with small particles, improving pellet durability. Since, the roller mill has less effect on fiber particle size (Koch, 1996), large fibers might have induced a weak spot in the pellet due to their stiffness and elasticity (Thomas *et al*., 1998). In accordance, Zimonja *et al*., (2008) reported higher pellet durability with inclusion of fine fibers than without fibers but inclusion of coarse fibers affected pellet quality negatively. Usually, small particles offer less resistance while passing through the pellet die than coarse particles. Lower consumption of energy when pelleting hammer milled, compared to roller milled material, confirm this (Table 1). Also, large fiber particles produced by roller milling have higher coefficient of friction and low treatability by pressing (Thomas *et al*., 1998; Kulig, 2007), increasing energy consumption. However, lower energy consumption for the pelleting of coarse hammer milled feeds contradicts above explanation. This may be due to two reasons; firstly, a higher water absorption capacity by fine particles (Hemmingsen *et al*., 2008) and secondly, a slow absorption of water by insoluble fibers. So, under the same conditioning, more water could be available for lubrication during pelleting by coarse fiber particles, consuming less energy. However, Zimonja *et al*., (2008) found similar values of energy consumption for fine and coarse fibrous material.

Conclusion

Roller milling produced a more uniform particle size distribution with fewer fines than the hammer milling of barley. However, hammer milling gave more durable pellets with less

energy consumption than roller milling. Pelleting increased homogeneity by modifying differences in particle size distribution both between and within hammer and roller milling

References

Allen, M.S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83, 1598–1624.

Anderson, S., 1994. Large Rotor High Speed Hammermills: Beyond Screen Size. Feed Managem. 45, 20-22.

ASAE, 2008. Method of determining and expressing fineness of feed materials by sieving. ASAE standard S319.4. St. Joseph, MI: American Society of Agricultural and Biological Engineers.

Beauchemin, K.A., McAllister, T.A., Dong, Y., Farr, B.I. & Cheng, K.J., 1994. Effects of mastication on digestion of whole cereal grains by cattle. J. Anim. Sci. 72, 236–246.

Boyles, S. L., Anderson, V. L. & Koch, K. B., 2001. Feeding barley to cattle. Beef Information, Ohio State University

Hemmingsen A.K.T., Stevik A.M., Claussen I.C., Lundblad K.K., Prestlokken E., Sorensen M. & Eikevik T.M., 2008. Water adsorption in feed ingredients for animal pellets at different temperatures, particle size, and ingredient combinations. Dry. Technol. 26, 738-748.

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.

Koch, K., 1996. Hammermills and rollermills. MF-2048 Feed Manufacturing, Department of Grain Science and Industry, Kansas State University. 8 pp.

Kulig, R., 2007. Effect of conditioning methods on energy composition during pelleting. Kom. Mot. Energ. Roln. 7A, 52-58.

Nocek, J.E. & Tamminga, S., 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci., 74, 3598–3629

Svihus, B., Kløvstad, K.H.., Perez, V., Zimonja, O., Sahlström, S., Schüller, R.B., Jeksrud, W.K. & Prestløkken, E., 2004. Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarsenesses by the use of roller mill and hammer mill. Anim. Feed Sci. Technol. 117, 281-293

Thomas, M., van Vliet, T. & van der Poel, A.F.B., 1998. Physical quality of pelleted animal feed. 3. Contribution of feedstuff components. Anim. Feed Sci. & Tecnol. 70, 5

Waldroup, P.W., 1997. ASA Technical Bulletin. Vol. PO34

Zimonja, O., Hetland, H., Lazarevic, N., Edvardsen, D.H. & Svihus, B., 2008. Effects of fibre content in pelleted wheat and oat diets on technical pellet quality and nutritional value for broiler chickens. Can. J. Anim. Sci. 88, 613–622.

Separating the effects of pre-ensiled chemical and microbial composition on silage fermentation and aerobic stability

K. Mogodiniyai Kasmaei, R. Spörndly & P. Udén
Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Box 7024, 750 07 Uppsala, Sweden Correspondence:
Kamyar.mogodiniyai.kasmaei@slu.se

Introduction

Ensiling results of different forage types usually differ. For instance, legume silages usually contain more acetic acid, butyric acid and ammonia-N and are aerobically more stable than maize silages (Muck & O'Kiely, 1992; O'Kiely & Muck, 1992; Wilkinson & Davies, 2013). However, as to what extent these differences are related to chemical or microbial composition is yet unknown. In recent work by Mogodiniyai Kasmaei *et al*. (2015), a new ensiling methodology was introduced that enables sterilization of forages and inoculation with original microfloras. In the present experiment, we have further utilized this methodology to separate the confounding effects of microbial and chemical composition on silage fermentation and aerobic stability.

Materials and Methods

Samples used were one second-cut of a mixed timothy (*Phleum pratense*)-meadow fescue (*Festuca pratensis*) sward harvested at early maturity (grass), one second-cut red clover (*Trifolium pratense*) (clover) at late flowering and one whole-crop maize (*Zea mays*) (maize) at early dent. Perennial forage samples were collected from fields around Uppsala, Sweden (59°51′N, 17°37′E) and maize sample was collected from southern Sweden (55°41′N, 14°10′E). Samples were collected in the autumn of 2013. The perennial forage samples were chopped to a length of 3-5 cm by a stationary chopper and the maize sample was chopped by a precision chopper before being frozen at -20°C.

Sampling procedure

An amount of 3 kg of frozen samples was divided into two replicates which were thawed at room temperature before sampling of 150 g for microbial isolation and 500 g for drying at 60°C in a forced draught oven for 18 h.

Sterilization

The dried samples were milled on a hammer mill to pass a 1-mm screen before weighing 130 g in glass beakers. Samples covered loosely with aluminium foil were sterilized by the heating procedure described by Mogodiniyai Kasmaei *et al*. (2015), i.e. heating at 60°C for 3 h followed by heating at 103°C for 15 h in a forced draught oven. Thereafter, samples were tightly sealed with aluminium foil and were transferred into a desiccator to reach the room temperature.

Microbial isolation procedure

A volume of 900 mL of 0.25-strength Ringer solution fortified with Tween®80 (Merck KGaA, Darmstadt, Germany) at 0.5 mL/L (O'Brien *et al*., 2007) was added to 150-g samples. The suspensions were kept on a bench for 30 min before being pummelled for 2 min in a laboratory stomacher (Seward 3500, Seward Ltd, Worthing, UK). Thereafter, an amount of 750 mL of the microbial solution, accounting for 75% of the total volume of Ringer solution and sample water, was centrifuged at 15,500 *g* for 90 min. The supernatant was discarded and

the pellet was re-suspended in 15 mL sterile 0.25-strength Ringer solution and stored at 4°C overnight.

Reconstitution and ensiling

To estimate the amount of forage fresh matter corresponding to the isolated microbes two assumptions were made: i) microbial population was completely removed from each 150-g forage sample and evenly distributed into the Ringer solution after stomaching, ii) based on our preliminary observations (data not shown), recovery proportion of microbial population after centrifugation was considered to be 90%. Further, half the hypothetical ratio of microbes:fresh matter in original forage samples was targeted at reconstitution and all samples were reconstituted to a DM content of 40%.

The microbial isolates (n=6) were each divided into 3 equal volumes. The 5-mL inocula were added to the calculated amounts of sterilized DM so that each forage type received each of the three kinds of inoculum. Final reconstitution was carried out with addition of sterile distilled water. An amount of ~65 g was then put in sterile glass tubes (20 and 3 cm in length and inner diameter, respectively) and sealed with water locks. Silos were kept at 20±2°C for 71 day.

Aerobic stability test

An amount of 30 g of silage was placed in glass filter crucibles. The crucibles were insulated in foam polyethylene insulation pipe (15 mm wall thickness). Pipes were covered with aluminium foil and the samples were kept at 20°C with sample temperature being recorded at 2 h intervals for 8 days.

Chemical analyses

The sterilized forage samples were subjected to N determination by the Kjeldahl method, with Cu as a catalyst. Extracted juice was analyzed for short chain organic acids and alcohols by HPLC and ammonia-N by flow injection analysis. The pH of silage and aerated silage samples was measured also on the extracted juice by a laboratory pH-meter (Metrohm 654, Metrohm AG, Herisau, Switzerland). The DM content of the silage samples were estimated after drying at 103°C and corrected for volatiles as described by Mogodiniyai Kasmaei *et al*. (2015).

Statistical analyses

The effect of inoculum type (n=3), forage type (n=3) and their interactions on fermentation quality and aerobic stability variables was tested by the General Linear Model procedure. Total number of observations was 18. The significant levels for the main and interaction effects were declared at $P < 0.05$ and $P < 0.10$, respectively. If significant, pairwise comparisons were made by the Tukey method.

Results and Discussion

The effects of forage type, inoculum type and their interaction on silage variables are depicted in Table 1. Butyric acid concentrations were below 1 g/kg DM and are therefore not shown. Formation of all the end-products was affected by the forage type. Clover silages had the highest contents of lactic and acetic acid. Silages made from maize had higher amounts of propionic, 2,3-butanediol and ammonia-N than grass silages. They also formed more ethanol than clover silages.

Inoculum type only affected the formation of ethanol where, the grass inoculum gave the highest ethanol concentration. This is surprising considering that counts and species of epiphytic lactic acid bacteria (LAB) differ among forage types (Andrieu & Gouet, 1991; Li *et al*., 1992). It may suggest that differences in fermentation quality of silage crops are mostly attributed to their chemical composition. It should be interesting to find out whether this also can be seen within the same forage type. An elaborate chemical analyses (e.g. water activity, amino acid and sugar composition) is then needed as chemical variables commonly measured (e.g. dry matter, crude protein, water soluble carbohydrates) poorly explained variations in fermentation results (Mogodiniyai Kasmaei *et al.,* 2013). Clover samples inoculated with grass and maize inocula had the highest concentration of acetic acid, with inoculation having no negative effects on lactic acid formation.

Table 1 The effect of forage, inoculum and their interaction on silage variables

Treatment	pH	Lactic acid	Acetic acid	Propionic acid	Ethanol	2,3-butanediol	Ammonia-N
		g/kg DM					g/kg N
Forage (F)							
Grass	4.12^{b}	40.3^{b}	12.4^{b}	2.6^{b}	4.3^{ab}	0.9^{b}	13.6^{b}
Clover	4.55^{a}	57.0^{a}	26.7^{a}	3.0^{a}	3.4^{b}	2.0^{ab}	15.5^{ab}
Maize	3.94^{c}	27.5^{c}	10.7^{b}	3.0^{a}	5.0^{a}	3.0^{a}	20.1^{a}
Inoculum (I)							
Grass	4.28^{a}	40.8	17.1	2.9	6.6^{a}	1.5	15.4
Clover	4.25^{a}	39.5	15.9	2.9	3.3^{b}	2.2	17.7
Maize	4.08^{b}	44.6	16.8	2.8	2.8^{b}	2.2	16.1
SEM[1]	0.02	1.52	0.51	0.06	0.33	0.35	1.57
Interaction							
Grass×Grass	4.20	36.1^{def}	12.1^{c}	2.6	7.0^{ab}	0.2	12.7
Grass×Clover	4.18	39.5^{cde}	12.4^{c}	2.7	3.0^{c}	1.0	16.3
Grass×Maize	3.99	45.4^{bcd}	12.8^{c}	2.6	2.8^{c}	1.4	11.7
Clover×Grass	4.59	62.5^{a}	28.5^{a}	3.1	3.9^{bc}	1.2	14.6
Clover×Clover	4.59	51.0^{abc}	23.2^{b}	2.9	3.8^{bc}	2.5	13.7
Clover×Maize	4.47	57.6^{ab}	28.4^{a}	3.0	2.6^{c}	2.4	18.2
Maize×Grass	4.05	23.8^{f}	10.7^{c}	2.8	8.9^{a}	3.0	18.9
Maize×Clover	3.99	28.1^{ef}	12.1^{c}	3.2	3.0^{c}	3.0	23.0
Maize×Maize	3.78	30.7^{def}	9.2^{c}	2.8	3.0^{c}	3.8	18.3
SEM[1]	0.03	2.64	0.88	0.11	0.57	0.61	2.71
P value							
F	<0.01	<0.01	<0.01	0.01	0.03	0.01	0.04
I	<0.01	0.10	0.25	0.48	<0.01	0.31	0.59
F×I	0.22	0.06	0.01	0.16	0.01	0.75	0.47

[1]Standard error of mean; ^{abcdef}values with different superscribed letters within 'F', 'I' or 'F×I' and analyte differ (P<0.05 for 'F', 'I' and P<0.1 for 'F×I').

Data from the aerobic stability test is shown in Table 2. One of the replicates of grass with maize inoculum was discarded as the thermometer had been misplaced. The highest temperature and the greatest pH increase were observed in maize silages. At the same time, the maize inoculum had the best scores for the aerobic stability variables (Table 2).

Table 2 The effect of forage, inoculum and their interaction on temperature and pH rise (pH after – pH before) of silages aerated for 8 d (mean ambient temperature=20.2°C)

Treatment	Maximum temperature (°C)	pH increase
Forage (F)		
Grass	20.9^{b}	0.73^{b}
Clover	20.6^{b}	0.03^{c}
Maize	21.7^{a}	1.78^{a}
Inoculum (I)		
Grass	21.4^{a}	1.58^{a}
Clover	21.3^{a}	0.93^{b}
Maize	20.7^{b}	0.03^{c}
SEM[1]	0.1	0.07
Interaction		
Grass×Grass	21.5^{abc}	2.00^{b}
Grass×Clover	20.9^{bc}	0.20^{c}
Grass×Maize	20.4^{c,A}	0.00^{c}
Clover×Grass	20.5^{c}	0.03^{c}
Clover×Clover	20.6^{c}	0.01^{c}
Clover×Maize	20.7^{c}	0.04^{c}
Maize×Grass	22.0^{ab}	2.72^{a}
Maize×Clover	22.3^{a}	2.58^{ab}
Maize×Maize	20.8^{bc}	0.05^{c}
SEM[1]	0.2	0.12
P value		
F	<0.01	<0.01
I	0.01	<0.01
F×I	0.02	<0.01

[1]Standard error of mean; ^{abc}values with different superscribed letters within 'F', 'I' or 'F×I' and analyte differ (P<0.05 for 'F', 'I' and P<0.1 for 'F×I'); ^{A}SEM=0.3.

The results found here could be of a great potential in inoculant research. For instance, inoculating the difficult-to-ensile legume crops with strains of LAB obtained from grass or maize forages could be tested. Considering the effects of maize inoculum on silage pH (Table 1) and aerobic stability variables (Table 2), further characterization of maize LAB is warranted. It should be kept in mind the results presented were obtained from one forage sample of the forage types investigated and hence, caution needs to be undertaken when extrapolating. More studies of this kind are therefore needed before drawing firm conclusions.

Conclusions

Fermentation quality was affected by forage type but only to a very limited degree by inoculum type. Inoculation of clover sample with grass or maize inocula increased formation of acetic acid, but had no negative effect on lactic acid production. The maize inoculum improved aerobic stability. The study showed that the possibility to separate confounding effects of chemical and microbial composition can result in new insights as well as new research questions.

References

Andrieu, J. P.& Gouet, J., 1991. Variation and modification of epiphytic microflora on standing crops and after their harvest for silage. In: Pahlow P. & Honig H. (eds.), Forage Conservation Towards 2000, Proc. Conf. Europ. Grassl. Fed. Institute of Grassland and Forage Research, Braunschweig, Germany, pp. 287-289.

Lin, C.J., Bolsen, K.K., Brent, B.E. & Fung, D.Y.C., 1992. Epiphytic lactic acid bacteria succession during the pre-ensiling and ensiling periods of alfalfa and maize. J. Appl. Bacteriol. 73, 375-387.

Moodiniyai Kasmaei, K., Rustas, B.-O., Spörndly, R. & Udén, P., 2013. Prediction models of silage fermentation products on crop composition under strict anearobic conditions: A meta-analysis. J. Dairy Sci. 96, 6644-6649.

Moodiniyai Kasmaei, K., Passoth, V., Spörndly, R. & Udén, P., 2015. A new sterilization and inoculation method in silage research. Grass Forage Sci. doi: 10.1111/gfs.12153.

Muck, R.E. & O'Kiely, P., 1992. Aerobic deterioration of lucerne (*Medicago sativa*) and maize (*Zea mais*) silages-Effects of fermentation products. J. Sci. Food Agric. 59, 145-149.

O'Brien, M., O'Kiely, P., Forristal, P. D. & Fuller, H. T., 2007. Quantification and identification of fungal propagules in well-managed baled grass silage and in normal on-farm produced bales. Anim. Feed Sci. Technol. 132, 283-297.

O'Kiely, P. & Muck, R.E., 1992. Aerobic deterioration of lucerne (*Medicago sativa*) and maize (*Zea mais*) silages-Effects of yeasts. J. Sci. Food Agric. 59, 139-144.

Wilkinson, J.M. & Davies, D.R., 2013. The aerobic stability of silage: key findings and recent developments. Grass Forage Sci. 68, 1-19.

The effect on silage quality of air ingression during fermentation in experimental silos

R. Spörndly & A. Persson
Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Feed Science Division, BOX 7024 Uppsala, Sweden.
Correspondence: Rolf.Sporndly@slu.se

Introduction

The stability of silage after opening the silo can be defined as the time before silage temperature has risen >2˚C above ambient temperature (O'Kiely, 1993). Yeast is commonly regarded as the organism initiating microbial growth, responsible for the observed temperature rise (Daniel *et al.*, 1970; Jonsson & Pahlow, 1984). Yeast growth on crops starts already during wilting and continues during the storage period if air infiltrates the silage (Henderson, 1993). Deterioration of silage is initiated by different species of yeast and depends on whether the ensiling environment is anaerobic or aerobic. During slightly aerobic ensiling conditions during the storage period, the genera *Candida* and, later during fermentation, *Wickerhamomyces anomalus* (also named *Hansenula anomala* and *Pichia anomalus*), were reported to be dominant. These genera are lactate utilizers and if exceeding 10^5 cfu/g DM, the silage is likely to deteriorate faster than if *Saccaromyces sp.,* which only ferment galactose and glucose, dominate (Jonsson & Pahlow, 1984).

There are also differences between crops in aerobic stability. Lucerne is more stable than maize (O'Kiely & Muck, 1992) and clover silages have been reported to be more stable to air exposure than grass silages (Pahlow *et al.*, 2001). O'Kiely & Muck (1992) found that lucerne ensiled in laboratory silos was stable in an aerobic environment for more than 7 days, and did not react to added yeast extracts from deteriorated silages. In the most stable silages, the inoculated yeast counts decreased rapidly from $>10^6$ to 10^2 cfu/g within 48 hours after opening the silo. These findings indicate that the most stable silages under aerobic conditions either lack essential nutrient for yeast growth or there are compounds present inhibiting yeast growth. The authors also found that the potentially inhibitory factor was not present in the fresh forage and appeared first after fermentation (O'Kiely & Muck, 1992).

Undissociated lactic, acetic and propionic acid are known to inhibit growth of yeast (Moon, 1983; O'Kiely & Muck, 1992). At low pH, a higher number of undissociated molecules of lactic and acetic acids pass into the yeast cell by passive diffusion and H^+ ions are released inside the yeast cell. To avoid death, the cell has to remove the H^+ ions. Under aerobic conditions, the yeast cell is capable of removing H^+ ions by active transport. However, at anaerobic conditions the yeast cell does not get sufficient energy from fermentation of sugars to be able to remove H^+ ions (Cassio *et al.*, 1987).

When evaluating aerobic stability of silage, ensiling in completely air-tight laboratory silos are often used in combination with the measurement of temperature rise after that the silo has been opened and the silage emptied into a container open in both ends. To obtain a more rapid temperature rise, DLG has guidelines for testing of silage additives for approval of DLG quality label (DLG, 2006). They stipulate 8 hours air inlet in the silo at day 14 and 7 prior to the actual opening of the silo. In the present paper, an alternative method is evaluated, to simulate the effects of silos in practice with a continuous entrance of small amounts of air.

Materials and Methods

Samples of 4-7 kg fresh crop of a second harvest (July 15 to July 19) were collected from 7 different farms in Sweden in 2014. The participating farms were located in the southern and middle parts of Sweden. Samples were taken outside the silos when the transport wagons or choppers arrived from the field and unloaded the fresh crop.

The samples were placed in plastic bags and stored in insulated boxes together with ice packs during transport to the laboratory. Samples were kept in the cooled boxes overnight, except for samples from two farms which were collected and transported to the laboratory the same day. Storage and transportation times varied between one hour and 30 hours. If the ice packs tended to melt during this time, they were replaced with new ones resulting in an approximate storing temperature of 0 to 5°C.

Samples of the fresh crop were taken for chemical analysis (frozen at -18°C) and for enumeration and identification of yeast. The crops were then packed in laboratory scale silos consisting of 1649-mL glass jars. One sample from each farm was ensiled in four replicates of which two were made in intact silos and two were made in silos adapted with two 6-mm ventilation holes - one in the lid and one in the silo wall. The intact silos were kept sealed during the complete storage period of 100 days. Silos with holes were plugged with rubber stoppers and once a week, the stoppers were removed for two hours to simulate air leakage. After opening the silos, samples were taken for chemical analysis and for enumeration of yeasts.

Both silos and lids were carefully washed and sprayed with ethanol (70%) and dried upside down on paper towels before being filled. The density of the crop in the silos was adjusted after the DM content of the crop estimated by a rapid microwave oven method. Density was 144 kg DM/ kg^3 at DM content of 20%, and 245 kg DM/ m^3 at a DM content of 52%. The silos with air inlet had a density of 80% of the density in the air tight silos (DLG, 2006). The silos were sealed with lids fitted with water-filled gas locks and were weighed on day 0, 3, 7, 14, 28, 56, 78, 90 and on the day of opening to monitor weight loss during storage.

Yeast counts of fresh crops and silages were performed after a three-day cultivation on MEA agar at 25°C and, to limit bacterial growth, streptomycin sulphate, penicillin G and chloramphenicol were applied. A detailed procedure is described by Spörndly & Persson (2015).

Samples for chemical analysis were thawed and 130-300 g fresh weight was spread on aluminium trays. The samples were pre-dried in a heating cabinet for 18 hours at 60˚C. After drying, the samples were stabilized in open air for approximately four hours and were then weighed again and ground with a hammer mill (KAMAS Slagy 200, Malmö, Sweden) to pass a 1-mm particle size screen. The fresh crop was analysed for DM, ash, crude protein (CP), digestible organic matter using rumen fluid, (VOS, Swedish method of *in vitro* organic matter digestibility (IVOMD)) and water soluble carbohydrates (WSC). Silages were analysed for DM, ash and WSC content.

Dry matter content was determined by drying the milled samples at 103°C for 16 h. Ash content was determined by incineration in a muffle furnace at 550°C for three hours. Concentration of CP was determined by using 2020 digester and 2400 Kjeltec Analyser unit (FOSS Analytical A/S Hilleröd, Denmark) (Nordic Committee on Food Analysis, 1976). Estimation of organic matter digestibility content of forages was determined by the VOS

method (Lindgren, 1979) and estimation of metabolisable energy content was done from these values. Content of WSC was determined by an enzymatic-spectrophotometric method (Larsson & Bengtsson, 1983).

After the storage period the silage was taken out of the silo with a metal hook, put in a plastic bag, thoroughly mixed and sampled. The hook was sterilized by flaming with ethanol (99%) between every silo emptying. For the aerobic stability test, silage was loosely filled into PVC pipes of 1320 ml volume fitted with a piece of geo textile (a material which air can easily pass) at the bottom by a rubber band. The PVC pipes were sterilized by ethanol (70%) and the geo textile was autoclaved for 20 minutes in 121˚C and 10 minutes drying time. The pipes were placed in a block of Styrofoam with holes and thermocouples were placed in the middle of the silage. The pipes were placed in a climate controlled room (20˚C) with relative air humidity of 80% to prevent the silage from drying out. The silos were covered with Styrofoam with small holes in it to enable air penetration. The temperature was logged every second hour for ten days.

The effect of treatment (air or no air ingress during fermentation) and DM content on aerobic stability was tested using analysis of variance and in the General Linear Models (GLM) procedure in SAS, and calculating least square means (LSM). Means were considered statistically different if $P<0.05$.

Results and Discussion

The chemical composition and microbial colonies in the fresh crop of the seven farms is presented in Table 2. DM content varies from 21 to 53% with WSC content from 35 to 108 g/kg DM. The number of microbial colonies was fairly equal, log 5.0 to 5.8 CFU/g.

Table 1 Content of dry matter, ash, water soluble carbohydrates (WSC), in vitro digestibility of organic matter (IVDOM), crude protein (CP) and number of microbial colonies in fresh crop from 15 farms. The first eight farms were sampled during the first harvest and farm 9-15 during the second harvest

Farm	DM %	Ash g/kg DM	WSC g/kg DM	IVDOM g/kg DM	CP g/kg DM	Microbial colonies log cfu/g
9	40.2	96	75	877	190	5.1
10	21.5	94	57	801	158	5.4
11	18.8	95	35	862	190	5.6
12	52.8	62	108	732	110	5.8
13	35.9	83	102	832	132	5.6
14	30.1	98	106	834	131	5.6
15	28.1	101	107	848	141	5.0

At time of counting, all visible colonies were counted assuming them being yeast. However, when a closer microscopy examination was performed, most colonies from the fresh crop were moulds and bacteria. Penicillin, streptomycin and chloramphenicol added to the substrate did not prevent the growth of moulds and bacteria effectively when samples from fresh crop were cultivated. Yeast colonies on the plates were white, light yellow or pink.

Table 2 illustrates the status after ensiling for 100 days. Ventilation of the silos did not affect final WSC content and DM losses were only slightly higher than in air-tight silos in all cases except one ($P>0.10$). Ventilation did, however, have a significant effect on yeast counts at opening of the silos ($P<0.05$). In contrast to the fresh crop, colonies growing in the MEA agar with penicillin, streptomycin and chloramphenicol were only yeasts.

Table 2 Water soluble carbohydrate (WSC) content, dry matter (DM) loss, number of yeast colonies at opening silos and number of hours until silage reached temperature 2˚C above ambient temperature during aerobic storage after opening the silos

	WSC g/kg DM silage		**Weight loss day 90 % of initial DM**		**Yeast count in silage log cfu/g**		**Time >2°C h**	
Farm number	**Air tight**	**Ventilated**	**Air tight**	**Ventilated**	**Air tight**	**Ventilated**	**Air tight**	**Ventilated**
9	5	5	2.7	3.1	<1.7	5.9	>240	63
10	1	1	6.2	4.6	<1.7	2.0	>240	234
11	2	0	3.1	3.9	<1.7	3.2	205	98
12	44	43	1.6	2.2	<1.7	5.9	>240	226
13	18	19	2.5	2.9	<1.7	5.9	>240	36
14	27	34	2.3	2.7	<1.7	5.6	>240	64
15	18	12	2.1	3.4	3.7	5.6	87	12

The effect of ventilation was manifested during the aerobic phase. Time until the temperature rose 2°C was shorter (Table 2) and on average, the temperature rose 2.7˚C in air-tight silos and 13.5˚C in ventilated silos (data not shown). WSC content decreased by 32.6% (s.d. 33.11%) in air-tight and 51.6% (s.d. 36.44%) in ventilated silos after opening of silos ($P<0.05$). Also DM loss during the aerobic phase (10 days) was increased ($P<0.05$) by 15% for the air tight silos and 30% for the ventilated silos (Figure 1).

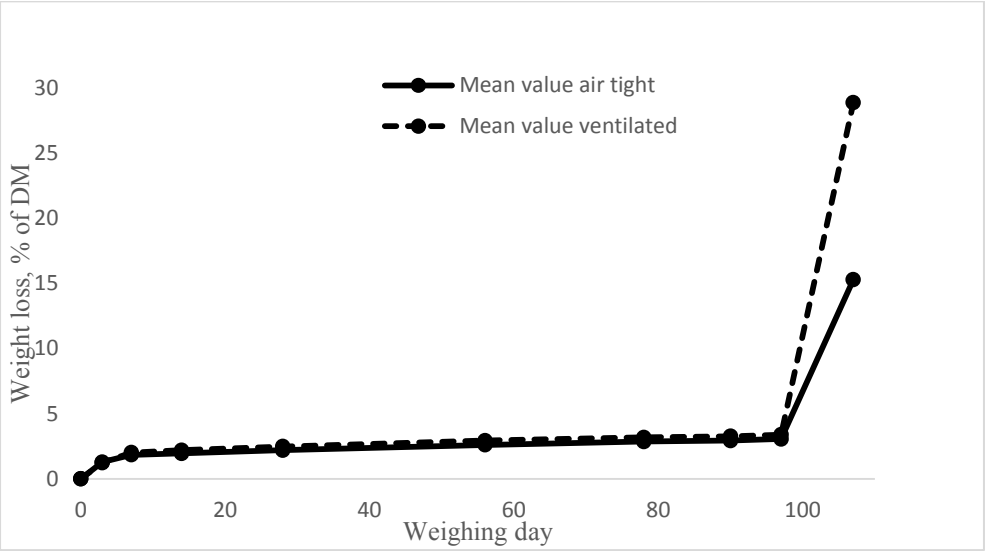


Figure 1 Comparison of weight loss between air-tight and ventilated silos during aerobic storage stability test, loss in percentage of dry matter

Ensiling under air-tight conditions obviously killed the yeast present on the fresh crop. Yeast in silage from air-tight silos was only detected in one of the samples from the seven farms while high amounts of yeast were detected in silages from all farms when using ventilated silos. The aerobic phase lasted for 10 days with little or no yeast growth in the air-tight silos. Therefore, it is not likely that yeast growth would have occurred had they been subjected to air only during a limited time at 7 and 14 days before opening as stipulated by DLG (DLG, 2006). This indicates that the system of continuously aeration of silos during the total ensiling period could be a more sensitive method to investigate aerobic stability.

Conclusions

Laboratory scale silos were either closed or ventilated 2 hours per week during the ensiling and storage period of 100 days. No differences in DM losses or WSC content were observed during the ensiling period but silage in ventilated silos contained more yeast. After opening the silos, the temperature rose faster and to higher levels and the DM losses were higher in ventilated silages. Ventilating silos regularly during the ensiling and storage periods seems to be an effective method to study aerobic stability in laboratory scale silos.

References

Cassio, F., Leao, C. & van Uden, N., 1987. Transport of lactate and other short-chain monocarboxylates in the yeast Saccharomyces cerevisiae. Appl. Environm. Microbiol. 53, 509–513.

Daniel, P., Honig, H., Weise, F. & Zimmer, E., 1970. Wirkung von propionsaure bei der Grunfuttersilierung. Wirtschaftseigene Futter, 16, 239–252. Cited in: McDonald, P., Henderson, N., Heron, S. (1991). The Biochemistry of Silage. Marlow, UK: Chalombe Publ.

DLG, 2006. DLG-Richtlinien für die Prüfung von Siliermitteln auf DLG-Gütezeichen--Fähigkeit (DLG guidelines for the test of silage additives for approval of DLG quality lables). DLG (www.dlg.org/home-en.html), DLG Commission for Silage Additives, Frankfurt a.M., Germany, p. 19.

Henderson, N. & Heron, S., 1991. The Biochemistry of Silage. Marlow, UK: Chalombe Publ.

Henderson, N., 1993. Silage additives. Animal Feed Sci. Technol. 45, 35–56.

Jonsson, A. & Pahlow, G., 1984. Systematic classification and biochemical characterization of yeasts growing in grass silage inoculated with Lactobacillus cultures. Animal Res. Developm. 20, 7–22.

Larsson, K. & Bengtsson, S., 1983. Bestämning av lättillgängliga kolhydrater i växtmaterial. SLL metod 22.

Lindgren, E., 1979. The nutritional value of roughages determined in vivo and by laboratory methods. Swedish University of Agricultural Sciences, Department of Animal Nutrition, Report 45. Uppsala

Moon, N. J., 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. J. Appl. Bacteriol., 55, 453–460.

Nordic Committee on Food Analysis. 1976. Nitrogen. Determination in Food and Feed according to Kjeldahl, 6 (Third Edition).

O'Kiely, P., 1993. Influence of a partially neutralised blend of aliphatic organic acids on fermentation, effluent production and aerobic stability of autumn grass silage. Irish J. Agric. Food Res. 32, 13–26. Cited in: Cherney, J. H. and Cherney, D. J. R. (2003). Assessing silage quality. In: D.R. Buxton, Muck, R. E., and Harrison, J. H. (Eds) Silage science and technology. pp 141–198. Amercian Society of Agronomy Inc., Madison, Wisconsin, USA.

O'Kiely, P. & Muck, R. E., 1992. Aerobic deterioration of lucerne (Medicago sativa) and Maize (zea mais) Silages—Effects of Yeasts. J. Sci. Food Agric. 59,139–144.

Pahlow, G., Rammer, C., Slottner, D. & Tuori, M., 2001. Ensiling of legumes. In: Wilkins, R. J., Paul, C. (Eds) Proc. Legume Silages for Animal Production: LEGSIL, Braunschweig, 2001. pp 27–30. Braunschweig.

Spörndly, R & Persson, A., 2015. Yeast species in Swedish forages. In: Udén et al (Eds), 2015. 6th Nordic Feed Science Conference, pp 66-70.

Yeast in fresh crop and silage from 15 Swedish farms and its impact on silage aerobic stability

R. Spörndly & A. Persson
Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Feed Science Division, BOX 7024 Uppsala, Sweden.
Correspondence: Rolf.Sporndly@slu.se

Introduction

Yeast is commonly regarded as the organism initiating growth upon silo opening and responsible for silage temperature rise (Daniel *et al.*, 1970; Jonsson & Pahlow, 1984). Yeast growth on crops starts already during wilting and continues during the storage period if air infiltrates in silage (Henderson, 1993). Yeasts are able to grow and multiply within the pH range of 3 to 8 (McDonald *et al.*, 1991). Wilkinson & Davies (2013) compiled factors that may increase the risk of yeast and mould presence on crops at harvest; 1) dead plant materials at the bottom of leys, 2) damaged crops from rain and/or wind during the last days of growth, 3) crops in advanced stages of maturity, 4) crops that have begun to reach senescence just before harvest, and 5) swaths that have been wilted for more than two days especially under poor weather conditions (Wilkinson & Davies, 2013). A recent study has shown an increase in numbers of yeasts, LAB and enterobacteria on the crop if the harvest date is delayed (Schenck & Müller, 2014). Different yeast species can be found in silage depending on when in the ensiling process sampling occurs and which crops are ensiled. In one study, the non-fermentative genera *Cryptococcus, Sporobolomyces, Rhodotorula, Torulopsis* and *Aurebasidium* were found on the fresh crop prior to ensiling (Jonsson & Pahlow, 1984). Three days after sealing, these species were replaced by different species of yeasts. If the ensiling was anaerobic, the dominant species belonged to the genus *Saccharomyces spp*. These yeasts are not able to utilize lactate but ferment galactose and glucose. In aerobic silages, the composition of yeast flora was dominated by *Candida lambica* and colonies of *Wickerhamomyces anomalus* and *C. krusei* were also present from day 49 to the end of the ensiling period. During silage aerobic stability test, the yeast counts rose from log 7 to log 9 and the pH value increased from 5 to > 7 (Jonsson & Pahlow, 1984).

Since it is reasonable to assume that yeast species vary with location we found it interesting to investigate the floras existing on typical leys in Sweden. The aim of the present study was to compare green forage crops from 15 random Swedish farms as to yeast prevalence and to study if a correlation exists between silage aerobic stability and yeast species. A second aim was to describe how the forages were produced and relate aerobic stability to forage management factors.

Materials and Methods

Samples of 4-7 kg fresh crop were collected from 15 different farms in Sweden in 2014. Eight samples were from the first harvest (May 28 to June 9) and seven samples were from the second harvest (July 15 and July 19). The farms were located in the southern and middle parts of Sweden. The samples were taken outside the silos when the transport wagons or choppers arrived from the field and unloaded the harvested forages. The samples were transported to our laboratory within 30 hours while being kept at 4°C. The crops were packed in laboratory glass silos of 1649-mL with packing density being adjusted to forage dry matter (DM) contents. Densities were 144 kg DM/ kg^3 for DM content of 20%, and 245 kg DM/ m^3

for DM content of 52% (DLG, 2006). The silos were sealed with a lid fitted with water-lock and were weighed on day 0 and at opening on day 100 to determine weight loss during storage. At opening, silages were subjected to aerobic stability test. Silage samples were loosely filled into sterile PVC pipes (1320 mL) which had been fitted with a piece of geo textile (a material which air can easily pass) at the bottom. The pipes were placed in a block of Styrofoam with holes and thermocouples were placed in the middle of the silage. Samples were kept at 20˚C with relative air humidity of 80% to prevent a fast drying out. The silos were covered with Styrofoam with small holes in it to enable air penetration. The temperature was logged every second hour for ten days.

The fresh crops and the silages were analysed for DM, Ash, CP and WSC content with standard methods as described by Spörndly & Persson (2015). For microbial analyses, an amount of 30 g forage sample was placed in a stomacher bag and 270 mL of autoclaved ¼ - strength Ringer solution (Merck, Darmstadt, Germany) was added. Samples were pummelled in a laboratory stomacher (Stomacher 3500, Seward Ltd, Worthing, West Sussex, UK) for 60 seconds twice. Serial 10-fold dilution was prepared from microbial solution before plating on MEA agar fortified with streptomycin sulphate (30 mg/L) and penicillin G (30 mg/L). For samples from the second harvest, an additional set of substrates was prepared where chloramphenicol replaced streptomycin. Colonies of yeasts were counted after three days of aerobic incubation at 25°C and the average colony number was determined according to Niemelä (1983). Colonies from the fresh crop were streaked on MEA-plates to ensure pure yeast colonies. A total number of 16 colonies from the first harvest and 40 colonies from the second harvest were grown in test tubes with 3 mL Yeast Extract Peptone Dextrose (YPD)-medium (Becton Dickinson Company, Sparks, Maryland, USA). The suspensions were incubated on a shaking table at 25°C for 48 hours. The YPD-medium had a concentration of 10 g yeast extract/L, 20 g bacteriological peptone/L and 20 g glucose/L. Thereafter, 600 µL was pipetted into cryo tubes (2 ml) and mixed with approximately 600 µL glycerol and put in a freezer at -70°C. Sample preparation for DNA sequencing which includes DNA extraction, PCR, gel electrophoresis and purification of PCR-products were done by the Department of Microbiology of the Swedish University of Agricultural Sciences in Uppsala. Sequencing was carried out by Macrogen (Amsterdam, Netherlands). The sequences were compared with sequences in NCBI's (National Center for Biotechnology Information, Bethseda, Maryland, USA) database using BLAST® (Basic Local Alignment Search Tool; www.ncbi.nlm.nih.gov/BLAST) (Altschul *et al.*, 1990).

A set of forage management factors were collected at each farm and the influence of such factors on aerobic stability were tested, when applicable, by the General Linear Model (GLM) procedure in SAS. The effect was considered statistically significant if $P < 0.05$ and tendency if $0.05 \leq P \leq 0.10$. Pearson correlation coefficients were calculated between chemical variables in fresh crop and silage, yeast counts in fresh crop and silage, ensiling losses, aerobic stability and temperature increase during aerobic test as well as correlations between these variables and management factors, using the procedure CORR in SAS.

Results and Discussion

In all farms, the ley consisted of a mixture of perennial grasses and red and white clover. Six farms were fertilized with liquid manures and in four farms commercial fertilizers were used. Three farms were also fertilized with a combination of liquid manure and commercial fertilizer. The amount of liquid manures spread ranged from 20 to 30 tonnes per hectare. The

fields were fertilized before each harvest except for one farm which was fertilized only before the first harvest. The age of the swards varied between one and four years. In none of the farms herbicides were used. In all farms, white silage film was used to cover bunker silos but eight farms additionally also used micro foil.

Use of manure as fertilizer tended to result in a higher yeast count in the fresh crop (P=0.08). Silages made from the first harvest had a tendency for longer stability than silages from the second cut (11.3 vs. 8.6 days) (P=0.06). The temperature of the silages from the first harvest only rose on average 0.14°C while the temperature of the silages from the second harvest rose on average 3.3°C.

The method used for quantifying the yeast resulted in number of colonies in the fresh crop ranging from log 4.6 to log 5.9 cfu/g (Table 1).

Table 1. Content of dry matter (DM), water soluble carbohydrates (WSC), number of microbial colonies in fresh crop and after ensiling, and aerobic stability after opening the silos at 15 farms. Farm 1-8 were sampled during the first harvest and farm 9-15 during the second harvest.

Farm	DM in fresh crop (%)	WSC in fresh crop (g/kg DM)	WSC in silage (g/kg DM)	Number of microbial colonies in fresh crop (log cfu/g)	Number of microbial colonies in silage (log cfu/g)	Time (h) until temperature +2°C above ambient temperature
1	25.0	83	0.3	4.6	3.2	213.4
2	25.5	109	1.0	4.7	3.0	181.6
3	23.7	109	4.3	5.6	<1.7	>288
4	33.2	108	13.7	5.6	2.05	>288
5	22.2	81	1.3	5.9	<1.7	>288
6	23.3	70	0	5.9	1.7	>288
7	33.7	149	18.8	5.5	2.4	>288
8	24.2	68	0	5.0	<1.7	>288
9	40.2	75	5.0	5.1	<1.7	>240
10	21.5	57	0.8	5.4	<1.7	>240
11	18.8	35	1.8	5.6	<1.7	205.9
12	52.8	108	43.9	5.8	<1.7	>240
13	35.9	102	18.2	5.6	<1.7	>240
14	30.1	106	26.6	5.6	<1.7	>240
15	28.1	107	17.9	5.0	3.7	87.9

For the green forage samples, the use of penicillin, streptomycin and chloramphenicol in the growth media was not effective in preventing mould and bacterial growth. However, this was not the case for silage samples.

The fresh forage samples from the two harvests contained 15 different species of yeast (Figure 1). The two most common yeast species found were *Rhodosporidium babjeave* and *Rhodotorula glutenis* found in 6 and 5 farms, respectively.

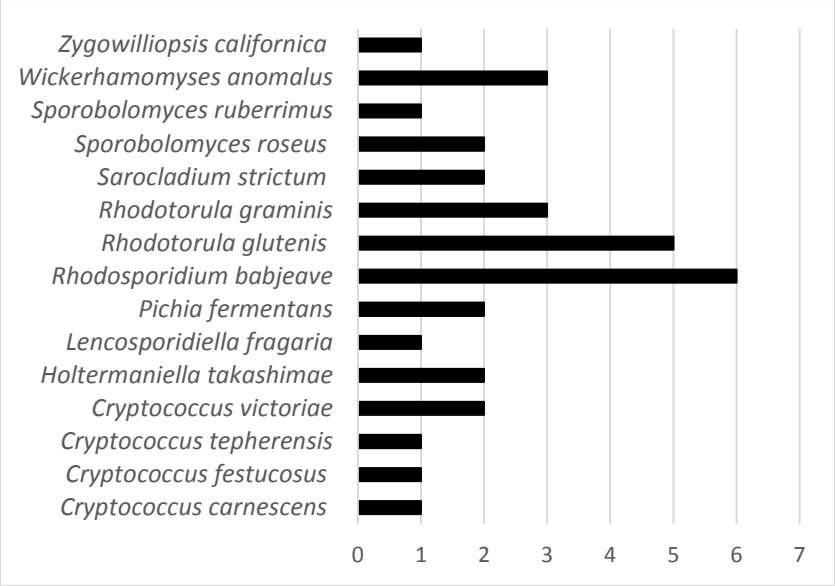


Figure 1. Number of farms per yeast species present in fresh crop from 1st and 2nd harvest at a total of 15 farms.

The correlation analyses showed higher DM losses at lower silage DM and WSC contents. The ash content was positively correlated with the yeast counts in fresh forages and was negatively correlated with the aerobic stability (Table 2). Silage yeast count was negatively correlated with storage stability.

Table 2. Correlation coefficients between yeast count in fresh crop and in silage, silage concentration of dry matter (DM), water soluble carbohydrates (WSC) and ash, and silage DM loss, aerobic stability (time lapse before 2˚C increase above the ambient temperature) and temperature increase during the aerobic phase

	Yeast count in fresh crop	**Yeast count in silage samples**	**DM loss**	**Aerobic stability**	**Temperature increase during aerobic storage**
Yeast count in fresh crop		NS	NS	0.47 P<0.07	NS
Yeast count in silage samples	NS		NS	-0.61 P<0.02	0.43 P<0.11
Silage DM	NS	NS	-0.57 P<0.03	NS	NS
Silage WSC	NS	NS	-0.65 P<0.009	NS	NS
Silage ash	0.55 P<0.03	NS	NS	-0.56 P<0.03	0.49 P<0.06

Conclusions

The method used to cultivate silage yeasts is not applicable for fresh crops as bacterial growth cannot be prevented in the growth medium by the antibiotics commonly used. The most frequently seen genera of yeasts in fresh crops were *Rhodosporidium* and *Rhodotorula*. Silage yeast counts and aerobic stability were negatively correlated.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J., 1990. Basic local alignment search tool. Journal of Molecular Biology, 215, 403–410.

Daniel, P., Honig, H., Weise, F. & Zimmer, E., 1970. Wirkung von propionsaure bei der Grunfuttersilierung. Wirtschaftseigene Futter, 16, 239–252. Cited in: McDonald, P., Henderson, N., Heron, S. (1991). *The biochemistry of silage*. Marlow, UK: Chalcombe publications.

DLG., 2006. DLG-Richtlinien für die Prüfung von Siliermitteln auf DLG-Gütezeichen--Fähigkeit (DLG guidelines for the test of silage additives for approval of DLG quality lables). DLG (www.dlg.org/home-en.html), DLG Commission for Silage Additives, Frankfurt a.M., Germany, p. 19.

Henderson, N., 1993. Silage additives. Animal Feed Sci. Technol. 45, 35–56.

Jonsson, A. & Pahlow, G., 1984. Systematic classification and biochemical characterization of yeasts growing in grass silage inoculated with Lactobacillus cultures. Animal Res. Developm. 20, 7–22.

McDonald, P., Henderson, N. & Heron, S., 1991. The biochemistry of silage. Marlow, UK: Chalcombe publications.

Niemelä, S., 1983. Statistical evaluation of results from quantitative microbiological examinations. Nordisk Metodik-Kommitté för Livsmedel (NMKL), 1.

Schenck, J. & Müller, C. E., 2014. Microbial Composition before and after Conservation of Grass-Dominated Haylage Harvested Early, Middle, and Late in the Season. Journal of Equine Veterinary Science, 34, 593–601.

Spörndly, R & Persson, A., 2015. Yeast species in Swedish forages. In: Udén *et al*. (Eds), 2015. 6th Nordic Feed Science Conference, pp 60-65.

Wilkinson, J. M. & Davies, D. R., 2013. The aerobic stability of silage: key findings and recent developments. Grass and Forage Science, 68, 1–19.

Aerobic stability of crimped barley ensiled with organic acids

A. Seppälä[1], M. Mäki[1], S. Orkola[2] & M. Rinne[1]
[1]*Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland.*
[2]*Taminco Finland Oy, (a subsidiary of Eastman Chemical Company), Tammasaarenkatu 1, 00180, Helsinki, Finland.*
Correspondence: Arja.Seppala@luke.fi

Introduction

Benefits of storage of high moisture grains in Nordic conditions have been already proven some decades ago (Palva *et al*. 2005; Jaakkola *et al*., 2005). No drying cost, less dependency on weather and an extended harvesting season are the main arguments that keep the method increasing in popularity. The method has become more common on farms with large herd sizes and total mixed ration (TMR) feeding.

It has been recommended to crimp high moisture grains and ensile them at moisture contents (MC) between 350 and 450 g/kg (Palva *et al*., 2005). When airtight ensiled, pH drops close to or below 4. Organic acids like formic, acetic and propionic acids in undissociated forms, either added or produced in fermentation, prevent growth of yeasts and moulds. Counts of yeasts and moulds on ensiled crimped grains (e.g. < 3 log cfu/g; Seppälä *et al*., 2015) are usually less than values typically detected in freshly harvested grains (e.g. 4.2 - 6.5 log cfu/g Olstorpe *et al*., 2010), resulting in an improved aerobic stability of ensiled grains.

Ensiling crimped grains at lower MC (250 – 160 g/kg) has recently become more common, which could mean an increased risk of deterioration. Olstorpe *et al*. (2010) collected samples of ensiled crimped barley grains from seven Swedish farms where grains had been rolled and ensiled in plastic tubes (2 meter diameter, 10 - 50 meter length) without any additives. When the grains had an intermediate MC (170 – 230 g/kg), most samples (9 out of 12) had mould counts above 4 log cfu/g. Seppälä *et al*. (2012) showed that the addition of propionic acid based additives at 3 L/t on crimped barely grains of similar MC can reduce mould counts to below 3 log cfu/g.

Unwillingness to use corrosive products has resulted in the development of buffered silage additives where part of the formic acid or propionic acid is in salt form. Buffered products may not be as effective in dropping the pH as pure acids. Therefore, we aimed at comparing the effects of different mixtures of organic acids and their salts and application levels on aerobic stability and microbial profiles of crimped barley grain silages.

Materials and Methods

Two batches (differing in moisture content, MoMo = more moisture, LeMo= less moisture) of fully ripened spring barley grain (Marthe) were obtained from Loimaa, Finland in August 10, 2014 and crimped by a Murska 1400 s2x2 mill (Aimo Kortteen Konepaja, Ylivieska, Finland). For each tested additive (Table 1), three replicates of 5 kg were weighed and additive applied using a bottle with perforated cap. From each replicate, three glass jars (1.7 L) were filled: one for measuring temperature change until day 23 or 24 of ensiling (short ensiling) and the follow up aeration for 15 days, one for pH determination and microbial analysis at day 113 of ensiling (long ensiling) and one for monitoring aerobic stability (40 days) of unsealed silos after long ensiling. The temperature change was measured by placing a thermocouple wire in the middle of each silo. The temperature was recorded at 10-minute intervals using a data logger.

Table 1 Tested additives and their application levels

Treatment	Composition	Application level (L/t)
Control	-	0
BL1	Formic acid, propionic acid, ammonium formiate, sodium formiate, surfactants, water	3
		5
BL2	Propionic acid, ammonium propionate, sodium benzoate, water	4
		6
		8
BL3	Propionic acid, ammonium propionate, water	4
		6
BL4	Propionic acid, ammonium propionate, water	3
		5*

*The higher application level of BL4 was dosed only on the low moisture grain.

Amount of moulded grain was visually evaluated when emptying the silos after each aerobic test period. Additionally the microbial quality of the crimped grain prior to ensiling and immediately after 113 days ensiling was measured using culture methods.

Statistical calculations were made using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Model pH= TREATMENT was used in PROC GLM and contrasts were performed. TREATMENT combined factors (barley batch, type of additive and dosage level) and had 19 levels. Temperature difference (TempDiff) curve between the silo and ambient was calculated for each replicate. Analysis of variance (PROC GLM) was conducted in each time point separately for both Mo-Mo and LeMo using model TempDiff = ADDITIVE + APPLICATION ADDI-TIVE*APPLICATION. The classifying independent variables were ADDITIVE (type of additive) and APPLICATION (application level of additive). Predicted mean and its 95 % confidence limits were plotted as figures.

Results and Discussion

Barley prior to ensiling

Hectolitre weight of the two barley batches were 66.4 kg (MoMo) and 69.8 kg (LeMo). Prior to crimping, the grains had a MC of 273 (MoMo) or 190 g/kg (LeMo) and crude protein contents of 142 (MoMo) or 114 (LeMo) in g/kg DM, while NDF content was 154 g/kg DM in both barley batches. Microbial counts of the grains prior ensiling are shown in Table 2.

Table 2 Microbial counts (log cfu/g) of the grains prior to ensiling

Grain batch	Enterobacteria	Total count of aerobic microbes	Lactic acid bacteria	Yeasts	Moulds
MoMo	7.1	7.9	4.8	6.1	5.1
LeMo	6.3	7.6	4.3	6.2	4.6

MoMo = barley batch having moisture 273 g/kg, LeMo = barley batch having moisture 190 g/kg.

Moisture content, pH and undissociated acids

After 113 ensiling days, the MoMo barley had a moisture content of 246 g/kg and LeMo had a moisture content of 184 g/kg. Additive treatments were able to drop the pH of the ensiled barley compared to the control treatment, Table 3 ($p < 0.001$). The amounts of undissociated propionic, formic and benzoic acids were calculated taking into account the dosage, pKa and molecular weight of each acid and measured silage pH (Table 3). In earlier trial (Seppälä *et al*. 2012) the amount of propionic acid measured in control treatments was negligible (in maximum 0.11 g/kg DM) when MC was below 300 g/kg, and thus the dosed propionic acid was assumed to be the only source of propionic acid. The calculated amount of undissociated benzoic acid was in all cases below 0.13 mol/t (not shown).

Table 3 The calculated amounts of undissociated propionic and formic acids (mol/t) in ensiled crimped barley when additive dosage and pH were taken into account.

		Dosage		Undissociated	
Batch	Additive	level L/t	pH	Propionic acid	Formic acid
MoMo	Control	0	6.3	0.0	0.0
	BL1	3	5.3	2.5	1.2
	BL1	5	4.9	8.3	5.5
	BL2	4	5.6	6.5	0.0
	BL2	6	5.8	7.0	0.0
	BL2	8	5.6	12.6	0.0
	BL3	4	5.2	15.8	0.0
	BL3	6	5.0	33.7	0.0
	BL4	3	5.5	8.1	0.0
LeMo	Control	0	5.9	0.0	0.0
	BL1	3	5.2	3.1	1.5
	BL1	5	4.8	8.6	6.0
	BL2	4	5.6	5.8	0.0
	BL2	6	5.6	9.8	0.0
	BL2	8	5.5	15.5	0.0
	BL3	4	5.1	18.2	0.0
	BL3	6	4.9	36.8	0.0
	BL4	3	5.3	11.1	0.0
	BL4	6	5.0	35.8	0.0

MoMo = barley having moisture 246 g/kg, LeMo =barley having moisture 184 g/kg. BL1-BL4 = blend of organic acids (formic acid, propionic acid, benzoic acid and their salts).

Barley with more moisture

After the short ensiling (23 or 24 d) MoMo was prone to rapid moulding and only the strongest additive treatments (BL3 dosage levels 4 or 6 l/t) were able to prevent moulding during the aerobic phase. During the first 24 days of ensiling, the temperature of control silages stayed one degree above the ambient temperature contrary to BL3 6 l/t treated silages where no temperature rise was observed (Figure 1). The BL3 6 l/t treated silages also showed no temperature rise during aeration. Long ensiling (113 d) clearly improved aerobic stability of untreated MoMo grain silages as the onset of heating was detected after two weeks of silo opening (Figure 2). BL3 6 l/t treated MoMo showed no visual moulding when silos were

emptied after 40 days of aeration. Other additive treatments were intermediate between the control and the BL3 6 l/t treatment in their ability to prevent the growth of moulds (results not shown).

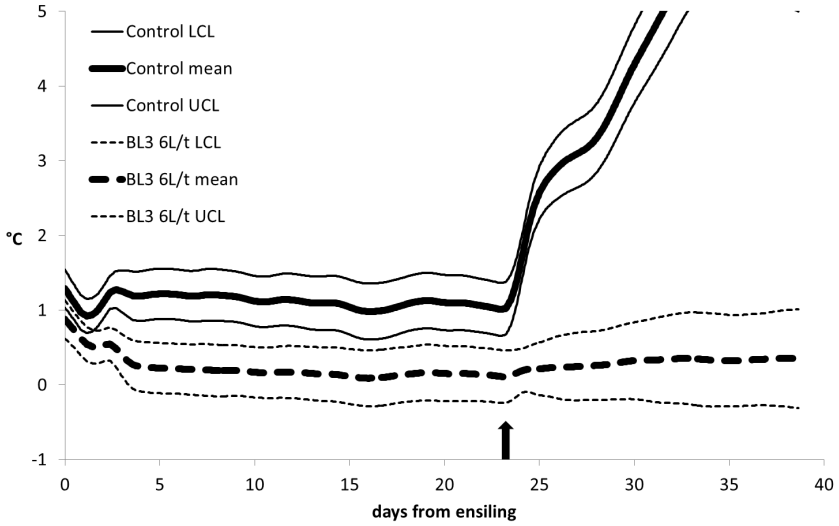


Figure 1 Temperature difference between the barley (moisture content 246 g/kg) and ambient during the first 24 days of ensiling and during the aerobic period after silo opening (arrow). Additive treatments: Control = without additive, BL3 6l/t: blend of propionic acid, ammonium propionate and water dosed 6 l/t. LCL = lower 95 % confidence limit, UCL = upper 95 % confidence limit.

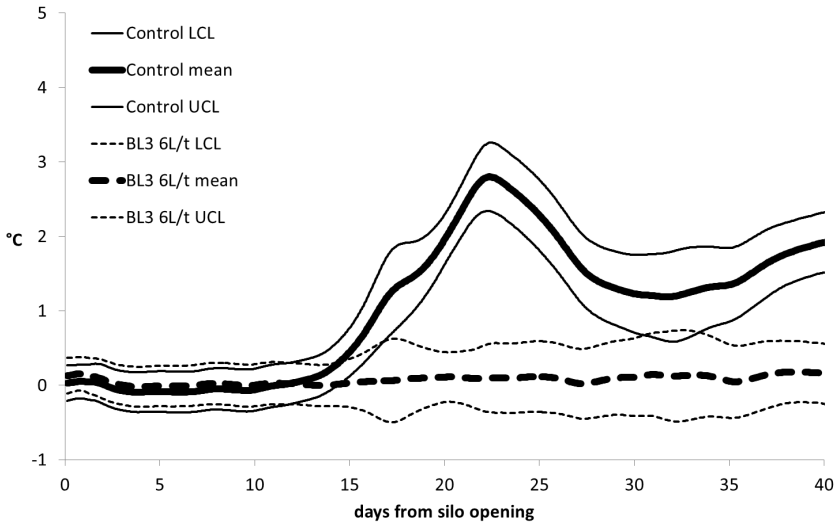


Figure 2 Temperature difference between the barley (moisture content 246 g/kg) and ambient during aerobic exposure after silo opening (ensiling period 113 days). Additive treatments: Control = without additive, BL3 6l/t: blend of propionic acid, ammonium propionate and water dosed 6 l/t. LCL = lower 95 % confidence limit, UCL = upper 95 % confidence limit.

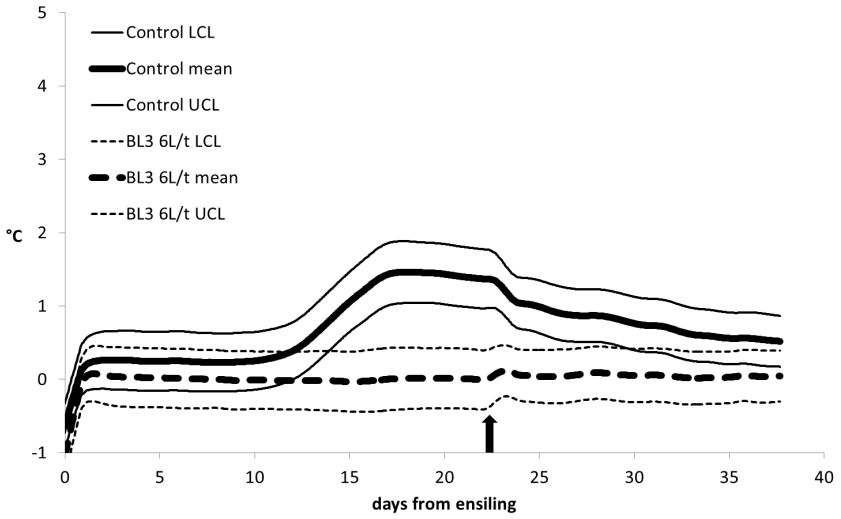


Figure 3 Temperature difference between the barley (moisture content 184 g/kg) and ambient during the first 24 days of ensiling and during the aerobic period after silo opening (arrow). Additive treatments: Control = without additive, BL3 6l/t: blend of propionic acid, ammonium propionate and water dosed 6 l/t. LCL = lower 95 % confidence limit, UCL = upper 95 % confidence limit.

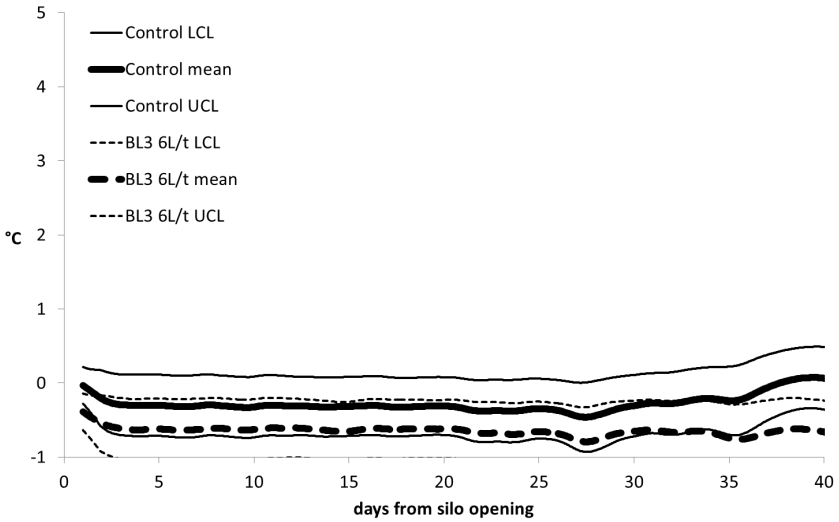


Figure 4 Temperature difference between the barley (moisture content 184 g/kg) and ambient during aerobic exposure after silo opening (ensiling period 113 days). Additive treatments: Control = without additive, BL3 6l/t: blend of propionic acid, ammonium propionate and water dosed 6 l/t. LCL = lower 95 % confidence limit, UCL = upper 95 % confidence limit.

Barley with less moisture

Barley LeMo was not generally prone to moulding as only the barley without additive or low level of BL1 showed moulding after short ensiling, as well as the barley without additive after the long ensiling period. Microbial growth in LeMo was more likely restricted by the low moisture content as reflected by the different shape of temperature curves of control treated LeMo barley compared to control treated MoMo barley (Figures 1 - 4). The additive treated silages did not show any significant temperature change.

Microbial counts

The long ensiling time was not able to totally kill the yeasts and moulds when no additive was used. Control treated ensiled MoMo had yeasts 2.2 - 4.8 cfu/g and moulds at maximum 4.4 cfu/g. One sample of control treated ensiled LeMo had yeasts 4.1 and moulds 2.0 cfu/g while the two other samples had yeasts and moulds below 2.0 cfu/g. All the additive treated ensiled barley samples had mould and yeasts counts below detection limit (2.0 cfu/g) when sampled just after silo opening..

Conclusions

Blends of organic acids were able to restrict growth of moulds in ensiled barley grains of 240 – 190 g/kg moisture contents. A strong restricting effect was achieved when the level of undissociated propionic acid was above 12.6 mol/t. A long ensiling period in combination with the use of organic acids was able to reduce the numbers of yeasts and moulds below 2 log cfu/g. When grains were ensiled for 24 days, application of additives was necessary to avoid heating upon silo opening.

References

Jaakkola, S., Saarisalo, E. & Kangasniemi, R., 2005. Ensiled high moisture barley or dry barley in the grass silage-based diet of dairy cows. In: edited by: R.S. Park, M.D. Stronge. Silage production and utilisation: Proceedings of the XIVth International Silage Conference, a satellite workshop of the XXth International Grassland Congress, July 2005, Belfast, Northern Ireland. Wageningen: Wageningen Academic Publishers. p. 184.

Olstorpe M., Schnurer J. & Passoth V., 2010. Microbial changes during storage of moist crimped cereal barley grain under Swedish farm conditions. Anim. Feed Sci. Tech. 156, 37-46.

Palva, R., Kirkkari, A-M. & Teräväinen, H. (Eds.) 2005. Viljasadon käsittely ja käyttö: Viljan tuoresäilöntä. Maaseutukeskusten Liiton julkaisuja 1012: Tieto tuottamaan 108.

Seppälä, A., Nysand, M., Mäki, M., Miettinen, H. & Rinne, M., 2012. Ensiling crimped barley grain at farm scale in plastic tube bag with formic and propionic acid based additives. In: Proceedings of the XVI International Silage Conference Hämeenlinna, Finland, 2-4 July 2012 / Edited by K.Kuoppala, M.Rinne & A.Vanhatalo. MTT Agrifood Research Finland, University of Helsinki. p. 436-437. www.mtt.fi/isc

Seppälä, A., Heikkilä, T., Miettinen, H., Mäki, M. & Rinne, M., 2015. Ensiling crimped high moisture barley with organic acids or lactic acid bacteria strains. Accepted to XVII Silage Conference, Piracicaba, Brazil, July 1-3, 2015.

Effect of acid-based additives and air stress on composition and aerobic stability of crimped maize grain ensiled in lab-scale silos

T. Pauly[1] & H. Hjelm[2]
[1]Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, VHC, Ulls väg 26, 756 51 Uppsala; [2]Perstorp AB, 28480 Perstorp, Sweden.
Correspondence: Thomas.Pauly@slu.se

Introduction

Crimping (i.e. ensilage of rolled, moist grain) is a suitable alternative to combine harvesting for grain intendeded to be used on-farm and when moist weather prevails during harvest. Farmers are recommended to apply additives during crimping. This restricts growth of fungi (yeasts & moulds) during storage and feed-out counteracting heating and moulding - the common cause of crimped grain deterioration. The most prominent factor controling fungal growth in fermented feeds is the amount of air (oxygen) that leaks into the silo or bag. That means, if no air is allowed to leak into the silo, fungal growth will be inhibited. Since there are no farm-scale silos which are completely air-tight, application of an additive with antifungal properties is advisable. Such an additive will increase the feed's tolerance to oxygen leakage and thus help to secure a high hygienic feed quality and minimizes nutrient losses.

In this lab-scale trial we studied the effect of air leakage and additives on fermentation characteristics and aerobic stability of crimped maize grain.

Materials and Methods

Maize grain was combine harvested in southern Sweden at 27% moisture content (MC) and was sent (cold storage at 6-9°C) about 650 km north to Uppsala, where the grain was stored at 4-6°C for 27 days before it was crimped (crimper: Murska 350 S2, Aimo Kortteen Konepaja Oy, Ylivieska, Finland, www.murska.fi/en) and ensiled in 1.7 -L glass jars with water-filled siphons (gas vents) mounted on the silo lids (Fig. 1). Siphons allowed some air ingress in silos driven by fluctuations in atmospheric pressure. Three samples of the grain were analysed for MC, crude protein (CP), water activity (a_w), pH and viable counts of lactic acid bacteria (LAB), yeasts and moulds. MC was determined by drying at 103°C for 6 hours and subsequent cooling in a desiccator, a_w was determined with an a_w-meter (model 5803; G.Lufft, Fellbach, Germany) and viable counts of LAB on Rogosa agar (Merck 1.05413, pH 5.5, pour plates, anaerobic cultivation at 30°C) and yeast and moulds on DG18 agar (Merck 1.00465, pH 5.6, surface spread, aerobic cultivation at 25°C).

Figure 1 Lab-scale silos with siphons and with stoppers removed for air stress treatment.

The 5 additive treatments represented 4 acid based products plus an untreated control. ProSid MI700 (Perstorp AB, Sweden) contained 66% propionic acid, 26% propionic acid glycerol esters, 2% formic acid and 1% glycerol, Crimpstore 2000S (Kelvin Cave Ltd., UK) contained over 60% formic acid, 10% propionic acid and 2.2% benzoic acid and finally propionic acid was applied at >99% purity. Recommended application rates for grain with 27% MC were 5.0 mL/kg for Crimpstore 2000S and 4.0 mL/kg for ProSid MI 700

(no recommended rates available for propionic acid). All additives in this trial were applied at the same rate: 4.0 mL/kg fresh matter (FM) equivalent to 4.0 L/metric tonne FM.

The three air treatments consisted of: 1) no air leakage, 2) one stopper in the jar bottoms and one on the lid. Stoppers (6.0 mm hole ø) were removed for 2 hours once each week (Monday) and 3) three stoppers in the jar bottoms and one in the lid were removed 3 times per week (Monday, Wednesday, Friday) for 2 hours.

An experimental plan of the study is shown in Table 1.

Table 1 Experimental plan showing treatments and number of lab-silos (= silo). Additives were applied at 4.0 mL per kg of crimped maize (27% MC)

Additive treatments	Air treatment: level of air leakage		
	no leakage	1 time/week	3 times/week
Control (C)			
ProSid MI700 (MI)			
Crimpstore 2000S (CS)			
Propionic acid (PA)			

Additives were applied to pre-weighed portions of grain filled in large, transparent plastic bags (1.15 x 0.45 m) using sterile Falcon bottles (50 mL) and ethanol treated atomizers of spray bottles. First, about half of the additive was sprayed over the grain which was spread out inside the bag, then contents were mixed by shaking the closed, inflated bag for approx. 20 s. Thereafter, this procedure was repeated with the remaining additive. The treated grain was left to rest for approx. 30 minutes before filling the autoclaved glass jars. A relatively low fill density was chosen to facilitate the movement of air during air stress treatments (density: 595 kg FM/m^3 or 434 kg DM/m^3).

Silos were stored for 62 days at 20±1°C and 70% relative humidity. During storage, all silos were frequently weighed and assessed for moulding with the help of a mould score system (Table 2). Mould scoring was regarded more useful than viable counts to assess the extent of mould growth since with mould counts are not correlated to mass. Weight losses during storage were calculated in % of the initial grain DM weight after silo filling.

Table 2 Mould score system based on the occurrence of mould growth visible through the glass jar.

Score	Visible mould occurrence in sample
0	No mould
1	<2 small colonies (<5 mm)
2	<10% of sample mouldy
3	10-30% of sample mouldy
4	30-50% of sample mouldy
5	>50% of sample mouldy

After 62 days of storage, silos were opened and sampled for DM, pH, ammonia-N, organic acids (formic, acetic, propionic, lactic, butyric) and alcohols (ethanol, 1,2-propanediol, 2,3-butanediol). Organic acids and alcohols were determined by HPLC according to Ericson & André (2010), ammonia-N using flow-injection analysis (ISO method 11732:2005) and DM and MC by drying as with the fresh grain. In addition, all samples were exposed to an aerobic

stability test that was planned for a period of 14 days but was extended to 29 days (ambient temperature 18.2±0.2°C). Grain samples (825 g FM) were aseptically transferred from silos to ethanol-treated PVC-pipes, which were covered with a piece of autoclaved geotextile at the bottom. PVC pipes were inserted into a Styrofoam block for insulation. The block was covered (top + bottom) with 10 mm thick Styrofoam boards. Air could pass through the PVC pipes via 2 holes (10 mm Ø), one at the bottom and one at the top of each pipe. Grain temperatures were recorded every 2nd hour via sensors (thermo-couples, type T) inserted into the centre of each pipe. An increase of temperature indicated the onset of aerobic microbial activity. Maximum grain temperature and time (days) until grain had reached +3°C above ambient temperature were used as indicators of aerobic stability.

The data was analysed statistically using the software package SAS 9.3 (SAS, 2014). Since most of the data were not normally distributed, the collected data could not be analysed by analysis of variance as planned. Instead the data was first ranked (PROC RANK) and then, the ranked data was analysed with PROC GLM (a method of least squares to fit general linear models). Results can be expected to be similar to the Wilcoxon Kruskal-Wallis test (acc. to U. Olsson, pers. comm.), a test often applied with data that is not normally distributed.

Results and Discussion

Analyses made on the freshly crimped grain (Table 3) showed a moisture content of 27% and relatively high viable counts of LAB, yeasts and moulds. A water activity of 0.97 indicated that fermentation activity in the crimped grain was restricted by the lack of available water.

Table 3 Composition of crimped maize (means of 3 samples) before ensilage.

MC (% FM)	a_w	pH	CP (% DM)	LAB (cfu/g FM)	Yeasts (cfu/g FM)	Moulds (cfu/g FM)
27	0.97	6.44	9.7	5.9×10^5	4.8×10^5	1.8×10^5

When silages were sampled 62 days later, pH values had decreased from 6.4 to 4.1-4.2 (Table 4). The formation of organic acids to accomplish this pH reduction was relatively small (se Table 4: 'All'), probably owing to the low buffering capacity of maize grain. Because of the restricted fermentation, weight losses were low. Losses were highest in control silages (C) but increased in all additive treatments with increasing quantities of air leakage (air stress).

Fermentation in Crimpstore 2000S-treated grain (CS) was not only restricted by the low a_w but even by the high proportion of formic acid in this additive. The concentration of propionic acid after 62 days was close to zero as in control silages (Table 4). This indicates a low anti-fungal activity of this product, since formic acid in the range shown in Table 4 is not a very efficient fungicide.

ProSid MI700 (MI) and propionic acid (PA) appeared to produce silages very similar in composition. Lactic acid, but not acetic or propionic acid, decreased in response to the intensity of air stress. Lactate concentrations, after the most intense air stress (3/w), were about 80% of the air stress-free treatment. This indicates that about 20% of the lactate was probably metabolized by yeasts or other aerobic microbes in the presence of oxygen.

The most compelling reason to apply an additive at crimping is to avoid problems with heating and moulding during feed-out. The aerobic stability test determines the sensitivity of the fermented feed to deteriorate upon exposure to air. Results of this test are shown in Table 5 together with mould scores assessed before and after the stability test (29 d).

Table 4 Composition of crimped maize after 62 days of storage. 'All' is the sum of all analysed fermentation products except ammonia. Each value represents the mean of 3 silo samples. Butyric acid, 1,2-propanediol and 2.3-butanediol were not detected

Addit.[a]	Air stress	MC	pH	Am-N	Lactic	Acetic	Formic	Propionic	All	Losses
		(% FM)		(% N)			(g/100 g FM)			(% DM)
C	no	27.3	4.16	3.1	1.46	0.3	<0.01	0.02	1.94	0.47
C	1/w	27.2	4.21	3.1	1.24	0.32	<0.01	0.02	1.63	0.6
C	3/w	27.6	4.5	2.8	1.17	0.05	<0.01	0.02	1.25	0.88
MI	no	27.3	4.09	2.8	1.22	0.17	<0.01	0.15	1.55	0.16
MI	1/w	27.4	4.18	2.7	1.04	0.18	<0.01	0.16	1.37	0.17
MI	3/w	27.3	4.22	2.7	0.98	0.19	<0.01	0.16	1.33	0.3
CS	no	27.6	4.2	4.7	0.65	0.04	0.32	0.02	1.02	0.05
CS	1/w	27.5	4.43	4.2	0.48	0.03	0.26	0.02	0.78	0.1
CS	3/w	27.8	5.27	4.7	0.46	0.01	0.13	0.02	0.61	0.34
PA	no	27.4	4.09	2.5	1.22	0.17	<0.01	0.18	1.57	0.12
PA	1/w	27.6	4.18	2.3	1.04	0.18	<0.01	0.18	1.41	0.13
PA	3/w	27.6	4.2	2.5	0.97	0.18	<0.01	0.18	1.32	0.24
Probabilities of effects:										
Additive:		*0.06*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Air stress:		*0.15*	*<0.001*	*0.19*	*<0.001*	*<0.001*	*<0.001*	*0.04*	*<0.001*	*<0.001*
Additive x Air:		*0.66*	*0.004*	*0.18*	*0.11*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

[a]C=Control ; MI=ProSid MI700; CS=Crimpstore 2000s; PA=propionic acid.

Table 5 Results of the aerobic stability test that started right after silo opening. Mould scores were assessed at silo opening (before) and after the stability test (after). Values represent means of 3 samples

		Aerobic stability test		Mould scores	
Additive[a]	Air stress	Max. temp.	Time	before	after
		(°C)	Days till +3°C	stability test	
C	no	25.5	22.8	0	4.7
C	1/w	48.5	3.11	0	5
C	3/w	50.6	0.15	0	5
MI	no	19.3	>29	0	0
MI	1/w	18.5	>29	0	0
MI	3/w	20.1	>29	0	0
CS	no	20.4	>29	0	1.3
CS	1/w	47.5	2.33	2	5
CS	3/w	48.8	0.87	3	5
PA	no	20.3	>29	0	0
PA	1/w	19.1	>29	0	0
PA	3/w	18.7	>29	0	0
Probabilities of effects :					
Additive:		*<0.001*	*<0.001*	*<0.001*	*<0.001*
Air stress:		*<0.001*	*<0.001*	*<0.001*	*<0.001*
Additive x Air:		*<0.001*	*<0.001*	*<0.001*	*<0.001*

[a]C=Control; MI=ProSid MI700; CS=Crimpstore 2000s; PA=propionic acid.

The stability test revealed the strong deteriorating influence of air leakage on aerobic stability. Control silages without air stress stayed close to ambient temperature for about 20 days until they slowly began to heat up and mould, while silages aerated once a week (1/w), heated after about 3 days and the intensively aerated silages (3/w) started to heat within 4-5 hours. Generally, the deteriorating effect on aerobic stability appeared to be larger between no air stress and air stress once a week than between air stress once and 3 times a week.

The effect of the CS additive was similar to the untreated control silages. CS did not result in more than marginal improvements in aerobic stability compared to the additive-free treatment. Moulding during air stress appeared to be stimulated by CS, compared to control silages. ProSid MI700 (MI) and pure propionic acid (PA) showed a very substantial improvement of aerobic stability and inhibited heating and moulding for more than 29 days even in intensively aerated grain silages.

Conclusions

- Air leakage during storage had a strong deteriorating impact on the rate of heating and moulding after silo opening.
- Grain treated with ProSid MI700 (MI) or with propionic acid (PA) showed no signs of heating or moulding during 29 days of aerobic exposure (both applied at 4 mL /kg). This applied even for the intensively aerated silages (3/w).
- If heating and moulding of fermented grains is an issue, an additive with high anti-fungal activity should be applied at the recommended level, particularly if air leakage of a unopened silo cannot be controlled.

References

Ericson, B. & André, J., 2010. HPLC applications for agricultural and animal science. Proc. of the 1st Nordic Feed Science Conference (June 22-23, 2010), Uppsala, Sweden, p.23-26.

ISO 11732:2005. Water quality - Determination of ammonium nitrogen - Method by flow analysis (CFA and FIA) and spectrometric detection. STD-40277, approved July 2005, Swedish Standards Institute (www.sis.se). 23 pp. http://www.sis.se/en/environment-health-protection-safety/water-quality/sewage-water/ss-en-iso-117322005

SAS, 2014. SAS Institute Inc. (www.sas.com), Cary NC, USA.

Performance and meat quality of growing bulls offered whole crop legume-cereal silages

M. Pesonen[1], M. Honkavaara[2] & A. Huuskonen[1]
[1]Natural Resources Institute Finland (Luke), Green Technology, Tutkimusasemantie 15, FI-92400 Ruukki, Finland. [2]Natural Resources Institute Finland (Luke), New Business Opportunities, Humppilantie, FI-31600 Jokioinen, Finland.
Correspondence: Maiju.Pesonen@luke.fi

Introduction

Producing whole crop small grain cereal silages provides an opportunity to improve the efficiency of forage production for ruminants under Northern European conditions. Especially in organic farming systems, annual legumes are often sown with cereals. However, there is paucity of published information on performance, carcass characteristics and meat quality of growing bulls when grass silage is replaced by whole crop legume-cereal silages. Therefore, our objective was to determine the effects of silage plant species (whole crop legume-cereals vs. grass) on animal performance, meat quality and meat fatty acid profile of growing Aberdeen Angus (AA) and Nordic Red (NR) bulls.

Materials and Methods

A feeding experiment was conducted in the experimental barn of Natural Resources Institute Finland in Ruukki. The experiment comprised in total 30 AA and 30 NR bulls. All animals, with an initial live weight (LW) of 477 (±38.7) (AA) and 363 (±65.9) (NR) kg, were purchased from commercial herds. At the start of the experiment the animals were on average 341 (±13.7) (AA) and 304 (±35.4) (NR) 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.

Experimental silages were produced at the experimental farm of Natural Resources Institute Finland in Ruukki (64°44'N, 25°15'E). The grass silage (GS) used was the regrowth from a timothy (*Phleum pratense*) sward, cut at heading stage of timothy (on August 9) using a mower conditioner, wilted for 24 h, and then harvested using a precision-chop forage harvester. Two legume-cereal mixtures used were: pea (*Pisum sativum*, cv. Florida) + wheat (*Triticum aestivum*, cv. Anniina) (PW) and faba bean (*Vicia faba*, cv. Fuego) + wheat (cv. Anniina) (FBW). The sowing rates of the cultivars were 138 kg pea with 75 kg wheat and 174 kg faba bean with 75 kg wheat per hectare. Both crops were harvested at 12 weeks after sowing using a direct-cut flail harvester. All silages were made in bunker silos and treated with a formic acid based additive applied at a rate of 5 L/tonne of fresh forage. According to botanical analysis, GS contained timothy [960 g dry matter (DM)/kg DM] and other plants (40). Respectively, PW contained pea (891), wheat (107) and other plants (2), and FBW contained faba bean (837), wheat (149) and other plants (14).

The bulls were fed a total mixed ration *ad libitum* (proportionate refusals of 5%). In the beginning of the experiment both AA and NR bulls were randomly allotted to the pens which were then randomly allotted to three feeding treatments (GSB, FBWB, PWB, four pens per diet). The compositions (g/kg DM) of these three diets were: GSB = grass silage (650) and rolled barley (350), FBWB = faba bean-wheat silage (650) and rolled barley (350), PWB =

pea-wheat silage (650) and rolled barley (350). The daily ration for the bulls included also 150 g of a mineral-vitamin mixture (Kasvuape E-Hiven, A-Rehu Ltd.). During the feeding experiment silage sub-samples were taken twice a week, pooled over periods of four weeks and stored at –20 ºC prior to analyses. The samples were analysed for DM, ash, crude protein (CP), neutral detergent fibre (NDF), indigestible NDF (iNDF), silage fermentation quality [pH, water-soluble carbohydrates (WSC), lactic and formic acids, volatile fatty acids] and 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 12 weeks and analysed for DM, ash, CP, NDF and iNDF. The metabolisable energy (ME), amino acids absorbed from small intestine (AAT) and protein balance in the rumen (PBV) values were calculated according to the Finnish Feed Tables (MTT, 2014).

The bulls were weighed on two consecutive days at the beginning of the experiment and before slaughter. The bulls were selected for slaughter based on age, and slaughtered in five batches. The target for average slaughter age was 500 days, and the slaughter age was used as the end point of the study. After slaughter the carcasses were weighed hot. The cold carcass weight was estimated as 0.98 of the hot carcass weight. Dressing proportions were calculated from the ratio of cold carcass weight to final LW. The carcasses were classified for conformation and fatness using the EUROP quality classification (EC, 2006). After the classification, the carcasses were chilled overnight below 7°C. One day after the slaughter, the pH-value of the loin (M. *longissimus dorsi*) was measured with a Knick 651 instrument with Inlab Solid electrode (Mettler Toledo) at the level of the 1st lumbar vertebra. Meat color of the loin was measured after a bloom time of half an hour with a Minolta Cr-200 handheld chroma meter (Minolta Camera Co., Ltd., Osaka, Japan). The marbling score of the loin (at the 1st lumbar vertebra) was evaluated visually by using a six-point scale (0=devoid to 5=abundant). The loin was cut at the level of the 1st lumbar vertebra, and a 3-kg loin sample between the 1st and the 5th lumbar vertebra was taken and vacuum packed for further analysis. Total ageing time of the loin samples was 12 days at 4°C. Thereafter, samples were analysed for drip loss, Warner-Bratzler shear force and for tenderness, juiciness and beef flavour (sensory analysis) as described by Pesonen *et al*. (2013). The fatty acid composition in intramuscular fat of the loin was analyzed using gas chromatography.

The results are shown as least squares means. The data were subjected to analysis of variance using the SAS GLM procedure (version 9.3, SAS Institute Inc., Cary, NC). The statistical model used was $y_{ijklm} = \mu + \delta_k + \alpha_i + \gamma_j + (\alpha \times \gamma)_{ij} + \theta_{ijm} + \beta x_{ijkl} + e_{ijklm}$, where μ is the intercept and e_{ijklm} is the residual error term associated with lth animal. α_i, γ_j and $(\alpha \times \gamma)_{ij}$ are the effects of ith diet (GSB, FBWB, PWB) and jth breed (AA, NR) and their interaction, respectively, while δ_k is the effect of the slaughtering batch (k=1,...,5) and θ_{ijm} is the effect of pen. The effect of pen was used as an error term when differences between treatments were compared because treatments were allocated to animals penned together. Initial live weight was used as a covariate (βx_{ijkl}) in the model. Differences between the treatments were tested using orthogonal contrasts: (1) AA vs. NR, (2) GSB vs. whole crop legume-cereal silage diets (FBWB + PWB), and (3) FBWB vs. PWB. Since the interactions between breed and feeding treatments were not statistically significant (P>0.10 for all variables), the P-values of the interactions are not presented.

Results and Discussion

Chemical composition and feeding values of the experimental feeds and total mixed rations used are presented in Table 1. The CP concentration of FBW and PW was 19 and 35% higher compared to GS, respectively. The grass silage had a higher NDF and lower iNDF concentration compared to FBW and PW. Further, GS had a 4% higher ME content than the whole crop legume-cereal silages. The fermentation characteristics of all three silages were good as indicated by the low pH value and the low concentrations of ammonia N in total N and total fermentation acids. Barley grain used in the experiment had typical chemical composition and feed values. Due to differences in composition of the experimental silages, FBWB and PWB rations included 14 and 25% more CP compared to GSB, respectively. Further, FBWB and PWB rations included less NDF and more iNDF compared to GSB.

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

	Feeds				Total mixed rations		
	GS	FBW	PW	Barley	GSB	FBWB	PWB
Dry matter (DM), g/kg feed	289	277	269	883	378	365	356
Organic matter (OM), g/kg DM	939	935	930	971	951	948	944
Crude protein, g/kg DM	129	154	174	107	121	138	151
Neutral detergent fibre (NDF), g/kg DM	580	465	427	210	451	376	351
Indigestible NDF, g/kg DM	101	148	148	37	79	109	109
Metabolisable energy, MJ/kg DM	10.1	9.7	9.7	13.2	11.2	10.9	10.9
AAT, g/kg DM	75	81	83	95	82	86	87
Protein balance in the rumen, g/kg DM	17	36	53	-36	-2	11	22
Digestible OM in DM, g/kg DM	629	608	608	821	696	683	683
Fermentation quality of the experimental silages							
pH	3.96	3.79	3.98				
Volatile fatty acids, g/kg DM	14	13	16				
Lactic + formic acid, g/kg DM	43	49	66				
Water soluble carbohydrates, g/kg DM	59	30	28				
In total N, g/kg							
NH_4N	43	49	66				
Soluble N	410	398	500				

GS = grass silage, FBW = faba bean-wheat silage, PW = pea-wheat silage, GSB = grass silage (650 g/kg DM) + rolled barley (350), FBWB = faba bean-wheat silage (650) + rolled barley (350), PWB = pea-wheat silage (650) + rolled barley (350), AAT = Amino acids absorbed from small intestine

During the experiment the total DM intake (DMI) of the AA bulls was 12% higher compared to the NR bulls (Table 2). However, DMI in relation to LW was 11% higher in the NR bulls compared to the AA bulls, which is in agreement with previously reported findings. It is stated that dairy breeds have a higher intake than beef breeds because the genetic selection for higher milk yield has resulted in dairy animals having a larger gastrointestinal tract and a higher feed intake capacity (Langholz, 1990). Due to higher total DMI, daily ME and CP

intakes of the AA bulls were higher compared to the NR bulls. Also feeding treatments affected intake parameters. The FBWB diet tended to increase DM and energy intakes of the bulls compared to GSB and PWB diets (Table 2). Crude protein intake was higher with whole crop legume silage feedings compared to GSB. As expected, the AA bulls grew faster than the NR bulls but there were no treatment differences among feeding treatments in LW gain or carcass gain. The carcass weight, dressing proportion, carcass conformation score and carcass fat score of the AA bulls were higher than the corresponding values of the NR bulls. Corresponding differences in growth and carcass characteristics between beef and dairy breeds has been demonstrated earlier by for example Kempster and Southgate (1984). The carcass weight of the FBWB bulls was 9% higher compared to the PWB bulls. It was probably due to differences in carcass weight that the FBWB bulls were better conformed than PWB bulls.

Feeding treatments had only minor effects on meat quality parameters (Table 3). The pH-value of *longissimus dorsi* muscle was slightly lower with whole crop legume silage feedings compared to GSB. In addition, whole crop legume silage feedings tended to increase marbling score compared to GSB. However, feeding did not affect drip loss, colour, shear force, sensory analysis or fatty acid profile of *longissimus dorsi* muscle. On the contrary, breed had clear effects on meat quality. The loin of the AA bulls had lower pH and WB shear force values and higher marbling score compared to the NR bulls. Further, muscle lightness (L), redness (a) and yellowness (b) values of the AA bulls were higher than those of the NR bulls. In the sensory analyses, the AA bulls got higher scores in tenderness and juiciness compared to the NR bulls. The loin samples of the AA-bulls contained a higher proportion of saturated fatty acids and tended to contain lower proportion of monounsaturated fatty acids compared to the NR bulls. The *n*-6/*n*-3 fatty acid ratio of the NR-bulls was 40% higher than the corresponding value of the AA-bulls, on average.

Conclusions

Breed differences between the AA and NR bulls in growth, carcass traits and meat quality were observed when the bulls were slaughtered at the age of 500 days. The results indicate that AA bulls produced meat with a lower n-6/n-3 fatty acid ratio compared to NR bulls. According to this study replacing moderately digestible timothy silage by whole crop legume-cereal silages in the diet did not have any remarkable effects on animal performance, carcass characteristics or meat quality of the growing bulls.

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.

Kempster, A.J. & Southgate, J.R., 1984. Beef breed comparisons in the U.K. Livest. Prod. Sci. 11, 491-501.

Langholz, H.J., 1990. High yielding cattle populations – concurring and compatible traits with special reference to reproductive efficiency (Review). Reprod. Domestic Anim. 25, 206-214.

MTT, 2014. Feed tables and nutrient requirements. MTT Agrifood Research Finland. Retrieved 8 April 2015, from http://www.mtt.fi/feedtables.

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.

Table 2 Intake, growth performance and carcass characteristics of the bulls fed different total mixed rations

Breed	Angus			Nordic Red			SEM	Orthogonal contrasts (*P*-values)		
Feeding	GSB	FBWB	PWB	GSB	FBWB	PWB		1	2	3
Number of observations	9	10	10	10	10	10				
Duration of the experiment, d	155	165	150	203	215	194	5.7	<0.001	0.75	0.02
Initial live weight, kg	481	473	478	373	366	349	11.7	<0.001	0.34	0.63
Final live weight, kg	708	713	707	609	622	570	14.7	<0.001	0.67	0.09
Slaughter age, d	496	503	495	504	522	500	8.2	0.17	0.56	0.12
Intake										
Dry matter (DM), kg/d	12.20	13.40	12.15	10.95	12.33	10.44	0.380	0.005	0.18	0.006
DM intake, g/kg live weight	20.5	22.6	20.5	22.6	25.2	23.1	0.82	0.01	0.12	0.04
Metabolizable energy (ME), MJ/d	136	143	132	123	132	113	4.1	0.005	0.85	0.01
Crude protein (CP), g/d	1457	1840	1834	1317	1688	1565	51.0	0.004	<0.001	0.25
Live weight gain, g/d	1479	1496	1569	1162	1197	1140	40.3	<0.001	0.42	0.84
Carcass gain, g/d	866	837	868	589	633	599	21.3	<0.001	0.74	0.95
Feed conversion										
Kg DM/kg carcass gain	14.2	16.1	14.1	18.9	19.8	17.9	0.29	<0.001	0.13	<0.001
MJ ME/kg carcass gain	159	172	154	211	212	194	3.1	<0.001	0.47	0.001
Carcass characteristics										
Carcass weight, kg	379	376	372	302	314	287	7.6	<0.001	0.62	0.02
Dressing proportion, g/kg	535	528	526	496	506	502	2.7	<0.001	0.94	0.30
Conformation, EUROP	8.4	8.5	7.7	4.9	5.1	4.9	0.20	<0.001	0.67	0.04
Fat score, EUROP	3.1	3.2	3.3	2.3	2.5	1.9	0.13	<0.001	0.91	0.09

GSB = grass silage (650 g/kg DM) + rolled barley (350), FBWB = faba bean-wheat silage (650) + rolled barley (350), PWB = pea-wheat silage (650) + rolled barley (350), orthogonal contrasts: 1 = Angus vs. Nordic Red, 2 = GS vs. whole crop legume-cereal silages (FBW + PW), 3 = FBW vs. PW, SEM = standard error of mean

Table 3 Meat quality and fatty acid profile (M. *longissimus dorsi*) of the bulls fed different total mixed rations

Breed	Angus			Nordic Red			SEM	Orthogonal contrasts (*P*-values)		
Feeding	GSB	FBWB	PWB	GSB	FBWB	PWB		1	2	3
Number of observations	8	8	8	8	8	8				
Quality of *longissimus dorsi* muscle										
pH (24 h *post mortem*)	5.57	5.53	5.52	5.66	5.58	5.61	0.026	0.008	0.009	0.91
Marbling score (0 = devoid, 5 = abundant)	1.56	2.31	1.97	1.06	1.56	1.22	0.295	0.004	0.08	0.71
Drip loss, %	0.22	0.22	0.19	0.27	0.33	0.36	0.031	0.01	0.32	0.98
Shear force, N/4 cm^2	48.8	49.6	48.1	58.8	54.6	63.0	4.11	0.007	0.98	0.82
Colour										
L (lightness)	36.2	36.9	36.8	34.4	34.6	33.9	0.77	<0.001	0.71	0.97
a (redness)	22.6	23.0	22.7	21.1	23.0	21.3	0.81	0.02	0.32	0.51
b (yellowness)	7.5	8.0	7.1	6.5	6.9	6.3	0.43	0.003	0.77	0.15
Sensory analysis										
Tenderness	5.8	5.7	5.9	5.1	5.2	5.0	0.27	0.009	0.95	0.56
Juiciness	5.7	5.7	5.8	5.4	5.5	5.4	0.15	0.02	0.82	0.59
Beef flavour	5.9	5.8	5.7	5.6	5.7	5.5	0.13	0.19	0.41	0.86
Fatty acid profile of *longissimus dorsi* muscle (% of total fatty acids)										
Saturated fatty acids	45.92	44.33	44.11	42.44	43.29	44.52	1.062	0.01	0.98	0.48
Monounsaturated fatty acids	46.27	47.20	47.72	48.99	47.41	46.79	1.001	0.06	0.91	0.96
Polyunsaturated fatty acids	6.71	7.52	7.38	7.53	8.18	7.89	0.677	0.27	0.55	0.39
*n*6/*n*3fatty acid ratio	3.01	3.19	3.09	4.32	4.26	4.47	0.134	<0.001	0.34	0.91

GSB = grass silage (650 g/kg DM) + rolled barley (350), FBWB = faba bean-wheat silage (650) + rolled barley (350), PWB = pea-wheat silage (650) + rolled barley (350), orthogonal contrasts: 1 = Angus vs. Nordic Red, 2 = GS vs. whole crop legume-cereal silages (FBW + PW), 3 = FBW vs. PW, SEM = standard error of mean, Sensory analysis: scale from 1 to 7, tenderness: 1 = very tough, 7 = very tender, juiciness: 1 = very dry, 7 = very juicy, beef flavour: 1 = very non beef like, 7 = very beef like.

Relationship between net energy intake by pregnant beef cows and dietary chewing index of roughage diets

M. Vestergaard Nielsen[1]*, M. Jardstedt[2], E. Nadeau[2], B. Markussen[3], A. Hessle[2], M. Falkesgaard[1] & P. Nørgaard[1]

[1]Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 3, 1870 Frederiksberg, Denmark. [2]Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Gråbrödragatan 19, SE-532 31 Skara, Sweden. [3]Laboratory of Applied Statistics, Faculty of Sciences, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark. Correspondence: mettevn@sund.ku.dk

Introduction

Mathematical models have proven to be a powerful tool for improving animal performance (Tedeschi *et al*. 2005). A new model for prediction of feed intake under Scandinavian conditions could help optimize beef cow feeding and, thereby, improve productivity in cow-calf herds. Nørgaard & Mølbak (2001) have presented a linear model describing net energy intake (NEI) relative to metabolic bodyweight (BW) as a linear function of the chewing index (CI) of the ration for lactating dairy cows, non-lactating non-pregnant cows, growing steers and bulls fed forages *ad libitum* and concentrates restrictively. They found that the NEI decreased with increasing dietary CI: $NEI=NE_0-b*CI$. The slope values were highly correlated with the squared intercept values (NE_0). Nørgaard & Mølbak (2001) hypothesized that the NE_0 was the theoretical maximum energy intake capacity of cattle. This model was able to describe feed intake over two very different physiological stages; lactation and growth. Hence, the model could possibly be used to describe feed intake of pregnant beef cows.

It has been observed that feed intake decreases in the late stages of pregnancy in dairy heifers (Ingvartsen *et al*. 1992) and there was also a tendency for a decreasing intake in pregnant beef cows (McGee *et al*. 2005). This is counterintuitive as requirements of cows rise with growth of the foetus, suggesting that intake should increase with increasing pregnancy, especially during late pregnancy. The decreasing intake could be related to hormonal changes of appetite (Ciccioli *et al*. 2003), increasing body condition score (BCS; Thompson *et al*. 1983) and the growing foetus limiting ruminal space (Forbes 1970; Robinson 1999).

The aim of this study was to modify the linear relationship between NEI and CI of feed rations as described by Nørgaard & Mølbak (2001) to be used for Scandinavian beef cows during pregnancy. Within the linear model, it was intended to test different variations of the model to describe the decreasing feed intake with increasing pregnancy, either as a direct effect of the week in pregnancy, or as an effect of change in BW during the pregnancy period.

Materials and Methods

The investigation was based on feed intake data from two experiments at Götala Beef and Lamb Research Centre, Swedish University of Agricultural Sciences, Skara, Sweden. The data consisted of weekly group mean feed intake values from 84 pregnant beef cows, distributed on seven different dietary treatments. In Exp. 1, the cows were housed in groups of four animals, in Exp. 2, the cows were housed in groups of three animals. Hereford and Charolais breeds were included in Exp. 1, whereas only Hereford cows were used in Exp. 2. In Exp. 1, the mean initial BW was 690 (sd=16) kg for the Hereford cows and 769 (sd=9) kg for the Charolais cows. In Exp. 2, the mean initial BW of the Hereford cows was 725 (sd=50) kg. The cows were weighed every week in Exp.1 and every second week in Exp. 2. All cows were multiparous, and all cows gave birth to one calf after the experiments, except for one

cow that lost her calf during Exp. 1 and three cows that gave birth to twins after Exp. 2. All cows were fed *ad libitum* roughage. The diets in Exp.1, which was conducted in 2012/2013, were either grass dominated silage (G1), reed canary grass silage (RC1) or whole-crop oat silage (WCO). The diets in Exp. 2, which was conducted in 2013/2014, were either *Festulolium* silage (FE; a cross between tall fescue and perennial ryegrass), grass dominated silage (G2), reed canary grass silage (RC2) or barley straw (BS; Table 1). The FE, BS and WCO diets were supplemented with urea and the BS diet was also supplemented with 0.50 kg dry matter (DM) rapeseed meal until eight weeks before expected calving and with 0.69 kg DM until calving. For all diets, the theoretical chopping length of the roughage was 70 mm.

Table 1. Nutritional characteristics[1] of the different diets in the two experiments represented as treatment means within experiment, with standard deviations in brackets. The number of observations for the treatment means is given by *n*

Exp.	Diet	NDF_r (g/kg DM) *n* = 2-3	$iNDF_r$ (g/kg NDF) *n* = 2-3	CP_r (g/kg DM) *n* = 2-3	$IVOMD_r$ (%) *n* = 1-2	NEL20 (MJ NEL20) *n* = 1-2	CI_{cor} (min/ MJ NEL20) *N* = 228
1	G1	585 (14.0)	223 (8.5)	83 (3.5)	77	5.3	16.6 (0.39)
1	RC1	651 (9.6)	311 (2.6)	119 (1.2)	63	4.3	24.2 (0.38)
1	WCO*	546 (47.5)	343 (18.7)	45 (4.2)	63	3.9	22.6 (1.80)
2	FE*	544 (9.6)	208 (8.9)	99 (2.6)	78 (1.2)	5.3 (0.10)	15.2 (0.62)
2	G2	571 (15.0)	280 (8.5)	109 (2.9)	70 (0.37)	4.7(0.06)	18.8 (0.33)
2	RC2	639 (10.6)	348 (12.0)	135 (12.0)	59 (1.16)	4.0 (0.04)	26.0 (0.59)
2	BS*†	772 (11.7)	255 (4.4)	72 (2.8)	63 (0.65)	3.7 (0.02)	31.7 (0.55)
Mean		626 (85.6)	276 (51.9)	89 (41.1)	68 (0.85)	4.5 (0.62)	22.6 (6.10)

[1] G1=Grass silage from Exp. 1, RC1=Reed canary grass silage from Exp. 1, WCO=Whole-crop oats silage, FE= *Festulolium* silage, G2=Grass silage from Exp. 2, RC2= Reed canary grass silage from Exp. 2, BS= Barley straw. NDF_r is neutral detergent fibre concentration of roughage (g/kg DM), $iNDF_r$ is indigestible NDF concentration of the roughage (g/kg NDF), CP_r is crude protein concentration of roughage (g/kg DM), $IVOMD_r$ is the *in vitro* organic matter digestibility of the roughage (% of organic matter). CI_{corr} is the CI values for the ration calculated by the NorFor method and corrected for BW and roughage NDF intake per BW. *Roughage supplemented with urea, †Roughage supplemented with rapeseed meal.

The NEI was calculated from the estimated apparent digestibility of crude protein, crude fat and carbohydrates at a standard daily DM intake of 20 kg (NEL20) as customary in the NorFor digestive kinetic model (Volden & Nielsen 2011) and expressed per kg metabolic BW (NEI; MJ NEL20 per kg $BW^{0.75}$per day), were BW is the mean BW in early pregnancy. The concentration of rumen indigestible neutral detergent fiber (iNDF) in Exp. 2 was estimated from the NDF concentration and *in vitro* organic matter digestibility based on the work by Eriksson (2010) and Åkerlind *et al*. (2011). The NorFor CI value of the roughages was estimated from the concentration of NDF, the iNDF concentration (g per kg NDF) and the theoretical chopping length (mm; Nørgaard *et al*., 2011). The CI values were corrected for BW at start of the experiments and for the deviations of roughage NDF intake from 0.7% of the BW (Nørgaard *et al*., 2011). The CI was expressed in min per MJ of NEL20. The rapeseed meal used in Exp. 2 was given a CI value of 4 min/kg DM as described by Nørgaard *et al*. (2011). The NEI was analysed as a function of corrected CI as described in Equation 1:

$$NEI = NE_0 - k*NE_0^2 *CI_{cor} + \varepsilon \quad \text{(Equation 1)}$$

where NEI is the net energy intake (MJ NEL20/day/kg $BW^{0.75}$), NE_0 is the intercept (MJ NEL20/d/kg $BW^{0.75}$), and is considered the theoretical intake capacity, $k*NE_0^2$ is the slope (unit for k is day*kg $BW^{0.75}$/min), which describes the reduction in energy intake with increasing CI, and CI_{cor} is the CI of the ration corrected as described above (CI; min/MJ NEL20). The unit of k is day*kg $BW^{0.75}$/min (an arbitrary unit) to achieve unit balance.

In the present investigation numerous variants were applied to the NE_0 and k of the model. NE_0 and k were tested as a linear function of week of pregnancy, and NE_0 were tested as a linear function of change in BW during pregnancy (Table 2, columns 1 and 2). The change in BW during pregnancy describes the accumulated change in BW from the start of each experiment (ΔBW). The different models were described by non-linear mixed effects modelling in R (version 2.15.2). The different model variations were compared by Bayesian information criterion (BIC; Schwartz, 1978), R-square and root mean squared prediction error (RMSPE; Bibby and Toutenburg 1977), but not on external data. The linearity of the models were evaluated by plots of normal quantile plots and by residual plots, and the systematic effects were tested by Walds test.

Results and Discussion

A visualization of NEI as a function of the corrected CI of the rations showed a pattern suggesting a linear relationship (Figure 1).

Table 2 shows that Model 6 had the highest R^2, the lowest BIC and a RMSPE closest to 0, thus, this model was the best estimate for describing Equation 1.

The standardized residual plot of Model 6 showed no pattern of distribution, and the normal quantile plot showed a linear relationship between quantiles of standard normal residuals versus standardized residuals. The estimates of Model 6 are presented in table 3.

Model 6 uses the accumulated change in BW to describe the change in theoretical intake capacity, NE_0, suggesting that the change in BW is a key factor in determining the change in the theoretical intake capacity of a pregnant beef cow, and that the theoretical intake capacity decreases with increasing ΔBW.

The cows in this study increased in BW over the pregnancy period, some of them as much as 140 kg (results not shown). This suggests that they were gaining BCS as well as increasing foetal BW. The cows that gained most BW decreased their feed intake, as supported by Model 6. In Model 6 NE_0 is a function of ΔBW, indicating that the metabolic capacity of the cows declined with increasing ΔBW. This could explain the observed decline in feed intake with increasing pregnancy (Ingvartsen *et al*. 1992, McGee *et al*. 2005) and further supports the findings of Thomson *et al*. (1983) that increased BCS of pregnant Angus-Hereford beef cows was associated with a lower energy requirement.

Conclusions

This study supports the hypothesis that NE intake of pregnant beef cows decreases linearly with increasing dietary chewing index. When the slope is proportional to the squared intercept, the theoretical intake capacity appears to be negatively related with accumulated BW changes. Change in BW with increasing pregnancy results in a decrease in theoretical intake capacity, and explains better the decrease in feed intake observed with increasing

pregnancy stage than week of pregnancy. Thus, the model proposed by Nørgaard & Mølbak (2001) expanded with change in BW could also be relevant for pregnant beef cows.

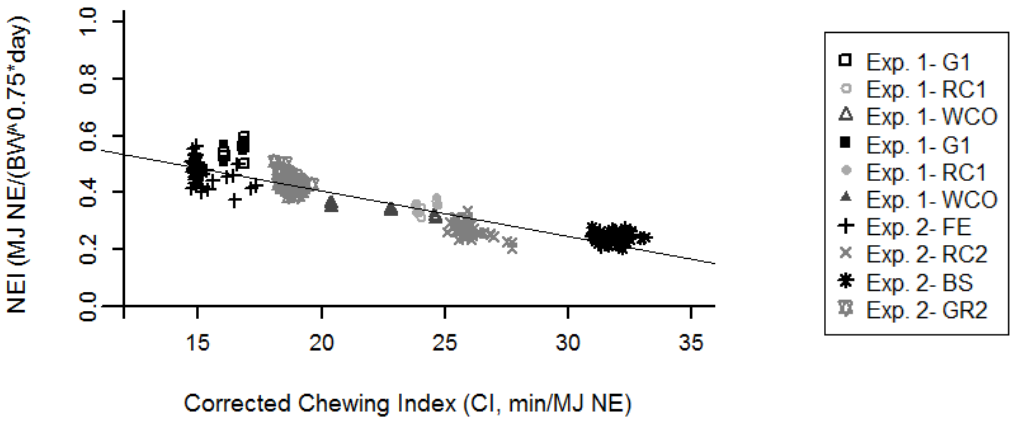


Figure 1. Daily NEI as a linear function of the dietary chewing index in pregnant beef cows fed concentrate restrictively and roughage *ad libitum*. In Exp.1, G1, RC1 and WCO correspond to the treatments grass silage, reed canary grass silage and whole crop oat silage. The open points represent the light breed, Hereford, and the closed points represent the heavy breed, Charolais. In Exp. 2, FE, G2, RC2 and BS correspond to the treatments *Festulolium* silage, grass silage, reed canary grass silage and barley straw. The line corresponds to a simple linear regression made on NEI=CIcor.

Table 2. Variations of the model NEI= NE_0- k*NE_0^2*CI_{cor}, by number (No) and accompanying fixed (F) and random (R) effects[1]. For each model the r-square (R^2), the Bayesian information criterion (BIC) and the root mean squared prediction error (RMSPE) are given

No	Model	R^2	BIC	RMSPE
1	NEI= NE_0- k(week)*NE_0^2*CI_{cor}+ε	0.87	-801	0.038
F	k = p + q*week			
R	$NE_{0,week,exp}$ = α + $A_{week,exp}$ + B_{exp}			
2	NEI= NE_0(week)- k*(NE_0(week))^2*CI_{cor}+ε	0.86	-797	0.039
F	NE_0 = n + m*week			
R	$k_{week,exp}$ = β + $A_{week,exp}$ + B_{exp}			
3	NEI= NE_0(week)- k(week)*(NE_0(week))^2*CI_{cor}+ε	0.87	-796	0.038
F	NE_0=n + m*week, k = p + q*week			
R	$NE_{0,exp}$ = α + A_{exp} , k=β + B_{exp}			
4	NEI= NE_0(week+ΔBW)- k(week)*(NE_0(week+ΔBW))^2*CI_{cor}+ε	0.87	-791	0.038
F	NE_0=n + m*week+l*ΔBW, k = p + q*week			
R	$NE_{0,exp}$ = α + A_{exp} , k=β + B_{exp}			
5	NEI= NE_0(ΔBW)- k(week)*(NE_0(ΔBW))^2*CI_{cor}+ε	0.85	-773	0.040
F	NE_0=n + m*ΔBW, k = p + q*week			
R	$NE_{0,exp}$ = α + A_{exp} , k=β+ B_{exp}			
6	NEI= NE_0(ΔBW)- k*(NE_0(ΔBW))^2*CI_{cor}+ε	0.88	-814	0.037
F	NE_0=n + m*ΔBW			
R	$NE_{0,exp}$ = α + A_{exp} , k=β + B_{exp}			
7	NEI= NE_0(ΔBW)- k*(NE_0(ΔBW))^2*CI_{cor}+ε	0.85	-776	0.041
F	NE_0=n + m*ΔBW,			
R	$NE_{0,period}$= α + A_{period}, k=β + B_{period}			

[1] The 'exp' describes the experiment, and 'week' describes the week of pregnancy. ΔBW describes the accumulated BW changes from the initial BW to the specific week in pregnancy. P,q,l,n and m are constants used to describe a linear function. The α and β are general values of NE_0 or k before applying random effect of experiment, or experiment and week within experiment, as noted by A and B.

Table 3. Estimated parameter values from the linear regression NEI= NE_0- k* NE_0^2*CI_{cor}, and the linear function of NE_0(ΔBW)=n+m*(ΔBW), model 6, expressed with fixed effects, standard error and significance for the pregnant beef cows.

Parameter	Estimate	SE	P-value
k (day*kg $BW^{0.75}$/min)	0.02941	0.00097547	<0.001
n (MJ NEL20/(day*$BW^{0.75}$)	0.85048	0.094823	<0.001
m (MJ NEL20/(day*$BW^{0.75}$)	-0.0003963	0.00020307	0.0522

References

Bibby, J. & Toutenberg, H., 1977. Prediction and Improvement Estimation in Linear Models. John Wiley, London, pp. 16–19.

Ciccioli, N.H., Wettemann, R.P., Spicer, L.J., Lents, C.A., White, F.J. & Keisler, D.H., 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J. Anim. Sci., 81, 3107–3120.

McGee, M., Drennan, M.J. & Caffrey P.J., 2005. Effect of suckler cow genotype on energy requirements and performance in winter and subsequently at pasture. Irish Journal of Agricultural and Food Research, 44, 157-171.

Eriksson, T., 2010. In vitro methods for indigestible Neutral Detergent Fiber (iNDF): Proc.1st Nordic Feed Science Conference, Uppsala, Sweden, 22-23 June, 2010.

Forbes, J., 1970. Voluntary food intake of pregnant ewes. J. Anim. Sci., 31(6), 1222-1227.

Ingvartsen, K.L., Andersen, H.R. & Foldager, J., 1992. Effect of sex and pregnancy on feed intake capacity of growing cattle. Acta Agric. Scand., Sect. A, Animal Sc., 42, 40-46.

Nørgaard, P. & Mølbak, L., 2001. Relation between dietary chewing index value and net energy intake in cattle fed concentrates restrictively and forage ad libitum. EAAP Publication. 103, 67-70.

Nørgaard, P., Nadeau, E., Randby, Å. & Volden, H., 2011. 11. Chewing index system for predicting physical structure of the diet. EAAP publications No. 130 (eds. H. Volden). Wageningen Academic Publishers, The Netherlands, 2011, p. 127-132.

Robinson, J., Sinclair, K., Randel, R. & Sykes, A., 1999. Nutritional Management of the Female Ruminant: Mechanistic Approaches and Predictive Models. In H.J. Jung, and G. C. Fahey Jr., (eds.), Nutritional Ecology of Herbivores, Proceedings of the Vth International Symposium on the Nutrition of Herbivores, 11-17 April 1999, San Antonio, TX, USA, p. 550-608.

Schwarts, G., 1978. Estimating the dimension of a model. The Annals of Statistics, 6 (2), 461-464.

Tedeschi, L. O., Fox, D. G., Sainz, R. D., Barioni, L. G., de Medeiros, S. R. & Boin, C., 2005. Mathematical models in ruminant nutrition. Scientia Agric., 62, 76-91.

Thompson, W.R., Meiske, J.C., Goodrich, R.D., Rust, J.R. & Byers, F.M., 1983, Influence of Body Composition on energy requirements of beef cows during winter. J. Anim. Sci., 56 (5), 1241-1252.

Volden, H. & Nielsen, N.I., 2011. 8. Energy and metabolizable protein supply. EAAP publications No. 130 (eds. H. Volden). Wageningen Academic Publishers, The Netherlands, 2011, p. 81-84.

Åkerlind, M., Weisbjerg, M., Eriksson, T., Tøgersen, R., Udén, P., Ólafsson, B.L., Harstad, O.M. & Volden, H., 2011. 5. Feed analyses and digestion methods. EAAP publications No. 130 (eds. H. Volden). Wageningen Academic Publishers, The Netherlands, 2011, p. 41-54.

What does the cow and NorFor say about sugar in the diet?

J. Bertilsson[1], T. Eriksson[1] & M. Åkerlind[2]
[1]*Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Box 7024, 750 07 Uppsala, Sweden.* [2]*Växa Sweden, Box 288, 75105 Uppsala, Sweden.*
Correspondence: Jan.Bertilsson@slu.se

Introduction

Dairy farming will need to become more sustainable and incorporate environment enhancing practices. Moves towards a more sustainable dairy production are impeded by the low efficiency of nitrogen conversion from plant to animal protein. This constraint is particularly problematic with ensiled forage. Provision of additional energy in form of water soluble carbohydrates (WSC) might be a way of increasing nitrogen efficiency especially if this is combined with legume protein. Studies on this was performed in the EU funded program SWEETGRASS (Grazing and ensiling of energy rich grasses with elevated WSC content for sustainable production of ruminant livestock, QLK-CT-2001-0498). Perennial ryegrass of a standard cultivar (cv. Fennema) was compared with a cultivar bred in the UK for enhanced WSC content (cv. AberDart). In this paper, we focus on comparisons between predictions from the Nordic feed evaluation system NorFor (Volden, 2011) and results from the SWEETGRASS trials with respect to production and rumen function.

Materials and Methods

The experiments took place at the Kungsängen Research Centre with tied-up multiparous cows of the Swedish Red Breed. The trials were carried out according to an incomplete change-over design (Year 1 and 2 resp.): 16 or 12 cows; 4 or 3 blocks; 4 treatments and 3 periods. The period lengths were 4 or 3 weeks for respectively year. The main recording of data took place during the last week of each period. Collection of urine and spot sampling of faeces were done on 8 cows each year. Four of the cows were rumen fistulated enabling extensive sampling of rumen content.

Pure swards of the two cultivars of perennial ryegrass (Standard and high WSC) and red clover (cv. Vivi) were established in one year and harvested for the first time in the following year. The crops were wilted 24 hours before baling and wrapped with 8 layers of plastic. Kofasil Ultra™ was used as an additive. In Year 1, the first and second cuts of both cultivars of grass and the first cut of red clover were used. In Year 2, poor winter survival of the rye-grass disabled the possibility to feed different qualities of silage.

The baled grass silages were mixed with red clover twice weekly and stored outdoors before feeding. In Year 1, the proportion was 75/25 grass/clover on DM basis. In Year 2, the different ryegrasses of both cultivars were first mixed into a basic grass silage mix. Then red clover was added, either in proportions of 75/25 or 50/50 (grass/clover). Household sugar (sucrose) was then mixed with the silages to create the targeted WSC contents of 10 and 20% on a DM basis.

In both years, silages were fed *ad lib.,* while concentrates were fed in fixed amounts. The concentrates were designed according to certified organic production and consisted of (per 100 kg as fed): oats 26, barley 32, rapeseed cake 12, field peas 30. Feed samples were taken daily during the collection period and frozen for later analyses. Faeces were spot sampled in morning and afternoon on five consecutive days, frozen directly and later mixed and analysed

individually. Total urine collections were performed using collection cups attached to a harness on the cow by rubber strings and urine was lead to a container with 10 % sulphuric acid ensuring pH below 3. Urine amount was weighed daily, and sub-samples were frozen for later analyses. Spot samples of rumen liquid were taken at different times covering all 24 hours of the day. These samples were frozen for later analyses of VFA and ammonia while pH was determined directly. Milk production was recorded on two consecutive days through Truetest^{TM} and milk samples were taken and analysed at each milking. All analyses (chemical and statistical) were performed by standard methods as indicated by Bertilsson & Murphy (2003) and Volden (2011). Indigestible NDF was estimated in vitro as described by Eriksson (2010).

Animal and feed characteristics and feed intake were used as inputs for theoretical calculations. The evaluation was performed with the Nordic feed system, NorFor® 2008, using the most recent equation set of the feed ration calculator (FRC version 1.81). . Calculations and estimates were made for rumen load index (the sum of sugar and rumen degraded starch divided by the sum of intake of NDF and sugar-free rest fraction) and the NorFor predicted milk protein yield, total excretion of nitrogen in faeces and urine and total digested NDF and nitrogen.

Results and Discussion

In Year 1 (Table 1a), there were no differences in WSC between cultivars, but there were significant differences between cuttings. While WSC content in the herbage was as high as 180 g x kg DM^{-1} in the high WSC ryegrass in the first cut, it was around 130 g after ensiling and finally after being mixed with 25% red clover, it was around 110 g (see Table 1a). The standard WSC ryegrass had a somewhat lower WSC content. There were only numerical differences in production results due to rye-grass cultivar but some significant although small intake differences, with largest intake from 1st cut. When fed 1^{-st} cut silage, the cows

Table 1a. Chemical composition of the diets in Year 1 in g kg^{-1} DM, unless otherwise stated. Means and standard deviations (within brackets)

	Standard WSC*		High WSC*		Concentrate
	1-st cut	2-nd cut	1-st cut	2-nd cut	
DM	468 (3)	370 (1)	440 81)	406 (2)	872 (1))
Ash	86 (3)	103 (1)	90 (1)	104 (2)	36 (2)
WSC	100 (3)	39 (12)	111 (14)	47 (4)	40 (2)
CP	161 (0.3)	171 (1)	159 (1)	161 (0.3)	173 (3)
NDF	404 (3)	434 (2)	413 (11)	422 (3)	202 (7)
iNDF, g/kg NDF	154	138	160	132	265
kdNDF, %/h	4.4	4.8	4.3	4.9	5.1

*Silages are mixtures of ryegrass and red clover, 75/25 on DM basis.

Table 1b Feed intake and milk production for cows fed different silages in Year 1 (kg x cow^{-1} x day^{-1}). LS-means, standard errors of the mean and p-values of differences

	Cultivar and cut							
	Standard WSC		High WSC			Difference (p<)		
	1-st cut	2-nd cut	1-st cut	2-nd cut	SEM	Cultivar	Cut	Cultivar*Cut
Silage DM	14.8	15.1	15.5	13.9	0.5	0.4	0.03	0.002
Concentrates DM	7.0	7.0	7.0	7.0	0.05	-	-	-
WSC	1.75	0.85	2.01	0.94	0.06	<0.001	<0.001	0.003
CP	3.58	3.78	3.54	3.44	0.5	0.0001	0.26	0.007
ECM	30.2	29.9	30.0	29.4	1.0	0.60	0.50	0.75

consumed ca. 1 kg more WSC compared to when fed the 2^{-nd} cut (Table 1b). There were tendencies for less N in urine and more in faeces when more WSC was consumed (figures not shown). It should, however, be noted that the CP intake was lower when WSC intake was higher (See Table 1b).

In Year 2**,** the different levels of WSC content in silage were created by mixing sugar into the silage mixtures. By this, the WSC content in the silage was raised from just above 100 g to 190-200 g x kg DM^{-1} (Table 2a). When adding sugar to the diet, the WSC intake was nearly doubled. Although the differences in production result were small, it is interesting to note that concentrate refusals increased (shown as lower concentrate intake, Table 2b) when WSC intake increased. Nitrogen partitioning to urine decreased, while there was an increase in faeces. N in milk as proportion of N in feed increased significantly from 0.23 to 0.26 with higher sugar content in the diet, as a mean across both clover inclusion levels (data not shown).

Table 2a Chemical composition of the diets in Year 2, g x kg DM^{-1} unless otherwise stated. Means and standard deviations (within brackets)

	Clover25[1] No sugar	Clover50[1] No sugar	Clover25[1] Plus sugar	Clover50[1] Plus sugar	Conc.
DM	320 (8)	325 (15)	347 (2)	334 (5)	874 (9)
Ash	89 (1)	87 (4)	82 (1)	84 (1)	33 (1)
WSC	115 (4)	102 (6)	198 (16)	189 (6)	49 (4)
CP	175 (3)	169 (4)	162 (2)	158 (4)	177 (0.2)
NDF	388 (8)	386 (9)	360 (9)	348 (8)	179 (5)
iNDF, g/kg NDF	192	210	176	207	265
kdNDF, %/h	5.3	5.3	5.6	5.6	5.1

[1]No. after 'clover' indicates red clover proportion of DM in silage.

Table 2b Feed intake and milk production when fed silage in Year 2 (kg x cow^{-1} x day^{-1}, n=12). LS-means, standard errors of the mean and p-values of differences

	Sugar and clover proportion							
	No sugar		Sugar				Difference (p<)	
	25%	50%	25%	50%	SEM	Sugar	Clover	Sugar*Clover
Silage DM	13.9	14.4	14.5	14.3	0.7	0.44	0.63	0.27
Concentrates DM	6.7	6.7	6.6	6.4	0.1	0.02	0.32	0.50
WSC	1.59	1.46	2.84	2.70	0.11	<0.0001	0.07	0.99
CP	3.50	3.56	3.37	3.47	0.14	0.20	0.39	0.65
ECM	27.1	26.8	26.6	28.1	1.0	0.63	0.47	0.82

In Year 2 there were generally no differences between treatments in production results (neither in ECM nor feed consumption), even though the sugar intake differed substantially in the experiment.

Higher sugar content in the diet lead to a significant reallocation of nitrogen from urine to faeces (data not shown). This might be explained by higher rumen microbial efficiency when sugar intake was higher. Higher sugar intake also led to lower pH in the rumen, lower concentration of branched VFA in the rumen and lower NDF digestibility (Figures 1 – 3). The decreased concentration of the branched VFA, isobutyrate and isovalerate, which are derived from leucine and valine (Van Soest, 1994), can be explained by that the rumen microorganisms prefer sugar as energy source over amino acids.

The rumen load index calculated in the Norfor system was strongly correlated to the sugar intake. Hence, the increased rumen load index, due to higher sugar intake, was also negatively

correlated with rumen pH (Figure 1) as well as the sum of isovalerate and isobutyrate (Figure 2).

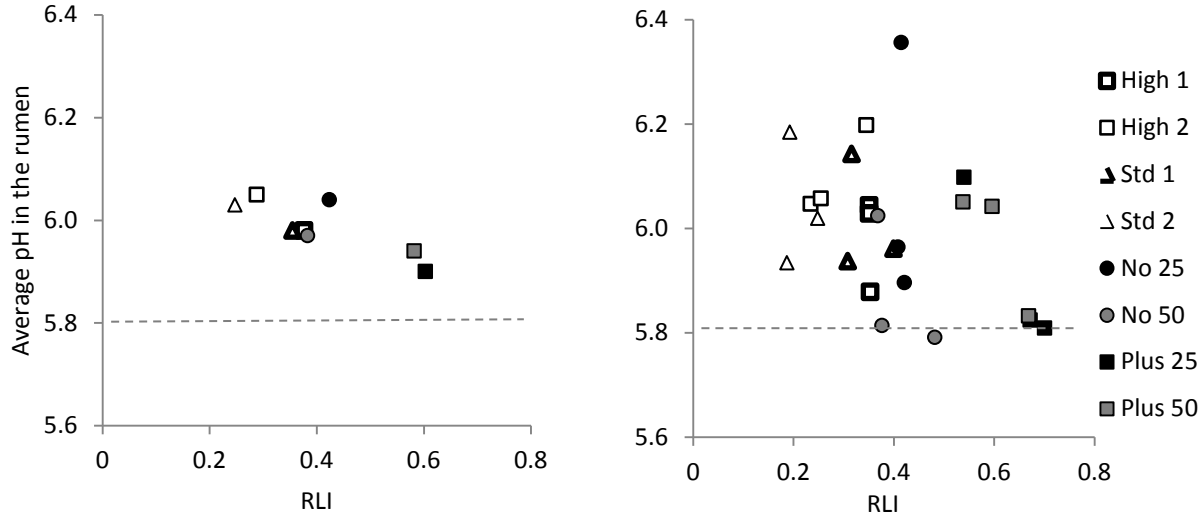


Figure 1 Average pH in the rumen (LSMEANS) as affected by the rumen load index in the diet (RLI) calculated in the NorFor system. Treatment means (left) and individual observations (right). pH less than 5.8 is regarded as a critical level (Nocek, 1997) and shown as a dotted line. The treatments Year 1 were ryegrass cultivars of High WSC and Standard WSC from 1st and 2nd cut (High 1; High 2; Std 1; Std 2). The treatments Year 2 were no or extra sugar added and included red clover were 25 or 50% on DM basis in the silage mixture (No25; No 50; Plus 25; Plus 50).

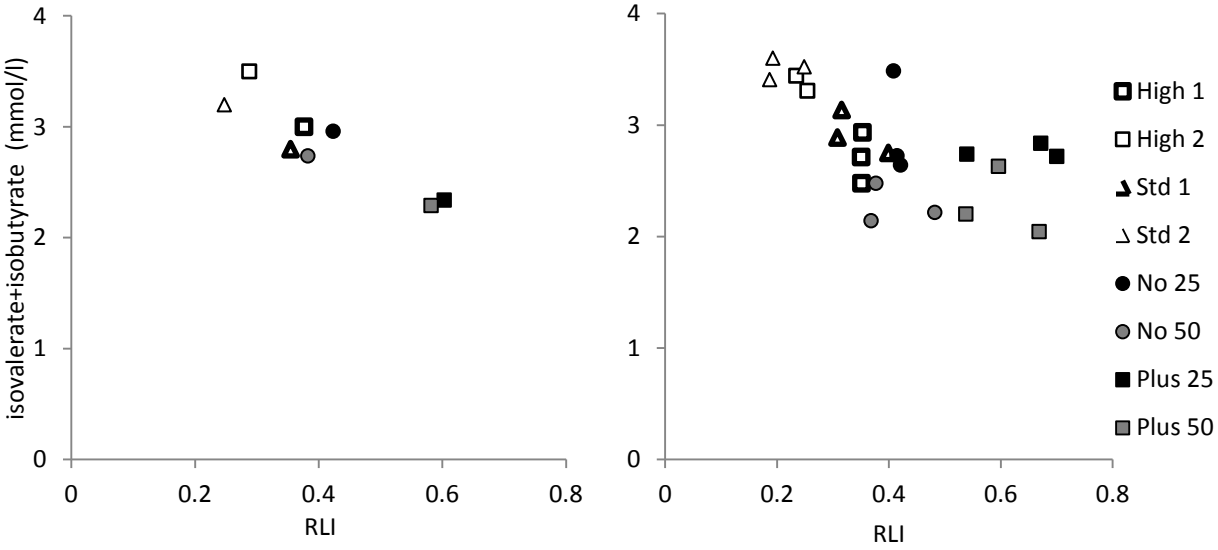


Figure 2 Ruminal concentrations of branched VFA, sum of isovalerate and isobutyrate, as affected by rumen load index. Treatment means, individual observations and legend details as in Figure 1.

NorFor predicted lower total digestibility of NDF when sugar intake was higher (Figure 3). A decrease in the degradation rate of the NDF in the rumen (kdNDF) was also predicted by an increased rumen load index

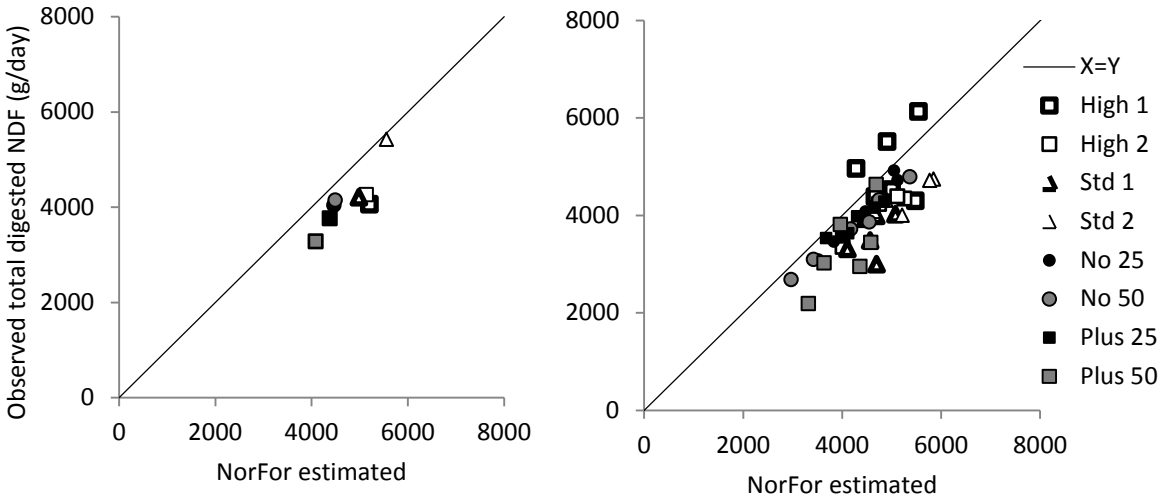


Figure 3 Total digestible NDF (g x day^{-1}) observed plotted against NorFor estimates. Treatment means, individual observations and legend details as in Figure 1.

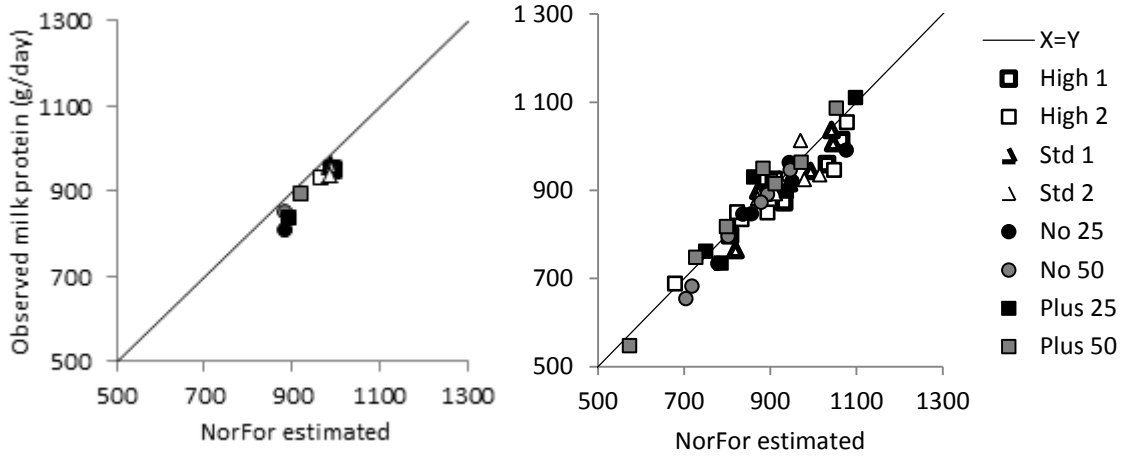


Figure 4 Observed milk protein production (g x day^{-1}) plotted against NorFor estimates. Treatment means, individual observations and legend details as in Figure 1.

NorFor estimated milk protein production well with an error of estimate of 40 g/d (Table 3). Similar results have also been shown by Broderick *et al.* (2013) on American experiments and Volden *et al.* (2011) on Nordic experiments.

Table 3 Accuracy and precision of NorFor estimated digested NDF and N, Nitrogen excreted in faeces and urine and milk protein production (g x day^{-1}) compared with observed individual results. MPE = Mean Prediction Error, RMSPE = Root Mean Square Prediction Error

	n	Intercept	Slope	R^2	MPE, %	RMSPE, g
Digested NDF	45	- 48	0.89	0.60	18	740
Digested N	45	11	0.96	0.58	8.5	32
Faecal-N	45	46	0.76	0.21	17	32
Urine-N	48	110	0.28	0.10	39	70
Milk protein	48	52	0.93	0.90	4.5	40

Conclusions

The effects on production from large dietary contrasts in sugar concentration were small. Rumen concentrations of branched VFA decreased with higher sugar intake, suggesting less fermentation of amino acids. The NorFor model predicted individual milk protein yields well. Rumen load index was correlated with treatment means for rumen pH, although individual observations displayed a considerable variation around the means.

References

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.

Broderick, G., Åkerlind, M & Nielsen, N., 2013. Using NorFor to balance diets for amino acids in lactating dairy cows. Nordic Feed Science Conference 4. SLU, Department of Animal Nutrition and Management, Report 287, Uppsala, Sweden, p 93-97.

Eriksson, T., 2010. In vitro methods for indigestible Neutral Detergent Fiber (iNDF). Nordic Feed Science Conference 1. SLU, Department of Animal Nutrition and Management, Report 274, Uppsala, Sweden, p 41-45.

Nocek, J., 1997. Bovine Acidosis: Implications on Laminitis. J. Dairy Sci. 80, 1005-1028.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. 2nd edition. Cornell University Press, Ithaca NY.

Volden, H. (ed.), 2011. NorFor – the Nordic feed evaluation system. 2011. EAAP publication No. 130. Wageningen Academic Publishers.

Effect of different rapeseed products and increasing glucosinolate intake on iodine concentration in cow milk

G. Trøan[1] & E. Prestløkken[1]
[1]*Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences. P.O. Box 5003. N-1432 Ås, Norway.*
Correspondence: Gisken.troan@nmbu.no

Introduction

Iodine is an essential part of the thyroid hormones triiodothyronine (T_3) and thyroxin (T_4), and the main storage of iodine in the body is in the thyroid gland. Adults have an iodine requirement of 150 µg/day (NNR, 2012). Because the thyroid hormones are important for the development of brain and neural tissue in the fetus and newborn child (Skeaff, 2011; Zimmermann, 2012), pregnant and lactating women have a higher need of iodine. Before 1950 endemic goiter was common, especially in inland areas of Norway (Frey *et al*., 1993). Therefore, in 1950 the Department of Agriculture required adding 2 mg I/kg to cattle feed. The high transfer of iodine from feed to milk combined with a high consumption of dairy products has made milk the primary iodine source for Norwegians (Frey *et al*., 1993; Dahl *et al*., 2003a; Dahl *et al*., 2004). In year 2000, reported milk iodine content was 98 and 232 µg/L in summer and winter milk, respectively (Dahl *et al*., 2003b). In year 2008, milk from 19 milk-collecting tours showed a reduction in iodine concentration of winter milk to only 122 µg/L (Haug *et al*., 2012). The reason for this dramatic reduction in milk iodine concentration is not known, but a main suspected is increased use of rapeseed product in concentrates for dairy cows.

Reduction in milk iodine concentration when feeding rapeseeds or rapeseed products to dairy cow is known (Papas *et al*., 1979; Emanuelson, 1989; Franke *et al*., 2009). The main explanation is that glucosinolates (GSLs) in the rapeseed by action of the enzyme myrosinase are degraded to toxic compounds like isothiocyanates (ICT), thiocyanates (SCN), nitriles and goitrin both during processing of the feed and during digestion of the feed in the animal (Fenwick *et al*., 1982). The sodium iodide symporter (NIS) is the main transport mechanism for transferring iodine from blood into the mammary and thyroid gland (Browngrant, 1957; Levy *et al*., 1997; Spitzweg *et al*., 1998). In the NIS, the SCN ion act as a competitive inhibitor of iodine (Vanderlaan & Vanderlaan, 1947; Browngrant, 1957; De la Vieja *et al*., 2000), reducing milk iodine content.

In dairy cow feeding, introduction of varieties of rapeseeds with low content of GSLs, and use of heat-treated rapeseed meals, have reduced the attention to GSL as a problem. However, the study of Franke *et al*. (2009) challenge this. In their study, iodine in milk was considerably reduced even though the rapeseed product used was low in GSLs. In 2008, when the study of Haug *et al*. (2012) was conducted, 83 415 metric tons of rapeseed products were imported, whereas the import in 2000 was only 2396 metric tons (Felleskjøpet, 2012). Most of the imported rapeseed products are used in dairy cow feeds. In addition, a high proportion of these rapeseed products are rapeseed cakes less heat-treated than traditional solvent extracted rapeseed meals.

To explore the influence of the various rapeseed products used in feed to dairy cows on milk iodine concentration, a three-year project started in 2012. This report give some preliminary results from two experiments carried out in this project. The project is founded by Fondet for

Forskningsmidler fra Landbruksprodukter (FFL), Tine BA and Felleskjøpet Fôrutvikling and administered through the Norwegian Research Council.

Materials and Methods

In both experiments, cows of Norwegian Red cattle in mid lactation with a milk yield of 27-28 kg/day were fed a diet consisting of 10 kg concentrates and grass silage *ad libitum*. The control concentrate consisted of barley, oat, wheat bran, molasses, dry fat and soybean meal. In the rapeseed containing concentrates, the soybean meal was exchanged with 20 % (Experiment 1) and 14 % (Experiment 2) (w/w) rapeseed cake or rapeseed meal. Iodine in the form of calcium iodate anhydrous ($Ca(IO_3)_2$) was included in the concentrate in a concentration of 3.5 and 5.5 mg per kg dry matter (DM) in Experiment 1 and Experiment 2, respectively.

Experiment 1- increasing level of rapeseed cake

The effects of increasing level of rapeseed cake were studied in 8 dairy cows in a 4 x 4 Latin square design, using 14 day periods. There were produced two concentrates, one control concentrate and an experimental concentrate with a rapeseed cake with a GSL content of 1.07 mmol GSL/kg. The two concentrates were used to compose four diets consisting of 0, 3, 7 and 10 % rapeseed cake of the total dry matter intake (DMI). Milk samples were taken on days 4, 7, 11, 13 and 14 of each period. Only samples from day 11, 13 and 14 were used in the calculations as iodine in milk has reached a steady state after 11 days (Franke *et al*. 2009).

Experiment 2- rapeseed products with different glucosinolate levels

The effects of rapeseed product and increasing GSL intake were studied in four dairy cows in a 4 x 4 Latin square design using 21 day periods. The four treatments included four different concentrates, one without any rapeseed product (control), one with rapeseed cake with low content of GLS (RSC-low), one with rapeseed cake with "high" content of GLS (RSC-high) and one with rapeseed meal (RSM). The two RSCs had a GSL content of 0.93 and 13.95 mmol per kg, whereas the GLS content in RSM was 6.05 mmol per kg. Separate morning and evening milk samples were taken at days 3, 4, 12, 13, 18, 19, 20 and 21 in each period. The samples from day 18 to 21 were used for the calculation of milk iodine content.

Analyses and statistics, Experiment 1 and 2

Glucosinolates in the rapeseed products and the concentrates were determined according to the ISO 9167:1-1992 method (ISO, 1992) using HPLC and photodiode array detection (HPLC-DAD). The iodine content in the silage and concentrate samples was determined according to Fecher *et al*. (1998), whereas iodine in milk samples was determined according to Nobrega *et al*. (1997). In all samples matrices, the concentration of the iodine was detected with an Agilent 8800 QQQ inductively coupled plasma mass spectrometer (ICP-MS) (Agilent Technologies, USA).

The data were analyzed using the MIXED procedure in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) with cow as random and period and treatment as fixed effects. In milk, iodine from different days were treated as repeated measurements.

Results and Discussion

Experiment 1

The total iodine intake of the cows was between 30-39 mg/day. The GSL intake was <0.01, 0.96, 2.23 and 3.19 mmol/day for rapeseed level 0, 3, 7 and 10 %, respectively. With increasing GSL intake, it was a significant ($P < 0.05$) decrease in iodine concentration in milk (Figure 1).

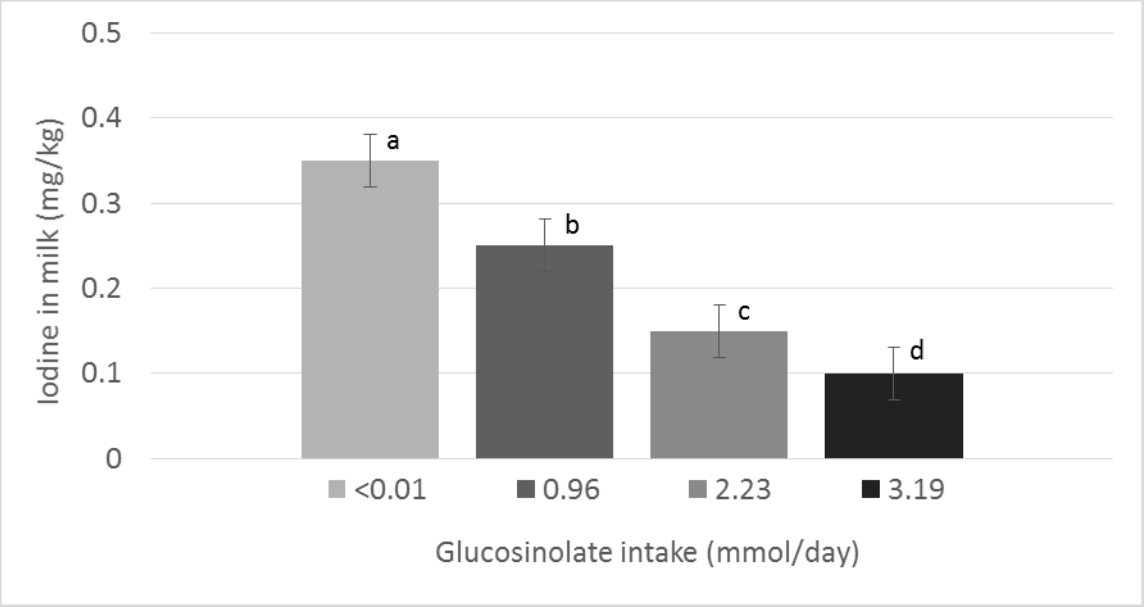


Figure 1. Iodine concentration in milk (mg/kg) at GSL intake of <0.01, 0.96, 2.23 and 3.19 mmol/day (LSmeans ± SEM (standard error of LSmeans) of day 11, 13 and 14, n = 24). [a-d] LSmeans with different superscripts differ ($P < 0.05$).

Experiment 2

The iodine intake in this experiment was between 50-57 mg/day per cow. There were significant differences in the iodine concentration in milk between the rapeseed free control diet and the three rapeseed containing diets (Figure 2). There were small differences in iodine content in milk between the rapeseed diets, but the RSC-high diet was significantly lower in milk iodine content than the RSC-low diet (Figure 2).

The concentration of GSLs in the feed used in the present studies was low compared to other studies (Papas *et al*., 1979; Laarveld *et al*., 1981a; Laarveld *et al*., 1981b; Hermansen *et al*., 1995). In spite of that, rapeseed products in the diet strongly reduced the transfer of iodine from feed to milk. Reduced milk iodine concentration when including rapeseed in the diet is in agreement with earlier experiments (Papas *et al*., 1978; Papas *et al*., 1979; Laarveld *et al*., 1981b; Franke *et al*., 2009). In contrast to our study, Papas *et al*. (1978; 1979) and Laarveld *et al*. (1981b) did not observe any differences between the different rapeseed levels when GSL intake of the cows raised from 20.4 up to as high as 314.2 mmol/day. Hermansen *et al*. (1995) state that increased GSL intake has no additive effect on milk iodine reduction, and that maximal reduction effect is achieved at less than 50 mmol GSL/day even with a low level of rapeseed in the diet. Subuh *et al*. (1995) suggested that the liver is capable of detoxifying GSL metabolites like nitriles to less toxic components like SCN up to a certain level. If that is true,

a threshold is probably induced at relatively low concentrations of GLS in the diet. Even though the results are not so clear in Experiment 1 they indicate that the reduction when going from 7 to 10% rapeseed cake in the diet is smaller and less significant than going from 3 to 7%. Together with the results in Experiment 2, this indicate that the maximal effect GSLs have on reducing iodine in milk is achieved at GSL intakes considerably lower than the 50 mmol/day Hermansen *et al*. (1995) state, and the 20.4 mmol/day indicated by Laarveld *et al*. (1981b). Metabolites of GSLs like SCN were not detected in the feed. Thus, the observed effects most likely is not explained by transformation of GLS to these compounds before ingestion of the rapeseed cake. However, although they were not detected in the feed, competitive inhibition by SCN in transferring iodine from blood into milk cannot be excluded as an explanation to reduced iodine content in milk.

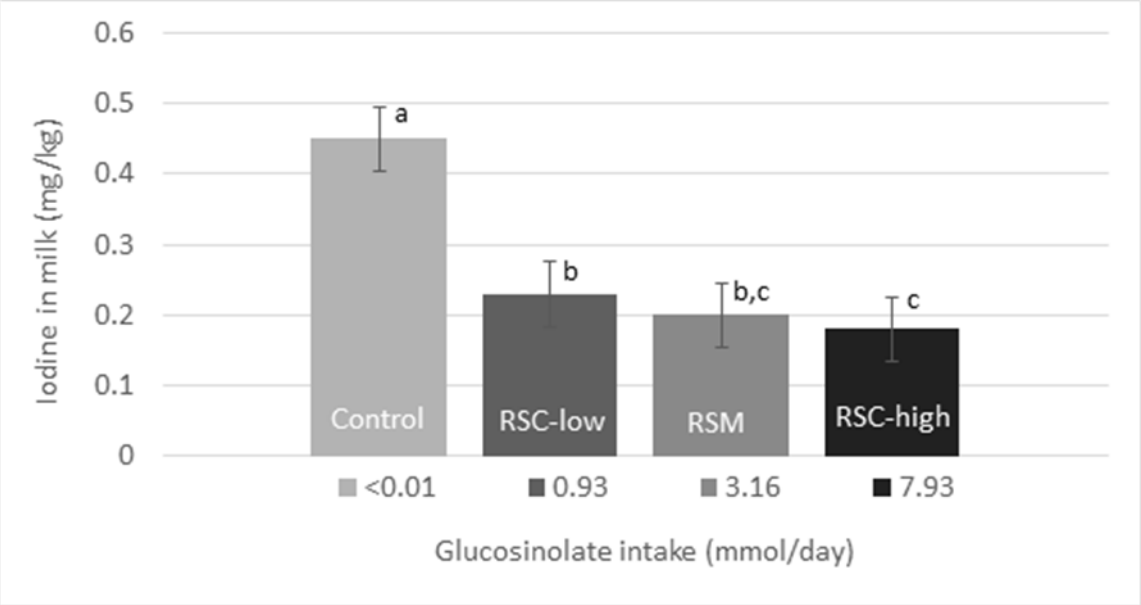


Figure 2. Iodine concentration in milk (mg/kg) at GSL intake of <0.01, 0.93, 3.16 and 7.93 mmol/day of the Control = without rapeseed products, RSC-low = rapeseed cake with low GSL content, RSM = rapeseed meal and RSC-high = rapeseed cake with "high" GSL content (LSmeans ± SEM of day 18 and 20, n = 8). [a-c] LSmeans with different superscripts differ ($P < 0.05$).

Conclusions

The rapeseed products used in the studies were low in GSLs and no GSLs metabolites were detected in the feed. In spite of that, iodine in milk was considerably reduced when using rapeseed products in the feed. Even though thiocyanate was not detected, competitive inhibition by thiocyante cannot be excluded as an explanation to reduced iodine content in milk.

References

Browngrant, K., 1957. The iodide concentrating mechanism of the mammary gland. J. Physiol. (Lond.). 135, 644-654.

Dahl, L., L. Johansson, K. Julshamn & H. M. Meltzer, 2004. The iodine content of Norwegian foods and diets. Public Health Nutr. 7, 569-576.

Dahl, L., H. M. Meltzer, J. A. Opsahl & K. Julshamn, 2003a. Iodine intake and status in two groups of Norwegians. Scand. J. Nutr. 47, 170-178.

Dahl, L., J. A. Opsahl, H. M. Meltzer & K. Julshamn, 2003b. Iodine concentration in Norwegian milk and dairy products. Br. J. Nutr. 90, 679-685.

De la Vieja, A., O. Dohan, O. Levy & N. Carrasco, 2000. Molecular analysis of the sodium/iodide symporter: Impact on thyroid and extrathyroid pathophysiology. Physiol. Rev. 80,1083-1105.

Emanuelson, M, 1989. Rapeseed products of double low cultivars to dairy cows. Effects of long-term feeding and studies on rumen metabolism. PhD Thesis. Department of Animal Nutrition and Management. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Fecher, P. A., I. Goldmann, & A. Nagengast, 1998. Determination of iodine in food samples by inductively coupled plasma mass spectrometry after alkaline extraction. J. Anal. At. Spectrom. 13, 977-982.

Felleskjøpet, 2012. Prosjekt norsk korn i kraftfôr [Norwegian grain in feed concentrates]. Available at: http://www.fk.no/Documents/Eksterne/NFK/kornpolitikk/Rapport-Norsk-korn-i-kraftfor.pdf (Accessed 10 January 2015).

Fenwick, G. R., R. K. Heaney, W. J. Mullin & C. H. VanEtten, 1982. Glucosinolates and their breakdown products in food and food plants. CRC Crit. Rev. in Food Sci. and Nutr. 18, 123-201.

Franke, K., U. Meyer, H. Wagner & G. Flachowsky, 2009. Influence of various iodine supplementation levels and two different iodine species on the iodine content of the milk of cows fed rapeseed meal or distillers dried grains with solubles as the protein source. J. Dairy Sci. 92, 4514-4523.

Frey H., B. Rosenlund, K. Try & I. Theodorsen, 1993. Urinary excretion of iodine in Norway, In: Delange F., ed. Iodine Deficiency in Europe. New York: Plenum Press, 297–300.

Haug, A., O. Taugbol, E. Prestlokken, E. Govasmark, B. Salbu, I. Schei, O. M. Harstad & C. Wendel, 2012. Iodine concentration in Norwegian milk has declined in the last decade. Acta Agric. Scand. Sect. A-Anim. Sci. 62, 127-134.

Hermansen, J. E., O. Aaes, S. Ostersen & M. Vestergaard, 1995. Rapsprodukter til malkekøer-mælkeydelse og mælkekvalitet (Rapeseed products for dairy cows-milk yield and milk quality). Statens Husdyrbrugsforsøg. Afd. for Forsøg med Kvæg og Får, Forskningscenter Foulum, Denmark. Report No. 29, 31 pp.

ISO, 1992. Method 9167:1. Rapeseed — Determination of glucosinolates content — Part 1: Method using high-performance liquid chromatography. ISO (the International Organization for Standardization), Geneva, Switzerland.

Laarveld, B., R. P. Brockman & D. A. Christensen, 1981a. The effects of the level of iodine in canola-meal concentrate on milk iodine and thiocyanate content and thyroid-function in dairy-cows. Can. J. Anim. Sci. 61, 625-632.

Laarveld, B., R. P. Brockman & D. A. Christensen, 1981b. The effects of Tower and Midas rapeseed meals on milk-production and concentrations of goitrogens and iodine in milk. Can. J. Anim. Sci. 61, 131-139.

Levy, O., G. Dai, C. Riedel, C. S. Ginter, E. M. Paul, A. N. Lebowitz & N. Carrasco, 1997. Characterization of the thyroid Na+/I− symporter with an anti-COOH terminus antibody. Proceedings of the National Academy of Sciences. 94, 5568-5573.

NNR, 2012. Iodine. In: Nordic Nutrition Recommendations 2012, Integrating Nutrition and Physical Activity. 5th ed. W. Becker, Alexander, J., Andersen, S., Aro, A., Fogelholm, M., Lyhne, N., eds. Nordic Council of Ministers, Copenhagen, Denmark. 583 - 590.

Nobrega, J. A., Y. Gelinas, A. Krushevska & R. M. Barnes, 1997. Direct determination of major and trace elements in milk by inductively coupled plasma atomic emission and mass spectrometry. J. Anal. At. Spectrom. 12, 1243-1246.

Papas, A., J. R. Ingalls & L. D. Campbell, 1979. Studies on the effects of rapeseed meal on thyroid status of cattle, glucosinolate and iodine content of milk and other parameters. J. Nutr. 109, 1129-1139.

Papas, A., J. R. Ingalls & P. Cansfield, 1978. Effects of Tower and 1821 rapeseed meals and Tower gums on milk-yield, milk-composition and blood parameters of lactating dairy-cows. Can. J. Anim. Sci. 58, 671-679.

Skeaff, S. A., 2011. Iodine Deficiency in Pregnancy: The Effect on Neurodevelopment in the Child. Nutrients. 3, 265-273.

Spitzweg, C., W. Joba, W. Eisenmenger & A. E. Heufelder, 1998. Analysis of Human Sodium Iodide Symporter Gene Expression in Extrathyroidal Tissues and Cloning of Its Complementary Deoxyribonucleic Acids from Salivary Gland, Mammary Gland, and Gastric Mucosa. J. Clin. Endocrinol. Metab. 83, 1746-1751.

Subuh, A. M. H., T. G. Rowan & T. L. J. Lawrence, 1995. Toxic moieties in ruminal and duodenal digesta and in milk, and hepatic integrity in cattle given diets based on rapeseed meals of different glucosinolate content either untreated or treated with heat or formaldehyde. Anim. Feed Sci. Technol. 52, 51-61.

Vanderlaan, J. E. & W. P. Vanderlaan, 1947. The iodide concentrating mechanism of the rat thyroid and it`s inhibition by thiocyanate. Endocrinology. 40, 403-416.

Zimmermann, M. B., 2012. The Effects of Iodine Deficiency in Pregnancy and Infancy. Paediatr. Perinat. Epidemiol. 26, 108-117.

Protein value of different seaweed species in dairy cows

U. Tayyab[1], M. Novoa-Garrido[2], M.Y. Roleda[2] & M.R. Weisbjerg[1],
[1]Department of Animal Science, Aarhus University Foulum, Blichers Allé 20, Postbox 50, DK-8830, Tjele, Denmark.
[2]Bioforsk, 1430 Ås, Norway.
Correspondence: usama.tayyab@anis.au.dk

Introduction

Limited land resources and increasing food demand puts pressure on agriculture and especially protein supply restricts increase in animal production. Indeed, it is no longer environmentally sustainable to increase the area of land used for farming, as this will further contribute to climate change (Steinfeld *et al.,* 2007). In order to support the needs of the rising world's population, farming has to intensify drastically so that more products are produced more rapidly from the same land area. On the other hand, marine waters hold the majority of the total world area and possess potential biomass, e.g. seaweeds, that could be utilized for feeding animals. These alternative feedstuffs can be used to decrease the dependency on conventional feedstuffs like soybeans. Use of seaweeds in animal feeding is not new; people living on coastal areas have fed their animals with seaweeds especially during lean feed seasons (Dunlop, 1953; Evans & Critchley, 2013). In the past, studies have focused on individual seaweeds or seaweed meal fed to small ruminants with respect to their nutritional value (Ventura & Castañón, 1998). In certain seaweed species, the crude protein (CP) content can reach 47% of the dry matter (DM) (Arasaki & Araski, 1983; Burtin, 2003). The nutritive value of seaweeds for ruminants varies widely and depends upon species, composition, and animal adaptation to that specific species. However, *in vivo* studies on protein digestibility of seaweed in dairy cows are scarce. The aim of this study was to evaluate the effect of season and seaweed species on the protein value for ruminants.

Materials and Methods

Three seaweed species (the green *Acrosiphonia sp*., the brown *Pelvetia canaliculata* and the red *Porphyra sp*.; hereafter *Acrosiphonia, Pelvetia* and *Porphyra,* respectively) were sampled in spring (March) and autumn (October and November) 2014 at the coast of Bodø in northern Norway.

Three dry rumen fistulated (#1C, Bar Diamond Inc., Parma, ID, USA) Danish Holstein cows were fed a standard ration (67:33 forage to concentrate ratio) at maintenance level and used for rumen incubations. Three duodenally canulated Danish Holstein dairy cows maintained on a 60:40 forage (grass and maize silage) to concentrate ratio (DM basis) diet were used for intestinal incubations of mobile bags. All cows had free access to fresh drinking water.

The samples were freeze dried before milling on a 1.5-mm screen with a cutter mill. The CP values were estimated as N×6.25 after N analysis by the Kjeldahl method. The samples were incubated in the rumen for *in situ* protein degradation at 8 time intervals (0, 2, 4, 8, 16, 24, 48 and 96 hours) using Dacron bags (38 µm pore size) according to the standard NorFor procedure (Åkerlind *et al.,* 2011). For the total tract digestibility, mobile bags (Dacron, 12 µm pore size) were ruminally pre-incubated for 16 h before pepsin-HCl treatment and incubation in the small intestine through the duodenal cannula, where after the bags were recovered in the faeces (Hvelplund & Weisbjerg, 2000).

Degradation profiles of CP were fitted assuming an exponential degradation profile including a lag time using PROC NLIN in SAS (9.4 version, SAS Institute Inc.).

Rumen degradable CP was estimated as effective rumen protein degradability (EPD) at 5% rumen fractional passage rate (Åkerlind *et al.,* 2011), but also including lag time:

EPD = a + (b(c/(c+kp)))×(exp(-(c+kp)×lt)), where a is soluble fraction, b is degradable but not soluble fraction, c is fractional rate of degradation, kp is fractional rate of passage (0.05/h), and lt is lag time (h).

Indigestible CP was estimated as the CP residue in mobile bags after faecal recovery. Intestinally degradable CP was estimated as rumen degradable CP minus indigestible CP. Rumen degradable, intestinal degradable and indigestible CP was reported as g per kg of original DM.

All data were analysed using PROC Mixed Model by SAS 9.4 version (SAS Institute Inc.) with species and season as fixed effects and cow as a random effect.

Results and Discussion

Crude protein concentrations in the seaweed, and CP (as g/kg original DM) degraded in the rumen and in the small intestine, and fully indigestible, are given in Table 1. The ash concentration in all three species was generally high and differed among species (P<0.0001), the high concentration was probably due to growing in sea water with high salt concentration. Concentrations of CP showed a large variation among species and season. Samples collected in the spring had higher (P<0.0001) CP contents than in autumn. The highest CP in DM was measured in *Porphyra* sampled in spring (37%) and lowest in *Pelvetia* sampled in autumn (8%). Both *Acrosiphonia* and *Porphyra* provided a high supply of rumen degradable protein, however *Acrosiphonia* had a higher indigestible part and thereby *Porphyra* had the highest supply of intestinally degradable protein. About 50% of total protein intake from *Porphyra* was degraded in the small intestine. *Pelvetia* had low rumen degradability (33%, average across both seasons) and, together with the low protein concentration, it only supplied 90 g rumen degradable CP/kg original DM. Pelvetia showed a negative small intestinal degradability.

Table 1 The ash and protein concentration in seaweed, and the amount of protein degraded in the rumen, in the small intestine, and indigestible (g/kg original DM)

Species	Season	Ash	CP	CP		
				Rumen degraded	Small intestinal degraded	Indigestible
Acrosiphonia	Autumn	126.7	285.9	120.1	82.7	83.1
Acrosiphonia.	Spring	170.8	333.1	153.2	102.5	77.4
Pelvetia	Autumn	210.1	75.0	21.8	-20.5	73.7
Pelvetia	Spring	218.5	105.3	37.9	-9.1	76.5
Porphyra	Autumn	106.5	320.6	121.8	162.5	36.3
Porphyra	Spring	149.2	372.2	152.6	185.0	34.6
P value	Specie	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Season	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

This might be due to methodological differences between the methods used for rumen and total tract mobile bag degradability, as e.g. pore size of the bags. *Pelvetia* did not fit the exponential curve model, which also might have affected the estimation of the effective rumen CP degradability. Species and season affected (P<0.0001) seaweed CP concentration and degradability.

Different *Porphyra* species are traditionally used by humans especially in Asia because of their high nutritive values. Crude protein concentrations found in the present study are in accordance with Hasan and Chakrabarti (2009). On the other hand, *Pelvetia* has been fed to pigs (Chapman & Chapman, 1980) but there is no evidence of earlier studies in cows. There is scarcely any information available in the literature about CP values and protein digestibility values of these seaweed species in dairy cows.

Conclusion

The three seaweed species investigated had higher protein content in spring than in autumn. Both *Porphyra* and *Acrosiphonia* can supply the rumen with high amounts of rumen degradable protein but, due to both a high protein concentration and a low indigestible part, *Porphyra* can also supply a high amount of digestible protein to the small intestine. *Pelvetia* protein had a very low degradability in the rumen and the rumen escapable protein was not degradable in the small intestine, therefore, *Pelvetia* should not be used to feed dairy cows.

References

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: NorFor-The Nordic feed Evaluation System. Wageningen Academic Publishers, pp 41-54.

Arasaki, S. & Arasaki, T., 1983. Low Calorie, high Nutrition: Vegetables from the Sea. Japan Publ., Tokyo.

Burtin, P., 2003. Nutritional value of seaweeds. Electr. J. Environm., Agric. Food Chem. 2, 498-503.

Chapman, V. J. & Chapman, D. J., 1980. Seaweeds and their uses. 3rd Ed., Chapman and Hall, London.

Dunlop, G., 1953. "Feeding of seaweed meal to lactating cows". Nature, 171, 439-440.

Evans, F. D. & Critchley, A. T., 2013. Seaweeds for animal production use. J. Appl. Phycol. 26, 891-899.

Hasan, M. R. & Chakrabarti, R., 2009. Use of algae and aquatic macrophytes as feed in small-scale aquaculture: a review (No. 531). Food and Agriculture Organization of the United Nations (FAO).

Hvelplund, T. & Weisbjerg, M. R., 2000. In situ techniques for the estimation of protein degradability and postrumen availability. Forage Evaluation in Ruminant Nutrition. CAB International, Wallingford, UK, pp 233-259.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M. & De Haan, C., 2007. Livestock's Long Shadow: Environmental Issues and Options. Food and Agriculture Organization, Rome, Italy.

Ventura, M. R. & Castañón, J. I. R., 1998. The nutritive value of seaweed (*Ulva lactuca*) for goats. Small Rumin. Res. 29, 325-327.

Effect of heat treated field beans on the performance of Swedish lactating dairy cows

M. Ramin[1] A. Höjer[1], F. Fogelberg[2], M. Hetta[1] & P. Huhtanen[1]
[1]*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden*
[2]*Swedish Institute of Agricultural and Environmental Engineering, box 7033, SE-750 07 Uppsala, Sweden*
Corresponding author: mohammad.ramin@slu.se

Introduction

In organic farming there is a high demand to use locally produced protein feeds for ruminants. However, organic milk production is, in contrast to crop production, a component of farming that has relatively low nitrogen efficiency as only a small part of the nitrogen fed to animals is found in the milk protein. Organic diets for dairy cows are to a large extent based on grasses and legumes with relatively high levels of rumen degradable protein (RDP) (Hedquist & Udén, 2006). On many farms, nitrogen efficiency is unnecessarily low and the cost for protein feed is relatively high due to overestimation of the marginal response to protein supplementation. There are, however, other options to influence quality and concentrations of protein in organic diets by using legumes such as field pea (*Pisum sativum*) and horse bean (*Vicia faba*). They have two advantages: they can be locally produced and can fix atmospheric N. Other benefits with peas are high digestibility and high energy concentration (high starch, low indigestible neutral detergent fibre; NDF) and they are therefore ideal substrates for microbial protein synthesis in the rumen.

One alternative to increase microbial protein (MP) input is using protein-rich feeds that are artificially protected from ruminal degradation. Various physical and chemical treatments have been used to pursue this objective. One of these is a controlled heat application, which allows protein degradation in the rumen to be reduced through denaturation and Maillard reactions, reducing solubility and protein degradation rate in the rumen (Moshtaghi Nia & Ingalls, 1995). There have been studies reporting responses on dairy cows by the use of rumen un-degradable protein (RUP) supplements, such as heat-treated soy protein (Faldet & Satter, 1991). Protein feeds, differing in RUP, showed to have different flows of RUP and total protein from the rumen (Brito & Broderick, 2007). A possibility to increase nitrogen efficiency is to heat-treat the protein feed. Heat-treatment may reduce protein solubility in the rumen and increase the availability of starch. This may then reduce the supply of nitrogen in the rumen and increase the amount of energy available for rumen microbes, as has been shown in international studies using heat-treated dehulled lupines (Barchiesi-Ferrari & Anrique, 2011; Mogensen *et al*., 2008). The results from the literature show that there is a need for applied studies exploring the possibilities to better utilise nitrogen in organic dairy production by altering the energy and protein concentration in the diets. Utilising locally produced feeds will avoid over-feeding of expensive protein concentrates and unnecessary losses of nutrients to the environment. At farms, a small scale roasting equipment can be used to heat-treat whole seeds. There is, however, a lack of feeding trials to study the effects of such treatment on locally produced protein feeds in Sweden. The first objective of this study was to evaluate if the feeding value of heat-treated field beans (FB) could be improved. The second objective was to compare different protein supplements, which could be used in organic farming, on the performance of lactating dairy cows fed a grass silage based diet.

Materials and Methods

Twenty-four lactating Swedish Red cows 95 days in milk (DIM) and average milk yield of 29.1 kg per day in the beginning of the experiment were used in a cyclic change-over trial with three 21-d experimental periods. Six diets were fed ad libitum as total mixed rations (TMR) and the feed intake was recorded using Insentec intake controlled feeders (Insentec B. V., Marknesse, the Netherlands). The control diet consisted of grass silage and dried rolled barley [60:40, dry matter (DM) basis]. In the experimental diets, barley was replaced with rapeseed expeller (RSE; 104 g/kg diet DM), or isonitrogenous supplements of peas (232 g/kg diet DM), untreated FB (UFB; 140 g/kg diet DM), heat-treated FB (TFB; 140 g/kg diet DM) or heat-treated FB, providing the same dietary MP concentrations as UFB (TFB-MP; 80 g/kg diet DM), (Table 1). The silage was a second cut with 181 g/kg DM of crude protein (CP) and 494 g/kg DM of NDF. Heat-treatment of FB was done with a farm-based roasting equipment (R-100E, Roastech, Blomfontein, South Africa). The machine was electrically (15 kW) heated and consisted of a drum with 3-mm holes. Roasting was conducted at a temperature of 140°C and a turning frequency of 50 Hz which gave a passage time of 7.5-8 minutes. At the outlet of the drum, a cooling container was mounted on the machine to allow air-cooling of the roasted product. The outlet of the machine was directly into bags so the heat treated FB could be collected and processed further.

Cows were milked twice a day (6 am and 3 pm) and were weighed on three consecutive mornings in the last days of each experimental period. Methane (CH_4) and carbon dioxide emissions were measured with the GreenFeed system (C-Lock Inc., Rapid City, SD, USA). Recordings of feed intake, milk production and gases were made the last 7 days in each period.

The data was analyzed with the MIXED procedure of Statistical Analysis Systems (SAS for Windows, version 9.3, SAS Institute, Cary, NC). The model included fixed effects of period, treatment and random effect of cow. For treatment comparison, the following contrasts were used: control *vs.* others, RSE *vs.* others excluding the control, UFB *vs.* peas, UFB *vs.* TFB-MP and UFB *vs.* TFB. The results are presented as least squares means.

Results

Protein supplementation had no effect (P>0.05) on DM intake (DMI); (18.8 vs. 18.2 kg/d), milk yield (23.8 vs. 23.5 kg/d) or energy corrected milk (ECM) yield (25.6 vs. 24.8 kg/d), as shown in Table 2. This was mainly because peas or FB supplemented diets did not increase milk and protein yield compared to the control diet. The RSE treatment increased milk (24.8 vs. 23.6 kg/d) and protein yield (913 vs. 863 g/d) compared to other protein supplements. Heat-treated FB had no effect on DMI, milk or protein yield compared to UFB. Milk nitrogen efficiency (Milk N/N intake) decreased and MUN increased with protein supplementation compared to the control diet (306 *vs*. 265 g/kg and 3.01 *vs*. 3.92 mmol/L, respectively). The RSE supplemented diet tended to decrease (P= 0.09) CH_4 production compared to other protein supplements (383 vs. 399 g/d) as given in Table 2.

Table 1 Ration ingredients and chemical composition (g/kg DM unless otherwise noted)

	Ration[1]					
Item[2]	CON	RSE	Pea	UFB	TFB	TFB-MP
Grass silage	600	600	600	600	600	600
Barley	400	296	168	260	260	320
Pea	0	0	232	0	0	0
Rapeseed	0	104	0	0	0	0
Field bean not-heat treated	0	0	0	140	0	0
Field bean heat treated	0	0	0	0	140	80
DM, g/kg of fresh matter	347	349	349	348	349	349
CP	159	187	187	181	183	176
Crude fat	26	33	24	25	25	25
NDF	400	415	365	395	390	393
iNDF	45	56	37	40	40	41
ME, MJ/kg of DM	11.8	11.8	12.1	11.9	12.0	11.9

[1]CON = control, RSE = rapeseed expeller, UFB = field bean not heat-treated, TFB = field bean heat-treated, TFB-MP = field bean heat-treated same metabolizable protein as UFB.
[2]DM = dry matter, CP = crude protein, NDF = neutral detergent fibre, iNDF = indigestible NDF, ME = metabolizable energy.

Discussion

In a meta-analysis conducted by Huhtanen *et al*. (2011), based on digesta flow and rumen metabolism studies, it was found that the effect of chemical and physical treatments to reduce crude protein CP degradability do not result in expected improvements in CP supply to the small intestine. Our study is in line with the findings of Huhtanen *et al*. (2011) since the performance of the dairy cows did not change by heat treating FB. Results from an organic farming study with high-yielding dairy cows indicate that toasting decreases effective rumen protein degradability for diets including toasted lupins, barley and soybeans (Mogensen *et al*., 2008). They reported that toasting of lupins tended to increase milk yield compared to the untreated lupins, whereas toasting of soybeans did not show any improvements. Our findings did not show any improvements on milk yield or ECM when FB was heat treated compared to the UFB diet. In agreement with our findings, several other experiments have shown that heat treatment does not affect milk fat (Pires *et al*., 1996) or milk protein content (Bertilsson *et al*., 1994). The discrepancy between studies on the effect of heat treatment of feeds and performance of dairy cows and supply of MP under organic farming conditions can vary among feeds. In an *in vitro* evaluation Vaga *et al*. (2014) found that treatment method (autoclave *vs.* oven), temperature and the length of treatment affected the concentration of utilizable CP. It should be noted that increasing temperature and treatment period will increase processing costs and may decrease the concentrations of essential amino acids and decrease intestinal digestibility of undegraded CP. Further studies are therefore required before making any definite conclusions.

Table 2 The effect of diet treatments on feed intake, milk yield and nutrient consumption of dairy cows

	Ration[1]							Contrasts (P – value)[2]				
Item	CON	RSE	PEA	UFB	TFB	TFB-MP	SEM[3]	C vs. O	R vs. O	UFB vs. PEA	UFB vs. TFB-MP	UFB vs. TFB
DMI, kg/d	18.2	19.0	19.0	18.7	18.7	18.6	0.37	0.13	0.33	0.58	0.80	0.98
CP intake, kg/d	2.90	3.55	3.44	3.35	3.32	3.15	0.072	<0.01	<0.01	0.33	0.04	0.79
NDF intake, kg/d	7.11	7.66	6.94	7.23	7.23	7.26	0.154	0.31	<0.01	0.17	0.90	0.99
AAT, kg/d	1.66	1.85	1.78	1.75	1.75	1.71	0.035	<0.01	<0.01	0.51	0.40	0.96
ME, MJ/d	219	224	231	223	223	222	4.50	0.18	0.98	0.17	0.87	0.97
Milk, kg/d	23.5	24.8	23.0	23.7	23.8	23.8	0.90	0.49	0.02	0.30	0.90	0.85
ECM, kg/d	24.6	26.6	24.9	25.8	25.8	25.3	0.91	0.18	0.17	0.40	0.97	0.62
Milk fat, g/kg	43.3	44.8	45.4	46.1	45.5	44.1	1.63	0.70	0.17	0.72	0.71	0.30
Milk protein, g/kg	37.6	37.3	36.6	36.9	36.9	37.5	0.53	0.07	0.049	0.29	0.44	0.07
Milk urea, mmol/L	3.01	3.79	3.94	3.90	4.42	3.57	0.154	<0.01	<0.01	0.16	0.80	0.03
FE[4], kg/kg	1.35	1.40	1.32	1.34	1.37	1.36	0.046	0.77	0.14	0.60	0.62	0.52
MNE[5], g/kg	306	264	250	255	266	288	8.95	<0.01	0.82	0.42	<0.01	0.09
CH_4, g/d	390	383	397	389	403	406	9.6	0.53	0.09	0.45	0.12	0.20
CH_4, g/DMI	21.4	20.4	21.0	20.8	21.6	22.0	0.57	0.71	0.11	0.78	0.10	0.22
CO_2, kg/d	11472	11839	11986	11786	12095	11716	217.0	0.03	0.75	0.40	0.76	0.19
CH_4/CO_2	0.034	0.033	0.033	0.033	0.033	0.035	0.0006	0.44	0.19	0.91	0.03	0.62

[1]CON = control, RSE = rapeseed expeller, UFB = field bean not heat-treated, TFB = field bean heat-treated, TFB-MP = field bean heat treated same MP as UFB, [2]C vs. O = CON vs. other diets, R vs. O = RSE vs. other diets excluding CON, [3]SEM = standard error of mean, [4]FE = feed efficiency (kg ECM) / (kg DMI), [5]MNE = milk nitrogen efficiency (milk protein yield × 6.38) / (protein intake × 6.25).

Conclusions

This study suggests that in organic farming no improvement on lactation performance of dairy cows were achieved by the inclusion of heat treated FB or peas as compared to a control diet without any protein supplement, provided that RDP requirements are met. Only RSE supplementation resulted in an improvement in animal performance compared to the control diet.

References

Brito, A. F. & Broderick, G. A., 2007. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. J. Dairy Sci. 90, 1816-1827.

Barchiesi-Ferrari, C. & Anrique, R., 2011. Ruminal degradability of dry matter and crude protein from moist dehulled lupin and extruded rapeseed meal. Chi. J. Agr Res.71, 430-436.

Bertilsson, J., Gonda, H. & Lindberg, J.E., 1994. Effect of level and degradability of rapeseed meal in rations for dairy cows. Acta Agric. Scand., A Anim. Sci. 44, 222–229.

Faldet, M. A. & Satter, L. D., 1991. Feeding heat-treated full fat soybeans to cows in early lactation. J. Dairy Sci. 74, 3047-3054.

Hedqvist, H. & Udén, P., 2006. Measurement of soluble protein degradation in the rumen. Anim. Feed Sci. Technol. 126, 1-21.

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.

Mogensen, L., Lund, P., Kristensen, T. & Weisbjerg, M. R., 2008. Effects of toasting blue lupins, soybeans or barley as supplement for high-yielding, organic dairy cows fed grass-clover silage ad libitum. Livest. Sci. 115, 249-257.

Moshtaghi Nia, S.A. & Ingalls, J.R., 1995. Influence of moist heat treatment on ruminal and intestinal disappearance of amino acids from rapeseed meal. J. Dairy Sci. 78, 1552-1560.

National Research Council., 2001. Nutrient requirements of dairy cattle. 7th rev. ed. National Academy of Science, Washington, DC.

Pires, A.V., Eastridge, M.L. & Firkins, J.L., 1996. Roasted soybeans, blood meal, and tallow as sources of fat and ruminally undegradable protein in the diet of lactating cows. J. Dairy Sci. 79, 1603–1610.

Vaga, M., Hetta, M. & Huhtanen, P., 2014. Effects of heat treatment on protein feeds evaluated *in vitro* utilizing the method utilizable protein. Pages 157 – 162 in the 5th Nordic Feed Science Proc., Uppsala, Sweden.

Estimation and evaluation of rumen microbial protein synthesis in NorFor and other feed evaluation systems

R. Hauge[1], M. Johansen[1] & N. I. Nielsen[2]
[1]Aarhus University, AU Foulum, Department of Animal Science, P.O. box 50, 8830 Tjele, Denmark. [2]SEGES, Cattle, 8200 Aarhus N. Denmark.
Correspondence: rasmushauge@icloud.com

Introduction

Protein supply for milk production in dairy cattle is affected by a range of variables such as passage rate, energy available for microbial growth as well as a sufficient N supply (Dewhurst *et al*., 2000; Younge *et al*., 2004) and protein quality (Broderick & Merchen, 1992), and is therefore difficult to estimate. It is of economic and environmental importance with a correct protein supply to the dairy cow since amino acids available for absorption in the small intestine (AAT) is one of the more expensive parts of a feed ration. Microbial protein can represent more than 70% of AAT (Oba & Allen, 2000) and a valid estimation of AAT from rumen microbial protein synthesis (RMPS) is therefore important. This paper will evaluate the estimation of RMPS in NorFor against independent in vivo data and by comparison with other estimation systems.

Materials and methods

The evaluation of the RMPS estimation in NorFor was done in two parts: one part compared different models for estimating RMPS and in the second part, estimations from NorFor was evaluated against *in vivo* data. In both parts, the NorFor model was used as implemented in the Danish Management System for cattle (DMS) (it.dlbr.dk).

Part 1

A comparison of NorFor (Volden, 2011) with three other models: CNCPS (cncpc.cornel.edu; Fox *et al*., 2004), the Danish/Nordic AAT/PBV system (Madsen *et al*., 1995) and a French model (Archimede *et al*., 1997) was done by use of variations of a typical Danish feed ration. Standard feedstuffs from the NorFor feed stuff table (FST) was used (norfor.info). In CNCPS, the feed rations were applied and feed information from the CNCPS feed database were modified with information from NorFor. The Archimede model was used as presented by Archimede *et al*. (1997):

(a) Microbial N ($g/LW^{0.75}$) = 0.22 (±0.22) +0.010 (±0.003)*OMI – 0.382 (±0.251)*C

where OMI is organic matter intake as $g/kg\ LW^{0.75}$,and C is the level of concentrate in the ration on DM basis as a fraction between 0 and 1. Both OMI and C were calculated using NorFor output. The AAT/PBV model was used as described in the Danish feed stuff table (Møller *et al*., 2005):

(b) RMPS = 125g of microbial amino acids per kg of fermented carbohydrate in the rumen

where fermentable carbohydrate input for the feedstuffs were taken from the Danish feed stuff table (Møller *et al*., 2005) with the following values: barley 740 g, canola meal 340 g, soybean meal 400 g, grass silage 520 g, corn silage 640 g of fermentable carbohydrates per kg of DM.

In NorFor and CNCPS, information on live weight, expected milk yield and DIM of 620 kg, 35 kg of ECM and 90 DIM were used. In the Archimede model, a live weight of 620 kg was also used. All results from the Archimede model and the AAT/PBV model were adjusted to an absorption of amino acids of 0.85 in the small intestine, as used in NorFor (Volden & Larsen, 2011). The RMPS from CNCPS were reported as % microbial metabolisable protein of total metabolisable protein.

The diets used for testing consisted of a forage part (50% grass silage, 50% corn silage on DM basis) and a concentrate part (54% barley, 28% canola meal, 18% soybean meal on DM basis (ration 1)) with a feeding level of 21.7 kg DM/day. Several feed rations with increasing concentrate proportion from 23 to 65 % of DM were made (23%, 34%, 46%, 53%, and 65%, on DM basis).

To evaluate how the models respond to different types of concentrate, the test was repeated where the concentrate part was changed to a high carbohydrate content with 75% barley, 7% soybean meal and 18% canola meal (ration 2).

Table 2 Chemical composition of Ration 1 and 2, calculated with feedstuffs from the NorFor feedstuff table (norfor.info)

	Concentrates of total ration DM (%)				
	23	34	46	53	65
*Ration 1**					
Starch (g/kg DM)	215	236	249	259	276
Rest carbohydrate*** (g/kg DM)	149	145	141	139	135
NDF (g/kg DM)	340	321	298	287	256
*Ration 2***					
Starch (g/kg DM)	247	278	313	332	366
Rest carbohydrate*** (g/kg DM)	141	134	125	121	113
NDF (g/kg DM)	338	318	294	282	259

* Forage part: 50% grass silage, 50% corn silage on DM basis, concentrate part: 54% barley, 28% canola meal, 18% soybean meal on DM basis;
** Forage part: 50% grass silage, 50% corn silage on DM basis, concentrate part: 75% barley, 7% soybean meal and 18% canola meal on DM basis;
*** Rest carbohydrate = 1000 – Ash – CP – Fat – NDF – Starch – Fermentation products.

The effect of DMI, ranging from 14 to 29 kg, was tested in the four models with a ration containing 32% grass silage, 32% corn silage, 19% barley, 10% canola meal, 6% soybean.

Part 2

Data from four in vivo studies, where RMPS was measured with omasal or duodenal sampling techniques, were collected. All studies contained at least four treatments, with variation in measured RMPS. Data regarding feed and chemical composition in each treatment was entered in NorFor. The estimation of RMPS was then compared with the RMPS measured in vivo. Data reported as microbial non ammonia nitrogen were multiplied by 6.25 to get microbial protein and adjusted for absorption in the small intestine with a coefficient of 0.85 (Volden & Larsen, 2011) and then presented as daily microbial amino acids absorbed in the small intestine (DMAAS).

Table 3 Overview of studies used for testing in NorFor

Study	Number of cows	Primary feedstuffs	Measuring technique	Treatments	Breed	Average DIM*	Average weight (kg)	Daily DMI (kg)
Younge *et al*. (2004)	4	Fresh grass or grass silage	Total purines	Fresh grass with/without concentrate, Wilted/unwilted silage with concentrate	Holstein/ Friesian	74	N/A**	18.2
Ahvenjarvi *et al*. (2002)	4	Grass silage Barley Canola meal	^{15}N	Silage with/without barley or canola	Finnish Ayrshire	167	571	16.4
Ahvenjarvi *et al*. (2006)	4	Grass/Barley Silage	Total purines	Grass vs. barley silage with concentrate	Finnish Ayrshire	57	606	21.0
Johansen (unpubl.)	4	Grass silage	Total purines	Silage varying from 28,3% DM to 72,5% DM	Danish Holstein	216	550	12.5

*DIM: Days in milk, **N/A: Not available

Results and **Discussion**

Part 1

Comparison of the four methods for estimation of DMAAS was done for 15 different feed rations. The results show that the AAT-PPV model does not react to a change in concentrate level (Figure 1). The Archimede model reacted with a small increase in DMAAS. Both NorFor and CNCPS are stabile with a small reduction when the concentrate level exceeds 50%.

When the concentrate part was increased in carbohydrate content and decreased in protein content (Figure 2), the Archimede model did not respond, whereas the AAT/PBV model showed a small increase in DMAAS. CNCPS kept a steady level at 1400 g/day until 55% concentrate and then dropped to 1100 g/day. NorFor showed the same drop, but in a more linear way than CNCPS. This illustrates that the Arhcimede model does not take into account what kind of concentrate that is used in the ration, as the results are similar when using a protein and carbohydrate rich rations, whereas the other models are responsive. An explanation for the decrease in DMAAS in NorFor can be the implementation of a variable RMPS efficiency (g microbial protein/kg rumen degraded organic matter). When the sum of starch and 'rest carbohydrate' concentration in the ration is outside the 18-32% range, the RMPS efficiency decreases (Volden, 2011). This factor is not used in any of the other models.

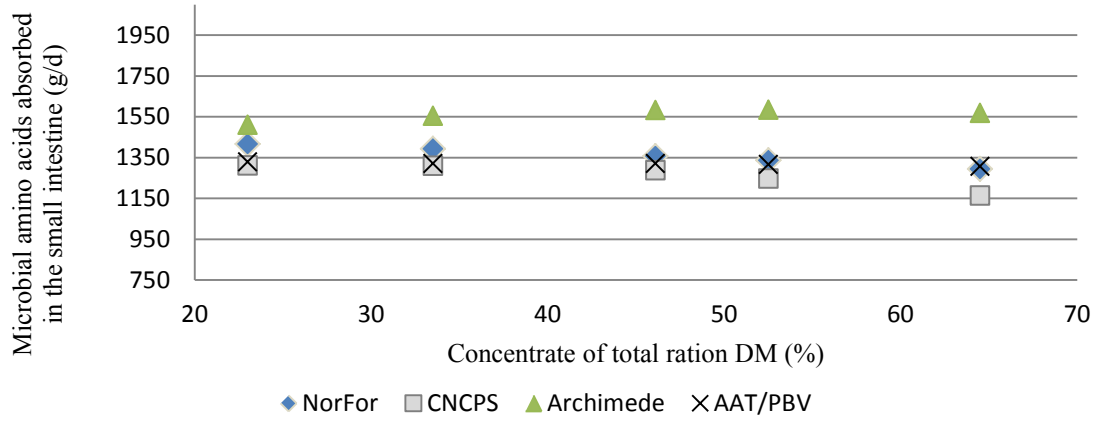


Figure 1 Estimation of microbial protein absorbed in the small intestine with varying concentrate levels where the forage consisted of 50% grass silage and 50% corn silage, and the concentrate consisted of 54% barley, 28% canola meal and 18% soybean meal on DM basis.

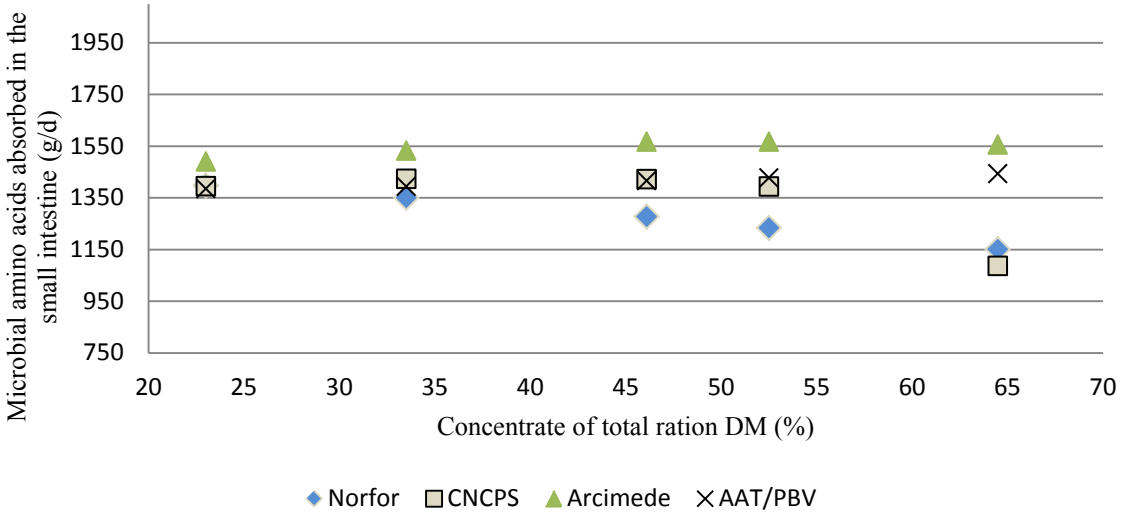


Figure 2 Estimation of microbial protein absorbed in the small intestine with varying concentrate levels where the forage consisted of 50% grass silage and 50% corn silage, and the concentrate consisted of 75% barley, 18% canola meal and 7% soybean meal on DM basis.

When using the same feed composition and changing DMI, impact on the DMAAS estimation varies. An increase in DMI caused a linear increase in DMAAS in all the models (Figure 3). NorFor reacted with the highest increase in DMAAS when increasing DMI, while the Archimede model showed the lowest increase. At typical DMI for Danish Holstein herds (22-25kg DM), the 4 models tended to agree more in their RMPS estimations than at low and high DM- intakes.

Part 2

The comparison of NorFor with in vivo studies showed a high correlation (r=0.93) between estimation in NorFor and measured DMAAS (Figure 4). Norfor showed an overestimation with data from Johansen (unpubl.) where the grass silage was the only feed stuff and the daily DMI was relatively low. DMAAS was better estimated for Ahvenjärvi et al. (2002) and

Ahvenjärvi et al. (2006) where the feed ration also included concentrates and daily DMI was higher. Estimation of DMAAS for Younge et al. (2004) did not correlate as well with measured values as for the two other studies. When using NorFor in DMS, it is not possible to change animal body weights from the standard 620 kg in the input. This affects ruminal passage rate calculated in NorFor and, therefore, true rumen degraded organic matter (Volden, 2011). The evaluation was to some extent limited by the data available for the individual feedstuffs in the studies, such as organic matter digestibility, starch and NDF contents.

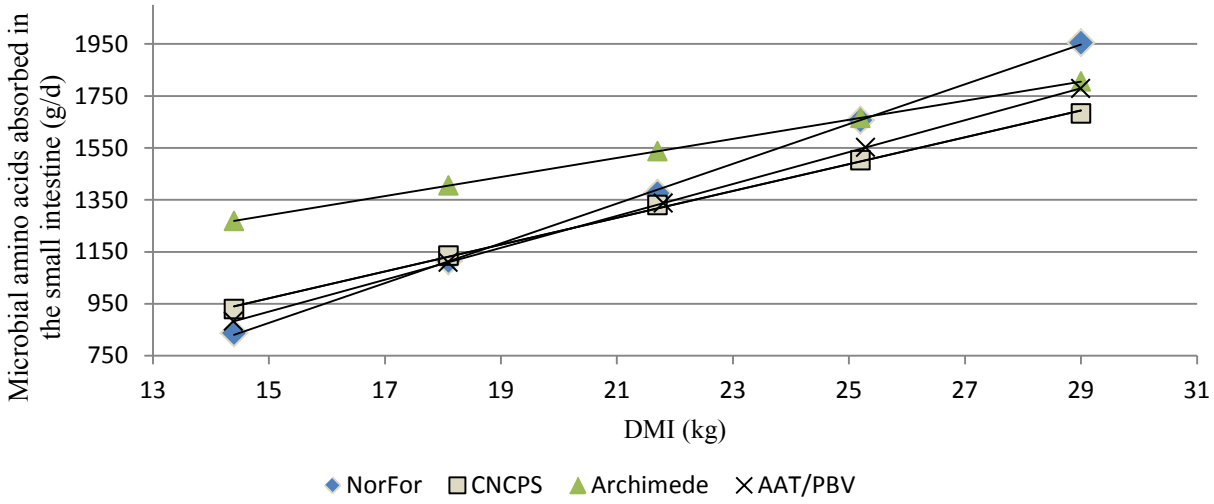


Figure 3 Estimation of microbial protein absorbed in the small intestine with varying DMI levels of the same ration (32% grass silage, 32% corn silage, 19% barley, 10% canola meal, 6% soybean meal).

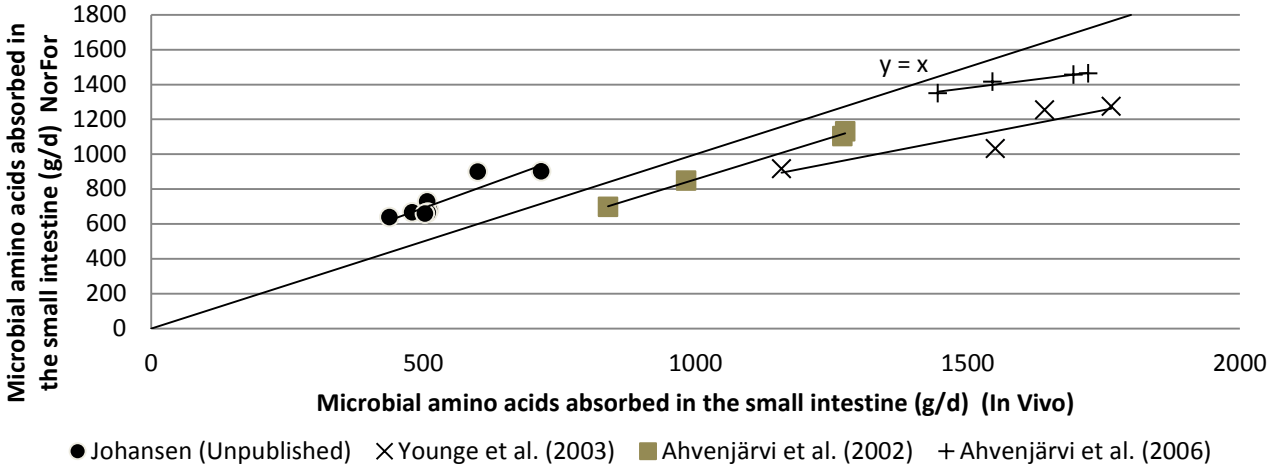


Figure 4 Comparison of microbial protein absorbed in the small intestine estimated in NorFor and measured in vivo.

Conclusion

The evaluations showed that NorFor has a more complex estimation of RMPS than the Archimede and the AAT/PBV models and is more responsive to changes in the concentrate composition as well as to DMI. NorFor agrees well with CNCPS when changing the concentrate to forage ratio using half protein and half carbohydrate concentrates, as well for a carbohydrate rich concentrate. The Archimede model had higher estimates of DMAAS for most of the diets and the AAT/PBV model disagreed with NorFor when the carbohydrate rich concentrate level in the feed was increased above 50%. With a concentrate level of 40% of DM and a daily DMI of 20 kg, the NorFor, CNCPS and the ATT/PBV, all models gave similar estimates of RMPS. Comparing NorFor estimations of RMPS with in vivo studies was successful in detecting changes in RMPS in the different feed rations examined (r=0.93).

References

Ahvenjarvi, S., Joki-Tokola, E., Vanhatalo, A., Jaakkola, S. & Huhtanen, P., 2006. Effects of replacing grass silage with barley silage in dairy cow diets. J Dairy Sci 89: 1678-1687.

Ahvenjarvi, S., Vanhatalo, A. & Huhtanen, P., 2002. Supplementing barley or rapeseed meal to dairy cows fed grass-red clover silage: I. Rumen degradability and microbial flow. J. Animal Sci. 80, 2176-2187.

Archimede, H., Sauvant, D. & Schmidely, P., 1997. Quantitative review of ruminal and total tract digestion of mixed diet organic matter and carbohydrates. Repro. Nutr. Devel. 37: 173-189.

Broderick, G. A. & Merchen, N. R., 1992. Markers for quantifying microbial protein synthesis in the rumen. J Dairy Sci. 75, 2618-2632.

cncpc.cornell.edu. CNCPS v6.5. http://cncps.cornell.edu/.

Dewhurst, R. J., Davies, D. R. & Merry, R. J., 2000. Microbial protein supply from the rumen. Anim. Feed Sci. and Tech. 85, 1-21.

Fox, D. G., Tedeschi, L. O., Tylutki, T. P., Russell, J. B., Van Amburgh, M. E., Chase, L. E., Pell, A. N. & Overton, T. R., 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Science and Technology 112, 29-78.

it.dlbr.dk. DMS. http://it.dlbr.dk/DLBRKvaegIT/DMS/DMS.htm.

Madsen, J., Hvelplund, T., Bertilsson, J., Spörndly, R., Olsson, I., Harstad, O., Volden, H., Tuori, M., Varvikko, T. & Olufsson, B., 1995. The AAT/PBV protein evaluation system for ruminants: a revision. Norwegian J. Agri. Re. 18.

Møller, J., Thøgersen, R., Helleshøj, M. E., Weisbjerg, M., Søegaard, K. R. & Hvelplund, T., 2005. Fodermiddeltabel 2005: sammensætning og foderværdi af fodermidler til kvæg.

norfor.info. NorFor Feedtable. http://feedstuffs.norfor.info/.

Oba, M. & Allen, M., 2000. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 3. Digestibility and microbial efficiency. J. Dairy Sci. 83, 1350-1358.

Volden, H., 2011. NorFor - The Nordic Feed Evaluation System.

Volden, H. & Larsen, M., 2011. NorFor - The Nordic feed evaluation system, Chapter 7 Digestion and metabolism in the gastrointestinal tract.

Younge, B. A., Murphy, J. J. & Rath, M., 2004. Nutrient metabolism in the rumen and milk production in cows fed on grass-silage and fresh grass based diets. Livestock Prod. Sci. 88, 43-54.

Predicting intake capacity of growing bulls

M. Åkerlind[1], B. Johansson[2], H. Stenberg[3], K. Zaralis[4], I. Schei[5], N.I. Nielsen[6] & E. Nadeau[2]
[1]Växa Sverige, BOX 7024, 750 07 Uppsala, Sweden; [2]Swedish University of Agricultural Sciences (SLU), Department of Animal Environment & Health, BOX 234, 532 23 Skara, Sweden; [3]Swedish Performance Testing of Beef Breeds, Lofta Tingstad 4,594 93 Gamleby, Sweden; [4]The Organic Research Centre, Elm Farm, Hamstead Marshall, Newbury, Berkshire RG20 0HR, UK; [5]Tine SA, Postboks 58,1431- Ås, Norway. [6]SEGES, Agrofood Park 15, 8200 Aarhus N, Denmark.
Correspondence: maria.akerlind@vxa.se

Introduction

Since the growing cattle model was introduced in the Nordic Feed Evaluation System, NorFor, new experiments have been performed and thereby these data can be used for improving the system. The feed intake model is based on individual fill values per feedstuff and an intake capacity per animal and correction factors according to concentrate share. When predicting feed intake the intake capacity and the total dietary fill value should be equal. The main objective was to evaluate the feed intake model on growing bulls in NorFor (Volden *et al.*, 2011) with new Swedish experimental data.

Materials and Methods

Data on feed intake, live weight (LW) and live weight gain (LWG) were collected from six experiments conducted at Götala Beef and Lamb Research Centre, SLU, Skara, Sweden and from two field studies at Vinö Säteri, Sweden. Five experiments were performed on young bulls of dairy breeds (100 to 268 kg LW) (Johansson *et al.*, 2011; 2013; 2015). One experiment, which was replicated over two years, was performed on finishing/growing bulls of dairy breeds (350 kg to 650 kg LW) (Nadeau *et al.*, 2013; Zaralis *et al.*, 2014). The field studies were performed on finishing bulls of beef breeds (300 to 600 kg LW) (Stenberg, 2013; Stenberg, 2014).

The bulls in the experiments were fed TMR *ad libitum* and the bulls in the field studies were fed silage *ad libitum* and concentrate restrictively from concentrate feeders. The feedstuffs were analysed for chemical composition according to the recommendations of NorFor (Åkerlind *et al*., 2011). All animals were housed in loose housing systems. Each study was divided into three or four periods of four to twelve weeks each. Table 1 shows that LW and LWG of the newly collected data ranged from 100 to 620 kg and from 400 to 2200 g per day, respectively. Data on previous Scandinavian experiments (Table 2), some of which were used for creating the previous feed intake model in NorFor, had LW that ranged from 113 to 568 kg and LWG from 538 to 1567 g per day.

The feed intake model for growing bulls in the NorFor System (Volden *et al*., 2011) was evaluated by data from the studies described above. The equation numbers presented hereafter are equal to the numbering presented by Volden (2011) and Volden *et al.* (2011). Observed feed intakes in the studies were transformed to intake of fill value (FV_intake; equation 10.6), based on dry matter intake (DMI) and fill value (FV) of each feedstuff. The FV of each individual feedstuff was calculated from *in vivo* organic matter digestibility (OMD), and concentrations of NDF, ammonia nitrogen (NH_3-N) and fermentation acids (lactic, acetic, propionic, and butyric acids) (equations 6.10 and 6.11; Volden, 2011). The FV_intake was calculated from the equation 10.6 according to Volden *et al.* (2011), which is

corrected for the proportion of concentrate (FV_SubR, equation 10.14) and the roughage metabolic regulation factor ((FV_MR, equation 10.15).

Table 1 Means and minimum and maximum values within parenthesis of live weight (LW), live weight gain (LWG), dry-matter intake (DMI) and dietary concentrate proportion from new Swedish experiments with bulls

	Young bulls of dairy breeds	Finishing bulls of dairy breeds	Finishing bulls of beef breeds
Breed codes[1]	HO, SR	HO, SR	CH, LI, SI
LW, kg	177 (100-268)	542 (450-620)	455 (291-600)
LWG, g/day	1248 (400-1712)	1417 (635-1886)	1942 (1730-2227)
DMI, kg/d	4.9 (2.8-7.7)	11.4 (9.9-12.7)	10.7 (8.3-12.9)
Concentrate, % of DMI	50 (12-68)	39 (36-42)	36 (31-46)
No of observations	55	32	27
Feeding strategy	TMR	TMR	Separate
References	Johansson *et al.*, 2011; 2013; 2015	Nadeau *et al.*, 2013; Zaralis *et al.*, 2014	Stenberg, 2013; 2014

[1]Breed codes: CH= Charolais; HO= Holstein; LI= Limousin; SI=Simmenthal; SR= Swedish Red

Table 2 Means and minimum and maximum values within parenthesis of live weight (LW), live weight gain (LWG), dry-matter intake (DMI) and dietary concentrate proportion from experiments with bulls presented by Volden *et al.* (2011)

	Danish	Norwegian	Swedish
Breed[1]	HO	NR	CRS, SR
LW, kg	268 (217-342)	382 (178-568)	236 (113-473)
LWG, g/day	1250 (1047-1481)	1107 (538-1567)	1134 (700-1420)
DMI, kg/d	5.9 (4.6-7.0)	7.3 (3.5 -9.9)	5.1 (2.6-8.4)
Concentrate, % of DMI	60 (22-95)	33 (14-37)	85 (24-96)
No of observations[2]	23	90	79
Feeding strategy	TMR	Separate	Separate
References	Damgaard & Hansen, 1992; Andersen *et al*., 1993a; Andersen *et al*., 1993b; Jørgensen *et al*., 2007; Kirkland *et al*., 2006	Berg, 2004; Berg & Volden, 2004; Selmer-Olsen, 1994; Matre, 1984	Martinsson, 1990; Olsson, 1987; Olsson, unpubl.

[1]Breed codes: CRS=crossbreds between dairy and beef breeds; HO= Holstein; NR=Norwegian Red; SR= Swedish Red. [2] No of experiments (exp) and treatments (trt) 5 exp 23 trtm DK; 10 exp 90 trt NO; 8 exp 79 trt SE.

The FV_intake of the consumed diets were compared to the NorFor predicted intake capacity (IC) of the animals. The IC of the bulls was calculated from their LW and LWG according to equation 10.9 by Volden *et al.* (2011). The difference between the IC and the FV intake (residuals) were tested against different parameters, i.e. the animal parameters LW and LWG and the feed parameters; the proportion of concentrate and dietary concentration of NDF, starch, crude protein, fatty acids and net energy.

The equation of the IC was further developed by the SOLVER function in Microsoft Excel 2010. The coefficients in the equation of IC, 10.9, were chosen by Excel as to minimize the sum of the squared differences between the observed FV_intake and the predicted IC. The accuracy and precision (R^2, mean square prediction error (MSPE)) of the equations of predicted IC were calculated according to Bibby & Toutenberg (1977).

If "PL" <= 6mm :

$FV_i = 0.22$

If "PL" > 6mm :

$$FV_j = \frac{0.86 - OMD \cdot 0.005}{0.94 + 0.56 \cdot e^{-0.000029 \cdot \left(\frac{NDF}{10}\right)^{2.9}}} \qquad 6.10$$

If "PL" > 6mm and grass and legume silage :

$$FV_j = \frac{0.86 - OMD \cdot 0.005}{0.94 + 0.56 \cdot e^{-0.000029 \cdot \left(\frac{NDF}{10}\right)^{2.9}}} \cdot \left(1 - \left(\frac{-0.000531 \cdot \left((TAF)^2 - 6400\right)}{100} + \frac{-4.765 \cdot \left(\ln(NH_3N) - \ln(50)\right)}{100}\right)\right) \qquad 6.11$$

where FV_i and FV_j are the fill values of i'th concentrate and j'th roughage respectively, PL is the particle length in mm, OMD is the *in vivo* organic matter digestibility in %, NDF is the neutral detergent fibre in g per kg DM, TAF is the total acids in feed in g per kg DM and NH_3-N is the ammonia nitrogen in g per kg total nitrogen. In equation 6.11 for grass and legume silages, values of TAF less than 80 should be set to 80, and values of NH_3-N less than 50 should be set to 50.

$$FV\_\text{int}ake = \sum_i DMI\_c_i \cdot FV_i + \sum_j DMI\_r_j \cdot FV_j \cdot FV\_SubR + FV\_MR \qquad 10.6$$

$$FV\_SubR = \left(\frac{0.9646 - 0.706 \cdot \frac{conc\_share}{100}}{1 - 0.7512 \cdot \frac{conc\_share}{100} - 0.2085 \cdot \left(\frac{conc\_share}{100}\right)^2}\right)^2 \qquad 10.14$$

$$FV\_MR = -\left(3.8301 - 2.6608 \cdot FV\_r - \frac{1.2158}{FV\_r}\right) \cdot \left(1 - e^{-0.029474 \cdot LW}\right) \qquad 10.15$$

where FV_intake is the total feed intake expressed as fill units. DMI_c_i is the intake of the i'th concentrate in kg DM per day, FV_i is the fill value of i'th concentrate, DMI_r_j is the intake of the j'th roughage in kg DM per day, FV_j is the fill value (calculated as equation 6.10 or 6.11) for the j'th roughage, FV_SubR is the roughage substitution correction factor, conc_share is the proportion of concentrate in the diet in % of DM , FV_MR is the roughage metabolic regulation factor, FV_r is the diet roughage fill value (calculated as equation 6.10) and LW is the live weight in kg.

$$IC = \left(0.006544 \cdot LW + 0.0007337 \cdot LWG + \frac{-20}{0.9552 \cdot LW}\right) \cdot exercize \qquad 10.9$$

where IC is the intake capacity, LW is the live weight in kg, LWG is the live weight gain in g per day and exercise is 1 for tied up and 1.05 for loose housed bulls.

Results and Discussion

Recent data from Sweden on feed intake by bulls (Table 1) showed that the predicted feed intake model by NorFor could be improved. Previous data collected from Scandinavian experiments on bulls (Table 2) fitted quite well to the NorFor predictions (Volden *et al*., 2011). The new data was unique in that LW had a wider range and the LWGs of the bulls were higher than in previous experiments.

The residuals (difference between IC and FV_intake) showed a trend of deviation with LW and LWG. The IC was overestimated for bulls with higher LW and with higher LWG (Figure 1). The dietary parameters tested had no effect on the residuals, as the slope and R^2 was close to zero (data not shown). Therefore, we concluded that the IC equation should be changed and not the equation for FV_intake, as the IC equation contains LW and LWG.

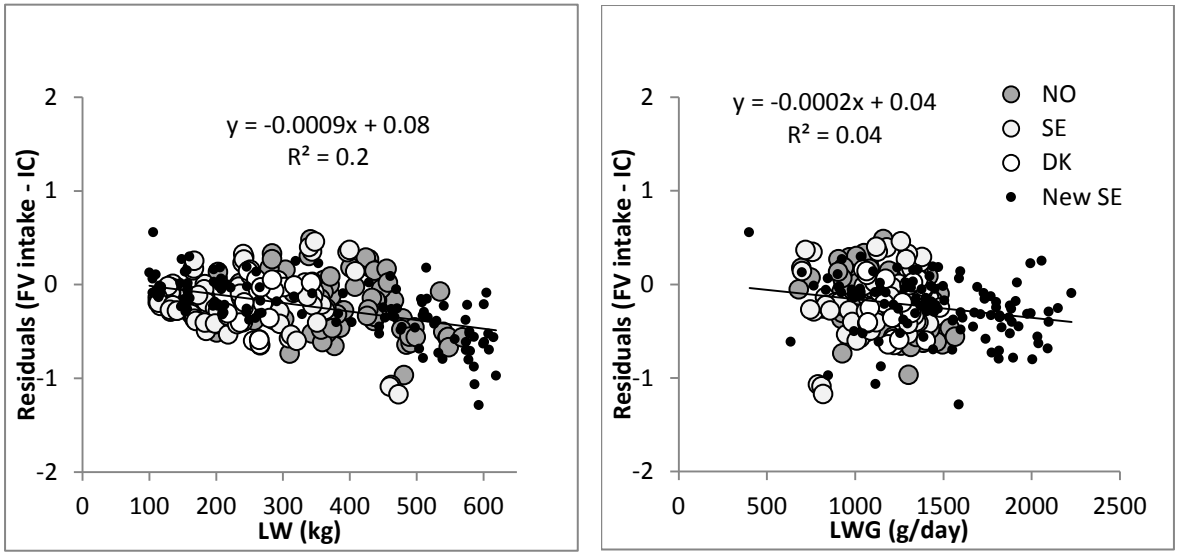


Figure 1 Residuals, the difference between observed FV intake and predicted intake capacity (IC) plotted against observed live weight (LW) and live weight gain (LWG). The filled circles of dark grey, light grey and white represent older experimental data from Norway (NO), Sweden (SE) and Denmark (DK) respectively, and the small black circles shows the results from new research from Sweden (input data shown in tables 1 and 2).

A new equation for intake capacity, as shown in Material and Methods, was established replacing equation 10.9.

$$IC = (0.005886 \cdot LW + 0.0006873 \cdot LWG) \cdot exercize \qquad \text{new 10.9}$$

where IC is the intake capacity, LW is the live weight in kg, LWG is the live weight gain in g per day and exercise is 1 for tied up and 1.05 for loose housed bulls.

The new equation 10.9 was introduced in a new version of the feed ration calculator (FRC) in the NorFor system the 26th of January, 2015 and since then it has been used by advisors and farmers in Denmark, Iceland, Norway and Sweden.

The accuracy and precision of the new equation for predicting the IC of bulls were improved compared to the previous equation (Figure 2; Table 3). The root mean square prediction error (RMSPE) value in Table 3 of 0.36 for the previous and 0.27 FV for the new equation can be translated to 0.8 and 0.6 kg DM, respectively, by dividing RMSPE value with the average FV of the diets which was 0.45 FV per kg DM. Hence, the RMSPE decreased by approx. 0.2 kg

DM. The coefficient of determination (R^2) did not increase with the new equation and there are possibilities to improve the predicted feed intake further.

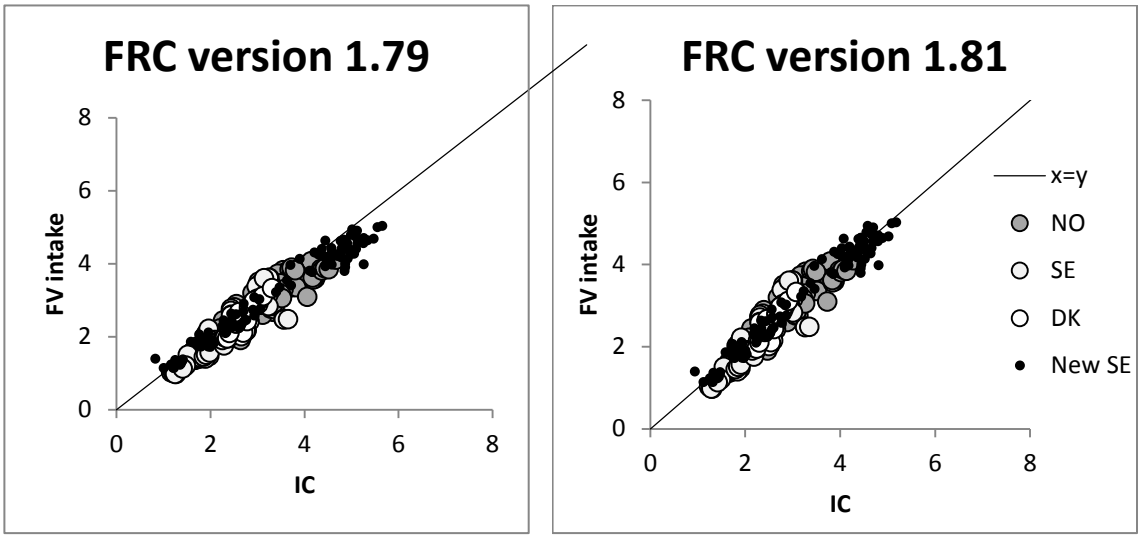


Figure 2 The intake capacity (IC) of bulls plotted to the total fill value of the ingested diets (FV intake). The black line shows when the x-axis is equal to the y axis (x=y) i.e. intercept is 0 and slope is 1. The filled circles of dark grey, light grey and white represent older experimental data from Norway (NO), Sweden (SE) and Denmark (DK) respectively, and the small black circles shows the results from new research from Sweden (input data shown in Tables 1 and 2). FRC version 1.79 and FRC version 1.81 are the equation sets in the feed ration calculator used in the NorFor system by advisors and farmers before and after 26th of January 2015, respectively. Intercept, slope and R^2 are presented in Table 3.

Table 3 Accuracy and precision of the ability to predict feed intake by the feed ration calculator (FRC) of the NorFor system for growing bulls before 26th of January 2015 (FRC version 1.79) and after (FRC version 1.81) using all experiments (Bibby & Toutenberg, 1977)

	FRC version 1.79	FRC version 1.81
No. of observations	296	296
Observed FV intake, FV	2.8	2.8
Predicted IC, FV	3.0	2.8
Regression		
Intercept	0.18	-0.0071
Slope	0.88	1.00
R^2	0.93	0.93
RMSPE[1], FV	0.36	0.27
Prediction error[1], %	12.7	9.6
MSPE	0.13	0.075
Proportion of MSPE		
Error of central tendency	0.29	0.00
Error of regression	0.15	0.00
Error of disturbance	0.56	1.00

[1] RMSPE divided by observed FV intake

The consequence of the new IC equation when optimizing rations for bulls by the NorFor system is that heavier and/or faster growing bulls have a somewhat lower IC compared to the

previous equation. The lower IC requires more energy dense diets, containing high-energy roughage and/or a higher concentrate proportions (Table 4).

Table 4 Optimised diets to growing bulls at different live weight (LW) and live weight gain (LWG) calculated with the original intake capacity (FRC version 1.79; Volden *et al*., 2011) and the revised one (FRC version 1.81). The LWGs corresponds to a carcass weight of 300 kg at 16 months of age

	FRC version 1.79		FRC version 1.81	
LW, LWG	Forage[1]	Concentrate[2]	Forage [1]	Concentrate[2]
Kg, g/day	kg DM	kg DM	kg DM	kg DM
100, 900	1.2	1.8	2.2	1.0
200, 1200	3.8	1.6	3.4	1.9
300, 1300	5.6	1.5	4.5	2.5
400, 1350	7.3	1.5	5.7	2.9
500, 1300	9.1	1.0	7.2	2.5
600, 1300	10.9	0.1	8.9	1.7

[1]Forage was average grass silage in Sweden (Växa Sverige): 73.5 % OMD, 149 g CP/kg DM and 483 g NDF/kg DM. [2]Concentrate consisted of ca 50% barley and 50% protein concentrate to bulls of 100 kg LW; 80% barley and 20% protein concentrate for bulls of 200 kg LW, and 100% barley for heavier bulls.

Conclusions

Our data from the recent experiments on growing/finishing bulls formed a good base for evaluating the NorFor system as it provided wider ranges of LW and LWG compared to earlier studies, where only one observation per treatment was available for a rather long measurement period. A new prediction model for IC in the NorFor system was established with improved precision and accuracy. The new IC is lower than the previous one, resulting in more energy dense diets. New data will continuously be added to the model.

References

Andersen, H.R., Foldager, J., Varnum, P.S. & Klastrup, S., 1993a. Effect of urea and soybean meal at two levels in a whole crop barley silage ration on performance, carcass and meat quality in young bulls. Forskningsrapport nr 4, Statens husdyrbrugsforsøg.

Andersen, H.R., Andersen, B.B., Madsen, P., Varnum, P.S & Jensen, L.R., 1993b. Effect of maize silage plus urea or soybean versus concentrate on performance, carcass, and meat quality of young bulls. Forskningsrapport nr 5, Statens husdyrbrugsforsøg.

Berg, J., 2004. Slutrapport. Produksjonsegenskapene hos slakteokser av Charolais x NRF, Blonde dáquitaine x NRF og NRF ved ulike fôrstyrkenivåer. Institutt for husdyr og akvakulturvitenskap, UMB. Intern rapport, pp. 11.

Berg, J. & Volden, H., 2005. Økologisk storfekjøtt. Buskap nr 4, p 16-17.

Damgaard, P & Hansen, S., 1992. Sammenligning af valset og pilleteret kraftfoder til ungtyre. LK-meddelelse nr 189. 3 pp.

Johansson, B., Fernqvist, T., Hessle, H., Nadeau, E., Arnesson, A. & Kumm, K.-I., 2011. Locally produced protein feeds for dairy bull calves. Proceedings of the 2nd Nordic Feed Science Conference, Uppsala 15-16 June. Swedish University of Agricultural Sciences, Dept. Animal Nutrition Management. Report 277, 38-42.

Johansson, B., Kumm, K-I. & Hessle, A., 2013. Närproducerade proteinfodermedel till mjölkraskalvar – del 2. Final report to the Swedish Farmer's Foundation for Agricultural Research.

Johansson, B., Hessle, A. & Kumm, K-I., 2015. Using clover/grass silage as a protein feed for dairy bull calves. Organic Agriculture, DOI: 10.1007/s13165-015-0113-7.

Jørgensen, K.F., Sehestedt, J. & Vestergaard, M., 2007. Effect of starch level and straw intake on animal performance, rumen wall characteristics and liver abscesses in intensively fed Friesian bulls. Animal 1,797-803.

Kirkland, R.M., Keady, T.W.J., Patterson, D.C., Kilpatrick, D.J. & Steen, R.W.J., 2006. The effect of slaughter weight and sexual status on performance characteristics of male Holstein Friesian cattle offered a cereal-based diet. Anim. Sci. 82, 397-404.

Martinsson, K., 1990. The effect of forage digestibility and concentrate supplementation on performance of finishing bulls. Swedish J. Agric Res. 20,161-167,

Nadeau, E., Zaralis, K., Kumm, K.-I., Nørgaard P. & Arnesson, A., 2013. Majsensilage till mjölkrastjurar – effekt av mognadsstadium och utfodringsstrategi på konsumtion, foderutnyttjande, slaktkroppskvalitet och ekonomi. Final report to the Swedish Farmer's Foundation for Agricultural Research. H0850375.

Olsson, I,, 1987. Effect of protein supply on the performance of intensively reared bulls – evaluation of the DCP and the Nordic AAT-PBV protein evaluation system. PhD dissert., Report164. Swedish University of Agricultural Sciences, Uppsala.

Selmer-Olsen, I., 1994. Feed intake, milk yield, live weight gain, with silage from big bales or silos. Poster. 45th Annual Meeting of EAAP, Edinburgh 5-8 September.

Stenberg, H., 2013. Vinö- ungtjurar 2012/13. www.taurus.mu. Taurus, Hörby, Sweden.

Stenberg, H., 2014. Vinö- ungtjurar 2013/14. www.taurus.mu. Taurus, Hörby, Sweden.

Volden, H., 2011. Feed calculations in NorFor. In: NorFor – The Nordic Feed Evaluation System. EAAP No. 130. Wageningen Academic Press, The Netherlands.

Volden, H., Nielsen, N.I., Åkerlind, M., Larsen, M., Havrevoll, Ø. & Rygh, A.J., 2011. Prediction of voluntary feed intake. In: NorFor – The Nordic Feed Evaluation System. EAAP No. 130. Wageningen Academic Press, the Netherlands.

Zaralis, K., Nørgaard, P., Helander, C., Murphy M., Weisbjerg, M.R. & Nadeau, E., 2014. Effects of maize maturity at harvest and dietary proportion of maize silage on intake and performance of growing/finishing bulls. Livest. Sci. 168, 89–93.

Effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on feed intake and growth performance in dairy calves

M. Pesonen & A. Huuskonen
Natural Resources Institute Finland (Luke), Green Technology, Tutkimusasemantie 15, FI-92400 Ruukki, Finland.
Correspondence: Maiju.Pesonen@luke.fi

Introduction

Influences of yeast culture supplementation on numerous growth and production traits have been studied in most ruminant age classes (e.g. Chaucheyras-Durand *et al*., 2008; Magalhães *et al*., 2008; Pinos-Rodríguez *et al*., 2008; Lascano & Heinrichs, 2009). However, results are somewhat inconsistent throughout the literature, partially because of confounding effects of ration composition, level of yeast culture inclusion, and source of yeast culture product tested. In addition, only a few studies have utilized pre-ruminant dairy calves. Therefore, the present experiment was designed to study the effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on feed intake, growth performance and health of dairy calves.

Materials and Methods

The present experiment included two batches of 20 Nordic Red bull calves each. The first batch started in April and the second in November 2013 in the experimental barn of Natural Resources Institute Finland in Ruukki. All calves were purchased from local dairy farms. The calves were housed in an insulated barn in four pens (3.0 × 3.5 m; 5 calves in each), providing 2.1 m^2/calf. The floor of the pen was 1/3 metal slats and 2/3 rubber mats. The ambient temperature of the insulated barn varied between 11 and 20°C in the winter (October–April) and between 15 and 23°C in the summer (May–September). In the beginning of the experiment, the calves in both batches were randomly allotted to pens (5 calves/pen) which were then randomly allotted to two feeding treatments. These two feeding treatments consisted of a concentrate containing 0% (control = C) or 0.1% (yeast = Y) supplemental yeast culture (Actisaf, *Saccharomyces cerevisiae* Sc 47, contains 1.0×10^{10} CFU/g S. *cerevisiae*) as a percentage of concentrate dry matter (DM). Both feeding treatments included 20 bull calves. In the beginning of the experiment, average live weight (LW) of the calves was 56 ± 3.0 kg (mean ± SD) and overall age 20 ± 2.5 days. During the whole experimental period (20 to 195 days of age), the calves had free access to water from an open water bowl (1 bowl/pen).

During the pre-weaning period (age 20 to 75 days), the calves received a milk replacer (MR) (at a dilution of 11.9% DM). The MR included (g/kg DM) skim milk powder (300), whey powder (283), vegetable oil (190), whey fractions (100), hydrolysed wheat protein (65), wheat starch (50) and vitamin-mineral premix (12). In both treatments, the MR was served by a computer controlled feeder (two pens/feeder; Stand Alone 2 Plus, Förster, Engen, Germany; programme: Kalbmanager 4.2). The feeding temperature of the MR was 37ºC. The calves were allocated to treatments at 20 days of age, and from days 20 to 62 the highest possible MR allowance of the calves was 7.0 L/d. All calves were weaned gradually from days 62 to 75 with the MR allowance being cut by reducing the number of MR portions per day. During the pre-weaning period, the calves had free access to concentrate and grass silage.

During the post-weaning period (age 75 to 195 days) the calves were fed grass silage *ad libitum*, but the amount of concentrate was restricted to 3 kg (air dry)/calf/d. The commercial

pelleted concentrate used during both pre- and post-weaning periods was supplied by A-Rehu Ltd (P.O. Box 908, FI-60061 Atria, Finland). The concentrate in the treatment C comprised (g/kg DM) barley (333), rapeseed meal (174), wheat (80), barley fibre (72), molassed sugar-beet pulp (70), soybean meal (50), oats (50), molasses (50), rapeseed cake (42), distilled solubles (40), $CaCo_3$ (20), vegetable oil mix (8), and vitamin, mineral and trace element premix (11). In the treatment Y the concentrate was otherwise the same, but comprised barley (332) and supplemental yeast culture (1) (*Saccharomyces cerevisiae* Sc 47). This dose of yeast culture was established according to Lascano & Heinrichs (2009). The grass silage used in the experiment was harvested from first-year stands grown in Ruukki, Finland (64°44′N, 25°15′E). The silage was prepared from primary growth of timothy (*Phleum pratense*) stand and harvested at early stages of maturity. The silage was cut using a mower conditioner, wilted for 5 h, harvested using a precision-chop forage harvester, ensiled in a bunker silo and treated with a formic acid-based additive applied at a rate of 5 L/tonne of fresh forage.

The silage and concentrates were offered separately from a box feeder with proportional refusals at 5% in *ad libitum* feeding, and the calves were fed three times per day (at 08:00, 12:00 and 18:00 h). The refused feed was collected and measured daily at 07:00 h. Daily solid feed intake was weighed penwise (i.e. average for five calves). Silage samples for chemical analyses were taken twice a week and pooled over periods of four weeks. Samples were analysed for DM, ash, crude protein (CP), ether extracts, starch and fermentation quality (pH, water soluble carbohydrates, lactic and formic acids, volatile fatty acids, soluble and ammonia-N content of N) as described by Pesonen *et al*. (2013). Concentrate and MR sub-samples were collected weekly, pooled over periods of 12 weeks and analysed for DM, ash, CP, ether extracts and starch. The metabolisable energy (ME) values were calculated according to the Finnish Feed tables (MTT, 2014). The calves were weighed on two consecutive days at the beginning of the experiment and thereafter every 14 days. The live weight gain (LWG) was calculated as the difference between the means of initial and final LW. Health parameters such as faecal consistency (normal or diarrhoea), bloat, movements, cough, inflammations, e.g., pneumonia, swollen joints and hair loss, were monitored daily.

The results were calculated across the two batches and are shown as least squares means. Pen (a group of five calves) was used as an experimental unit and thus, mean values for each pen were calculated. There were 4 pens/treatment (20 calves for both treatments). The average group feed intake and growth data were subjected to analysis of variance using the SAS MIXED procedure (version 9.3, SAS Institute Inc., Cary, NC). The statistical model used was $y_{jkl} = \mu + \beta_k + \alpha_j + (\beta \times \alpha)_{jk} + e_{jkl,}$ where μ is the overall mean, e_{jkl} is the random error term and y_{jkl} is the mean of five animals penned together (4 pens/treatment; l=1,…,4). α, β and $\beta \times \alpha$ are the fixed effects of treatment, batch and their interaction, respectively.

Results and Discussion

Chemical composition and feeding values of the experimental feeds are presented in Table 1. The commercial starter concentrate contained ME 12.4 MJ/kg DM and CP 198 g/kg DM, on average. The grass silage used was of good nutritional quality as indicated by the ME value as well as the CP content. The fermentation quality of the grass silage was good, as indicated by low pH values and low contents of ammonia N and fatty acids. The silage used was restrictedly fermented with a high residual water soluble carbohydrate concentration and a low lactic acid concentration. MR used in the present experiment had a typical chemical composition and feed values (Table 1).

Table 1 Chemical composition and feeding values of the experimental feeds

	Grass silage	Milk replacer	C	Y
Dry matter (DM), g/kg feed	287	943	856	865
Organic matter (OM), g/kg DM	932	910	920	922
Crude protein, g/kg DM	138	216	203	193
Ether extracts, g/kg DM	39	114	38	40
Starch, g/kg DM	5	100	313	295
Metabolisable energy, MJ/kg DM	10.6	17.4	12.4	12.4

C (control) = a concentrate containing 0% supplemental yeast culture, Y (yeast) = a concentrate containing 0.1% supplemental yeast culture (Actisaf, *Saccharomyces cerevisiae* Sc 47) as a percentage of starter dry matter, Fermentation quality of the grass silage: pH 3.96; volatile fatty acids 11 g/kg DM; lactic + formic acid 41 g/kg DM; water-soluble carbohydrates 56 g/kg DM; ammonia-N 39 g/kg total N; soluble N 423 g/kg total N

There were no treatment differences in feed intake of the calves during the pre-weaning and post-weaning periods or average during the experiment (Table 2). Since there were no differences in feed intake or diet chemical compositions and feeding values, also energy and nutrient intakes were at the same level for both treatments. Compared to the recent Finnish experimental data sets for dairy bull calves fed diets based on MR, grass silage, and concentrates in similar housing environments (Huuskonen *et al*., 2005; 2011), average DM intake of the calves during the pre-weaning and post-weaning periods was approximately 20% higher in the present experiment (1.67 kg and 5.23 DM/d, for pre- and post-weaning periods, respectively) than in those recent feeding trials (1.36 kg and 4.27 DM/d). The high feed intake measured in the present study probably implies a good palatability of the starter concentrate used and a good health of the calves in the present experiment.

The average LW of the calves was 101 and 247 kg at the end of the pre-weaning period and at age of 195 days, respectively. There were no treatment differences in the average LW, LWG or feed conversion rates of the calves (Table 3). Average LWG of the calves during the pre-weaning and post-weaning periods and during the entire experiment were 792, 1301, and 1132 g/d, respectively, values which are slightly higher compared to results by Huuskonen *et al*. (2005; 2011), with dairy bull calves fed diets based on MR, grass silage, and concentrates in similar housing environments (724, 1258, and 1080 g/d). The use of different starters did not affect health parameters of the calves in the present study (data not shown).

According to the literature, supplemental yeast products have been shown to improve performance of dairy ruminants, the most consistent effects being an increase in intake and milk production (El-Ghani, 2004; Jouany, 2006; Stella *et al*., 2007). Also in beef cattle or young ruminants, growth, intake and feed conversion have been reported to improve by yeasts supplementation (Lesmeister *et al*., 2004; Galvao *et al*., 2005). In neonatal dairy calves, Lesmeister *et al*. (2004) reported that inclusion of yeast culture (*Saccharomyces cerevisiae*) increased starter and total DM intake, average daily gain, and daily hip width change compared with a control treatment. However, results in dairy calves are inconsistent throughout the literature and, for example, Quigley *et al*. (1992) observed a significant decrease in DM intake with supplemental yeast culture. In addition, others have found decreased DM intake when brewer's yeast (Seymour *et al*., 1995) or live yeast (Wagner *et al*., 1990) were added to calf diets. Williams *et al*. (1991) indicated that yeast culture beneficially alters conditions detrimental to cellulolysis, by possibly influencing intake.

According to Chaucheyras-Durand *et al*. (2008), yeast responses vary depending on the strain of yeast used, the nature of the diet, and the physiological status of the animal. Chaucheyras-Durand *et al*. (2008) concluded that use of supplemental yeast culture appears particularly relevant when the digestive microbiota is challenged, for example, during feed transitions at weaning, allowed to graze, supplied with high-concentrate diets, or during periods of stress from high temperatures or during transportation.

Table 2 Daily feed and nutrient intakes of dairy calves fed diets with a concentrate containing either 0% (control = C) or 0.1% (yeast = Y) supplemental yeast culture (Actisaf, *Saccharomyces cerevisiae* Sc 47) as a percentage of starter dry matter (DM)

	Diets		SEM	*P*-value
	C	Y		
Pre-weaning (between 20 to 75 days of age)				
Milk replacer, kg DM/d	0.71	0.72	0.010	0.41
Concentrate, kg DM/d	0.69	0.67	0.065	0.76
Forage, kg DM/d	0.30	0.25	0.019	0.17
Total, kg DM/d	1.70	1.64	0.087	0.64
Metabolizable energy, MJ/d	24.1	23.5	1.08	0.71
Crude protein, g/d	334	318	16.5	0.52
Fat, g/d	121	120	3.8	0.99
Post-weaning (between 75 to 195 days of age)				
Concentrate, kg DM/d	2.48	2.46	0.030	0.71
Forage, kg DM/d	2.80	2.73	0.103	0.66
Total, kg DM/d	5.27	5.19	0.125	0.65
Metabolizable energy, MJ/d	60.4	59.4	1.36	0.65
Crude protein, g/d	890	852	18.7	0.23
Fat, g/d	204	206	4.8	0.83
Average during the experiment				
Milk replacer, kg DM/d	0.24	0.24	0.003	0.41
Concentrate, kg DM/d	1.88	1.86	0.041	0.73
Forage, kg DM/d	1.97	1.91	0.074	0.60
Total, kg DM/d	4.09	4.01	0.107	0.64
Metabolizable energy, MJ/d	48.3	47.5	1.20	0.65
Crude protein, g/d	705	674	17.0	0.27
Fat, g/d	177	178	0.004	0.87

Table 3 Live weights, live weight gains and feed conversion rates of dairy calves fed diets with a concentrate containing either 0% (control = C) or 0.1% (yeast = Y) supplemental yeast culture (Actisaf, *Saccharomyces cerevisiae* Sc 47) as a percentage of starter dry matter (DM)

	Feedings		SEM	*P*-value
	C	Y		
Live weight, kg				
Initial, at age of 20 days	57	55	3.3	0.71
At the end of pre-weaning	103	98	5.4	0.51
Final, at age of 195 days	249	244	8.5	0.71
Live weight gain, g/d				
Pre-weaning (between 20 to 75 days of age)	824	760	52.2	0.43
Post-weaning (between 75 to 195 days of age)	1298	1304	35.6	0.92
Average during the experiment	1141	1123	37.1	0.75
Feed conversion rates (kg DM / kg live weight gain)				
Pre-weaning	2.17	2.21	0.039	0.51
Post-weaning	4.08	4.02	0.121	0.75
Average	3.60	3.60	0.091	0.99
MJ / kg live weight gain				
Pre-weaning	30.7	31.6	0.78	0.49
Post-weaning	46.7	46.0	1.34	0.75
Average	42.6	42.6	1.04	0.98

Conclusions

No treatment differences were observed in DM or energy intakes, live weight gain, feed conversion rate or health of the calves. Thus, no evidence existed that *Saccharomyces cerevisiae* inclusion can enhance calf performance under the conditions of the present study. All calves in the present experiment were healthy and different results may be observed in calves facing greater disease challenge.

References

Chaucheyras-Durand, F., Walker, N.D. & Bach, A., 2008. Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. Anim. Feed Sci. Technol., 145, 5-26.

El-Ghani, A.A.A., 2004. Influence of diet supplementation with yeast culture (*Saccharomyces cerevisiae*) on performance of Zaraibi goats. Small Rumin. Res., 52, 223-229.

Galvao, K.N., Santos, J.E., Coscioni, A., Villasenor, M., Sischo, W.M. & Berge, A.C., 2005. Effect of feeding live yeast products to calves with failure of passive transfer on performance and patterns of antibiotic resistance in fecal *Escherichia coli*. Reprod. Nutr. Dev., 45, 427-440.

Huuskonen, A., Khalili, H., Kiljala, J., Joki-Tokola, E. & Nousiainen, J., 2005. Effects of vegetable fats versus lard in milk replacers on feed intake, digestibility, and growth in Finnish Ayrshire bull calves. J. Dairy Sci., 88, 3575-3581.

Huuskonen, A., Tuomisto, L. & Kauppinen, R. 2011. Effect of drinking water temperature on water intake and performance of dairy calves. J. Dairy Sci., 94, 2475-2480.

Jouany, J.P., 2006. Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. Anim. Reprod. Sci., 96, 250-264.

Lascano, G.J. & Heinrichs, A.J., 2009. Rumen fermentation pattern of dairy heifers fed restricted amounts of low, medium, and high concentrate diets without and with yeast culture. Livest. Sci., 124, 48-57.

Lesmeister, K.E., Heinrichs, A.J. & Gabler, M.T., 2004. Effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. J. Dairy Sci., 87, 1832-1839.

Magalhães, V.J.A., Susca, F., Lima, F.S., Branco, A.F., Yoon, I. & Santos, J.E.P., 2008. Effect of feeding yeast culture on performance, health, and immunocompetence of dairy calves. J. Dairy Sci., 91, 1497-1509.

MTT, 2014. Feed tables and nutrient requirements. MTT Agrifood Research Finland. Retrieved 8 April 2015, from http://www.mtt.fi/feedtables.

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.

Pinos-Rodríguez, J.M., Robinson, P.H., Ortega, M.E., Berry, S.L., Mendoza, G. & Bárcena, R., 2008. Performance and rumen fermentation of dairy calves supplemented with *Saccharomyces cervisiae*1077 or *Saccharomyces boulardii*1079. Anim. Feed Sci. Technol., 140, 223-232.

Quigley, J. D., III, Wallis, L.B., Dowlen, H.H. & Heitmann, R.N., 1992. Sodium bicarbonate and yeast culture effects on ruminal fermentation, growth, and intake in dairy calves. J. Dairy Sci., 75, 3531-3538.

Seymour, W. M., Nocek, J.E. &. Siciliano-Jones, J., 1995. Effects of a colostrum substitute and of dietary brewer's yeast on the health and performance of dairy calves. J. Dairy Sci., 78, 412-420.

Stella, A.V., Paratte, R.,Valnegri, L., Cigalino, G., Soncini, G., Chevaux, E., Dell'Orto,V. & Savoini, G., 2007. Effect of administration of live *Saccharomyces cerevisiae* on milk production, milk composition, blood metabolites, and faecal flora in early lactating dairy goats. Small Rumin. Res., 67, 7-13.

Wagner, D. G., Quinonez, J. & Bush, L.J., 1990. The effect of corn or wheat-based diets and yeast culture on performance, ruminal pH, and volatile fatty acids in dairy calves. Agri-Pract., 11, 7-12.

Williams, P.E.V., Tait, C.A.G., Innes, G.M. & Newbold, C.J., 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci., 69, 3016-3026.

DISTRIBUTION:
Sveriges Lantbruksuniversitet
Institutionen för husdjurens utfodring och vård
Box 7024
750 07 UPPSALA
Tel.018/672817

Margareta.Norinder@slu.se