Proceedings of the 5th Nordic Feed Science Conference, Uppsala, Sweden

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences Department of Animal Nutrition and Management

Rapport 290
Report

Uppsala 2014
ISSN 0347-9838
ISRN SLU-HUV-R-290-SE

Proceedings of the 5th Nordic Feed Science Conference, Uppsala, Sweden

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

Rapport 290
Report

Uppsala 2014

ISSN 0347-9838
ISRN SLU-HUV-R-290-SE

Published by:
Organising committee of the 5th 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,
Kungsängen Research Centre
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 celebrate the 5th anniversary of the Nordic Feed Science Conference which started in 2010. The aim of the conference has been to create an arena for Nordic feed scientists to meet and discuss ruminant and horse feeds and feeding. The 1st year, the conference drew a high number of participants (119) and after that, it has stabilized at about 60 (Figure 1). The number of contributions has varied from 25 to 44 and, outside Sweden, Denmark and Finland have been the most frequent contributors (Table 1). It is gratifying that in the last years, we have seen 2 to 3 joint Nordic papers. Maybe, this is a result of more cooperation among the Nordic countries.

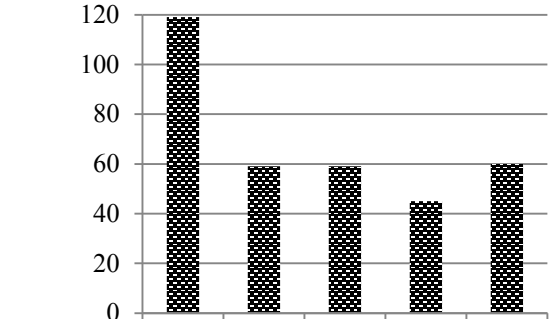

Figure 1 Number of participants

Table 1. Contribution to the Conference from different countries

Year	SE	DK	FI	NO	ISL	NORD	EST	LV	LT	Other	Total
2010	21	7	7	3	0	0	1	0	0	5	44
2011	17	4	2	0	0	0	0	2	0	0	25
2012	14	0	1	4	1	0	0	0	0	11	31
2013	15	4	1	1	0	2	1	0	0	0	24
2014	12	2	8	0	0	3	1	0	2	5	33
Mean	15.8	3.4	3.8	1.6	0.2	1.0	0.6	0.4	0.4	4.2	31.4

One topic of particular interest this year is protein evaluation. In ruminants, Session II deals exclusively with protein nutrition and in the evening session, we anticipate interesting discussions of the value of forage proteins. The two contributions on horses also deal with protein.

This year we are extremely happy to have three internationally renowned scientists speaking to us. Limin Kung from the Dairy Nutrition and Silage Fermentation Lab, University of Delaware, Karl-Heinz Südekum from the University of Bonn and (for the 4th time) Glen Broderick from Madison, Wisconsin. We wish to welcome these eminent scientists for coming all the way to join us in this conference.

Uppsala 2014-06-02

Peter Udén

Contents

Forage conservation and feed processing

Ruminant nutrition

Ecology of silage microorganisms

K. Mogodiniyai Kasmaei[1], V. Passoth[2] & Peter Udén[1]
[1] *Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Feed Science Division, Kungsängen Research Centre, 753 23 Uppsala, Sweden.* [2] *Swedish University of Agricultural Sciences (SLU), Department of Microbiology, 756 51 Uppsala, Sweden*
Correspondence: Kamyar.Mogodiniyai.Kasmaei@slu.se

Introduction

The biochemistry and microbiology of ensiling have been investigated in depth in several studies (McDonald et al., 1991; Rooke and Hatfield, 2003; Pahlow et al., 2003). It is, however, difficult to combine these results to address as to why the catabolic diversity in silage ecosystem is large and what are the driving forces for such complexity. As an example, certain lactic acid bacteria under the presence of air reduce O_2, by which, reactive oxygen species (e.g. superoxide, hydrogen peroxide) which are essentially detrimental to them are formed (Pahlow, 1991). In this work, we aim at dissecting factors of importance to the silage ecosystem and provide an overview of some of the catabolic pathways employed by silage microorganisms.

Respiration versus fermentation

Understanding similarities and differences between respiration and fermentation is essential for understanding causes and effects in the silage ecosystem. In both processes, chemical energy of substrates is released by means of oxidation-reduction (redox) reactions. Electrons are transferred from substrates to compounds with higher reduction potentials, *i.e.* the electron acceptors, by electron carrier molecules (*e.g.* nicotinamide adenine dinucleotide). A larger difference between reduction potential of electron donors and acceptors means a greater magnitude of the $\Delta G^{0'}$. In biological systems, O_2 has the highest reduction potential (Madigan et al., 2012). The half-reactions, *i.e.* oxidation of the substrate and reduction of the electron acceptor, always take place together so that the electron carrier molecules become again available and the process of energy release continues, a phenomenon referred as a balanced reaction (Madigan et al., 2012). It is therefore appeared that this process is heavily dependent on the presence of electron acceptor compounds in the system.

In fermentative pathways, the redox balance is reached by reduction of intermediates (*e.g.* pyruvate) that are formed from the up-taken substrates. This results in an incomplete oxidation and excretion of semi-catabolized substrates, *i.e.* fermentation end-products. In respiratory pathways, external electron acceptors (EEAs), *e.g.* O_2, or nitrate, are exploited instead. A further distinction between the two is that in fermentation, the released energy is stored via substrate level phosphorylation but in respiration, oxidative phosphorylation is also employed. This in turn gives substantial superiority to respiration regarding energy conservation efficiency.

Silage ecosystem

A low pH of silage causes stress to acid tolerant microorganisms while being detrimental to the others. This effect is the result of passive entry of short chain fatty acids (e.g. acetic and propionic acids) into the cell before dissociation and decreasing the intracellular pH to detrimental levels. Silage microorganisms employ different strategies to withstand a low pH. Active removal of H^+ is the most important defensive response (De Angelis and Gobbetti,

2004). It seems, therefore, under low pH conditions, silage microorganisms are in a greater demand of energy.

When air penetrates into a silo, growth of yeasts is enhanced (Jonsson and Pahlow, 1984). This increased population growth has been attributed to the lactate assimilating ability of certain species, collectively known as lactate assimilating yeasts. Firstly, such classification of silage yeasts (*i.e.* lactate *vs.* non-lactate assimilating) is an approximation because the response of different yeast species to lactate depends heavily on environmental conditions (*e.g*. N source and pH) and adaptation time (Middelhoven and Franzen, 1986). Secondly, other fermentation end-products, such as ethanol, can also be assimilated by yeasts when air is present. Therefore, this classification is incomplete and could be misleading. The increased growth is, however, explained by the fact that respiratory species (*e.g. Wickerhamomyces anomalus*) can switch from fermentation to respiration (Madigan et al., 2012), by which, the efficiency of energy conservation is improved.

A similar strategy of increasing energy gain can also be found in prokaryotic species. For instance, *Escherichia coli* is able to anaerobically respire when nitrate is available (Madigan et al., 2012). *Leuconostoc* spp. and *Lactobacillus plantarum* will ferment glucose to acetic acid instead of lactic acid under the presence of O_2 and nitrate, respectively (Rooke and Hatfield, 2003). Through this externally balanced fermentative pathway, two extra moles of ATP are gained. Lactic acid bacteria generally lack catalase for detoxification of reactive oxygen species (Pahlow, 1991; Sanders et al., 1999) but as discussed in the Introduction, they still opt reducing O_2. This phenomenon might be explained by the theory that silage microorganisms prioritize enhancement of energy gain due to an inflated energy demand. On standing forges, this inflation is owing to starvation, irradiation and climatic conditions and during ensiling, it is caused by the low pH.

Anaerobic condition together with the closed system of ensiling favor lactic acid bacteria as these conditions allow accumulation of lactic acid and, thereby, out-competition of pH sensitive microorganisms. The ability of silage microorganisms to produce anti-microbial compounds that directly target competitors, however, should not be ruled out. For instance, it is known that *L. plantarum*, *L. buchneri* and *W. Anomalus* have the ability to produce certain anti-microbial compounds (Gollop et al., 2005; Passoth et al., 2006; Olstorpe et al., 2011).

Different strategies used by silage microorganisms for increasing energy conservation efficiency can be summarized as shown in Figure 1.

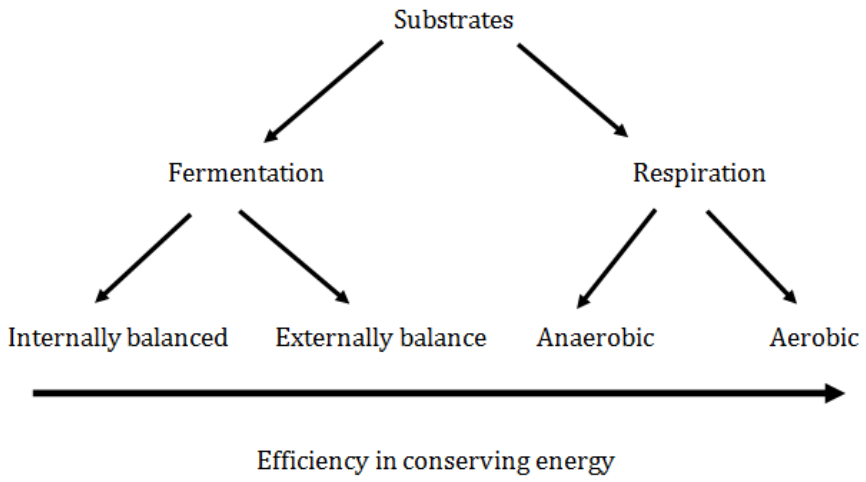

Figure 1 A summary over catabolic pathways used by silage microorganisms.

The metabolic ability of microorganism (*e.g.* yeasts cannot practice anaerobic respiration) and redox balance status (*i.e.* availability of EEAs) are two factors that determine pathway. Substrate availability and silage pH can also play important roles (McDonald et al., 1991). The complexity is even further increased by the fact that some microorganisms can utilize certain end-products during starvation. It was, for instance, found that under the presence of citrate, *L. plantarum* can ferment lactic acid, using citrate as the EEA (Lindgren et al., 1990).

Implications

Modeling silage quality from pre-ensiled forage composition could be of great benefit to farmers. Advice on the correct kind of additives, wilting strategies, etc. could eventually be improved. Such models can also play important parts in silage research and additive evaluations. However, most of the attempts in this regard (Wilkinson et al., 1983; Pitt et al., 1985; Mogodiniyai Kasmaei et al., 2013) have been largely unsuccessful. The majority of these modeling attempts, including both dynamic and static models, have not taken the role of microbial interactions into account, something which may have contributed to their failures. This has mainly been due to scarcity of the relevant data and/or underestimating the effect of this factor on ensiling results. For constructing more powerful silage models, data on microbial interaction seem to be absolutely necessary.

Improving the aerobic stability of silage upon opening has recently become of great interest. Inoculation with *L. buchneri*, a heterofermentative species, has been suggested (Kleinschmit and Kung, 2006). However, this strategy could lead to an increased dry matter loss, which is explained by a slow rate of pH decline that allows continuation of microbial catabolic activities and formation of CO_2 by heterolactic fermentation (Wilkinson and Davies, 2012). Application of chemical additives can be an alternative strategy to improve both aerobic stability and DM losses (Knicky and Spörndly, 2011). However, the cost and unsustainability are unfortunately disadvantages of this technique (Wilkinson and Davies, 2012). An increased knowledge of microbial ecology could improve silage research and result in a new generation of additives or even new storage strategies.

Conclusions

Silage microorganisms strive to enhance energy conservation efficiency. The strategy used is a constant adjustment of catabolic pathways to environmental conditions including substrate availability, redox balance and pH. A better understanding of microbial ecology is needed to improve silage research and technology.

References

De Angelis, M. & Gobbetti, M., 2004. Environmental stress responses in Lactobacillus: A review. Proteomics 4, 106-122.

Gollop, N., Zakin, V. & Weinberg, Z.G., 2005. Antibacterial activity of lactic acid bacteria included in inoculants for silage and in silages treated with these inoculants. J. Appl. Microbiol. 98, 662-666.

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

Kleinschmit, D.H. & Kung, L. Jr., 2006. A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. J. Dairy Sci. 89, 4005-4013.

Knicky, M. & Spörndly, R., 2011. The ensiling capability of a mixture of sodium benzoate, potassium sorbate, and sodium nitrite. J. Dairy Sci. 94, 824-831.

Lindgren, S.E., Axelsson, L.T. & McFeeters, R.F., 1990. Anaerobic L-Lactate Degradation by Lactobacillus-Plantarum. FEMS Microbiol. Lett. 66, 209-213.

Madigan, M.T., Martinko, J.M., Stahl, D.A. & Clark, D.P., 2012. Brock Biology of Microorganisms. Pearson Education, San Francisco.

McDonald, P., Henderson, A.R. & Heron, S.J.E., 1991. The Biochemistry of Silage. Chalcombe publications, Marlow, Bucks, UK.

Middelhoven, W.J. & Franzen, M.M., 1986. The Yeast flora of ensiled whole-crop maize. J. Sci. Food Agr. 37, 855-861.

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

Olstorpe, M., Jacobsson, K., Passoth, V. & Schnürer, J., 2011. Controlling fungal growth and mycotoxins in animal feed, In: Lacroix, C. (Ed.), Protective Cultures, Antimicrobial Metabolites and Bacteriophages for Food and Beverage Biopreservation. Woodhead Publishing Ltd., Cambridge, UK, pp. 225-239.

Pahlow, G., 1991. Role of microflora in forage conservation, In: Pahlow, G., Honig, H. (Eds.), Forage conservation towards 2000. Landbauforschung Völkenrode, Bruanschweig, Germany, pp. 26-36.

Pahlow, G., Muck, R.E., Driehuis, F., Oude Elferink, S.J.W.H. & Spoelstra, S.F., 2003. Microbiology of ensiling, In: Buxton, D.R., Muck, R.E., Harrison, J.E. (Eds.), Silage Science and Technology. Am. Soc.Agron.Inc., Madison, Wisconsin, USA, pp. 31-93.

Passoth, V., Fredlund, E., Druvefors, U.A. & Schnürer, J., 2006. Biotechnology, physiology and genetics of the yeast Pichia anomala. FEMS Yeast Res. 6, 3-13.

Pitt, R.E., Muck, R.E. & Leibensperger, R.Y., 1985. A quantitative model of the ensilage process in lactate silages. Grass Forage Sci. 40, 279-303.

Rooke, J.A. & Hatfield, R.d., 2003. Biochemistry of ensiling, In: Buxton, D.R., Muck, R.E., Harrison, J.E. (Eds.), Silage Science and Technology. Am. Soc.Agron.Inc., Madison, Wisconsin, USA, pp. 95-139.

Sanders, J.W., Venema, G. & Kok, J., 1999. Environmental stress responses in Lactococcus lactis. FEMS Microbiol Rev. 23, 483-501.

Wilkinson, J.M., Chapman, P.F., Wilkins, R.J. & Wilson, R.F., 1983. Interrelationships between pattern of fermentation during ensilage and initial crop composition, In: Smith, J.A., Hays, V.W. (Eds.), Proc.XIV Intern. Grassl. Congr., Kentucky, USA, pp. 631-634.

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

Managing the aerobic stability of silages

L. Kung Jr.
Dairy Nutrition & Silage Fermentation Laboratory, Department of Animal & Food Sciences, University of Delaware, Newark, Delaware 19716, USA
Correspondence: lksilage@udel.edu

Introduction

Prolonged infiltration of air during storage or feed out into the silage mass can lead to aerobic spoilage. Silage that is unstable when exposed to air heats rapidly and spoils, leading to a loss of DM and nutrients with the potential for production of undesirable compounds. Aerobic spoilage during storage often is responsible for a large portion of the total DM loss in forages conserved as silage. Losses of DM could be as high as 15 to 25% or even higher in areas of poor compaction. This review will focus on the processes involved during aerobic spoilage of silages, why aerobic spoilage is undesirable and ways to improve aerobic stability.

Aerobic stability of silages

When the active stage of ensiling is completed, the remaining microorganisms in the mass are relatively dormant because of the low pH and absence of oxygen. However, if silage is exposed to air, the result can be a chain reaction resulting in aerobic spoilage. Specifically, yeasts that are able to degrade lactic acid in the presence of air usually initiate this process. Examples of these organisms include *Candida krusei* (*Issatchenkia orientalis*) and *Pichia membranifaciens* (*C. valida*) (Inglis et al., 1999). Yeasts able to metabolize sugars (e.g. *Saccharomyces*) are also active and can exacerbate the spoilage process. Degradation of lactic acid specifically causes an increase in pH of the silage to a level that allows opportunistic bacteria (e.g. Bacilli) and molds (e.g. *Aspergillus, Fusarium,* and *Pencillium*) to then become active, furthering the spoilage process (McDonald et al., 1991). Aerobic microbial activity causes oxidation of nutrients resulting in the production of heat. Of particular concern is the development of pathogenic bacteria and molds in aerobic spoiling silages because of their potential for producing mycotoxins and causing other detrimental effects. In some cases, bacteria from the genus, Acetobacter may initiate aerobic spoilage in maize silages (Spoelstra et al., 1988). Grain crops (e.g. whole crop barley silages, maize silages, and high moisture maize-grain silage) are very prone to aerobic spoilage. Epiphytic populations of yeasts are found on all forage crops in the field. However, their numbers are not well correlated to aerobic stability because there is a mix of non-fermentative and fermentative species present. The ensuing fermentation and level of silage management ultimately determine the number of lactate-assimilating yeasts that may survive ensiling. A list of some factors affecting the aerobic stability of silages is shown in Table 1. The ambient temperature around the silage mass affects the rate of aerobic spoilage. When temperature is low, microbial activity is slowed or even stopped (e.g. in freezing weather). Warm climate stimulates microbial activity and thus, aerobic spoilage and it is the primary reason that more spoilage typically occurs in the summer than in the winter. High concentrations of residual sugars in silage can also lead to a higher probability of aerobic spoilage.

Impact of feeding aerobically spoiled silages to ruminants

Few studies have been conducted evaluating the effects of feeding aerobically spoiled silages to ruminants. Whitlock et al. (2000) reported that feeding spoiled corn silage from the

surface of a bunker silo depressed DM intake as the level of spoiled feed in the diet increased from 0 to 16% of ration DM. Recently, Windle and Kung (2013) reported that heifers fed a spoiling TMR consumed less DM than those fed a fresh total mixed ration (TMR).

Table 1 Some factors that may make silages more prone to aerobic spoilage

Factor	Effects	Examples
High sugar content or high natural population of yeasts	Yeasts use sugars as energy sources during fermentation	a) sugarcane
High DM content	a) High DM restricts fermentation and reduces acid production that could increase yeast survival during ensiling b) High DM crops are more difficult to pack and allow infiltration of air into the mass	a) Lucerne ensiled > 45 to 50% DM b) Maize silage ensiled > 40% DM
Poor pack density/porosity	Allows penetration of air into the silage mass	a) Fill rate too fast b) Insufficient compaction
Poor feeding management	Allows penetration of air into the silage mass	a) Slow silage removal b) Loose silage c) Uneven silage face d) Intermediate feeding piles e) Silage moved from one structure to another
Poor management of plastic and weights	Allows penetration of air into the silage mass	a) Torn silo covers b) Insufficient weight on plastic c) Plastic pulled back too far in advance
High ambient temperatures	Spoilage organisms grow faster in warmer weather	More spoilage in the summer than winter months
Addition of spoiled feeds to a TMR	Spoiled feeds bring spoilage organisms to the TMR	Spoiled wet distillers grains
Overly dominant homolactic acid fermentation	Limited production of organic acids that have antifungal properties	An extremely dominant homolactic acid fermentation caused by microbial inoculation

In contrast, the intake of cows that were fed a TMR containing aerobically spoiling high moisture corn was unaffected when compared to cows fed fresh corn but the former produced 3.2 kg less milk per cow (Hoffman and Ocker, 1998). When animals consume spoiled silages, the exact causes of reduced intake and/or performance are not fully understood. In the study of Whitlock et al. (2000), reduced DM intake probably occurred because of a lower nutrient digestibility of the silage. However, in the study of Windle and Kung (2013), nutrient composition, and in vitro digestion of NDF and starch of the diets was very similar and could not obviously explain the differences in observed intake. One major difference between diets

was that the fresh TMR contained 5.0 log yeasts/g, whereas the spoiled diet contained 7.8 log cfu of yeasts/g. Santos et al. (2011) added various levels of a pure culture of *I. orientalis* to *in vitro* ruminal fermentations and reported lower NDF digestibility as the amount of yeast in the culture increased, suggesting that undesirable spoilage yeasts may have direct effects on ruminal fermentation. Gerlach et al. (2012) reported negative correlations between concentration of ethyl lactate and ethanol with DM intake in goats but the strongest negative effect was related to silage temperature (as difference to ambient). Other variables that may contribute to depressions of intake include growth of molds in spoiled silage that may result in production of mycotoxins and effects of microbes or compounds on immune functions. Organoleptic properties (e.g., taste and smell) of spoiled feeds on intake have not been well studied. In addition to negative effects on animal performance, spoiled silages also potentially present a contaminant to the environment if the feed is spoiled to the extent that must be discarded.

Improving the aerobic stability of silages through management

Filling silos quickly with sufficient pack weight to maximize silage density and minimize porosity can minimize oxygen in a silo. After filling, silage should be covered with plastic as soon as possible and weighted down with tires (tires should be touching) or gravel bags to exclude air. The return on investment (labor and plastic) is extremely high for covering bunk and pile silos. Oxygen barrier plastics with low transmission rates for oxygen appear to be useful in minimizing the loss of nutrients at the silage/plastic interface (Borreani et al., 2007). This practice can also reduce the number of yeasts in silages and improve aerobic stability. Proper management for removal of silage from silos at the feed bunk with the use of mechanical equipment (e.g., block cutters and silo facers) can help producers to maximize profits and production. Enough silage should be removed between facings to minimize aerobic spoilage. Removal of silage should be such to minimize disruption of the silage face and loose silage on the ground between feedings. Extreme care should be taken to prevent air from penetrating between the plastic and reaching the silage mass during feed out and storage and this can be accomplished by stacking tires, or lining gravel bags on the plastic at the leading edge of the feeding face.

Improving the aerobic stability of silages with additives

Various chemical additives with antifungal properties have been used to enhance aerobic stability of silages such as organic acids. For example, buffered propionic acid-based products are commonly used in North America because they are less corrosive and safer to handle than the straight acid. It is the undissociated (protonated) form of organic acids that is responsible for their antifungal properties and its prevalence is dependent on pH. This fact unfortunately means that more acid is needed to be effective in crops that are naturally limiting in acids from silage fermentation (e.g. crops with more than 40% DM). At the pH of a standing crop of lucerne (about 6) only about 1% of propionic acid is in the undissociated form whereas, at a pH of 4.8, about 50% of the acid is undissociated. The undissociated acid functions by penetrating into microbial cells and disrupting cytosolic functions after dissociation. Undissociated acids also remain active on the surface of microorganisms and compete with amino acids for space on active sites of enzymes. Some weak acids may also alter permeability of microbial cells (Stratford and Anslow, 1998). Application of buffered propionic acid-based products in North America ranges from about 0.5 to 2 kg/t of wet forage, depending on the specific situation. In previous studies, we have found that, as

expected, the effectiveness of propionic acid based additives increases with higher application rates (Kung et al., 1998; Kung et al., 2000). Potassium sorbate and sodium benzoate have also been used to improve aerobic stability of maize silages. For example, treatment with 0.1% potassium sorbate also improved DM recovery and aerobic stability and lowered the final concentration of ethanol in maize silage (Teller et al., 2012). Knicky and Spörndly (2011) reported that an additive containing sodium benzoate, potassium sorbate, and sodium nitrate improved the aerobic stability of a variety of crops with DM > 35%.

Bacterial inoculants, based on homofermentative lactic acid bacteria are commonly added to silages to improve fermentation and increase DM and energy recovery. However, most of these inoculants are not very effective in inhibiting the growth of yeasts because they tend to maximize the production of lactic acid (poor antifungal activity) and decrease the accumulation of other organic acids that have good antifungal activity. Muck and Kung (1997) summarized the literature and found that treatment with classical homolactic acid-based inoculants improved aerobic stability about one third of the time, had no effect about one third of the time but made aerobic stability worse about one third of the time.

Lactobacillus buchneri, an obligate heterolactic acid bacterium, has been used as a silage inoculant to specifically enhance aerobic stability by converting moderate amounts of lactic acid to acetic acid in a variety of silages from maize, sorghum, barley, lucerne, ryegrass, orchard grass, etc. (Dreihuis et al., 1999a, Kung and Ranjit, 2001). Concerns relative to the potential of large DM losses from silages treated with *L. buchneri,* because of its heterolactic nature, have not been substantiated (Kleinschmit and Kung, 2006). Although, some have suggested that high levels of acetic acid in silages may depress intake, studies have shown that ruminants fed silages treated with *L. buchneri* consume similar amounts of DM as those fed untreated silages (Dreihuis et al., 1999b, Kung et al., 2003).

Conclusions

Aerobically spoiled silage is undesirable because of losses in nutrients and potential negative effects on animal performance and health. Good management and the use of various additives can help to minimize the incidence of aerobically spoiled silage.

References

Borreani, G., Tabacco, E. & Cavallarin, L., 2007. A new oxygen barrier film reduces aerobic deterioration in farm-scale corn silage. J. Dairy Sci., 90, 4701-4706.

Driehuis, F., Oude Elferink, S.J.W.H. & Spolestra, S.F., 1999a. Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with *Lactobacillus buchneri* inhibits yeast growth and improves aerobic stability. J. Appl. Microbiol., 87, 583-594.

Driehuis, F., Oude Elferink, S.J.W.H. & Van Wikselaar, P.G., 1999b. *Lactobacillus buchneri* improves the aerobic stability of laboratory and farm scale whole crop maize silage but does not affect feed intake and milk production of dairy cows. Pages 264-265 in Proc. XII Intl. Silage Conf. Uppsala, Sweden.

Gerlach, K., Weiß, K., Roß, F., Büscher, W. & Südekum, K.-H., 2013. Changes in maize silage fermentation products during aerobic deterioration and its impact on feed intake by goats. Agri. Fd. Sci., 22, 168–181.

Hoffman, P. C. & Ocker, S. M., 1997. Quantification of milk yield losses associated with feeding aerobically unstable high moisture corn. J. Dairy Sci. 80 (Suppl. 1), 234 (Abstr.).

Inglis, G. D., Yanke, L.J., Kawchuk, L.M. & McAllister, T.A., 1999. The influence of bacterial inoculants on the microbial ecology of aerobic spoilage of barley silage. Can. J. Microbiol., 45, 77-87.

Kleinschmit, D. H. & Kung, L., Jr., 2006. A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn, grass and small grain silages. J. Dairy Sci., 89, 4005-4013.

Knicky, M. & Spörndly, R., 2011. The ensiling capability of a mixture of sodium benzoate, potassium sorbate, and sodium nitrite. J. Dairy Sci., 94, 824–831.

Kung, L., Jr., & Ranjit, N.K., 2001. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. J. Dairy Sci., 84, 1149-1155.

Kung, L., Jr., Taylor, C. C., Lynch, M.P. & Neylon, J.M., 2003. The effect of treating alfalfa with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for lactating dairy cows. J. Dairy Sci., 86, 336-343.

McDonald, P., Henderson, N. & S. Heron, J. E. (Eds.), 1991. The Biochemistry of Silage. Second Edition. Chalcombe Publications, Bucks, England.

Muck, R. E. & Kung, L., Jr., 1997. Effect of silage additives on ensiling. Proceedings of the conference on Silage: Field to feedbunk. North American Conference Hershey, PA. NRAES-99.

Santos, M. C., Lock, A.L., Mechor, G.D. & Kung, L., Jr., 2011. Spoilage yeasts in silage have the potential to directly impact rumen fermentation. J. Dairy Sci. 94 (E-Suppl. 1), 207 (Abstr.).

Spoelstra, S. F., Courtin, M. G. & Van Beers, J.A.C., 1988. Acetic acid bacteria can initiate aerobic deterioration of whole crop maize silage. J. Agric. Sci., Cambridge., 111, 127-132.

Stratford, M. & Anslow, P.A., 1998. Evidence that sorbic acid does not inhibit yeast as a classic 'weak acid preservative'. Lett. Appl. Microb., 27, 203–206.

Teller, R. S., Schmidt, R.J., Whitlow, L.W. & Kung, L., Jr., 2012. Effect of physical damage to ears of corn before harvest and treatment with various additives on the concentration of mycotoxins, silage fermentation, and aerobic stability of corn silage. J. Dairy Sci., 95, 1428-436.

Whitlock, L. A., Wistuba, T.J., Seifers, M.K., Pope, R.V. & Bolsen, K.K., 2000. Effect of level of surface-spoiled silage on the nutritive value of corn silage diets. J. Dairy Sci. 83 (Suppl. 1), 110 (Abstr.).

Windle, M. C. & Kung, L., Jr., 2013. The effect of a feed additive on the feeding value of a silage-based TMR exposed to air. J. Dairy Sci. 91(E-Suppl. 1), 16 (Abstr.).

Woolford, M. K., 1975. Microbiological screening of the straight chain fatty acids (C_1-C_{12}) as potential silage additives. J. Sci. Food Agric., 26, 219-228.

Aerobic exposure of silages – effects on chemical composition and preference by goats

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

Introduction

Whole-crop maize, grass and legume silages play a significant role in the nutrition of ruminants world-wide. After silo opening, most silages are exposed to air for several days (silo face, fodder mix waggon, feeding trough) until being consumed by the animal. However, aerobic stability is often shorter than this interval and as a consequence, aerobic deterioration processes might have taken place before consumption. There is a lack of data concerning the impact of aerobic spoilage related changes on dry matter intake (DMI) and preference by ruminants.

Only few experiments focused on the impact of aerobic exposure of silages on DMI. A possible reason might be the difficulty of keeping silages at specific stages of deterioration for repeated use during feeding trials. As frost before harvest can have a major impact on silage digestibility (St-Pierre et al., 1983), freeze-thaw processing of silages for experimental purposes may also lead to significant changes in silage structure, digestibility and rate of volatilisation of fermentation products, especially in low-DM substrate. Therefore, freezing is not the best solution for preserving a given stage of aerobic spoilage of silages for feeding experiments when sensory characteristics and their influence on DMI are being evaluated.

Sensory characteristics, especially odour and taste play a significant role in the feeding behaviour of ruminants (Favreau-Peigné et al., 2013). The perception of odour is a very complex process with information being processed within a few moments. Most naturally occurring odours may comprise several hundreds of chemical components. The consequence is that the odour of a source specifies it but is not chemically identical with it (Persaud et al., 1996). Efforts to reproduce or mimic the complex process have been made, for example by creating chemosensor/electronic olfactory systems ("electronic nose", sensor array systems).

A broad range of chemical constituents can be detected in silages, often only by advanced analytical methods. Increasing numbers of volatile organic compounds that are of special relevance in ensiled feedstuffs, can be detected and quantified but they may also be closely interrelated (e.g., ethanol and ethyl esters), and/or complex interactions exist which hamper clear assignments to production variables. Silage fermentation characteristics have impact on DMI (e.g., Huhtanen et al., 2002; Eisner et al., 2006), but it is very unlikely that the presence of a single product determines preference for or avoidance against a specific forage in ruminants. Also microbiological analyses are important and help explaining differences in spoilage processes between different substrates. Nevertheless, chemical and microbiological analyses alone might fail in finding points during the course of oxygen ingress that determine the initiation of spoilage with a concurrent reduction in DMI.

Therefore, preference trials with aerobically exposed silages were carried out to get extended information and understanding of the animals´ reaction to changing forage qualities.

Materials and Methods

Between 2010 and 2013, a total of 18 preference trials were conducted to determine the impact of aerobic exposure of different silages on short-time DMI and preference behaviour by goats. Eight maize silages, eight grass silages (all differing in DM concentration, chop

length and density) and two lucerne silages (preserved with and without a chemical additive) were stored aerobically for eight days (d), respectively. Measuring of silage temperature and sampling for chemical (proximate constituents, fibre fractions and fermentation products), *in vitro* (gas production using the Hohenheim gas test) and microbiological analyses (yeasts, moulds, aerobic mesophilic bacteria and lactic acid bacteria) was conducted in a two-d interval scheme (d 0, 2, 4, 6 and 8 after silo opening). A detailed description of methods can be found in Gerlach et al. (2013; 2014). Additionally, in grass and maize silages a chemosensor system was used for measuring volatile compounds as described by Roß et al. (2012).

Additionally, samples of the homogenized silage heap from each day of aerobic exposure (d0, d2, d4, d6 and d8) were stored anaerobically in polyethylene bags (170 µm, 400 mm × 600 mm; Innovapac, Durach, Germany), which were evacuated and sealed with a single-chamber vacuum packing machine (MAX-F 46; Helmut Boss Verpackungsmaschinen, Bad Homburg, Germany). A single bag was used for each meal for each goat which was filled with 1.5 - 1.7 kg silage (requirements + buffer = 60 bags/d for each treatment). Bags were stored in a dark, dry and cool room (15°C) until used in the preference trial. Storage time of the silages in the vacuum bags ranged from 1 to 26 d depending on the day when fed.

In 18 trials with Saanen-type goats (n = 6 for maize silages, n = 5 for grass and lucerne silages, mean body weight 91 ± 12.4 kg), the impact of aerobic exposure on DMI and preference was studied by presenting each possible pairwise combination of aerobically stored forages (d0 - d8), each one lasting 21 d. During the experimental period, each forage combination was presented for 3 h, always allowing free choice between the treatments. The experimental design was conducted according to Buntinx et al. (1997). The statistical analysis was done using the SAS procedure Multidimensional Scaling and by analysis of variance.

Results and Discussion

One of the challenges of the experiment was to keep silage samples at specific stages of deterioration for their later use in preference trials several days to weeks after the period of aerobic exposure. For this purpose, silages were stored completely airtight in vacuum-sealed polyethylene-bags, as previously proposed by Pippard et al. (1996) as a method for obtaining and preserving uniform silages for feeding experiments. When storing different grass silages in evacuated bags for 18 days, no aerobic spoilage-related changes were observed and therefore, the methodology was judged as being suitable for preserving silages for use in trials of the type reported in this publication.

Aerobic stability and degree of changes in fermentation products differed remarkably between forages. Goats strongly differentiated between aerobically exposed silages, but with different magnitudes between silage types. In maize silages, avoidance of silages began at the d4 of exposure, in lucerne silages even earlier. In grass silages, the impact of oxygen was much less pronounced. When comparing d0- and d8-silages, the mean decline of DMI was 53, 35 and 61% for maize, grass and lucerne silages, respectively.

Although silages were analysed for a wide range of chemical components including more than 20 fermentation products, it was difficult to find substances being responsible for the decrease in preference. No fermentation product exhibited a close relation to DMI, i.e. a correlation coefficient > 0.6. Consequently, it appears from the experiments that assigning a decline in feed intake to single constituents is difficult if not futile; often the impact of

Table 1 Dry matter intake (g/3 h) of selected silages with different lengths of aerobic exposure (0-8 days) shown by goats, n=25/30

	Length of aerobic exposure (days)				
Substrate	0	2	4	6	8
Maize silage	715 a	657 a	467 b	444 b	256 c
Grass silage	566 a	561 a	630 a	509 a	272 b
Lucerne silage (untreated)	672 a	569 a	427 b	392 b	223 c

Means within rows bearing different superscripts (a, b, c and d) differ ($P < 0.05$).

individual compounds or their mixtures on preference is still unclear. Huhtanen et al. (2002) proposed that eventually a complex of fermentation products or still unidentified compounds might be responsible for effects on intake. The difficulty of identifying single constituents or understanding chemical reactions being responsible for preference or avoidance of a feed is emphasized by the fact that silage temperature expressed as difference to ambient temperature (Δ T) was by far the best single predictor in maize silages. The strong negative correlation between Δ T and DMI underlines its utiliability for practical silage management and the suitability of recommendations given by Spiekers et al. (2009).

One of the olfactory systems trying to reproduce the process of odour perception by animals was also evaluated in our grass and maize silage studies (Roß et al., 2012). Results demonstrated the possibility of objectively measuring forage quality with the help of sensor array systems under laboratory conditions. Nevertheless, it was difficult to guarantee constant measurement conditions and to clearly differentiate between silages. Furthermore, sensor array systems are known to respond very sensitively to temperature and humidity changes and signal drift over time and have only moderate reproducibility and high costs (Sankaran et al., 2012), which might impede the use of sensor arrays directly at the silo face. The fact that temperature showed a much closer correlation with DMI underlines the need for further modifications concerning the suitability for daily use (Gerlach, 2013). The taste, which also strongly influences feeding behavior (Favreau-Peigné et al., 2013), is at least partly neglected when using olfactory systems.

With the presented methodology, the impact of aerobic exposure of different silages on chemical characteristics and changes in DMI and preference behaviour can be characterised. The design of the preference trials lead to a sufficient number of observations in a manageable interval of time, therefore giving the possibility of using different forages and conducting several trials successively. The pairwise presentation of each possible combination of feeds allows the identification of even small differences in forage characteristics influencing DMI and preference.

The number of fermentation products and other silage characteristics that can be analysed and detected will likely continue to increase in the future; on the other hand the animal which perceives a multiple of these compounds by sensory impression and its feedback (shown by increased or decreased preference and intake) should not be neglected. Sensory characteristics of feedstuffs have a more important role than generally considered in the feeding behaviour of domestic ruminants (Favreau-Peigné et al., 2013). It is worth putting the animal itself and its preference behaviour in focus in further investigations, as it is the only possibility of covering all silage characteristics (sensory and post-ingestive feedback) the animals perceive.

References

Buntinx, S.E., Pond, K.R., Fisher, D.S. & Burns, J.C., 1997. The utilization of multidimensional scaling to identify forage characteristics associated with preference in sheep. J. Anim. Sci. 75, 1641-1650.

Eisner, I., Südekum, K.-H. & Kirchhof, S., 2006. Beziehungen zwischen Fermentationscharakteristika von Silagen und der Futteraufnahme von Milchkühen [Relationships between silage fermentation characteristics and feed intake by dairy cows]. Übers. Tierernährg. 34, 197-221 (in German with English summary).

Favreau-Peigné, A., Baumont, R. & Ginane, C., 2013. Food sensory characteristics: their unconsidered roles in the feeding behaviour of domestic ruminants. Animal 7, 806-813.

Gerlach, K., 2013. The aerobic deterioration of silages as estimated from chemical composition and dietary choice by goats. Dissertation, University of Bonn, 97 pp.

Gerlach, K., Roß, F., Weiß, K., Büscher, W. & Südekum, K.-H., 2013. Changes in maize silage fermentation products during aerobic deterioration and effects on dry matter intake by goats. Agric. Food Sci. 22, 168-181.

Gerlach, K., Roß, F., Weiß, K., Büscher, W., Südekum, K.H. & 2014. Aerobic exposure of grass silages and its impact on dry matter intake and preference by goats. Small Rumin. Res. 117, 131-141.

Huhtanen, P., Khalili, H., Nousiainen, J.I., Rinne, M., Jaakkola, S., Heikkila, T. & Nousiainen, J., 2002. Prediction of the relative intake potential of grass silage by dairy cows. Livest. Prod. Sci. 73, 111-130.

Persaud, K.C., Khaffaf, S.M., Payne, J.S., Pisanelli, A.M., Lee, D.-H. & Byun, H.-G., 1996. Sensor array techniques for mimicking the mammalian olfactory system. Sens. Actuators B Chem. 36, 267-273.

Pippard, C.J., Porter, M.G., Steen, R.W.J., Gordon, F.J., Mayne, C.S., Poots, R.E., Unsworth, E.F. & Kilpatrick, D.J., 1996. A method for obtaining and storing uniform silage for feeding experiments. Anim. Feed Sci. Technol. 57, 87-95.

Roß, F., Boeker, P., Büscher, W., Gerlach, K., Haas, T., Maack, C. & Südekum, K.-H., 2012. A chemosensor system for assessment of silage quality. Proc. XVI Int. Silage Conf., Hämeenlinna, Finland, pp. 111-112.

Sankaran, S., Khot, L.R., Panigrahi, S. & 2012. Biology and applications of olfactory sensing system: A review. Sens. Actuators B Chem. 171-172, 1-17.

Spiekers, H., Potthast, V. & Nussbaum, H., 2009. Erfolgreiche Milchviehfütterung, DLG-Verlags-GmbH, Frankfurt am Main, Germany.

St-Pierre, N.R., Bouchard, R., St-Laurent, G.J., Vinet, C. & Roy, G.L., 1983. Effects of stage of maturity and frost on nutritive value of corn silage for lactating dairy cows. J. Dairy Sci. 66, 1466-1473.

Effects of particle size and chemical additives on fermentation and aerobic stability of grass-clover silage

E. Nadeau[1] & H. Auerbach[2]
[1] *Swedish University of Agricultural Sciences (SLU), Department of Animal Environment and Health, 532 23 Skara, Sweden.* [2] *ADDCON EUROPE GmbH, Parsevalstrasse 6a, 06749 Bitterfeld-Wolfen, Germany*
Correspondence: elisabet.nadeau@slu.se

Introduction

Particle size of ensiled forages affects fermentation profile (McEniry et al., 2008) and forage particle size *per se* affects intake and performance of cattle and sheep (Rustas and Nadeau, 2011; Helander et al., 2014). Nadeau et al. (2012) observed positive effects on fermentation in long and chopped grass silage by chemical silage additives. However, as there is still lacking comprehensive knowledge of the influence of additives on the fermentation of *Clostridium*-infected forages differing in particle size, this study aimed at investigating the effects of chopping and additive use on the fermentation characteristics of grass-clover forage containing *Clostridium* spores at ensiling.

Materials and Methods

A second cut ley of grass -clover (50%/50% of dry matter (DM)) ley was mowed on July 17, 2012, wilted to 30% DM and subsequently put through a commercial baler (Deutz-Fahr 160). At harvest, the forage contained 361 g neutral detergent fibre, 130 g water-soluble carbohydrates (WSC) and 130 g crude protein per kg DM. The *in vitro* organic matter digestibility was 857 g/kg of organic matter. The epiphytic lactic acid bacteria (LAB) count was 300000 cfu/g fresh herbage, and clostridia were below the limit of detection of 10 MPN/g. Half of the material was left unchopped (long, 180 mm particle size) whereas the remaining portion was chopped to 17 mm particle size in a laboratory chopper before being ensiled. Soil containing *Clostridium* spores was added at 50 g per kg forage to give a theoretical inoculation rate of 90 MPN/g fresh matter to all the treatments, except for one treatment that served as the uninoculated control (UIC). Prior to ensiling of the forage contaminated with soil, the following salt-based chemical additives were applied: KOFASIL LIQUID (24.5% sodium nitrite; 16.4% hexamine, 2.5 mL/kg; KOFASIL LIQUID Plus (24.5% sodium nitrite, 5.0% sodium benzoate, 5.0% potassium sorbate, 2.5 mL/kg); KOFASIL LP (20.2% sodium nitrite, 13.5% hexamine, 5.0% sodium benzoate, 2.5 mL/kg), KOFASIL ULTRA K (16.5% sodium nitrite, 11.0% hexamine, 8.1% potassium sorbate, 2.2% sodium benzoate, 0.8% sodium propionate, 2.5 mL/kg); SAFESIL (18.0% sodium benzoate, 7.4% potassium sorbate, 5.0% sodium nitrite, 4.0 mL/kg). Additional treatments were tested by using the following acid-based additives: GrasAAT SX (40.0% formic acid, 20.0% sodium formate, 20.0% propionic acid, 1.0% benzoic acid, 1.0% sorbic acid, 4.0 mL/kg), PROMYR XR680 (48.8% formic acid, 18.4% propionic acid, 6.1% sodium, 4.0 mL/kg). The additives were diluted with water and applied manually by spraying at 10 mL/kg forage. A control infected with *Clostridium* spores (C) and the UIC received 10 mL of water per kg forage. Silages of the nine treatments for each particle size were prepared in triplicates and stored in 1.5-L glass jars at 25 °C for 173 days.

The loss of DM during fermentation was calculated according to Weissbach (2005). Fermentation products were analyzed by HPLC and GC and their concentrations were based on DM, which was corrected for the loss of volatiles during drying (Weiss and Kaiser, 1995;

Weiss, 2001; Weissbach and Strubelt, 2008). Ammonia-N concentration was determined colorimetrically by Scalar (CFSA) based on the Berthelot reaction and the content of ammonia-N in silages treated with KOFASIL LIQUID, KOFASIL LIQUID Plus, KOFASIL LP, KOFASIL ULTRA K and SAFESIL were corrected for the amount of N derived from hexamine (100%) and NO_2 (90%) based on previous studies (Zwierz and Weissbach, 1989; Knicky and Lingvall, 2004). Water soluble carbohydrates (WSC) were determined by the anthrone method according to Lengerken and Zimmermann (1991). Aerobic stability of the silages was measured for 20.4 days by wireless temperature loggers (Tinytag Talk 4014, Gemini, Chichester, UK) and expressed as the number of days before reaching a temperature of 2°C above ambient (Honig, 1990). Data were analysed as a completely randomized design in PROC GLM of SAS 9.3, with additive treatment and particle size as fixed factors, using three replicates per treatment. As some additives had similar effects on studied variables, contrasts were made from the nine treatments within each particle size. The contrasts used were UIC vs. C, the mean of the salts vs. C, the mean of the acids vs. C and the mean of the salts vs. the mean of the acids. To avoid the risk for mass significance, the non-significance level was lowered to $P > 0.01$ in the comparison of the means in the contrasts.

Results and Discussion

Table 1 Effects of particle size on fermentation and aerobic stability of grass-clover silages (n = 27).

Parameter	Particle size		SEM	*P* - value
	Chopped	Long		
DM (%)	30.8	30.9	0.13	ns
DM losses (%)	6.8	6.9	0.03	<0.01
WSC (% of DM)	0.7	0.8	0.019	<0.01
pH	4.16	4.13	0.005	<0.001
NH_3-N (% total N)	7.5	6.9	0.06	<0.001
Lactic acid (% of DM)	9.79	9.49	0.114	ns
Acetic acid (% of DM)	3.69	3.52	0.059	<0.05
Butyric acid (% of DM)	0.05	0.05	0.01	ns
Ethanol (% of DM)	0.38	0.34	0.007	<0.001
Propanol (% of DM)	0.63	0.56	0.013	<0.001
Aerobic stability (days)	18.4	19.3	0.12	<0.001

Even though there were significant interactions between additive treatment and particle size for some of the variables studied, the differences were not significant between particle sizes within additive treatment. Thus, the main effect of particle size across additive treatments is presented. Particle size had no effect on lactic acid and butyric acid concentrations (Table 1). All other tested parameters showed response to mechanical treatment but the magnitude was of minor relevance in terms of silage quality. When averaged over treatments, the silages showed good fermentation characteristics and very high stability upon exposure to air substantiating findings for grass silages by Nadeau et al. (2012).

The results presented in tables 2 and 3 demonstrate that the forage which was not inoculated with clostridia fermented well and had low DM losses regardless of particle size. This supports findings by Weissbach and Honig (1996) who set the critical value for good fermentation quality of silages from forages to a minimum of 100,000 epiphytic LAB/g,

which is lower than 300,000 epiphytic LAB found per g forage in this experiment. However, inoculation of the herbage with *Clostridium* spores resulted in butyric acid formation as observed by Kaiser et al. (2002). These authors demonstrated that not only the typically used parameters characterizing the fermentability of the crop – DM, ratio of water-soluble carbohydrates and buffering capacity– affect the quality of the fermentation process, but also the clostridia level, as well as the nitrate content.

Table 2 Effects of *Clostridium* inoculation and of chemical silage additives on fermentation, dry matter (DM) losses and aerobic stability of chopped grass-clover silage.

Parameter	Treatments				*P* contrasts			
	UIC[1]	C[2]	Salts	Acids	UIC *vs* C	Salts *vs* C	Acids *vs* C	Salts *vs* Acids
DM (%)	29.0	30.5	30.9	31.6	ns	ns	ns	ns
DM losses (%)	6.1	8.1	7.0	6.2	<0.001	<0.001	<0.001	<0.001
pH	4.08	4.39	4.18	4.02	<0.001	<0.001	<0.001	<0.001
WSC (% of DM)	2.46	0.32	0.43	0.70	<0.001	ns	<0.001	<0.001
NH_3-N (% of total-N)	8.1	10.5	6.8	7.5	<0.001	<0.001	<0.001	<0.001
Lactic acid (% of DM)	8.66	8.03	10.01	10.66	ns	<0.001	<0.001	ns
Acetic acid (% of DM)	2.75	4.14	4.08	2.97	<0.001	ns	<0.001	<0.001
Propionic acid (% of DM)	0.02	0.25	0.12	0.28	<0.001	<0.001	ns	<0.001
Butyric acid (% of DM)[3]	0.00	0.43	0.00	0.00	<0.001	<0.001	<0.001	ns
Ethanol (% of DM)	0.59	0.85	0.32	0.21	<0.001	<0.001	<0.001	<0.001
Propanol (% of DM)	0.01	0.86	0.77	0.46	<0.001	ns	<0.001	<0.001
Aerobic stability (days)	10.6	20.4	18.9	20.0	<0.001	<0.001	ns	<0.001

[1]UIC = uninoculated control, [2]C = control inoculated with *Clostridium* spores, [3]below detection limit of 0.01% of fresh matter.

Higher clostridia contamination increases the risk of undesired clostridia fermentation. Despite butyric acid formation, ammonia levels were still acceptable and the pH was low. This can be explained by the activity of saccharolytic clostridia in the early stages of fermentation, which is associated with only limited proteolysis (Kaiser et al., 1997) compared with the activity of proteolytic butyric acid bacteria at later stages of fermentation (Weiss and Kaiser, 2002). In addition to the presence of butyric acid, inoculated silages also contained higher acetic acid concentrations, thereby rendering them more stable upon exposure to air (Tables 2 and 3; Auerbach et al., 2013; Nadeau and Auerbach, 2013).

Obviously, inoculation with clostridia also had an impact on other metabolic pathways during the fermentation process as indicated by higher ethanol, propanol and propionic acid contents as compared to the UIC. Although 1,2-propanediol was not found in the silage, it most likely was produced by epiphytic or soil-originating *Lactobacillus buchneri* bacteria in combination with acetic acid from anaerobically degraded lactic acid. Further on, 1,2-propanediol was utilized by *Lactobacillus diolivorans*, to form 1-propanol and propionic acid (Oude-Elferink et al., 2001; Krooneman et al., 2002). These changes in metabolic pathways led to greater losses of WSC and DM in the C than in the UIC treatment.

Table 3 Effects of *Clostridium* inoculation and of chemical silage additives on fermentation, dry-matter (DM) losses and aerobic stability of long grass-clover silage.

Parameter	Treatments				*P* contrasts			
	UIC[1]	C[2]	Salts	Acids	UIC *vs* C	Salts *vs* C	Acids *vs* C	Salts *vs* Acids
DM (%)	28.3	31.1	31.1	31.5	<0.001	ns	ns	ns
DM losses (%)	6.2	8.4	7.0	6.4	<0.001	<0.001	<0.001	<0.001
WSC (% of DM)	2.02	0.57	0.67	0.62	<0.001	ns	ns	ns
pH	4.13	4.27	4.13	4.04	<0.001	<0.001	<0.001	<0.001
NH_3-N (% of total-N)	8.2	9.8	6.0	7.1	<0.001	<0.001	<0.001	<0.001
Lactic acid (% of DM)	8.18	7.81	10.26	9.05	ns	<0.001	<0.01	<0.001
Acetic acid (% of DM)	2.60	4.49	3.76	2.90	<0.001	<0.001	<0.001	<0.001
Propionic acid (% of DM)	0.01	0.17	0.10	0.22	<0.001	<0.001	<0.01	<0.001
Butyric acid (% of DM)[3]	0.00	0.46	0.00	0.00	<0.001	<0.001	<0.001	ns
Ethanol (% of DM)	0.48	0.71	0.29	0.19	<0.001	<0.001	<0.001	<0.001
Propanol (% of DM)	0.03	0.88	0.66	0.40	<0.001	<0.001	<0.001	<0.001
Aerobic stability (days)	10.5	20.4	20.4	20.4	<0.001	ns	ns	ns

[1]UIC = uninoculated control, [2]C = control inoculated with *Clostridium* spores, [3]below detection limit of 0.01% of fresh matter.

Both the salts and the acids inhibited the growth of clostridia as indicated by no butyric acid formation and an increased lactic acid production while the acetic acid and ethanol concentrations were decreased, indicating a more homolactic fermentation. This resulted in lower pH in the silages treated with the chemical additives compared with the C treatment (Tables 2 and 3). The more extensive acidification in the treated silages resulted in less proteolytic activity with a lower ammonia-N concentration in the additive-treated silages compared to the C treatment. The improved fermentation resulted in less losses of DM in the silages treated with the chemical additives compared to the C treatment (Tables 2 and 3). Similar positive effects on silage fermentation and on the extent of proteolysis by chemical additives have previously been reported (Auerbach et al., 2012). The aerobic stability was high in all silages.

The acid-treated silages had lower concentrations of acetic acid and ethanol and lower pH and DM losses than the salts in both chopped and long silages (Tables 2 and 3). However, the lactic acid concentration was higher in the silage treated with the salts compared with the acid treatment when ensiled as long forage. Also, the salts decreased the proteolysis of protein as indicated with a lower ammonia-N concentration in both chopped and long silages treated with the salts compared to the acids which is in agreement with results by Nadeau et al. (2012).

Conclusions

The influence of particle size on fermentation and aerobic stability was less pronounced than that of chemical additives. As contamination of forage by *Clostridium*-containing soil cannot always be avoided under practical farming conditions, strategic use of chemical additives is

strongly advised in order to alleviate the adverse effects on fermentation characteristics by *Clostridium* bacteria.

Acknowledgements

The authors are deeply indebted to Agroväst, Skara, Sweden for funding this experiment and to Annika Arnesson, SLU, Skara, for technical support during the trial. Dr. Kirsten Weiss, Humboldt University, Berlin, Germany, is acknowledged for conducting the analyses of fermentation products in the silages.

References

Auerbach, H., Nadeau, E. & Weiss, K., 2012. Benefits of using silage additives, in: Auerbach, H., Lückstädt, C., Weissbach, F. (Eds.), The future of silage preservation. Proceedings of the 1st International Silage Summit, 12 November, Leipzig, Germany, pp. 75-144. ISBN: 978-0-9573721-6-0.

Auerbach, H., Weiss, K., Theobald, P. & Nadeau, E., 2013. Effect of inoculant type on dry matter losses, fermentation pattern, yeast count and aerobic stability of green rye silages, in: Mair, C., Kraft, M., Wetscherek, W., Schedle, K. (Eds.), 12. BOKU-Symposium Tiernernährung. Universität für Bodenkultur, 11 April, Wien, Austria. pp. 179-185.

Helander, C., Nørgaard, P., Arnesson, A., Nadeau, E. & 2014. Effects of chopping grass silage and of mixing silage with concentrate on feed intake and performance in pregnant and lactating ewes and in growing lambs. Small Rumin. Res.116, 78-87.

Honig, H., 1990. Evaluation of aerobic stability. Grass Forage Reports, Special issue 3, 76-82.

Kaiser, E., Weiss, K. & Zimmer, J., 1997. Zum Gärungsverlauf bei der Silierung bei nitratarmem Grünfutter. 1. Mitteilung: Gärungsverlauf in unbehandeltem Grünfutter (On the course of fermentation in silages from low-nitrate forages. First part: The course of fermentation of untreated forages). Arch. Tierern. 50, 87-102.

Kaiser, E., Weiss, K. & Polip, I., 2002. A new concept for the estimation of the ensiling potential of forages, in: Gechie, L.M., Thomas, C. (Eds.), Proc. 13th Int. Silage Conf., 11-13 Sept., Auchincruive, Scotland, pp. 344-357.

Knický, M. & Lingvall, P., 2004. Ensiling of high wilted grass-clover mixture by use of different additives to improve quality. Acta Agric. Scand., Sect. A, Animal Sci. 54, 197-205.

Krooneman, J., Faber, F., Alderkamp, A. C., Oude Elferink, S.J. H.W., Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J.C. & Vancanney, M., 2002. *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. Intern. J. Syst. Evolution. Microbiol. 52, 639-646.

Lengerken, von, J., Zimmermann, K., 1991. Handbuch Futtermittelprüfung (Handbook feed evaluation) . Deutscher Landwirtschaftsverlag Berlin, 1. Auflage.

McEniry, J., O´Kiely, P. Clipson, N.J.W., Forristal, P.D. & Doyle, E.M. 2008. The microbiological and chemical composition of silage over the course of fermentation in round bales relative to that of silage made from unchopped and precision-chopped herbage in laboratory silos. Grass Forage Sci. 63, 407-420.

Nadeau, E., Arnesson, A. & Auerbach, H., 2012. Effects of additive and particle size on fermentation characteristics and aerobic stability of grass silage, in: Kuoppala, K., Rinne, M., Vanhatalo, A. (Eds.), Proc. XVIth Int. Silage Conf., 2-4 July, MTT Agrifood Research & University of Helsinki, Hämeenlinna, Finland, pp. 380-381.

Nadeau, E. & Auerbach, H., 2013. Additive type affects dry matter losses, fermentation pattern, aerobic stability and clostridia counts of baled red clover-grass silage. Article no. 35. III Int. Symp. Forage Quality Conserv. July 22-23, Campinas, SP, Brazil.

Oude Elferink, S.J.W.H., Krooneman, J., Gottschal, J.C., Spoelstra, S.F., Faber, F. & Driehuis, F., 2001. Anaerobic conversion of lactic acid to acetic acid and1,2-propanediol by *Lactobacillus buchneri*. Appl. Environm. Microbiol. 67(1), 125-132.

Rustas, B.O. & Nadeau, E., 2011. Chopping of whole-crop barley silage improves intake and live weight gain of young dairy steers. Livest. Sci. 141, 80-84.

Weiss, K., 2001. Gärungsverlauf und Gärqualität von Silagen aus nitratarmem Grünfutter (Course of fermentation and fermentation quality of silages made from low-nitrate forages). Dissertation. Humboldt-Universität zu Berlin.

Weiss, K. & Kaiser, E., 1995. Milchsäurebestimmung in Silageextrakten mit Hilfe der HPLC (Determination of lactic acid in silage extracts by HPLC). Das wirtschaftseigene Futter. 41, 69-80.

Weiss, K. & Kaiser, E. 2002. Fermentation patterns in silages from herbage low in nitrate, in: Gechie, L.M., Thomas, C. (Eds.), Proc. 13th Int. Silage Conf., 11-13 Sept., Auchincruive, Scotland, pp. 414-415.

Weissbach, F., 2005. A simple method for the correction of fermentation losses measured in laboratory silos, in: Park, R.S., Stronge, M.D. (Eds.), Silage production and utilization, Proc. 14th Int. Silage Conf., July, Belfast, Northern Ireland, p. 278.

Weissbach, F. & Strubelt, C., 2008. Correcting the dry matter content of grass silages as substrate for biogas production. Landtechnik 63(4), 210-211.

Weissbach, F. & Honig, H., 1996. Über die Vorhersage und Steuerung des Gärungsverlaufs bei der Silierung von Grünfutter aus extensivem Anbau (On the prediction and the control of the course of fermentation in silages from extensively grown forages). Landbauforschung Völkenrode, FAL, 46, 10-17.

Zwierz, P.M. & Weissbach, F. 1989. Investigations into preservative residues in silage and milk, Proc. Int. Symp. Production, evaluation and feeding of silage, 12-16 June, Rostock, Germany, pp. 185-195.

Effects of different moisture contents and additives on the quality of baled oat silage

G. Q. Zhao*, F.C. Qin, T. Jiao, J.J. Hou & X.D. Song
College of Pratacultural Science, Gansu Agricultural University, Lanzhou 730070, China
Correspondence: zhaogq@gsau.edu.cn

Introduction

In recent years, effects of additives on quality of alfalfa, smooth vetch, ryegrass silage have been studied in China (Ma et al., 2010; Chen et al., 2013; Zhang et al., 2009). Additives such as lactic acid bacteria (Re, 2009), urea (Zang et al., 2012), corn flour (Guo et al., 2005) and others have been used in silage making. Besides additives, moisture content of the raw material is also an important factor affecting silage quality. High water contents could increase nutrient losses and the risk of badly fermented silage. In contrast, low water contents might cause substantial nutrient losses due to suboptimal compaction and increased growth and activity of fungi (Wang et al., 2007).

Oat (*Avena sativa L.*) is an important feed in high altitude regions of NW China. It is commonly used to make hay, but frequent rains during autumn make it difficult to get high quality hay (Liu et al., 2007). Oat in these areas is therefore more suitable to preserve as silage. Research on either hay or silage making is insufficient (Zhao et al., 2004). There are no reports on suitable water contents and the use of additives for oat silage. Therefore, the objectives of this paper was to study the effects of different moisture contents and additives on the quality of baled oat silage, on the nutritive and fermentation quality and on viable counts of some microbial groups in oat silage, providing guidance for oat silage making in this region.

Materials and methods

The experiment was conducted in Xiahe County, which is located in Gannan Tibetan Autonomous Prefecture (E102°83′, N35°23′, 2517 m altitude).The maximum temperature was 29.7°C, the minimum -24.1 °C with an annual average temperature of 3.9°C; annual rainfall was 489 mm. The soil organic matter content was 1.98%, total N 1.18 g/kg, available nitrogen 60 mg/kg, available phosphorus 57 mg/kg, and soil pH 7.84.

The oat cultivar Longyan No.3 (*Avena sativa L.*) was provided by the College of Pratacultural Science, Gansu Agricultural University. The seeding rate was 255 kg/ha. Before planting, phosphate ammonia was applied at the rate of 225 kg/ha. Corn flour, urea, Synlac Dry (bacterial inoculant, from Yaxin Biological Bcience and Technology Company, China), and Sila-Max 200 (bacterial inoculant from Ralco Nutrition Company, USA) were used as additives.

Oat was harvested at milk stage with a disc mower and was wilted to target moisture contents of 45-50% (A_1) and 65-70% (A_2). It was then treated with corn flour (B_1), urea (B_2), Synlac Dry (B_3), Sila-Max 200 (B_4) or baled directly without additives (CK). The amount of additives were all based on fresh weight of raw material, namely 4%, 0.4%, 0.0002% and 0.00025%, respectively (Table 1). Oat was baled with a round baler and 6 bale replicates per treatment were produced. Bales weighed approximately 50 kg and were wrapped with 5 layers of stretch film as soon as baled. Bales were sampled 40 d after baling.

Table 1 Experimental design of the oat silage trial

Moisture content	Additives				
	Control （CK）	Corn flour （B_1）	Urea (B_2)	SynlacDry (B_3)	Sila-Max200 (B_4)
45%-50% （A_1）	A_1CK	A_1B_1	A_1B_2	A_1B_3	A_1B_4
65%-70% （A_2）	A_2CK	A_2B_1	A_2B_2	A_2B_3	A_2B_4

Before sampling, the stretch film was removed from the bales and 200 g samples were collected from 3-4 locations of the bale and composited for each bale. Samples were dried at 105°C for 15 min then kept at 65°C until stable weight. After masceration, the samples were sieved through 40 mesh sieve and sealed in zip-lock bags. Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ammonia-N and soluble sugars (WSC) contents were determined (Yang 1998).

For the detection of organic acids, 20 g silage was mixed with 180 mL deionized water and kept for 24 h at 4°C, then filtered through 4 layer filter paper, before passing a 0.22 μm membrane. HPLC (Agilent 1260 and G1321B ultraviolet fluorescence detector) was used to determine the contents of lactic acid (LA), acetic acid (AA) and acrylic acid (PA) and butyric acid (BA). Chromatographic conditions: SB-AQ C18 column (4.6 × 250 mm), mobile phase (methanol): the mobile phase (0.01 mol/L $(NH_4)_2HPO_4$, pH 2.70) was 3 : 97, velocity was 1 mL/min; detection wave length 210 nm, injection volume 20 μL and temperature was 25°C.

Viable counts were determined by mixing 20 g silage with 180 mL of 0.85% sterilized saline. A flat gradient dilution method was used to determine the counts of the following microbial groups: lactic acid bacteria (LAB) were quantitatively determined by using MRS media, generally aerobic bacteria (Bac) by using defined agar medium and moulds and yeast (M&Y) by using Tiger red agar medium.

The PC programs SPSS18.0 and SAS JMP10 were used to perform a 2×5 factorial analysis of variance.

Results and analysis

Moisture contents and additives had both a significant impact on most oat silage parameters (Table 2). Significant moisture content × additive interactions were observed on all indicators except Bac. Compared with those parameters of A1 treatment, after adding corn flour (B_1), CP, WSC, LA, M&Y and LAB of A2 treatment did not change much, while NDF, ADF and NH_3-N values were reduced 8.28%, 8.28%, 8.99%, respectively (Table 3, 4 & 5). CP content increased 15.81%, the LA, AA, PA, Bac and LAB remained stable, but M&Y decreased 13.33%. Synlac Dry had little impact on CP, ADF, AA, PA, M&Y, Bac and LAB, but LA increased 21.69%; meanwhile, NDF and NH_3-N dropped 6.01% and 10.78%, respectively. After adding Sila-Max 200, CP, LA, AA, PA, M&Y, LAB of A2 treatment maintained stable, WSC increased while NDF, ADF and M&Y reduced 9.06%%, 18.84% and 14.05%, respectively, compared with those of A1 treatment . Compared to A1, CK under A2 had a lower content of NDF and a higher LA (increasing 62.71%). A2 (65%-70%) is more conducive to oat silage.

Oat silage quality was significantly affected by additives (Tables 3, 4, 5). With the high moisture level (A2 treatment), CP, NDF and ADF contents of oat silage with corn flour did not change compared with the control, while WSC increased by 21.22% and LA and AA

Table 2 F-values of variables including interactions

Analyses	df	Moisture content	Additive	Moisture content*Additive
		F Value	F Value	F Value
CP	1,4	6.55*	20.06**	3.13*
NDF	1,4	59.35**	3.20*	5.43**
ADF	1,4	30.88**	4.60**	7.72**
WSC	1,4	56.91**	95.46**	167.57**
LA	1,4	2.54	232.36**	29.78**
AA	1,4	41.14**	45.32**	6.83*
NH_3-N	1,4	7445.02**	31761.36**	3605.83**
M&Y	1,4	1.05	19.60**	14.15**
Bac	1,4	0.31	18.83**	2.14
LAB	1,4	0.34	18.03**	3.11*

Note: * and ** indicate significant differences at $p<0.05$ and $p<0.01$, respectively

decreased by 53.13% and 30.00%, respectively; microbial groups did not change. Urea treatment increased CP by 22.78%, ADF increased a little while LA and WSC were reduced by 32.29% and 10.53%, respectively. Levels of NH_3-N and M&Y increased significantly but no remarkable change was observed on LAB counts. In contrast with the control under A2 treatment, CP, volatile fatty acid, M&Y and Bac did not change after adding Synlac Dry, but WSC increased by 43.97% and LAB counts increased significantly; ADF and NH_3-N were reduced 3.34% and 61.52%, respectively. Sila-Max 200 increased oat silage CP, WSC, LA and AA by 11.61%, 19.24%, 40.63% and 43.33%, respectively, compared with the control; while NDF, ADF and NH_3-N decreased 4.77%, 7.59%, and 46.09%; LAB increased significantly, M&Y and Bac decreased 19.57% and 10.93%, respectively.

Table 3 Nutritional composition of oat silages (mean ± SD)

Item	CP (%DM)		NDF (%DM)		ADF (%DM)		WSC (%DM)	
A_1CK	9.56±0.01	bc	55.41±0.33	bcd	34.77±1.27	bcd	10.10±0.17	a
A_2CK	9.13±0.22	bcd	54.73±0.97	bcde	34.78±0.60	cbd	6.55±0.07	d
A_1B_1	9.26±0.21	bc	58.71±0.33	a	37.80±0.71	ab	8.31±0.06	c
A_2B_1	9.04±0.31	bcd	53.85±1.30	cde	33.71±0.91	cd	7.94±0.05	c
A_1B_2	9.68±0.33	bc	56.45±0.19	bcd	37.75±0.38	ab	7.75±0.20	c
A_2B_2	11.21±0.58	a	55.28±0.47	bcd	37.28±1.65	abc	5.86±0.05	e
A_1B_3	7.75±0.09	e	56.06±0.01	abc	35.37±0.97	bcd	7.95±0.8	c
A_2B_3	8.41±0.40	de	52.69±0.86	de	33.62±0.77	cd	9.43±0.22	b
A_1B_4	9.84±0.10	abc	57.31±0.24	ab	39.60±0.55	a	6.32±0.07	de
A_2B_4	10.19±0.00	ab	52.12±0.19	e	32.14±0.63	d	7.81±0.05	c

Note: Different letters in the same column indicate significant difference ($P<0.05$).

Under A_2 moisture treatment, CP content of Sila-Max 200 treatment was similar with that of urea treatment, 12.72% and 21.17% higher than that of corn flour and Synlac Dry treatment;

their NDF and ADF contents were the lowest, with the highest LA and AA content; meanwhile, their M&Y counts were 19.08% and 14.33% higher than the corn flour and the Synlac Dry treatment. Sila-Max 200 had similar Bac counts with the Synlac Dry treatment, but 12.59% and 7.86% lower than the corn flour and urea treatment; its LAB counts were highest, followed by Synlac Dry, both significantly higher than the corn flour treatment (Table 5).

Table 4 Organic acids and ammonia nitrogen of oat silages (mean ± SD)

Item	LA（%DM）		AA（%DM）		PA（%DM）		NH_3-N（%TN）	
A_1CK	0.59±0.01	de	0.42±0.01	a	0.32±0.01	bc	10.90±0.07	f
A_2CK	0.96±0.01	b	0.30±0.02	bc	0.27±0.02	cde	20.87±0.07	c
A_1B_1	0.52±0.02	ef	0.29±0.01	bcd	0.41±0.02	a	13.12±0.02	d
A_2B_1	0.45±0.01	f	0.21±0.01	e	0.31±0.02	bcd	11.94±0.26	e
A_1B_2	0.72±0.02	cd	0.36±0.02	ab	0.34±0.02	abc	38.87±0.11	b
A_2B_2	0.65±0.01	de	0.29±0.01	bcd	0.37±0.03	ab	68.68±0.14	a
A_1B_3	0.83±0.02	bc	0.28±0.01	cde	0.23±0.01	de	9.00±0.09	g
A_2B_3	0.65±0.01	de	0.22±0.01	de	0.23±0.01	de	8.03±0.02	h
A_1B_4	1.25±0.08	a	0.40±0.02	a	0.23±0.02	de	7.55±0.23	h
A_2B_4	1.35±0.02	a	0.43±0.01	a	0.18±0.01	e	11.25±0.23	ef

Note: Butyric acid (BA) was not detected in any treatment, therefore not listed.

The moisture content of the fresh forage had a significant impact on silage fermentation. Yan et al. (1996) found that LAB counts were sensitive to moisture content. Too high or too low moisture contents are detrimental to silage quality. Generally, suitable moisture content for oat silage is considered to be around 65%. The results of this study indicate that 65%-70% moisture content can lead to a better silage quality than 40%-50%. The content of CP and organic acids of oat silage with higher moisture level (65%-70%) were similar to the one with the lower level (45%-50%), but NDF and ADF were significantly reduced. On the other hand, although M&Y under some additive treatments was influenced by moisture content, LAB counts (approx. 10^8/g) were always much higher than for M&Y (approx. 10^3/g). No significant difference was observed for LAB and Bac counts (Table 5). These results suggest that the effects of moisture content on oat silage nutrients was not just caused by its effect on microorganism quantity, it may also have been caused by differences in cell decomposition rates and dissolved substances, thereby affecting microbial metabolic rate and metabolic pathways.

The aim of using additives is to create suitable conditions for a favourable fermentation so that the LAB propagate rapidly, accelerate the fermentation process and thus help to improve the fermentation quality of silage (Guo et al., 2005). Adding Sila-Max 200 or Synlac Dry increased LAB counts and improved silage fermentation by reducing pH and improving fermentation conditions (Driehuis & Vanw, 2000). Meanwhile, large number of LAB secreted a lot of enzymes to effectively degrade plant tissues which decreased NDF and ADF levels. The addition of urea and corn flour did not improve this early stage of fermentation, and did not decrease NDF and ADF values significantly. Our results were not in agreement with Yang and Zhang's (2005) results, namely that urea had a dilution effect on degradation of fresh forage. In contrast, our results showed that adding urea reduced the WSC level and

Table 5 Microbial counts (log_{10} cfu/g) of treated oat silages (mean ± SD)

Item	M&Y		Bac		LAB	
A_1CK	3.19±0.08	ab	4.32±0.09	a	7.70±0.10	e
A_2CK	3.27±0.04	a	4.21±0.02	ab	8.05±0.03	cde
A_1B_1	2.89±0.03	bc	4.19±0.03	a	7.92±0.16	de
A_2B_1	3.25±0.03	a	4.29±0.07	ab	8.15±0.13	bcde
A_1B_2	3.00±0.05	ab	4.12±0.01	ab	8.32±0.10	abc
A_2B_2	2.60±0.15	c	4.07±0.06	ab	8.37±0.15	abcd
A_1B_3	3.20±0.03	ab	3.77±0.02	cd	8.79±0.06	a
A_2B_3	3.07±0.04	ab	3.98±0.07	bc	8.61±0.07	ab
A_1B_4	3.06±0.05	ab	3.56±0.07	d	8.45±0.05	abc
A_2B_4	2.63±0.04	c	3.75±0.08	cd	8.69±013	a

increased NDF and ADF levels. Urea eventually decomposes to NH_3, which dissolves in water to form ammonium hydroxide and weakens the lignin bonds. But, this reaction may need a longer period than 40 d to affect silage composition. Meanwhile, the degradation of WSC leads to an increase in NDF and ADF.

It is generally believed that remaining oxygen at the beginning of the fermentation makes proteinases retain vitality and decomposes protein into ammonia and amine (Kung et al., 2003). In addition, under aerobic condition, anaerobic bacteria such as clostridia and intestinal bacteria decompose amino acid, resulting in increasing protein loss (Herson et al., 1986). Sila-Max 200 and Synlac Dry treatments significantly increased LAB counts at the beginning of the fermentation when oxygen was still present. As LAB multiplied, the growth of other aerobic microbes was significantly inhibited, pH reduced and the content of NH_3–N decreased significantly.

There are more than 20 varieties of LAB which are active during silage fermentation. They can be grouped into homolactics and heterolactics. Li et al.(2004) believed homolactic bacteria to be more effective than heterolactics, while Guo's (2006) study showed the opposite result. MacDonald et al. (1991) also pointed out that metabolic processes differ among LAB. Although the applied LAB quantities of Synlac Dry and Sila-Max 200 treatments were not significantly different in our experiment, the latter produced substantially higher LA , CP and volatile fatty acids (VFA) levels, and lower NDF and ADF levels than that of Synlac Dry. This may have been due to the different LAB strains contained in Synlac Dry and Sila-Max 200. As LAB strains have different metabolic pathways during lactic acid fermentation, choosing the most efficient LAB strains is an important prerequisite to ensure good fermentation. In addition, silage inoculants containing only one strain may not able to meet practical needs as multiple LAB strains might be able to.

To achieve an appropriate level of soluble carbohydrates in forages is a prerequisite for good silage fermentation (Yu et al., 2009). Due to high soluble sugar content and low buffer capacity, oats can be used to make high-quality silage without any additive. In this study, BA was not detected in any treatments including the control, indicating that unwanted butyric acid fermentation was well controlled and fermentation quality was good. However, by using appropriate additives better silage quality was achieved with lower pH and NH_3-N, and higher LA levels (Re, 2009).

Conclusions

Moisture content had a significant effect on oat silage quality. At 65%-70% moisture content, CP, organic acids, M&Y and LAB of additive-treated oat silages were comparable to those at 45%-50% moisture content; while NDF, ADF, WSC and M&Y levels were reduced and NH_3-N increased, which made the wetter the better silage.

Although oat ensiles easily without additives, they could accelerate the fermentation process and reduce nutrient losses. Compared with the non-bacterial additives such as corn flour and urea, LAB-based additives silage gave better silage quality and among the two tested, Sila-Max 200 gave better results than Synlac Dry.

References

Chen, P. F., Bai, S. Q., Yang, F. Y. et al., 2013. Effects of additive and moisture content on fermentation quality of smooth vetch silage. Acta Pratacult. Sinica, 22, 80-86.

Driehuis, F. & Vanw, P. G., 2000. The occurrence and prevention of ethanol fermentation in high-dry-matter grass silage. J. Sci. Food Agric., 80, 711-718.

Guo, X. S., Zhou, H. & Yu, Z., 2006. Effect of different additives on aerobic stability of silage. China Dairy Cattle 9, 18-20.

Guo, Y., Yang, Q. J. & Wang, H.B., 2005. Effects of different levels of corn flour the nutrient composition of alfalfa silage. Feed and Husband., 6, 20-22.

Herson, S.E., Edwards, R.A. & McDonald, P., 1986. Changes in the nitrogen components of gamma irradiated and inoculated ryegrass. J. Sci. Food Agric., 37, 979-985.

Kung, L.Jr., Taylor, C.C., Lynch, M.P. & Neylon J.M., 2003. The effect of treating alfalfa with *Lactobacillus 40788* on silage fermentation, aerobic stability, and nutritive value for lactating dairy cows. J. Dairy Sci. 86, 336-343.

Li, J.X., Gao, J., Wang, J.H. et al., 2004. Study of lactic acid bacteria biomass in silage fermentation. Feed. Ind. Mag., 25, 48-51.

Liu Z., Wu, G., Ren, Q. et al., 2007. Sustainable development of animal husbandry based on oat in alpine grassland area. Pratacult. Sci., 24, 67-69.

Ma, C.H., Xia, Y.J., Han, J., et al., 2010. Effects of different additives on the quality of alfalfa silage. Acta Pratacult. Sinica, 19, 128-133.

Re, M.N., 2009. Effects of Lactobacillus additives on silage quality of Qing Hai Tibet Plateau Oats. J. Anhui Agri. Sci. 37, 146- 149.

Wang, L., Li, X.L. & Zhang, X., 2007. The effect of different water contents and additive mixtures on alfalfa silage. Acta Pratacult. Sinica16, 40-45.

Yan, T.D., Patterson, C. & Gordon, F.J., 1996. The effects of bacteria inoculation of unwilted and wilted grass silages on silage fermentation, rumen microbial activity, silage nutrient degradability, and digestibility. Proc. of the 11th Intern. Silage Conf., IGER, Aberystwth, UK. p. 98-99.

Yang, F.Y. & Zhang, Y.W., 2005. A review on the urea-treated silage. China Dairy Cattle 5, 23-25.

The effect of silage additive on fermentation quality, clostridia and aerobic stability of a white lupin-wheat silage

W. König[1], M. Lamminen[1], K. Weiss[3], T.T. Tuomivirta[2], S. Sanz Muñoz[2], H. Fritze[2], K. Elo[1], L. Puhakka[1], A.Vanhatalo[1] & S. Jaakkola[1]
[1]Department of Agricultural Sciences, P.O. Box 28, FI-00014 University of Helsinki, Finland; [2]Finnish Forest Research Institute (Metla), Vantaa Unit, P.O. Box 18, FI-01301 Vantaa, Finland; [3)]U Berlin, Invalidenstraße 42, 10115 Berlin
Correspondence: walter.konig@helsinki.fi

Introduction

Bi-crop cultivation has a positive effect on dairy production, farm economy and environment (Hauggaard-Nielsen et al. 2008). White lupin is one potential legume bi-crop to be used as whole crop silage because of its high yield (Fraser et al. 2005, Azo et al 2012). Using grain as bi-crop for legumes improves ensiling quality of legumes, which are considered as difficult to ensile due to their low dry matter (DM) content and high buffering capacity (McDonald et al. 1991). During the last 20 years, there has been intensive development of technologies to identify microbial species and estimate their abundancies using polymerase chain reaction (PCR) (Takeuchi et al. 1997). Information from DNA sequences of 16S gene and its copy numbers can be used to estimate microbial diversity and abundance in various types of samples (Bagge et al. 2009, Weimer and Stevenson 2012). The ensiling experiment was conducted to study the effects of different types of additives on the quality of silage made on bi-crop differing in white lupine to wheat ratio and maturity.

Materials and Methods

The trial was conducted in 2012 at the University of Helsinki. The white lupin (*Lupinus albus*, var. Ludic) and spring wheat (*Triticum aestivum, var.* Amaretto) seed density was 36 seeds/m^2 and 360 seeds/m^2, respectively. The growth was fertilized with 60 kg N/ha. The bi-crop was cut with scissors at two growth stages: 96 days (G1) and 110 days (G2) after sowing. At G1 wheat was at the beginning of the dough stage and at G2 at the end of the dough stage. Two mixtures of lupin and wheat were made before ensiling: mixture 1 (M1)1/3 lupin + 2/3 wheat and mixture 2 (M2) 2/3 lupin + 1/3 wheat in fresh weight. The mixtures were treated with three different silage additives: 1) formic acid (FA) 4 l (100%)/tn; 2) mixture of sodium nitrite (0.75 kg/t) and hexamethylentetramine (hexamine) (0.5 kg/t) (NaHe); 3) *Lactobacillus plantarum* 1x10^6cfu/g forage (LAB). The control (CON) was without any treatment. The forages were ensiled in 1.5 l glass silos with three replicates per treatment. Silos were opened after 100 (G1) and 101 (G2) days, respectively. The aerobic stability of the silages was measured over 12 days. Sample temperature changes compared with the ambient temperature were monitored using data loggers.

The contamination of silages with clostridia was determined by PCR. For this, silage samples were stored at -20°C. From 2 to 3 grams of silage was weighed for each DNA extraction. Silage samples were homogenized in 10 ml milk-lactic-acid-glucose medium. Homogenates were centrifuged using 10 000 g for 15 min at 23°C. Approximately 200 ng of pellet per sample was collected for DNA extraction. The DNA extraction was conducted using Macherey-Nagel NucleoSpin® Soil kit (Macherey-Nagel GmbH & Co. KG**,** Germany). Species-specific primers were designed for *Clostridium perfringens*, *C. tyrobutyricum*, *C. sporogens*, *C. butyricum*. Quantitative real-time PCR was performed by using Rotor-Gene 6000 (Corbett Research, Australia) with Maxima SYBR Green qPCR Master Mix (Thermo

Scientific, USA). The lengths of PCR products varied from 254 to 285 bp. All silage samples were analysed in duplicates and average number of *Clostridium* genome copies were calculated per gram of silage. The used DNA extraction protocol should extract DNA from bacterial spores. Thus, the number of genome copies includes both bacteria and spores.

Statistical tests of silage quality parameters were done for G1M1, G1M2, G2M1 and G2M2 silages separately. Normally distributed variables were tested with ANOVA (SAS 9.3 mixed procedure). Sums of squares for additive effects were separated using orthogonal contrasts: 1) CON vs additives; 2) LAB vs FA and NaHe; 3) FA vs NaHe. Non-normal distributed data were tested with the Kruskall-Wallis analysis (SPSS, version 21). Differences between the treatments were analysed by pairwise testing.

Results and Discussion

The raw material compositions for different mixtures are given in Table 1. All additives improved silage quality compared to the control. The performance of the additives varied between the different growth stages and mix ratio of white lupin and wheat (table 2 and 3). Comparison of the additives revealed their different functionality. All feed samples were aerobically stable. The raw material of this trial was contaminated with clostridia spores despite the fact that the samples were collected unsoiled. This can be deduced from the high amounts of clostridia in the silages. Whole crop raw materials may be more prone to butyric acid fermentation than grasses or forage legumes. The contamination with clostridia was also measured in the numbers of PCR-copies in a gram of silages. From four tested species of clostridia, *C. tyrobutyricum* was the most abundant in this trial.

Table 1 Raw material composition (g/kg DM, if not otherwise stated)

	Growth stage 1				Growth stage 2			
	White lupin	Spring wheat	Mix 1	Mix 2	White lupin	Spring wheat	Mix 1	Mix 2
Dry matter, g/kg	163	379	307	235	138	359	285	212
Ash	70.2	96.2	91.6	84.2	68.7	88.1	85.0	79.7
Crude protein	184	71	91	123	182	61	81	114
NDF	390	520	497	460	436	525	510	486
WSC[1]	126	84	91	103	84	35	43	56
Starch	30	183	152	113	23	255	218	154
Buffer cap., mekv/kg DM	709	297	370	488	687	296	359	466

[1]Water soluble carbohydrates; in MIX 1 white lupin - spring wheat ratio 1:2 and MIX 2 ratio 2:1 of fresh weight.

Table 2 The effect of additive on silage quality and clostridia (expressed as log10 copies/g) (g/kg dry matter, if not otherwise stated). First growth stage

	Silage additive[1]					Statistical significance[1]		
	CON	FA	NaHe	LAB	SEM	CON vs additives	LAB vs FA and NaHe	FA vs NaHe
MIX 1								
Dry matter, g/kg	290	311	313	309	6.3	0.020	0.677	0.801
pH	4.53	4.28	5.01	3.75	0.028	<0.001	<0.001	<0.001
WSC[2]	29.2	109	113	23.9	5.70	<0.001	<0.001	0.622
Lactic acid	32.5	2.9	17.2	53.0	1.92	0.006	<0.001	<0.001
Acetic acid	3.24	4.59	10.48	4.75	0.417	<0.001	<0.001	<0.001
Butyric acid	37.69[a]	6.01[ab]	0.00[b]	0.00[b]	1.297	non-normally distributed		
Ethanol	25.23	3.78	2.32	6.37	0.554	<0.001	0.001	0.100
Ammonia N, g/kg N	188	66	107	62	5.2	<0.001	0.005	<0.001
Ammonia N, g/kg N[3]	188	66	25	62	5.0	<0.001	0.025	<0.001
Clostridia total, lg c/g[4]	5.61	4.92	2.48	2.07	0.665	0.013	0.081	0.032
C.tyrobutyr., lg c/g[4]	5.57	4.90	2.50	2.00	0.669	0.014	0.072	0.035
MIX 2								
Dry matter, g/kg	226	237	240	245	4.1	0.016	0.238	0.556
pH	4.60	4.06	4.67	3.83	0.094	0.005	0.002	0.002
WSC[2]	14.2	87.2	112	21.3	17.1	0.017	0.006	0.335
Lactic acid	45.9	24.9	38.4	75.3	7.54	0.974	0.002	0.244
Acetic acid	7.23	6.79	11.71	7.27	0.718	0.140	0.054	0.001
Butyric acid	43.09	9.32	0.54	0.00	3.201	<0.001	0.244	0.089
Ethanol	28.3	5.92	5.42	12.9	0.001	<0.001	0.004	0.815
Ammonia N, g/kg N	241	64	127	60	10.3	<0.001	0.023	0.003
Ammonia N, g/kg N[3]	241	64	37	60	10,3	<0.001	0.480	0.104
Clostidia total, lg c/g[4]	5.66	4.68	3.67	1.93	0.974	0.082	0.097	0.486
C.tyrobutyr., lg c/g[4]	5.63	4.67	3.67	1.93	0.970	0.084	0.097	0.487

[1]CON=no additive, FA=formic acid, NaHe=hexamethylentetramine and sodium nitrite mixure, LAB=*Lactobacillus plantarum;* [2] water-soluble carbohydrates; [3] deducted all nitrogen applied through additive; [4] copies per silage gram (fresh weight). In MIX 1 white lupin and spring wheat ratio 1:2 and in MIX 2 ratio 2:1 of fresh weight.

Table 3 The effect of additive on silage quality and clostridia (expressed as log10 copies/g) (g/kg dry matter, if not otherwise stated). Second growth stage

	Silage additives[1]					Statistical significance		
	CON	FA	NaHe	LAB	SEM	CON vs additives	LAB vs FA and NaHe	FA vs NaHe
MIX 1								
Dry matter, g/kg	315	311	317	327	5.3	0.583	0.090	0.434
pH	4.05	4.69	4.20	4.08	0,047	0.001	<0.001	<0.001
WSC[2]	12.7	33.6	39.1	15.0	2.26	<0.001	<0.001	0.125
Lactic acid	41.9	3.8	32.7	34.7	2.37	<0.001	<0.001	<0.001
Acetic acid	6.37	4.85	9.36	4.26	1.103	0.873	0.068	0.020
Butyric acid	4.44	11.6	0.00	5.69	1.526	0.475	0.960	<0.001
Ethanol	5.80	3.99	1.61	4.35	0.005	0.002	0.003	0.009
Ammonia N, g/kg N	129	128	124	107	7.5	0.298	0.069	0.728
Ammonia N, g/kg N[3]	129[a]	128[ab]	36[b]	107[ab]	7.3	non-normally distributed		
Clostridia total, lg c/g[4]	5.48	5.90	2.46	6.41	0.773	0.548	0.046	0.014
C.tyrobutyr., lg c/g[4]	5.50	3.67	1.87	6.43	0.757	0.122	0.004	0.131
MIX 2								
Dry matter, g/kg	218	226	229	245	9.5	0.200	0.175	0.857
pH	3.93	4.20	3.96	4.00	0.146	0.501	0.693	0.279
WSC[2]	15.3	65.9	30.5	13.4	8.82	non-normally distributed		
Lactic acid	70.4	5.1	57.8	45.8	8.41	0.008	0.201	0.002
Acetic acid	10.3	8.3	11.7	13.4	2.40	0.770	0.286	0.347
Butyric acid	5.16	8.70	0.43	6.51	3.992	0.991	0.700	0.181
Ethanol	11.3	3.41	3.57	7.55	0.869	<0.001	0.005	0.901
Ammonia N, g/kg N	155	112	138	130	21.8	non-normally distributed		
Ammonia N, g/kg N[3]	155	112	44	130	21.7	non-normally distributed		
Clostridia total, lg c/g[4]	5.60	4.02	2.59	6.53	0.401	0.030	0.000	0.036
C.tyrobutyr., lg c/g[4]	5.67	3.73	2.57	6.53	0.341	0.011	<0.0001	0.042

[1]CON=no additive, FA=formic acid, NaHe=hexamethylentetramine and sodium nitrite mixure, LAB=*Lactobacillus plantarum;* [2] water-soluble carbohydrates; [3] deducted all nitrogen applied through additive; [4] copies per silage gram (fresh weight). In MIX 1 white lupin and spring wheat ratio 1:2 and in MIX 2 ratio 2:1 of fresh weight.

Control vs additives

The values of the control silage quality parameters were clearly worse than those of silages treated with an additive (P<0.001). The pH value close to 4 of the control silages of G2 did not prevent high amounts of butyric acid and ammonia but the average of quality parameters did not differ from the treated silages (P>0.05), because also FA and LAB samples were of

poor quality. The higher amount of clostridia PCR-copies in the control ($P<0.05$) compared with additive treated silages underlined the poor quality of the control silages.

Lactic acid bacteria vs chemical additives

The results of the LAB treatment were good on G1 but poor on G2. Apart from G2M2-mixtures the pH value of the LAB silages was lower ($P<0.01$) than that of the silages treated with chemical additives. The PCR-copies of clostridia in LAB silages on G2 were significantly higher ($P<0.001$) than in silages treated with chemical additives.

Formic acid vs sodium nitrite and hexamine

Both chemical additives restricted fermentation and therefore no difference in WSC content was observed. The amount of lactic acid was higher in the NaHe silages except in G1M2-mixture. The average content of butyric acid was clearly higher in FA samples. However, a significant difference was observed only in G2M1-mixtures ($P<0.001$) as a consequence of high variation of butyric acid content in the replicates of all other mixtures. Ammonia content of NaHe-silages were higher compared with MH-feeds on the first growth stage ($P<0.05$). When adding the NaHe-additive it was assumed that the added nitrogen compounds were completely broke down to ammonia. G1M1 feeds of NaHe showed lower ammonia values than the FA-feed ($P<0.05$) when the total nitrogen amount was corrected by the ammonia of the added additive. In other feeds the differences were not significant.

The quality of FA silages was poor. Silage pH-value increased during storage time, so that the highest pH was measured on growth stage 2 of the mixture 1silage (pH 4.69). Formic acid antimicrobial effect is based on the non-dissociated acid molecule. The pk_a-value of formic acid is 3.78. At this pH-level 50 % of the formic acid molecules are dissociated. The sum of PCR clostridia copies in FA silages was higher in G1 mixture 1 and in G2 both mixtures than in NaHe silages ($P<0.05$). In this research, the bactericidal effect of formic acid was not sufficient due to the high pH of the silages, thus, the ensiling result was likely based on acidification which, however, was not sufficient. The best silage quality was achieved by NaHe treatment. NaHe feed quality was good even when feed pH was quite high. The chemical preservatives of NaHe affect directly the growth of undesired microbes. Thus, the quality of NaHe-silages was not connected to low pH in the same way as the quality of silages treated with other additives.

Conclusions

Excluding NaHe treatment, inhibition of butyric acid fermentation was not successful due to the difficult ensiling properties of the raw material. Despite the better ensiling characteristics of wheat, compared with white lupin, more wheat in the mixture did not improve ensilability enough for all the additives to be effective. Lactic acid bacteria based additives worked well only on the early growth stage when the WSC content was high. Water-soluble carbohydrates and lactic acid fermentation allowed silage pH to drop down sufficiently to prevent butyric acid fermentation .The direct acidic effect of formic acid was not sufficient to prevent butyric acid fermentation. On the other hand, the dosage level prevented lactic acid bacteria activity and formation of lactic acid, which would have made it possible to maintain a low pH. The action of a mixture, based on hexamine and sodium nitrite, impaired directly the activity of undesirable microbes, resulting in silages with almost no butyric acid. The additive prevented also the activity of lactic acid bacteria and lactic acid production in some samples, resulting

in silages with relative high pH values. However, pH was not a suitable criterion here for assessing the efficacy of hexamine and sodium nitrite treated feeds in this research.

References

Azo, W.M., Lane, G.P.F., Davies, W.P. & Cannon, N.D., 2012. Bi-cropping white lupins (*Lupinus albus* L.) with cereals for wholecrop forage in organic farming: The effect of seed rate and harvest dates on crop yield and quality. Biol. Agric. Hortic. 28, 86-100.

Bagge, E., Sternberg Lewerin, S. & Johansson, K-E., 2009. Detection and identification by PCR of *Clostridium chauvoei* in clinical isolates, bovine faeces and substrates from biogas plant. Acta Vet. Scand. 51, 8.

Fraser, M.D., Fychan, R. & Jones, R., 2005. The effect of harvest date and inoculation on the yield and fermentation characteristics of two varieties of white lupin (*Lupinus albus*) when ensiled as a whole-crop. Anim Feed Sci.Technol. 119 307–322.

Hauggaard-Nielsen et al. 2008 Grain legume–cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. Renew. Agric.Food Syst. 23, 3–12.

McDonald, P., Henderson, A R. & Heron, S. J. E., 1991. *The Biochemistry of Silage*. 2nd edition. Chalcombe Publications, Marlow, UK. 340 p.

Takeuchi, S., Hashizume, N., Kinoshita, T., Kaidoh, T. & Tamura, Y., 1997. Detection of *Clostridium septicum* hemolysin gene by polymerase chain reaction. J. Vet. Medic. Sci. 59, 853–855.

Weimer, P.J. & Stevenson, D.M., 2012. Isolation, characterization, and quantification of *Clostridium kluyveri* from the bovine rumen. Appl. Microbiol. Biotech. 94, 461–466.

Protein value in organic grass-clover silages from spring and summer growth

A.K. Bakken[1], M. Vaga[3], M. Hetta[3], Å.T. Randby[4] & H. Steinshamn[2]
[1,2]Norwegian Institute for Agricultural and Environmental Research (Bioforsk), [1]N-7512 Stjørdal and [2]N-6630 Tingvoll, Norway. [3]Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, Box 4097, S-904 03 Umeå, Sweden. [4]Norwegian University of Life Sciences, Box 5003, N-1432 Ås, Norway. Correspondence: anne.kjersti.bakken@bioforsk.no

Introduction

In organically grown grass-clover swards, there is usually considerable differences in the content of clover between spring and summer growths. Forage from the first cut is dominated by grasses and has a lower content of crude protein (CP) and an easily digestible carbohydrate fraction (Steinshamn and Thuen, 2008). The yields from regrowths contain on the other hand, more legumes, more indigestible cell walls and more CP. At feeding, these qualitatively different forages, might be utilized complementary according to the animal demands. To realize their potential alone or together and explore their complementarity, their respective values as protein and energy sources have to be determined. Knowing that energy preservation and prevention of protein degradation during wilting and ensiling are critical for the final feed value, we have investigated gains and losses obtained by restricted versus extensive fermentation of the crops. The initial results presented here express feed value according to NorFor (Volden, 2011).

Materials and Methods

The plant material was harvested from a mixed crop of *Phleum pratense, Festuca pratensis, Lolium perenne, L. boucheanum* and *Trifolium pratense*. The spring growth was harvested at late stem elongation of the dominating grass species, *P. pratense*, and the summer growth 614d° afterwards (base temperature 0°C). The content of *T. pratense* was 30% of dry matter in spring and 76% in summer growth.

The herbage was wilted indoors for 24 hours before ensiling with different types of additives (4 mL/kg FM) in evacuated and sealed polyethylene bags. The additives were 1) water (Control treatment (C)), 2) formic acid (FA, 850 g/kg), and 3) lactic acid bacteria (LAB) (Kofasil® Lac, Addcon Europe). The dosage of LAB corresponded to 10^5 cfu per g FM.

Dried (60°C) samples of herbage and silages were analysed for ash, CP, buffer soluble CP (sCP) (Hedquist and Udén, 2006), neutral detergent fibre (NDF) (Mertens et al., 2002) and water soluble carbohydrates (WSC) (Larsson and Bengtsson, 1983). Freshly frozen silage samples were analysed for pH, and content of organic acids and ethanol (Ericsson and André, 2010) and NH_4-N. The oven DM contents of the silages were corrected for volatile losses according to NorFor. Concentrations of indigestible NDF (iNDF) were determined by a 288 h *in situ* incubation (Huhtanen et al., 1994). Organic matter digestibility (OMD) was calculated from iNDF and NDF concentrations (Huhtanen et al., 2013). Net energy lactation (NEL_{20}), metabolizable protein (calculated as amino acids absorbed in the small intestine (AAT_{20})) and protein balance in the rumen (PBV) were calculated according to NorFor at daily intake of 20 kg DM (Volden, 2011). The concentration of constituents in herbage were modelled using the procedure MIXED in SAS (SAS Institute inc., 1999) with growth (spring or summer) and wilting as fixed factors and replicate (1-3) as random. For silages, the model included growth and silage additive as fixed factors and replicate (1-3) as random.

Results and Discussion

The crop harvested in spring growth, was more digestible and contained less CP and more WSC than the summer growth dominated by mature red clover (Table 1). All silages were well fermented as evaluated from pH and the concentration of NH_3-N. No single sample contained more than 72 g NH_3-N/kg N, and none had pH higher than 4.50. According to the content of organic acids, addition of LAB caused the most extensive fermentation (140 g/kg DM) and FA the least (57 g/kg DM). Protein solubility increased already during wilting in spring growth, but not in summer growth (Table 1). The CP fraction was further solubilized during fermentation (Table 2), and the soluble proportion was higher in silages from spring growth than in silages from summer growth ($P<0.001$), and higher in extensively fermented (C, LAB) compared to restrictedly fermented (FA) silages ($P<0.001$). The restriction of silage fermentation caused by application of formic acid contributed positively to energy preservation and protein feed value (NEL_{20}, AAT_{20}), compared to treatments causing more extensive fermentation (Table 2).

Table 1 Organic matter digestibility (OMD) and content of dry matter (DM), ash, crude protein (CP), soluble CP (sCP), water soluble carbohydrates (WSC), neutral detergent fiber (NDF) and indigestible NDF (INDF) in a fresh and wilted grass-clover crop harvested in spring and summer growth. N=3

Herbage,	DM	Ash	CP	sCP	WSC	NDF	iNDF	OMD
growth (G) and wilt (W)	g/kg	g/kg DM						
Spring growth, fresh	163	70	101	32	264	391	59	769
Spring growth, wilted	235	76	114	39	221	396	67	759
Summer growth, fresh	139	99	133	40	112	361	113	707
Summer growth, wilted	232	102	138	40	87	383	121	695
SEM	5.0	1.5	2.8	0.9	5.3	7.3	3.0	4.0
Sign. effect of ($P\leq0.05$)	G, W	G, W	G, W	G, W, G×W	G, W	G	G, W	G, W

Conclusions

Fresh and wilted herbage from a summer growth dominated by red clover contributed more, and more stable CP than spring growth dominated by grasses did. Preserved as silage, it still supplied less metabolizable protein (AAT_{20}) because of its lower digestibility. Whether it has a potential as a complementary forage to silages from spring growth with a lower CP content needs to be evaluated *in vivo*. Further investigations which are presently conducted by the authors, will reveal if the quality and protein value of summer growth silages may be improved by more frequent or appropriate timing of harvests.

Acknowledgements

The study was financed by the Norwegian Agricultural Agreement Research Fund, Tine BA, the County governors and councils in Sør- and Nord-Trøndelag, and the Norwegian Agricultural Extension Service**.**

Table 2 Organic matter digestibility (OMD) and content of dry matter (DM), ash, crude protein (CP), soluble CP (sCP),) metabolizable protein (MP), protein balance in the rumen (PBV) and net energy for lactation (NEL_{20}) in wilted silages made from spring and summer growth of a grass-clover crop and according to type of additive.

Silage,	DM	Ash	CP	sCP	OMD	AAT_{20}	PBV	NEL_{20}
growth and additive	g/kg	g/kg DM						MJ/kg DM
Spring growth (n=9)	246	84	124	72	760	80	7	5.9
Summer growth (n=9	237	111	155	70	699	67	56	5.2
SEM	4.9	1.0	3.5	1.9	3.0	0.4	3.4	0.03
Significance, $P\leq$	NS	0.01	0.01	NS	0.001	0.001	0.01	0.001
Control (n=6)	243	99	142	77	731	69	41	5.5
Formic acid (n=6)	240	97	137	62	729	83	13	5.7
Lactic acid bacteria (n=6)	242	98	140	76	729	69	41	5.6
SEM	4.5	0.9	3.4	2.0	3.1	0.4	3.1	0.04
Significance, $P\leq$	NS	0.05	NS	0.001	NS	0.001	0.001	0.05

References

Ericson, B., André, J. & 2010. HPLC-applications for agricultural and animal science. Proceedings from the 1st Nordic Feed Science Conference, Uppsala, Sweden. Report 274, Swedish University of Agricultural Sciences, Dept. of Animal Nutrition and Management. pp. 23-26.

Hedqvist, H. & Udén, P. 2006. Measurement of soluble protein degradation in the rumen. Animal Feed Science and Technology 126, 1-21.

Huhtanen, P., Kaustell, K. & Jaakkola, S., 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Animal Feed Science and Technology 48, 211-227.

Huhtanen, P., Jaakkola, S. & Nousiainen, J., 2013. An overview of silage research in Finland: from ensiling innovation to advances in dairy cow feeding. Agricultural and Food science 22, 35-56.

Larsson, K. & Bengtsson, S., 1983. Bestämning av lätt tillgängliga kolhydrater i växtmaterial. Metodbeskrivning 22. Uppsala: Statens Lantbrukskemiska Laboratorium, Sweden.

Mertens, D.R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C.S., Jones, D., Kaplan, D., Kim, G.N., Kobata, S., Main, D., Moua, X., Paul, B., Robertson, J., Taysom, D., Thiex, N., Williams, J. & Wolf, M., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative Study. Journal of AOAC International 85, 1217-1240.

SAS institute Inc., 1999. SAS/STAT® User's guide, Version 8, Cary, NC. 3884 pp.

Steinshamn, H. & Thuen, E., 2008. White or red clover-grass silage in organic dairy milk production: Grassland productivity and milk production responses with different levels of concentrate. Livestock Science 119, 202–215.

Volden, H., 2011. Feed calculations in NorFor, in: Volden, H. (Ed), NorFor – The Nordic feed evaluation system – EAAP 130, Wagenigen Academic Publishers, The Netherlands, pp. 55-58.

Dynamics of gas formation during ensilage

M. Knicky[1], H-G. Wiberg[2], F. Eide[3] & B. Gertzell[3]
[1]*Department of Animal Nutrition & Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences, S-753 23 Uppsala, Sweden;* [2]*Dept. Analyses & Environmental service, Coor Service Management, S-251 09 Helsingborg, Sweden;* [3]*AB Hanson & Möhring, P.O. Box 222, S-301 06 Halmstad, Sweden*
Correspondence: martin.knicky@slu.se

Introduction

The formation of a whole spectrum of gasses occurs during ensiling process. The formation of gasses is undesirable, because it is often a sign of undesirable processes in silages, and causes concern about the impact on the global environment. Formation of CO_2 is the most abundant during ensiling and its volume can reach up to 80% of total gasses produced during the first 60 ensiling days (Peterson et al., 1958). Processes where CO_2 occurs as a by-product are less effective in transformation of substrate to main fermentation products which results in higher ensiling losses (McDonald et al., 1991). Therefore, the formation CO_2 can be considered as a measure of ensiling efficiency and ensiling losses. Besides CO_2, the formation of toxic N oxides also occurs during ensiling. Research concerning nitrogenous gas formation during ensiling is sporadic and often incomplete (Spoelstra, 1985). Since silage additive addition affects the fermentation pattern, the aim of the study was to monitor the formation of various gasses from silages treated with different silage additives.

Materials and Methods

A grass ley (70% timothy) was harvested on August 24th 2013, nearby Helsingborg, southern Sweden. The forage crop was directly harvested using a precision chopper (Claas Jaguar 690). Standard analyses to determine composition of the fresh forage (FF) such as dry matter (DM), buffering capacity, ash, water soluble carbohydrates (WSC), pH, neutral detergent fibre (NDF), metabolisable energy (ME), crude protein (CP), and hygienic quality (enterobacteria, clostridia spores) were performed. The forage was divided in three fractions. One was left untreated while the rest was treated with either of two silage additives; one with a bacterial inoculant (*E. faecium, L. plantarum, L. buchneri*) at the rate of 250000 cfu/g FF, and the second one with Safesil at the rate of 3 L/ton FF. Forages were ensiled in steel laboratory silos (vol. of 25 L). Each treatment consisted of 6 replicates. Bottom and lids of silos were equipped with stoppers with tubes allowing collection of gas and silage liquids. Two forms of gas collection were applied. First, the escaping gasses in three silos of each treatment were collected into Tedlers bags (Supelco), which were regularly changed. Gasses collected in bags were analysed for N_2, H_2, O_2, CO, CH_4 by gas chromatographaphy. Separation was done on a packed column (40/60 mesh, 4 m, OD 1/8) in a Perkin Elmer Clarus 580 gas chromatograph using TCD detector. The second set of three silos of each treatment was connected to distilled water baths (regularly changed) in which CO_2, NO, and NO_2 were absorbed. The gases absorbed in water were analysed using ion chromatography by use of a UV detector according to ISO 10304-1. Gas collections were done during14 days (bacterial treatment for 30 days). At the end of storage (120 days), silages samples were extracted and standard analyses (DM, volatile fatty acids, lactic acid, ethanol, pH, NDF, ME, crude protein, lactic acid bacteria, clostridia spores, aerobic stability) were performed to determine silage quality.

Results and Discussion

The chemical and microbiological composition of the forage, prior to ensiling, is presented in Table 1. Chemical composition of fresh forage represented a common composition found in third cut grass crops in Sweden. Calculated fermentation coefficient of 39 characterizes the forage as slightly above the limit for a difficult crop to ensile (Weissbach, 1974). The analyses of microbiological contamination revealed high counts of enterobacteria and particularly of clostridia spores.

Table 1 Chemical and microbiological compositions of fresh forage (n=2)

Analyses	Unit	
DM	%	23.8
Ash	% of DM	8.6
CP	% of DM	13.8
WSC	% of DM	11.3
NDF	% of DM	55.9
ME	MJ/kg DM	10.9
Buffering capacity	g LA/100 g DM	6.0
Enterobacteria	log cfu/g FM	3.4
Clostridia spores	log cfu/g FM	4.7
pH of forage mass		6.2
Fermentation coefficient		39

DM-dry matter; FM-fresh matter; CP-crude protein; WSC-water-soluble carbohydrates; NDF-neutral detergent fiber; ME-metabolisable energy.

Results of chemical analyses of the silages displayed variation in chemical composition (Table 2). Silages treated with bacterial inoculant had significantly higher pH, propionic acid, acetic acid, and ethanol contents but lower concentration of lactic acid than other silage treatments

Table 2 Chemical composition of silages at the end of storage (n=3)

	DM	pH	NH_3-N	Lactic acid	Acetic acid	Propionic acid	Ethanol
	%		% TN	% DM			
Control	24.3	4.0	6.6	10.1	1.7	0.03	0.5
Inoculant	24.1	4.4	7.1	3.9	5.5	0.45	0.8
Safesil	25.1	4.0	6.2	8.7	1.8	<0.04	0.3
$LSD_{0.05}$		0.02	1.24	0.38	0.20	0.02	0.05
P-value		0.001	0.3	0.001	0.001	0.001	0.001

It is assumed that the effects were caused by *L. buchneri* in the silage innoculant which is known to form acetic acid at the expence of lactic acid (Reich & Kung, 2010). As a concequence of the high levels of acetic acid, silage treated with bacterial innoculant were found to be significanly more aerobicaly stable than the untreated control silage (Table 3). This study confirmed early findings (Knicky & Spörndly, 2009, 2011) that Safesil is efficient to secure a proper ensiling process and in improving the aerobic stability of silage.

Table 3 Microbiological composition and aerobic stability of silages at the end of storage (n=3).

	Time (hours) until temp. aerated silages increased 3°C	Max. temp (°C)	Max. temp. increase (°C)	pH after stab.	Yeasts (lg cfu/g)
Control	72	31.8	13.9	6.1	3.6
Inoculant	166	19.6	1	4.5	<1.7
Safesil	166	19.3	0.3	4.1	<1.7
$LSD_{0.05}$	16.8			1.37	0.32
P-value	0.001			0.02	0.001

Gas analyses revealed significantly higher formation of total gasses in control silage and bacterial innoculant treated silage than in the Safesil treated silage (Figure 1). This could be explained by differences in fermentation intensity and variation in bacterial composition among the silage treatments. A high gas formation in bacterially inoculated silages is assumed to be a consequence of addition of bacterial microflora which intensified the fermentation process. On the other hand, Safesil possesses a rather selective inhibitory property which restricts particularly undesirable fermentation processes. This probably caused a lower gas formation in Safesil treated silages. The proportion of CO_2 of total gass was 0.56 for Safesil, 0.65 for bacterial inoculant, and 0.68 for control silage and similar to Peterson et al. (1958).

All silages displayed a similar pattern in development of gas over time. The highest formation of gasses was observed approximatelly between 11-29 hours of the ensiling period. This peek of gas formation corresponds to the period of the most intensive fermentation, according to Pahlow et al. (2003). Another increase in gas formation was observed in bacterialy innoculated silages after ca. 300 hours of ensiling. This increase in CO_2 formation was observed only in these silages (Figure 2). This phenomenon is regarded to be the consequence of *L. buchneri* activity, which has the ability to convert lactic acid into acetic acid under unearobic conditions (Oude Elfering et al., 2001) and where CO_2 is formed as a bi-product of this conversion.

Due to lack of formation of ensiling liquid, it was impossible to follow degradation of nitrate during ensiling process. Neverheless, the formation of NO (Figure 3) and NO_2 (Figure 4) seemes to follow the degradation of nitrate and nitrite during fermentation, as described by Spoelstra (1985) and demonstrated by Knicky & Lingvall (2005) and Knicky & Spörndly (2009). The study of Peterson et al. (1958) showed a similar pattern of NO formation as in the present study. Surpisingly, the formation of all NO_x gasses revealed no statistical differences between Safesil and the other silages. A higher formation of NO was expected due to the presence of Na-nitrite in Safesil. The lack of N_2O measurement as well as monitoring of ammonia formation during fermentation process makes it difficult to explain the lack of variation in NO_x formation.

Conclusions

All silages were well fermented; however, bacterially inoculated silages contained increased concentrations of acetic and propionic acid and ethanol. Both additive treated silages possessed improved aerobic stability. Control and bacterially inoculated silages produced more gas than Safesil treated silages, mainly due to an increased proportion of CO_2. The formation of NO_x gases displayed no significant differences among other treatments.

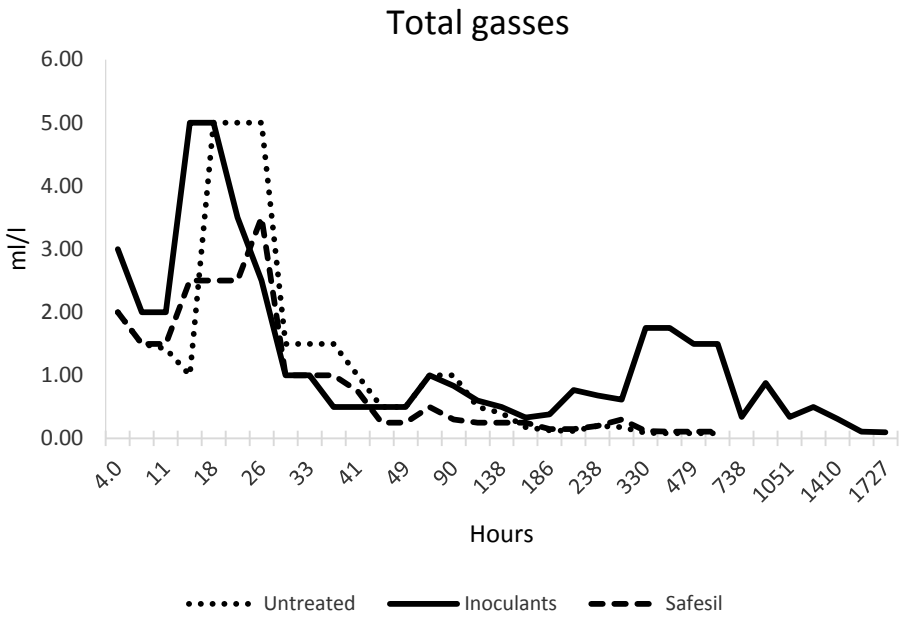

Figure 1 The sum of all gasses formed in silages during measurement. (n=3)

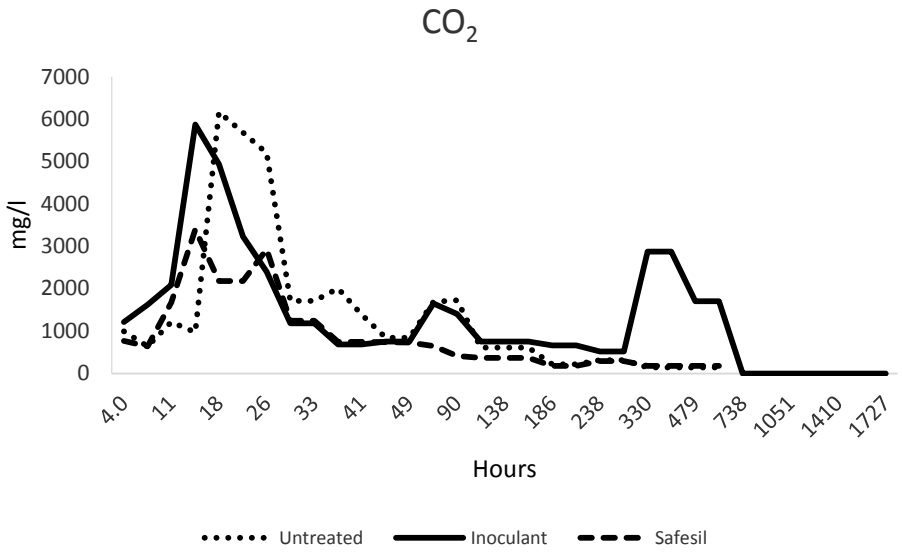

Figure 2 The formation of CO_2 in silages during measurement. (n=3)

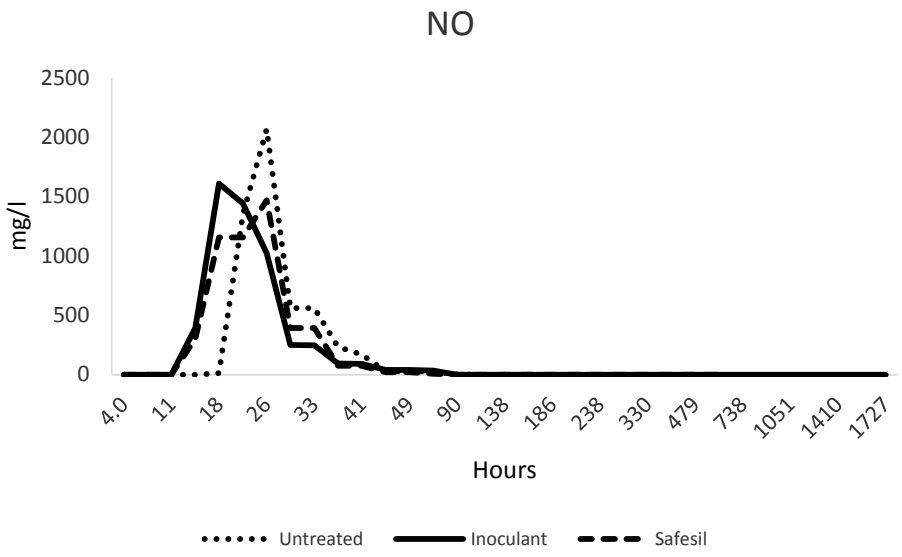

Figure 3 The formation of NO in silages during measurement. (n=3)

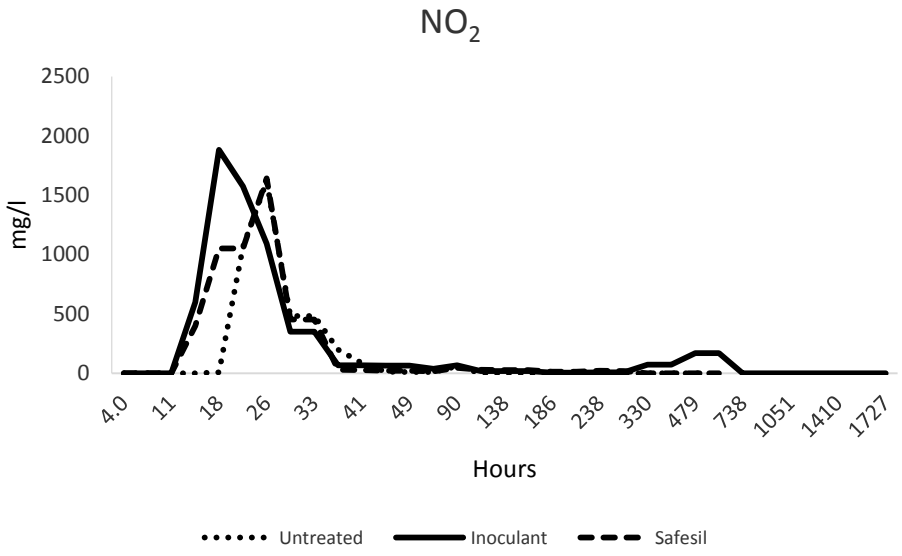

Figure 4 The formation of NO_2 in silages during measurement. (n=3)

References

Knicky, M & Lingvall P. 2004. Ensiling of high wilted grass-clover mixture by use of different additives to improve the hygienic quality. Acta Agr.Scand., Section A, 54, 197-205.

Knicky, M. & Spörndly, R., 2009. Sodium benzoate, potassium sorbate and sodium nitrite as silage additives. J. Sci. Food Agric. 89, 2659-2667.

Knicky, M. & Spörndly, R., 2011. The ensiling capability of a mixture of sodium benzoate, potassium sorbate, and sodium nitrite. J. Dairy Sci., 94, 824-831.

McDonald, P., Henderson, A. R., & Heron, S. J. E., 1991. The Biochemistry of Silage. Chalcombe Publications, Marlow, Bucks, UK.

Oude Elfering, S.W.J.H., Krooneman, J., Gottschal, J.C., Spoelstra, S.F., Faber, F. & Driehuis, F., 2001. Anaerobic Conversion of Lactic Acid to Acetic Acid and 1,2-Propanediol by *Lactobacillus buchneri.* Appl. Environ. Microbiol. 67**,** 125-132.

Pahlow, G., Muck, R.E., Driehuis, F. & Oude Elferink, S.J.W.H. 2003. Microbiology of ensiling. Ed. Buxton, D.R., Muck, R.E. & Harrison, J.H. 2003. Silage science and technology, Madison, Wisconsin, USA.

Reich, L.J. & Kung, L. Jr., 2010. Effects of combining Lactobacillus buchneri 40788 with various lactic acid bacteria on the fermentation and aerobic stability of corn silage. J. Anim. Feed Sci. Technol., 159, 105-109.

Spoelstra, S.F., 1985. Nitrate in silage (review). *Grass Forage Sci. 40*, 1-11.

Weissbach, F., Schmid, L. & Hein, E., 1974. Method of anticipation of the run of fermentation in silage making based on chemical composition of green fodder. Pages 663-673 in Proc 12th Intern Grassland Congress, Moscow, Russia, 11-20 June.

Peterson, W.H., Burris, R.H., Sant, R. & Little, H., 1958. Production of toxic gas. (Nitrogen oxides) in Silage Making. Agricultural Food Chemistry, 6, 121-126.

Mycotoxins in haylage

J. Schenck[1,2], C. Müller[1] & R. Spörndly[1]
[1] *Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Feed Science Division, Kungsängen Research Centre, 753 23 Uppsala.*
[2]*Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, BOX 7026, 750 07 Uppsala, Sweden*
Correspondence: Jessica.Schenck@slu.se

Introduction

Silage with high dry matter content, also referred to as haylage, is a common forage in feed rations for horses in Sweden (Enhäll *et al*., 2012). As water activity is low in haylage, lactic acid fermentation is restricted resulting in high content of residual water soluble carbohydrates and no or a very small pH-decrease as opposed to silage (Finner, 1966; Müller, 2005). If haylage bales are not sufficiently air-tight, this environment may favour mould growth in the forage. Mould growth may increase the risk for mycotoxin presence in the feed as well, but there is scarce information about formation of mycotoxins in haylage and in horse feeds in general (Liesener *et al.,* 2010). Moulds do not regularly produce mycotoxins, but may occur as secondary metabolites under certain environmental conditions (Samson *et al*., 2002). The aim of this study was therefore to examine mycotoxin presence in randomly selected haylages in Sweden and Norway, and to investigate if any correlations existed between mycotoxin and mould presence.

Materials and Methods

Haylage samples from in total 100 farms in Sweden and Norway were analysed for chemical composition, mould growth and mycotoxins. Sampling of haylage was performed at 77 different Swedish farms during two years (2010 and 2011), and at 23 Norwegian farms during 2011. The farms were evenly distributed over the countries. Samples for mould cultivation were taken in three ways as described by Schenck et al. (2013). In short, they were: i) direct plating of samples taken from visible fungal growth on bale surface, ii) direct plating of plant material from drilled core samples and iii) dilution plating where core samples were mixed with peptone water and the dilutions cultured. Two substrates (malt extract agar (MEA) and Dichloran 18% glycerol agar plates (DG-18) (Merck, KGaA, Darmstadt, Germany)) and at two incubation temperatures (25 and 37 °C) were used for culturing. Samples were plated within 48 hours after sampling, and were incubated for ten days. For mycotoxin analysis, core samples were used. These samples were ground after freeze-drying and analysed by LC-MS/MS as described by Rasmussen et al. (2010) at National Food Institute, Division of Food Chemistry, Technical University of Denmark. Eleven mycotoxins, including patulin, nivalenol (NIV), deoxynivalenol (DON), 3-acetyldeoxynivalenol (15-ACDON)), gliotoxin, alternariol, HT-2 toxin, T-2 toxin, zearalenone (ZEA), beauvericin (BEAU) and enniatin B (ENN B), were analyzed. Lowest detection limits were: 371 µg patulin/kg, 122 µg NIV/kg, 20 µg DON/kg, 35 µg 15AC-DON/kg, 41 µg gliotoxin /kg, 10 µg alternariol/kg, 5 µg HT-2 toxin/kg, 8 µg T-2 toxin/kg, 5 µg ZEA/kg, 10 µg BEAU/kg and 10 µg ENN B/kg.

Chemical variables were analysed as described by Müller (2005).

Correlation calculations between presence of mycotoxins and moulds in haylage samples were performed using PROC CORR statement (SAS, 2014). The probability to find mycotoxins was also tested with PROC LOGISTIC model (SAS, 2014), where variables such as presence of mould (any species or *Fusarium* spp. in particular) and of chemical composition such as dry matter, crude protein, ash, NDF, pH, ammonia nitrogen, lactic acid, acetic acid, propionic acid, butyric acid, ethanol and 2,3-butanediol were included. In all statistical calculations, farm was used as experimental unit and effects were considered as statistically significant when $P<0.05$.

Results and Discussion

One or more mycotoxin(s) were found in haylage from fifty farms, while no mycotoxins were detected in the remaining haylage samples (50) (Figure 1). The most frequently detected mycotoxin was ENN-B (31 farms) followed by BEAU (16 farms) and DON (12 farms).These mycotoxins are all known to be produced by *Fusarium* species. Minimum, maximum and mean mycotoxin concentrations are reported in Table 1. The level of the second most common mycotoxin found in the present study (BEAU) was low, compared to a recently presented survey from Korea, where the average level of concentrate feeds for cattle was 720 µg/kg (Kyung et al 2010). Wheat bran was identified as the concentrate ingredient containing the highest amount BEAU ranging from 340 to 1100 µg/kg.

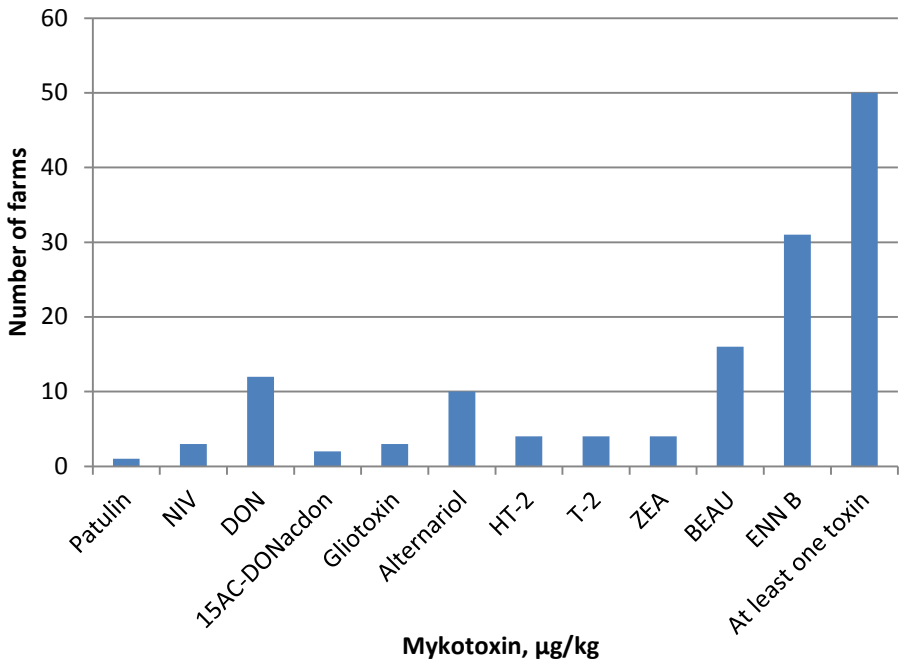

Figure 1 Mycotoxins detected in haylage sampled from 100 farms in Sweden and Norway and number of farms where each mycotoxin was present.

Mould was detected in haylage samples from 49 farms with method ‘’', 74 farms with method ‘ii’, and 56 farms with method ‘iii’. The most common mould genera were *Pencillium* spp. and *Artrinium* spp. *Fusarium* spp. were only detected in haylage from five farms.

Table 1 Mycotoxins detected in haylage samples from 100 Swedish and Norwegian farms (µg/kg). Values only represent samples where the mycotoxins were detected

Mycotoxin	Minimum	Maximum	Mean	SD
Patulin	Nd	Nd	Nd	Nd
NIV	Nd	Nd	Nd	Nd
DON	69	479	238	134.7
15- ACDON	70	288	179	154.1
Gliotoxin	44	57	51	9.2
Alternariol	11	1452	212	501.7
HT-2	19	78	35	28.8
T-2	8	11	9	1.5
ZEA	8	8	8	-
BEAU	11	988	248	376.8
ENN B	10	283	56	83.9

Nd = value below lower limit of detection (see text for lower detection limits)

There was no correlation between mycotoxin presence and mould occurrence (r=0.03). Thus, finding visible mould at the bale surface or from core samples will not mean that mycotoxins are present. An analysis of the magnitude of total mould growth (log CFU/g) established with the dilution method, and the prevalence of mycotoxins, resulted in an indication that the risk of finding mycotoxins was higher when mould numbers were higher (r=0,25; P<0,05). However, higher mould counts were not correlated to higher mycotoxin concentrations (r=0.03). This is in contrast to the findings of Legzdina and Buerstmayr (2004) who studied *Fusarium* head blight and mycotoxin presence in barley, where the percentage of visually infected barley spikes was positively correlated with the content of DON and 15-ACDON.

It was notable that no correlation was found between occurrence of *Fusarium*-toxins (NIV, DON, BEAU and ENN-B) and presence of *Fusarium* spp.-moulds (*Fusarium culmorum, Fusarium equiseti, Fusarium graminearum and Fusarium poae*) in the haylage samples. However, the small number of haylage samples where *Fusarium*-moulds were detected may partly explain this. Also, presence of mycotoxin may be a result of previous and not present active mould growth, meaning that the product of the mould is detected but not the mould itself.

The difficulty of using other variables as predictors of mycotoxin presence was further highlighted when analysing the data with the LOGISTIC procedure, where the statement SELECTION=FORWARD was used, allowing all chemical variables and mould occurrence that met the significance level of P<0,05 to enter the model. No variable appeared to statistically increase or decrease the odds ratio to find mycotoxins in the haylage.

Conclusions

Mycotoxins were detected in 50 % of sampled haylage bales in Sweden and Norway. *Fusarium* spp. mycotoxins were the most common mycotoxins present. Neither conventional mould analysis by culturing nor chemical variables were able to indicate if mycotoxins were

present. However, in bales where moulds occurred, higher mould counts correlated weakly with the presence of mycotoxins.

References

Enhäll, J., Nordgren, M. & Kättström, H., 2012. Hästhållning i Sverige 2010. The Swedish Board of Agriculture, Report 2012:1. Jönköping, Sweden.

Finner, M.F., 1966. Harvesting and handling low-moisture silage. Transact. ASAE 9, 377-381.

Legzdina, L. & Buerstmayr, H., 2004. Comparison of infection with *Fusarium* head blight and accumulation of mycotoxins in grain of hulless and covered barley. J Cereal Sci 40, 61-67.

Liesener, K., Curtui, V., Dietrich, R., Märtlbauer, E. & Usleber, E. 2010. Mycotoxins inn horse feed. Mycotox. Res. 26, 23-30.

Kyung-Eun, L., Byung Hee, K. & Chan, L., 2010. Occurrence of Fusarium mycotoxin beauvericin in animal feeds in Korea. Anim. Feed Sci. Technol., 157, 190-194

Müller, C. E., 2005. Fermentation patterns of small-bale silage and haylage produced as a feed for horses. Grass Forage Sci. 60, 109-118.

Rasmussen, R.R., Storm, I.M.L.D., Rasmussen, P.H., Smedsgaard, J. & Nielsen, K.F., 2010. Multi-mycotoxin analysis of maize silage by LC-MS/MS. Anal. Bioanal. Chem. 397, 765-776.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg & O., 2002. Introduction to food- and airborne fungi. 6th ed. Centralbureau voor Schimmelcultures, Utrecht, The Netherlands.

SAS, 2014. SAS 9.3. SAS institute Inc. Cary. NC. USA

Energy efficient and competitive on farm storage of moist feed grain - preliminary results

N. Jonsson[1], J. Blomqvist[2] & M. Olstorpe[2]
[1]*JTI – Swedish Institute of Agricultural and Environmental Engineering, P.O. Box 7033, S-750 07 Uppsala, Sweden,* [2]*Department of Microbiology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden*
Correspondence: Nils.Jonsson@jti.se

Introduction

Feed grain is the principal source of energy (40-80%) in animal feeds. Preparation of on-farm feed grain is profitable if a high quality feed is produced. Fungi are the major factor restricting the storability of feeds and fungal activity may lead to a decrease in nutritional value and loss of palatability, and to health hazards for animal consuming the feed and people handling it. Storage fungi are quite common in feed grain preserved on Swedish farms (Jonsson & Pettersson, 1999). Depending on preservation method used, 20-60% of the farms produced feed grain contaminated with storage fungi. Many of the storage fungi are potential producers of mycotoxins. High-temperature drying is probably the safest preservation method but involves high investment and energy costs. An alternative is sealed storage of the grain in silos, which is an environmentally more beneficial method and can be used up to moisture contents (MC) of 26 %. This method only requires about 2% of the energy consumed in high-temperature drying (Pick et al., 1989). Sealed storage relies on perfect sealing in combination with the anaerobic conditions caused by respiration of the grain itself and the adhering microorganisms. However, it is difficult to achieve fully anaerobic conditions since leakage, the removal of grain and great diurnal changes in gas pressure result in gas being exchanged between the stored and the surrounding atmosphere (Ekström, 1992; Newman, 1971; Druvefors et al., 2002). An experience is that grain stored in airtight silos usually deteriorates when stored until the following summer. A way of prolonging storage time is the use of propionic acid at levels of 3-8 l/tonne, depending on water content (Ekström, 1992). Another method for grain with high MC (>30 %), increasingly used, is storage of crimped grain in silo-bags. Here, the grain is converted into a stable livestock feed by fermentation (Finch et al., 2002). With this method, growth of fungi and bacteria typically occurs for only a short period during initial stages of fermentation after which lactic acid production and alcoholic fermentation pre-dominate (Salovaara, 1998). Pathogenic and spoilage organisms may be inhibited by production of organic acids, hydrogen peroxide, carbon dioxide and/or antimicrobial substances, and also by lowering the pH (Ouwehand, 1998). A group of bacteria, which have been identified in moist stored grain are *Enterobacteriaceae* (Olstorpe et al., 2012). Many members of this family are a normal part of the gut flora of humans and other animals, while others are found in water or soil, or are parasites on a variety of different animals and plants. This group include EHEC, a type of *Escherichia coli,* associated with severe human health problems. Lowering the number of these bacteria in feeds has been shown to reduce their frequency in the food chain (Brooks et al., 2001). A problem with a preservation method of high MC grains is that during favourable harvest conditions, the MC will decrease with up to 5% units per day (Gunnarsson et al., 2012) and only a part of the stored grain will be protected by fermentation, unless water is added during the crimping. Drier conditions will favour the development of fungi and one of the most important spoilage fungi in airtight stored grain is *Penicillium roqueforti*. This fungus can grow at low partial pressures of oxygen (>0.14%) (Magan and Lacey, 1984), high levels of carbon dioxide and

low temperatures and is a potential mycotoxin producer (Häggblom, 1990; Pitt and Hocking, 1999).

In the present study, the hygienic quality of moist crimped barley and wheat (in mixture or separate) stored in silo-bags on three Swedish farms were evaluated during two successive years. As in an earlier study, which showed that the microbial flora in grain stored in this kind of system is highly diverse and not predictable (Olstorpe et al., 2010), the microbial flora were identified and enumerated. Compared with the earlier study in which the storage ended in March, the objective was to follow the grain quality from harvest until July the following year. The evaluation of the storage system will also include technology and economy.

Materials and Methods

Experimental design and feed components

Three Swedish farms, two in Uppland and one in Hälsingland, took part in the study. All farmers used a bagging machine combined with a crimper: Murska with rollers (Farm 1 and 3) or Bagmaster with discs (Farm 2), and an auger for packing the crimped grain into the silo-bag. Both years, the harvest period started in August (Uppsala) and ended in October (Hälsingland). In most cases, the grain was harvested (wheat or barley, separate or in mixture), crimped and packed into silo-bags during the same day. In Uppland, both farms (Farm 1 and 2) added propionic acid to the grain (3-5 l/ton) as an extra precaution. The silo-bags had a diameter of 2 (Farm 1 and 3) or 2.4 m (Farm 2) and a length of up to about 50-60 m, containing up to 200 ton. To ensure proper fermentation of the grain, the tubes were not opened until after 3-4 weeks of storage. After opening, a layer of about 0.5 m was removed daily from the face. Grain samples were collected from each farm at harvest (start), in October/November, December, March/April, May, June and July by JTI. In connection to the sampling in November to July, gas-composition (CO_2 and O_2; GA2000 Gas Analyser Geotechnical Instruments Ltd, UK) and the temperature (TSI VelociCalc 9565, TSI Incorporated, MN, USA) of the grain were measured. Information of average daily temperatures during the storage periods were received from the Swedish Meteorological and Hydrological Institute.

Analytical methods

Moisture content was determined by drying samples at 103 ◦C for 16 h and water activity of the grain samples by an a_w-meter (AquaLab Series 3TE, Decagon Devices Inc., Pullman, WA, USA). The pH of the liquid obtained after mixing aliquots of 100 g grain with 100 ml deionized water and shaking for 10 minutes at room temperature was measured using a laboratory pH meter (Radiometer, Copenhagen, Denmark).

Microbial quantification

Samples for microbial quantification, etc. were collected from the surface where the grain was unloaded (3 points). From these samples, aliquots of 20 g of barley were suspended in 180 ml of peptone water (2 g/l) and homogenized with a stomacher as previously described (Petersson et al., 1999). The samples were serially diluted and spread on selective agar plates to enumerate and isolate LAB, yeasts, moulds, , Enterobacteriaceae. LAB were quantified by anaerobic incubation at 30°C for 48 h on de Man Rogosa Sharp (MRS) agar (Oxoid Ltd., Basingstoke, UK) supplemented with 100 g/l Delvocid (Gist-brocades B.V., Delft, The Netherlands). A GasPack system (Becton Dickinson; Sparks, MD, USA) was used during the

incubation to obtain an anaerobic environment. Yeasts were enumerated on Malt Extract Agar (MEA) (Oxoid Ltd.) plates, supplemented with 100_g/l chloramphenicol (Sigma–Aldrich, Stockholm, Sweden). Plates were incubated at 25°C for 2–4 days. Moulds were quantified on Dichloran-Glycerol (DG18) Agar after incubation at 25°C for 5-7 days. Enterobacteriaceae were enumerated on Violet Red Bile Glucose (VRBG) Agar (Oxoid Ltd.) incubated at 37°C for 24 h, by the pour plate method (1 ml sample overlaid with culture media; Lyberg et al., 2008; Olstorpe et al., 2010). Colonies were counted and mean cfu/g cereal grain was calculated. Moulds were identified both based on morphological characteristics and sequencing (Leong et al 2012, Samson et al. 2004).

Yeast and LAB strain conservation

To identify the dominating yeasts and LAB flora in the storage systems, 10 yeasts and 10 LAB colonies were randomly selected from quantification plates at the start and end of the storage period. Yeast isolates were conserved by mixing 1 ml of an overnight culture of the yeast isolate with 1 ml glycerol and frozen at -80°C. LAB strains were conserved in a cryo solution and frozen as described by Olstorpe et al. (2008).

DNA extraction, amplification and identification of microorganisms

Yeast and lactic acid bacteria and mould DMA were extracted separately according to Leong et al. (2012). For sequencing of the chosen yeast and LAB isolates, the D1/D2 region of the 26S subunit respectively 16S region of the ribosomal DNA were amplified using NL1/NL4-primers (yeast) and 16S/16Sr-primers (LAB) (Olstorpe et al. 2008). PCR for mould sequencing was done according to Leong et al (2012). A volume of 21 µl of RNase free water, 1 µl of each primer and 2 µl of either extracted DNA (yeast, moulds) or cell suspension (LAB) were pipetted in a Illustra PuReTaq Ready-to-go PCR bead-tube. PCR was run according to Olstorpe et al (2008, 2010). The amplified sequences were sent to Macrogen Europe Inc (Amsterdam, The Netherlands) and the isolates were identified by sequence comparison against the NCBI´s database (http://blast.ncbi.nlm.nih.gov/Blast).

Results and Discussion

Preliminary results show considerable variations in MC and gas composition in the stored grain, Table 1. Elevated levels of oxygen could usually be linked to damage of the silage casing caused by birds or by the unloading tractor. The addition of propionic acid resulted in lower microbial activity and thus usually resulted in higher oxygen content and lower content of carbon dioxide.

The addition of propionic acid, the same quantity regardless of MC in the grain, reduced the occurrence of yeasts, moulds and *Enterobacteriaceae*, often to below the detection level. The presence of lactobacilli remained mostly constant. When propionic acid was not used, the prevalence of lactobacilli usually increased substantially while yeasts and *Enterobacteriaceae* decreased slightly, and the presence of moulds decreased to below the detection limit. Traffic within the farm would need more attention. Often, the transport of feed and fertilizer crossed each other with an increased risk of contamination of the feed with manure. Farm 3 also lacked a paved open area (concrete/asphalt) for the storage of the silo-bags, which increase the risk of soil/manure contamination in connection with handling of the feed.

Table 1 Preliminary results concerning treatments, gas composition and the development of microorganisms in crimped moist feed grain during storage in silo bags on three Swedish farms

Farm 1 (Uppland) (mixture of wheat and barley)		
	2011/2012	2012/2013
MC, % at harvest	17-26	24-39
Propionic acid, l/ton	3-4	3-4
$O_{2,\%}$ 1m from unloading point	6-20	4-21
$O_{2,\%}$ 10 m from unloading point	0,6-14	0-19
$CO_{2,\%}$ 1m from unloading point	3-17	0-20
$CO_{2,\%}$ 10 m from unloading point	13-29	3-53
Yeasts, (log_{10}CFU/g)	< 2	< 2
Lactic acid bacteria, (log_{10}CFU/g)	3- 6	< 2 - 7
Moulds, (log_{10}CFU/g)	< 2	< 2 - 2.3
Enterobacteriaceae, (log_{10}CFU/g)	< 1	< 1 – 1.5
Farm 2 (Uppland) (mixture of wheat and barley)		
MC, % at harvest	17-36	19-22
Propionic acid, l/ton	5	4
$O_{2,\%}$ 1m from unloading point	5-21	0-21
$O_{2,\%}$ 10 m from unloading point	2-17	0-21
$CO_{2,\%}$ 1m from unloading point	0.7-18	0-23
$CO_{2,\%}$ 10 m from unloading point	9-18	0.3-18
Yeasts, (log_{10}CFU/g)	< 2 – 6	< 2- 3
Lactic acid bacteria, (log_{10}CFU/g)	< 2 – 6	< 2- 3
Moulds, (log_{10}CFU/g)	< 2 – 4	< 2- 2
Enterobacteriaceae, (log_{10}CFU/g)	< 2 – 5	< 1- 3

Table 1 (Continued)

Farm 3 (Hälsingland) (barley)	2011/2012	2012/2013
MC, % at harvest	17-27	20-34
Propionic acid, l/ton	0	0
$O_{2,\%}$ 1m from unloading point	5-17	4-21
$O_{2,\%}$ 10 m from unloading point	0-0.4	0-21
$CO_{2,\%}$ 1m from unloading point	8-34	0-30
$CO_{2,\%}$ 10 m from unloading point	27-54	1-83
Yeasts, (log_{10}CFU/g)	< 2 – 6	< 2 – 6
Lactic acid bacteria, (log_{10}CFU/g)	< 2 – 8	2 – 7
Moulds, (log_{10}CFU/g)	< 2 – 5	< 2
Enterobacteriaceae, (log_{10}CFU/g)	2 – 7	< 1 – 7
Farm 3 (Hälsingland) (wheat)		
MC, % at harvest		29-43
Propionic acid, l/ton		0
$O_{2,\%}$ 1m from unloading point		0-21
$O_{2,\%}$ 10 m from unloading point		0-17
$CO_{2,\%}$ 1m from unloading point		0.4-68
$CO_{2,\%}$ 10 m from unloading point		12-98
Yeasts, (log_{10}CFU/g)		< 2 – 5
Lactic acid bacteria, (log_{10}CFU/g)		6 – 8
Moulds, (log_{10}CFU/g)		< 2 - 3
Enterobacteriaceae, (log_{10}CFU/g)		< 1 – 5

References

Brooks, P.H., Beal, J.D. & Niven, S.J., 2001. Liquid feeding of pigs: potential for reducing environmental impact and for improving productivity and food safety. Rec. Adv. Anim. Nutr. Aust. 13, 49–63.

Druvefors U., Jonsson, N., Boysen, M. & Schnürer, J., 2002. Efficacy of the biocontrol yeast *Pichia anomala* during long-term storage of moist feed grain under different oxygen and carbon dioxide regimens. FEMS Yeast Res. Aug;2(3):389-94.

Ekström, N., 1992. Lufttät lagring av fuktig foderspannmål. Meddelande nr 439, JTI, Uppsala.

Finch, H.J.S., Samuel, A.M., Lane, G.P.F. & Wiseman, A.J.L., 2002. Cereals. In: Lockhart and Wiseman's Crop Husbandry including Grasslands. Woodhead Publishing Limited, Cambridge, England, pp. 259–302.

Gunnarsson, C., de Toro A., Jonsson N. & Lundin G., 2012. Cereal harvesting- strategies and costs under variable weather conditions. JTI-rapport 403, Lantbruk och industri.

Jonsson, N. & Pettersson, H., 1999. Evaluation of different cereal grain conservation methods based on hygienical quality. Swedish Institute of Agricultural and Environmental Engineering, Uppsala, Sweden, Report 263 (in Swedish).

Lyberg, K., Olstorpe, M., Passoth, V., Schnürer, J. & Lindberg, J.E., 2008. Biochemical and microbiological properties of a cereal mix fermented with whey, wet wheat distillers' grain or water at different temperatures. Anim. Feed Sci. Technol. 144, 137–148.

Magan N. & Lacey J., 1984. The effect of gas composition and water activity of field and storage fungi and their interactions. Trans. Br. mycol. Soc. 82, 305-314.

Newman, G., 1971. Investigation of a pressure equalising system in sealed silos. Journal and proceedings of the Institute of Agicultural Engineering, UK, volume 25 no 4, pp 158-160.

Olstorpe, M., Lyberg, K., Lindberg, J.E., Schnürer, J. & Passoth, V., 2008. Population diversity of yeasts and lactic acid bacteria in pig feed fermented with whey, wet wheat distillers' grains or water at different temperatures. Appl. Environ. Microbiol. 74, 1696–1703.

Olstorpe, M., Axelsson, L., Schnürer, J. & Passoth, V., 2010. Effect of starter culture inoculation on feed hygiene and microbial population development in fermented pig feed composed of a cereal grain mix with wet wheat distillers' grain. J. Appl. Microbiol. 108, 129–138.

Ouwehand, A.C., 1998. Antimicrobial components from lactic acid bacteria. In: Salminen, S., Von Wright, A. (Eds.), Lactic Acid Bacteria Microbiology and Functional Aspects. Marcel Dekker Inc., New York, NY, USA, pp. 139–159.

Pick, E., Noren, O. & Nielsen, V., 1989. Energy Consumption and Input Output Relations in Field Operations. Food and Agricultural Organization of the United Nations, Rome, Italy.

Salovaara, H., 1998. Lactic acid bacteria in cereal based products. In: Salminen, S., Von Wright, A. (Eds.), Lactic Acid Bacteria Microbiology and Functional Aspects. Marcel Dekker Inc., New York, NY, USA, pp. 115–137.

Samson, R.A., Hoekstra, E.S. & Frisvad, J.C., 2004. Introduction to Food- and Airborne Fungi, 7th edition. Centraalbureau voor Schimmelculture, Institute of the Royal Netherlands Academy of Arts and Sciences, Utrecht, The Netherlands.

Agro-food industry wastes conversion into safer feed stock by using lactic acid bacteria

V. Krungleviciute[1], E. Bartkiene[1], J. Kantautaite[1], G. Juodeikiene[2], J. Damasius[2], V. Baliukoniene[1], B. Bakutis[1]
[1]*Lithuanian University of Health Sciences, Tilzes str. 18, 47181 Kaunas, Lithuania*
[2] *Kaunas University of Technology, Radvilenu str. 19, 50254 Kaunas, Lithuania*
Corresponding author: vitakrungleviciute@gmail.com

Introduction

Wheat and barley are popular products for human consumption. From the manufacture of them high amount of by-products remain, which are good source for animal feeding. A higher economical advantage could be achieved with the use of wastes or by-products from agro-food industries, such as the flour milling or groat industries. These products are potential resources of new safer feed stocks with high nutritional and biological values.

The aim of this study was to evaluate the possibilities of an alternative substrate for *Pediococcus acidilactici* multiplication and to adapt multiplied strains for wheat and barley cereal industry by- product fermentation, as a potential resources to produce safer feed stocks with a high amount of live lactic acid bacteria (LAB).

Results and Discussion

We found that potato juice could be used as an alternative substrate for *P. acidilactici* cultivation. After 72 h of fermentation, a pH of samples 4.1±0.01 was achieved, a total titratable acidity (TTA) of 8.92±0.06°N (Neiman degrees) and a LAB cell concentration of 9.6±0.07 log10 colony forming units (cfu) per ml.

By spray drying at 150°C or lyophilization (-48°C), the dried powders could be used on farms as LAB starters for safe feed fermentation because the powders contained viable cell concentrations of 9.18±0.09 log10 cfu/g and 9.04±0.07 log10 cfu/g, respectively.

Fermentation of cereal by-products (n=7) decreased pH to 4.06±0.01, increased the TTA to 11.9±0.10°N and LAB counts to 9.3±0.3 $\log_{10}$ cfu/g, reduced the occurrence of aflatoxin, zearalenone, deoxynivalenol and T-2 toxin. Biogenic amine concentrations were changed by fermentation: phenylethylamine was reduced to 14.9 to 58.2%, putrescine from 46.8 to 97.9%, cadaverine from 69.4 to 100% and spermidine from 14.9 to 58.2% in the fermented cereal by-products, relative to original concentrations.

Also, *P. acidilactici* grown on a wheat/barley (10/90; w/w) substrate produced a very small amount of D-lactic acid and mainly L-lactate. The lactic acid bacteria count of fermented and dried cereal by-products (n=7) after 90-day storage contained 6.7 log10 cfu/g.

Conclusions

The *P. acidilactici* multiplied on an alternative substrate such as potato juice can be used for cereal industry by-product fermentation, as a potential resource to produce safer feed stocks with high amounts of live lactic acid bacteria with beneficial effects on animal health.

Should rumen microbial protein formation be maximized?

G. A. Broderick
Emeritus Professor, University of Wisconsin-Madison and US Dairy Forage Research Center, Agricultural Research Service-USDA, Madison, Wisconsin 53706, USA

Introduction

Ruminants are efficient users of diets poor in amount and quality of crude protein (CP) because rumen microbes synthesize the majority of their needs for metabolizable protein (MP) and essential amino acids (EAA). In addition, microbial utilization of ammonia allows the feeding of nonprotein N (NPN) compounds, such as urea, plus enables ruminants to utilize urea-N that is recycled to the rumen (Lapierre and Lobley, 2001). This latter mechanism captures some N that would otherwise be excreted in the urine. The CP in grass and legume silages is largely in the form of NPN; microbial protein synthesis converts silage NPN to MP utilized by the cow. Lactating dairy cows have the highest EAA requirements among domestic ruminants because of high production of milk protein. Although dairy cattle can utilize diets with relatively poor quality CP, they typically excrete 2 to 3 times more N in manure than they secrete in milk. Nitrogen inefficiency necessitates supplementing large amounts of protein, which increases production costs and contributes to environmental pollution. Increasing microbial protein formation in the rumen is an important way to improve N efficiency.

"Optimizing" microbial protein formation in the rumen

The EAA pattern of microbial protein is of better quality than most dietary ingredients commonly fed to domestic ruminants (Broderick, 1994; Schwab, 1996). Notably, the proportion of Met and Lys in microbial protein is very similar to that in the proteins in lean tissue and milk (NRC, 2001). Although there is evidence that His might be limiting in lactating cows fed diets containing mostly rumen-degraded protein (RDP), animals that depend largely on microbial protein synthesis (MPS) for their EAA (Korhonen et al., 2000), most abomasal infusion studies indicate little evidence that a single EAA is limiting under these conditions (Schwab et al., 2003). However, the total CP in rumen microbial cells contains from 20% (NRC, 2001) to perhaps 33% (Clark et al., 1992) non-AA N (e.g., N in nucleic acids and cell-wall components). Thus, conversion of good quality feed proteins into microbial protein may actually impair N utilization, despite an overall improvement in EAA pattern, because of a net reduction in EAA supply. There is evidence of linear increases in MPS in response to increasing dietary RDP (Broderick et al., 2010) while maximal milk protein secretion occurs at less than maximal MPS (Reynal and Broderick, 2005; Olmos Colmenero and Broderick, 2006). Hence, MPS should be "optimized" rather than "maximized".

Value of NPN in ruminant diets

A number of experiments have shown that ruminants can be moderately productive on diets in which virtually all CP is supplied as NPN (e.g., Virtanen, 1966). Data from Bryant and Robinson (1962) indicated that ammonia-N was more important than N from amino acids (AA) and peptides for growth of many pure cultures of rumen bacteria. However, Oltjen (1969) summarized results from studies using purified diets showing that replacing CP from the readily degradable soy protein with that from urea reduced gain, feed efficiency and N retention in beef steers and heifers. These effects may be partly attributed to rumen escape of isolated soy protein but one may infer that much of the difference was due to depressed MPS

on the urea-based diet. Satter and Slyter (1974) fed diets to mixed rumen organisms in continuous culture fermenters in which CP content was increased above 4% (DM basis) by adding only urea. In 3 experiments, ammonia concentrations remained at ~1 m*M*, and microbial protein yield increased linearly, until dietary CP reached ~13%. At that point, protein outflow from the fermenters stopped increasing and ammonia concentration climbed rapidly; microbial protein yield did not increase above a mean ammonia concentration of ~2 m*M* (Satter and Slyter, 1974). This value was adjusted to about 3.6 m*M* (5 mg ammonia-N/dL) for a safety margin. Schaefer et al. (1980) found that ≤1 m*M* ammonia (1.4 mg N/dL) gave 95% of maximum growth for 9 of 10 pure cultures of rumen bacteria studied. Results reported in both studies called into question the value of feeding NPN in many situations. There has been much debate during the intervening years about whether 5 mg N/dL was in fact the upper limit for ammonia utilization in vivo. For example, Mehrez et al. (1977) infused urea into the sheep rumen and found that in situ barley DM digestion increased with increasing ammonia until reaching ~20 mg ammonia-N/dL. Odle and Schaefer (1987) conducted similar studies in cattle and observed maximal rates of in situ DM disappearance at 12 and 6 mg ammonia-N/dL for barley and maize. Rumen concentration of diaminopimelic acid, now a rarely used marker for bacterial protein, increased with urea addition to a high maize diet until ammonia-N reached 8.5 mg/dL (Kang-Meznarich and Broderick, 1980). Higher optimal ammonia concentrations may be required under some circumstances because the physical association of bacteria with particulate matter results in low ammonia levels in these localized niches (Odle and Schaefer, 1987). Moreover, rumen ammonia varies greatly, so a mean 5 mg ammonia-N/dL over the day will be the resultant a wide a range of concentrations (Dixon, 1999).

Amino-N stimulates microbial protein formation

Ammonia is formed partly from deamination of AA derived from rumen protein degradation and ammonia production parallels formation of peptides and free AA. This confounds the explanation of whether MPS is responding to ammonia alone, or also to the peptides and AA that also gave rise to ammonia on natural diets. Work by Lee Baldwin's group at UC-Davis shed considerable light in this area with a number of in vitro studies. Maeng et al. (1976) found that replacing 25% of urea-N with N from a mixture of 18 protein AA maximized the yield of microbial DM per unit carbohydrate fermented on glucose, starch and cellobiose; greater proportions of AA-N appeared to reduce microbial yields. Argyle and Baldwin (1989) showed that adding only 1 mg/L of a blend of protein AA plus 1 mg/L of peptides from trypsin digested casein (trypticase) more than doubled in vitro cell yield of mixed rumen organisms. They also found progressively lower response to 10 and 100 times greater addition of AA and peptides. Furthermore, Argyle and Baldwin (1989) found that mixtures of different classes of AA did not increase bacterial cell yield when added to a medium containing ammonia and peptides (trypticase); greater cell yield was only obtained with addition of a complete mixture of the protein AA. Other data indicated that in vitro MPS increased when degradation rate of protein sources in the medium increased from 0.01 to 0.14/h, but altered little with further increase in rate up to 0.54/h (Hristov and Broderick, 1994).

Dramatic responses to RDP supplementation also have been observed in vivo. Chikunya et al. (1996) found that replacing urea CP with casein CP in sheep fed a grass hay diet (with rumen OM digestibility of ~32%) increased MPS 10%, but the same replacement in a sugar beet pulp diet (with rumen OM digestibility of ~51%) increased MPS 82%. Kalscheur et al. (2003) held rumen-undegraded protein (RUP) supply constant but increased RDP by replacing

treated soybean meal with solvent soybean meal; they observed significant increases in yield of milk, fat and protein at equal DM intake, although N efficiency declined. Recent in vivo results demonstrated significant linear depression in yield of milk, fat and protein when RDP from urea replaced RDP from soybean meal; these production effects appeared to be caused largely by depressed rumen outflow of non-ammonia N (NAN), essential AA and total AA due to reduced efficiency of microbial protein synthesis (Broderick and Reynal, 2009). Kozloski et al. (2000) similarly observed a linear decline in MPS as more and more soybean meal CP was replaced by urea CP. On the other hand, Ahvenjärvi et al. (2002) attributed the increased flow of NAN to elevated RUP when they supplemented rapeseed meal to dairy cows fed a grass silage diet. But overall, at least under some conditions, NPN sources cannot provide all of the RDP and RDP from true protein is required to optimize microbial protein formation in the in vivo rumen.

Stouthamer (1973) computed theoretical energetic efficiencies of microbial cell growth based on known metabolic pathways and estimated yields of 28.8 or 28.6 g OM/mol of ATP for organisms growing on media with N coming from, respectively, ammonia only or AA. However, Cruz Soto et al. (1994) reported that adding AA or peptides to the medium increased specific growth rates 2 to 44 fold for several pure cultures of rumen bacteria. Based on several in vitro studies, Russell et al. (1992) estimated that addition of AA mixtures to rumen incubations would improve cell yields by 18%, which seems more consistent with in vivo observations. Wallace's group at the Rowett Research Institute has explored possible mechanisms for these increased yields. Adding AA mixtures, and particularly mixed peptides (trypticase), resulted in substantial reduction in proportions of individual AA in microbial protein that derived from de novo synthesis (Atasoglu et al., 2004). Deletion of single AA from a complete mix of protein AA slowed gas production when microbes were grown on a mixture soluble carbohydrates (Atasoglu et al., 2003) or xylan (Guliye et al., 2005), but did not alter protein yield in either study. It is not clear what causes the disparity between experimental observations and theoretical estimates and whether the AA and peptides present in silages would have similar affects. Russell (2007) speculated that AA and peptides reduced "energy spilling" during cell growth. The Wallace group (Cruz Soto et al., 1994) concluded that degree of stimulation by AA and peptides was related to carbohydrate fermentation rate, with greater responses occurring with more rapidly fermented substrates.

Value of silage CP for microbial protein formation

Charmley and Veira (1990) suppressed proteolysis in ensiled alfalfa using a 2-min steam-treatment, which reduced silage NPN from 65 to 40% of total N without altering NDF or ADIN. When this alfalfa was fed to sheep, flow of protein N from the rumen increased from 22 to 27 g/d; about 60% of this increase was due to more microbial protein. These workers also reported that growth efficiency increased from 22 to 37 g microbial NAN/kg OM apparently digested in the rumen. Thus, breakdown of the forage protein in the silo clearly depressed microbial protein formation. Formic acid addition to direct-cut, hay-crop silages is used widely in the U.K. but has been thought unnecessary for forages wilted to greater than 35% DM, such as those commonly harvested in North America. However, Nagel and Broderick (1992) found that applying formic acid to alfalfa wilted to 40% DM reduced silage NPN by one-third and increased milk yield 3.4 kg/day and protein yield 110 g/day when fed to early lactation cows. The results of Charmley and Veira (1990) suggested that the improved production observed by Nagel and Broderick (1992) was at least partly due to improved microbial protein formation in the rumen. Alfalfa harvested in 3 trials from the same swards

as either 40% DM silage or hay in small rectangular bales averaged 2.5% less CP (20.6 versus 18.1%) when harvested as hay (Broderick, 1995; Vagnoni and Broderick, 1997). However, despite leaf protein losses, feeding forage as hay rather than silage resulted in greater milk protein secretion and little or no response to supplementing with RUP. In vitro studies conducted with samples of forages collected during these 3 in vivo trials indicated that alfalfa hay and silage had similar RDP and RUP contents but that hay gave rise to greater in vitro yields of microbial protein (Peltekova and Broderick, 1996). We speculated that similar effects would happen with grass silages but evidence from the literature does not support this contention. Protein utilization in cows was not greater on hay compared to silage from the same sward (Shingfield, 2002). Murphy et al. (2000) also reported similar milk and protein yields in cows fed either timothy-meadow fescue silage or hay.

Supplementing with fermentable energy

What Murphy et al. (2000) did observe was a trend for increased protein yield, and a highly significant increase in milk protein concentration, with greater dietary starch content on both grass silage and hay. Feeding fermentable carbohydrates to match silage RDP supply will stimulate microbial protein formation and improve N utilization. However, there are substantial differences among starch sources (Herrera-Saldana et al., 1990), and within grains due to processing, in the rates of energy release in the rumen. Effects of processing on extent of rumen digestion of maize starch are much greater than on total tract digestibility (Owens et al., 1986; Owens et al., 1997). We found that grinding high moisture maize through a 1-cm screen, reducing mean particle size from 4.3 to 1.7 mm, greatly enhanced rates of in vitro ammonia uptake by rumen microbes; finer grinds (using screens as small as 0.2-cm) did not further increase ammonia uptake (Ekinci and Broderick, 1997). Feeding the ground high moisture maize (1.7 mm mean particle size) to lactating cows increased milk and protein yield by 2.4 and 0.12 kg/day compared to unground high moisture maize. Much the same thing happens with dry maize, except at smaller particle sizes. Processing dry shelled maize to reduce mean particle size from 3.5 to 0.6 mm increased rumen starch digestibility from 54 to 70% (Remond et al., 2004). In a large meta-analysis of literature data, Huhtanen et al. (2008) found that metabolizable energy intake was the main factor explaining milk yield responses in cows fed grass silage based diets; silage CP content had a small but significant negative affect on milk yield in this analysis.

There are “optimal” levels of carbohydrate and forage for supporting rumen protein synthesis and milk production. The Cornell model (Sniffen et al., 1992) predicts reduced formation of microbial protein per unit of fermented energy when rumen pH falls below 6.2. This effect would be particularly problematic in ruminants fed high forage diets where NDF contributes much of the energy. Rumen pH in high producing dairy cows fed large amounts of fermentable concentrate may remain below 6.0 for much of the day, often averaging well below 6.2 overall (Ekinci and Broderick, 1997; Weimer et al., 1999). De Veth and Kolver (2001) reported that, when pH in rumen continuous culture fermenters was reduced from 6.3 to 5.4 for periods of 0, 4, 8, and 12 hours per day, there was a negative linear relationship between microbial protein yields and the length of time pH was at 5.4. It is important to confirm in vivo the pH at which high concentrate feeding begins to reduce utilization of dietary NPN or otherwise depresses microbial protein formation and to quantify the magnitude of these effects. Depression of fiber digestion at low rumen pH can aggravate the problem of depressed milk fat yield by reducing formation of acetate (e.g., Dixon and Stockdale, 1999), a major milk fat precursor. Adding high moisture maize to an 80% alfalfa

silage, 20% concentrate diet to increase concentrate to (% alfalfa silage DM/% concentrate DM) 65/35, 50/50, and 35/65 gave quadratic responses in DM intake and fat corrected milk (FCM; Valadares et al., 2000). Intake and FCM yield were maximal at 51% concentrate; maximum fat yield occurred at 43% concentrate. However, milk and protein yield responses were not quadratic but linear and both were still going up at 35% forage and 65% concentrate. Moreover, purine derivative excretion in the urine, an indirect measure of rumen protein formation, also gave a linear response despite low rumen pH and other signs of over-feeding of concentrate (Valadares et al., 1999). The lactating cow's demand for energy is substantial but short-term reversal trials, such as the Valadares studies (1999, 2000), could mask adverse effects on rumen and animal health occurring with long-term feeding of excessive concentrate.

In North America and much of Europe, maize silage is commonly fed as a major portion of the ration. This high energy "forage" is low in CP and, thus, can be fed to dilute hay-crop forages containing highly degradable protein (such as legume or grass silages). Dhiman and Satter (1997) replaced 1/3 or 2/3 of the dietary alfalfa silage with maize silage. Compared to 100% of the forage from alfalfa, milk yield was 6% higher over the whole lactation when 2/3 of the dietary forage was alfalfa silage and 1/3 was maize silage; there also were comparable improvements in N efficiency. Feeding forage as 1/3 alfalfa silage and 2/3 maize silage slightly reduced production and substantially increased the need for dietary protein concentrate. Brito and Broderick (2007) assessed the effects of step-wise replacement of alfalfa silage with maize silage. The greatest improvement in N efficiency, without loss of production of milk, fat and protein, occurred at about 50% of the forage from alfalfa silage and 50% from maize silage, mainly because intake declined when dietary alfalfa was reduced. As discussed, replacing some dietary starch with very rapidly fermenting sugars may enhance rumen capture of degraded N. Maize starch was replaced with sucrose (Broderick et al., 2008), or dried molasses or liquid molasses (Broderick and Radloff, 2004), in three separate feeding studies; basal diets were formulated from alfalfa and maize silages plus high moisture maize and soybean meal and averaged 2.6% total sugars in dietary DM. An overall analysis of the data from the three trials indicated maxima for total sugars (on a DM basis) were 6.8% for DM intake and 4.8% for protein yield. However, the positive production effects of sugar feeding in these three trials were primarily driven by increased feed intake.

Summary and conclusions

Dairy cows excrete substantially more N in manure than they secrete in milk, increasing milk production costs and environmental N pollution. Although the AA pattern of microbial protein produced in the rumen is of good quality, 20 to 33% of the CP equivalent in microbial cells is in the form of non-AA N (such as nucleic acids). Optimizing (as opposed to maximizing) microbial protein formation, by providing the appropriate N compounds in the rumen, will improve the protein status of the lactating cows and other productive ruminants. NPN can replace only part of the dietary RDP because RDP from peptides and AA stimulates microbial protein synthesis (both in amount and efficiency of protein formed per unit of energy fermentation). The effects of providing RDP in the form of AA and/or peptides are complex and the mechanism of these improvements in protein yield is not clear. Reducing dietary NPN by suppressing protein degradation in the silo also improves efficiency of microbial protein formation. Providing fermentable energy as processed grain and whole-crop grain silages (e.g., maize silage) is an effective way to improve rumen microbial protein

formation from silage NPN. This increases utilization of silage CP and animal performance while reducing N losses to the environment.

References

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

Argyle, J. L. & R. L. Baldwin. 1989. Effects of amino acids and peptides on rumen microbial yields. J. Dairy Sci., 72, 2017-2027.

Atasoglu, C., A. Y. Guliye & R. J. Wallace. 2003. Use of a deletion approach to assess the amino acid requirements for optimum fermentation by mixed micro-organisms from the sheep rumen. Anim. Sci. 76, 147-153.

Atasoglu, C., A. Y. Guliye & R. J. Wallace. 2004. Use of stable isotopes to measure de novo synthesis and turnover of amino acid-C and -N in mixed micro-organisms from the sheep rumen in vitro. Brit. J. Nutr. 91, 253–261.

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

Broderick, G. A. 1994. Quantifying forage protein quality. In G. C. Fahey, Jr., M. D. Collins, D. R. Mertens and L. E. Moser (ed.) Forage Quality, Evaluation, and Utilization. American Soc. Agron., Madison, WI, pp. 200-228.

Broderick, G. A. 1995. Performance of lactating dairy cows fed either alfalfa silage or alfalfa hay as the sole forage. J. Dairy Sci. 78, 320-329.

Broderick, G. A. & W. J. Radloff. 2004. Effect of molasses supplementation on the production of lactating dairy cows fed diets based on alfalfa and corn silage. J. Dairy Sci. 87, 2997-3009.

Broderick, G. A. & S. M. Reynal. 2009. Effect of source of rumen-degraded protein on production and ruminal metabolism in lactating dairy cows. J. Dairy Sci. 92, 2822-2834.

Broderick, G. A., P. Huhtanen, S. Ahvenjarvi, S. M. Reynal & K. J. Shingfield. 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle--A meta-analysis. J. Dairy Sci. 93, 3216-3230.

Broderick, G. A., N. D. Luchini, S. M. Reynal, G. A. Varga & V. A. Ishler. 2008. Effect on production of replacing dietary starch with sucrose in lactating dairy cows. J. Dairy Sci. 91, 4801-4810.

Bryant, M. P. & I. M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. 84, 605-614.

Charmley, E. & D. M. Veira. 1990. Inhibition of proteolysis in alfalfa silages using heat at harvest, Effects on digestion in the rumen, voluntary intake and animal performance. J. Anim. Sci. 68, 2042-2051.

Chikunya S., C. J. Newbold, L. Rode, X. B. Chen & R. J. Wallace. 1996. Influence of dietary rumen-degradable protein on bacterial growth in the rumen of sheep receiving different energy sources. Anim. Feed. Sci. Tech. 63, 333-340.

Clark, J. H., T. H. Klusmeyer & M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75, 2304-2323.

Cruz Soto, R., S. A. Muhammed, C. J. Newbold, C. S. Stewart & R. J. Wallace. 1994. Influence of peptides, amino acids and urea on microbial activity in the rumen of sheep receiving grass hay and on the growth of rumen bacteria in vitro. Anim. Feed Sci. Technol. 49, 151-161.

De Veth, M. J. & E. S. Kolver. 2001. Diurnal variation in pH reduces digestion and synthesis of microbial protein when pasture is fermented in continuous culture. J. Dairy Sci. 84, 2066-2072.

Dhiman, T. R. & L. D. Satter. 1997. Yield response of dairy cows fed different proportions of alfalfa silage and corn silage. J. Dairy Sci. 80, 2069-2082.

Dixon, R. M. 1999. Effects of addition of urea to a low nitrogen diet on the rumen digestion of a range of roughages. Australian J. Agric. Res. 50, 1091-1097.

Dixon, R. M. & C. R. Stockdale. 1999. Associative effects between forages and grains, Consequences for feed utilization. Australian J. Agric. Res. 50, 757-773.

Ekinci, C. & G. A. Broderick. 1997. Effect of processing high moisture corn on ruminal fermentation and milk production. J. Dairy Sci. 80, 3298-3307.

Guliye, A. Y., C. Atasoglu & R. J. Wallace. 2005. Assessment of amino acid requirements for optimum fermentation of xylan by mixed micro-organisms from the sheep rumen. Anim. Sci. 80, 353-360.

Herrera-Saldana, R., R. Gomez-Alarcon, M. Torabi & J. T. Huber. 1990. Influence of synchronizing protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. J. Dairy Sci. 73, 142-148.

Hristov, A. & G. A. Broderick. 1994. In vitro determination of ruminal protein degradability using [15N]-ammonia to correct for microbial nitrogen uptake. J. Anim. Sci. 72, 1344-1353.

Huhtanen, P., M. Rinne & J. Nousiainen. 2008. Effects of silage soluble nitrogen components on metabolizable protein concentration, A meta-analysis of dairy cow production experiments. J. Dairy Sci. 91, 1150-1158.

Kalscheur, K. F., R. L. Baldwin VI, B. P. Glenn & R. A. Kohn. 2003. Milk production of dairy cows fed differing concentrations of rumen-degraded protein. J. Dairy Sci. 89, 249-259.

Kang-Meznarich, J. H. & G. A. Broderick. 1980. Effects of incremental urea supplementation on ruminal ammonia concentration and bacterial protein formation. J. Anim. Sci. 51, 422-431.

Korhonen, M., A. Vanhatalo, T. Varvikko & P. Huhtanen. 2000. Responses to graded postruminal doses of histidine in dairy cows fed grass silage diets. J. Dairy Sci. 83, 2596-2608.

Kozloski, G. V., H.M.N. Ribeiro-Filho & J.B.T. Roch. 2000. Effect of the substitution of urea for soybean meal on digestion in steers. Canadian J. Anim. Sci. 80, 713-719.

Lapierre, H. & G. E. Lobley. 2001. Nitrogen recycling in the ruminant. A review. J. Dairy Sci. 84(E. Suppl.), E223-E236.

Maeng, W. J., C. J. Van Nevel, R. L. Baldwin & J. G. Morris. 1976. Rumen microbial growth rates and yields. Effect of amino acids and protein. J. Dairy Sci. 59, 68-79.

Mehrez, A. Z., E. R. Ørskov & I. McDonald. 1977. Rates of rumen fermentation in relation to ammonia concentration. Brit. J. Nutr. 38, 437-443.

Murphy, M., M. Åkerlind & K. Holtenius. 2000. Rumen fermentation in lactating cows selected for milk fat content fed two forage to concentrate ratios with hay or silage. J. Dairy Sci. 83, 756-764.

Nagel, S.A. & G.A. Broderick. 1992. Effect of formic acid or formaldehyde treatment of alfalfa silage on nutrient utilization by dairy cows. J. Dairy Sci. 75, 140-154.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Odle, J. & D. M. Schaefer. 1987. Influence of rumen ammonia concentration on the rumen degradation rates of barley and maize. Brit. J. Nutr. 57, 127-138.

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

Oltjen, R. R. 1969. Effects of feeding ruminants non-protein nitrogen as the only nitrogen source. J. Anim. Sci. 28, 673-682.

Owens, F. N., D. S. Secrist, W. J. Hill & D. R. Gill. 1997. The effect of grain source and grain processing on performance of feedlot cattle. A review. J. Anim. Sci. 75, 868-879.

Owens, F. N., R. A. Zinn & Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. J. Anim. Sci. 63, 1634-1648.

Peltekova, V. D. & G. A. Broderick. 1996. In vitro ruminal degradation and synthesis of protein on fractions extracted from alfalfa hay and silage. J. Dairy Sci. 79, 612-619.

Remond, D., J. I. Cabrera-Estrada, M. Champion, B. Chauveau, R. Coudure & C. Poncet. 2004. Effect of corn particle size on site and extent of starch digestion in lactating dairy cows. J. Dairy Sci. 87, 1389–1399.

Reynal, S. M. & G. A. Broderick. 2005. Effect of dietary level of rumen-degraded protein on production and nitrogen metabolism in lactating dairy cows. J. Dairy Sci. 88, 4045-4064.

Russell, J. B. 2007. The energy spilling reactions of bacteria and other organisms. J. Mol. Microbiol. Biotechnol. 13, 1-11.

Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest & C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets. I. Ruminal fermentation. J. Anim. Sci. 70, 3551-3561.

Satter, L. D. & L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Brit. J. Nutr. 32, 199-208.

Schaefer, D. M., C. L. Davis & M. P. Bryant. 1980. Ammonia saturation constants for predominant species of rumen bacteria. J. Dairy Sci. 63, 1248-1263.

Schwab, C. G. 1996. Rumen-protected amino acids for dairy cattle. Progress towards determining lysine and methionine requirements. Anim. Feed Sci. Tech. 59, 87-101.

Schwab, C. G., R. S. Ordway & N. L. Whitehouse. 2003. Amino acid balancing in the context of MP and RUP requirements. Pages 10-25 in Proc. Four-State Applied Nutrition and Management Conference.

Shingfield K. J., S. Jaakkola & P. Huhtanen. 2002. Effect of forage conservation method, concentrate level and propylene glycol on intake, feeding behavior and milk production of dairy cows. Anim. Sci. 74, 383–397.

Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox & J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. J. Anim. Sci. 70, 3562-3577.

Stouthamer, A. H. 1973. A theoretical study on the amount of ATP required for synthesis of microbial cell material. Ant. van Leeuwenhoek 39, 545-565.

Vagnoni, D. B. & G. A. Broderick, G. A. 1997. Effects of supplementation of energy or ruminally undegraded protein to lactating cows fed alfalfa hay or silage. J. Dairy Sci. 80, 1703-1712.

Valadares, R.F.D., G. A. Broderick, S. C. Valadares Filho & M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82, 2686-2696.

Valadares Filho, S. C., G. A. Broderick, R.F.D. Valadares & M. K. Clayton. 2000. Effect of replacing alfalfa silage with high moisture corn on nutrient utilization and milk production. J. Dairy Sci. 83, 106-114.

Virtanen, A. I. 1966. Milk production of cows on protein-free feed. Science 153, 1603-1614.

Weimer, P. J., G. C. Waghorn, C. L. Odt & D. R. Mertens. 1999. Effect of diet on populations of three species of ruminal cellulolytic bacteria in lactating dairy cows. J. Dairy Sci. 82, 122-134.

Biological limits to N utilization in dairy cows

S. Ahvenjärvi[1], T. Stefanski[1], A. Vanhatalo[2] & P. Huhtanen[3]
[1]MTT Agrifood Research Finland, Animal Production, 31600 Jokioinen, Finland,[2]University of Helsinki, Department of Agricultural Sciences, P.O. Box 28, 00014 University of Helsinki, Finland,[3] Swedish University of Agricultural Sciences (SLU), Department of Agricultural Research for Northern Sweden,S-901 83 Umeå, Sweden.
*Correspondence: seppo.ahvenjarvi@*mtt.fi

Introduction

Dairy cow diets are often supplemented with protein rich ingredients to satisfy alleged protein requirements. Increases in dietary crude protein (CP) concentration often increase milk yield but consistently decrease the efficiency of N use for milk protein synthesis (Broderick, 2003; Ipharraguerre and Clark, 2005). The conflict of interest between economically profitable and environmentally friendly decision-making was clearly highlighted in a recent meta-analysis by Huhtanen et al. (2011). Daily milk yield responses to supplementary protein were between 2.1 to 3.7 kg kg^{-1} increase in CP intake, whereas the responses in milk protein yield were only between 98 to 136 g kg^{-1} increase in CP intake. These results suggest that while milk yield responses may be economically profitable they are biologically very inefficient because in average case 86 to 90% of high quality feed protein will be lost as N in urine and faeces. In Finland, over the next few years dairy business will be facing increasing pressures to improve the efficiency of N use because Finland has committed to reduce ammonia emissions by 20% relative to year 2005 by the year 2020. Based on substantial experimental evidence by far the most efficient means to improve N use efficiency is to reduce dietary CP concentration (Broderick, 2003; Huhtanen and Hristov, 2009). The objective of the current paper is to present some previous findings on ruminal N metabolism underlying N utilization for milk protein synthesis.

Utilization of protein vs. non-protein N

In our previous study (Stefanski et al., unpublished), ruminal metabolism of ammonia-N and feed protein were studied using ^{15}N labelled N sources. Labelled ammonium-N, soluble and insoluble rapeseed meal protein were introduced as a pulse dose into the rumen of lactating dairy cows. Cumulative secretion of ^{15}N in milk over the course of 108 h post dose indicated that 19, 20, and 22% of labelled N administered as ammonium-N, soluble and insoluble rapeseed meal protein was utilized for milk protein synthesis (MNE_{15N}), respectively. These differences between non-protein-N, soluble protein and insoluble protein were smaller than expected and could be attributed to moderate CP concentration in the diet (155 g/kg DM) and low ruminal ammonium-N concentration (6.0 mg/100 mL). Low dietary CP and ruminal ammonia-N concentrations favour efficient utilization of rumen degradable N for microbial protein synthesis and result in small net absorption and outflow of ammonia-N from the rumen. In addition, the efficiency of N recycling as urea into the rumen increases with decreases in dietary CP concentration (Marini and Amburgh, 2003). On a low CP diet (91 g/kg DM) approximately 43% of the N recycled into the gastrointestinal tract was used for microbial N synthesis, whereas only 6% was utilized on a high CP diet (213 g/kg DM; Marini and Amburgh, 2003).

In our study, the efficiency of N use (milk N/N intake) determined for the whole diet was 32% (MNE_{diet}). The difference between MNE_{diet} and MNE_{15N} could be attributed to deposition of labelled N in tissue protein that turns over slower than 108 h period of milk

protein synthesis accounted for. Following this hypothesis MNE_{15N} should indicate the extent of milk protein that was synthesized either directly or via rapid turnover body tissue pools, whereas the difference between MNE_{diet} and MNE_{15N} should indicate the proportion that was derived from slow turnover body tissues. Based on such comparison, MNE_{15N} accounted for 59 to 69% of MNE_{diet} suggesting that between 60 to 70% of milk protein was synthesized from amino acids absorbed from the gastrointestinal tract, whereas 30 to 40 % of milk protein was derived from body tissues.

The relationship between cumulative secretion of ^{15}N labelled ammonia-N in milk as a proportion of a single dose administered into the rumen of lactating dairy cows (Figure 1), compiled from three separate studies, points out a substantial effect of dietary CP concentration on the efficiency of N utilization. In ruminants, the efficiency of urea recycling and the proportion of recycled N used for microbial protein synthesis are heavily dependent on dietary CP concentration (Marini and Van Amburgh, 2003). Studies on the renal function of sheep have indicated that animal physiology has an important role in conservation of N (Leng et al., 1985). Sheep fed a low N diet retained urea by the mechanism of decreases in renal glomerular filtration rate and increases in tubular transport capacity for urea (Leng et al., 1985).

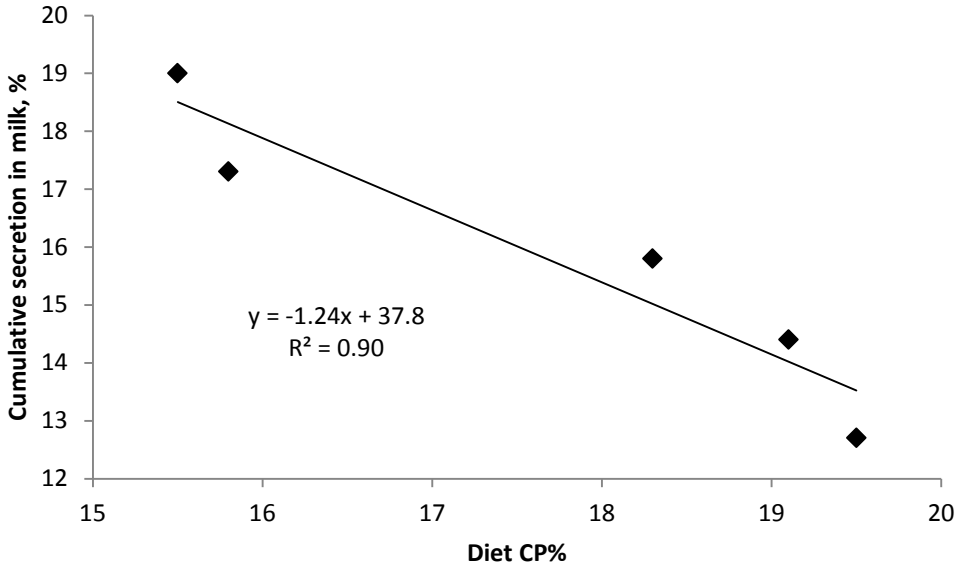

Figure 1 Cumulative secretion of ^{15}N labelled ammonia-N in milk as a proportion of a single dose introduced into the rumen. Data from Hristov and Ropp, 2003; Hristov et al., 2004; Stefanski et al. unpublished.

Critical rumen ammonia-N concentration

Our previous study (Ahvenjärvi and Huhtanen, unpublished) was designed to identify the critical dietary CP and rumen ammonia levels below which ruminal fiber digestibility and microbial N synthesis are decreased relative to optimal conditions. Dairy cows were offered a

total mixed ration that was formulated slightly deficient in terms of rumen degradable protein (CP 127 g/kg DM, PBV -3 g/kg DM). The basal diet was supplemented with increasing levels or urea infusion (17, 33, 49 and 66 g/d of N) into the rumen to increase ammonia-N supply to rumen microbes. On the basal diet, average rumen ammonia-N and milk urea concentrations remained rather low 2.7 mmol/l and 9.5 mg/100 ml, respectively. In spite of low rumen ammonia-N concentrations cows didn't exhibit responses to intraruminal urea infusions in terms of feed intake, NDF digestibility or energy corrected milk yield. However, increases in milk protein yield ($P = 0.08$) and faecal N excretion ($P = 0.05$) indicated that microbial N synthesis slightly increased as a response to urea infusion. Interestingly, rumen ammonia-N concentration didn't exhibit any pattern of increase until the highest level (66 g/d) was achieved. This inflection point in rumen ammonia-N concentration was achieved at a dietary CP concentration of 140 g/kg DM. In contrast to rumen ammonia, milk urea concentrations increased already on the lowest levels of urea infusion (Figure 1). Lack of response in rumen ammonia-N concentration may be explained by active ammonia transportation into microbial cells with subsequent ammonia-N gradient between intracellular and extracellular space (Russell and Strobel, 1987). Therefore, rumen ammonia-N concentration may not be a satisfactory indicator of N deficiency in the rumen.

The relationship between urea infusion levels and increases in N excretion indicated that 0.67 ($R^2 = 0.99$) of urea-N was excreted in urine, 0.14 ($R^2 = 0.69$) in feces and 0.22 ($R^2 = 0.78$) was secreted in milk. These estimates represent marginal utilization of supplementary ammonia-N provided as continuous infusion into the rumen. Compared with marginal utilization of soybean meal protein for milk protein synthesis (98 g kg^{-1}), reported by Huhtanen et al. (2011), the marginal utilization of ammonia-N in the current study was approximately two times higher. This indirect comparison clearly points out that N utilization is more dependent on dietary CP concentration than the quality of protein. The proportion of supplementary N utilized for microbial N synthesis is higher than the responses in milk N output owing to inevitable losses in the intermediary metabolism of absorbed amino acids. Assuming 0.80 of rumen microbial N entering the intestines is true protein, the digestibility of this true protein is 0.80, and the efficiency of use of metabolizable protein to milk protein synthesis is 0.67 (NRC, 2001) the maximum efficiency of microbial protein utilization of milk protein synthesis would be 0.43. Assuming as in the current study, 0.22 of supplementary urea-N is transferred to milk N then, calculating back to the proportion used by the rumen microbes (0.22/0.43), this suggests that 0.51 of urea-N was captured by rumen microbes. This figure represents both immediate capture of urea-N to microbial N and also recycled ammonia-N returned to the forestomach via hepatic urea synthesis. This estimate is in close agreement with the mean values for urea-N kinetics estimated by Lapierre and Lobley (2001). These authors estimated that typically 0.33 of hepatic urea flux is eliminated in urine, whereas 0.67 enters the digestive tract. Of the latter, 0.50 is reabsorbed as anabolic-N (microbial N), whereas 0.40 is reabsorbed as ammonia-N and 0.10 is lost in feces.

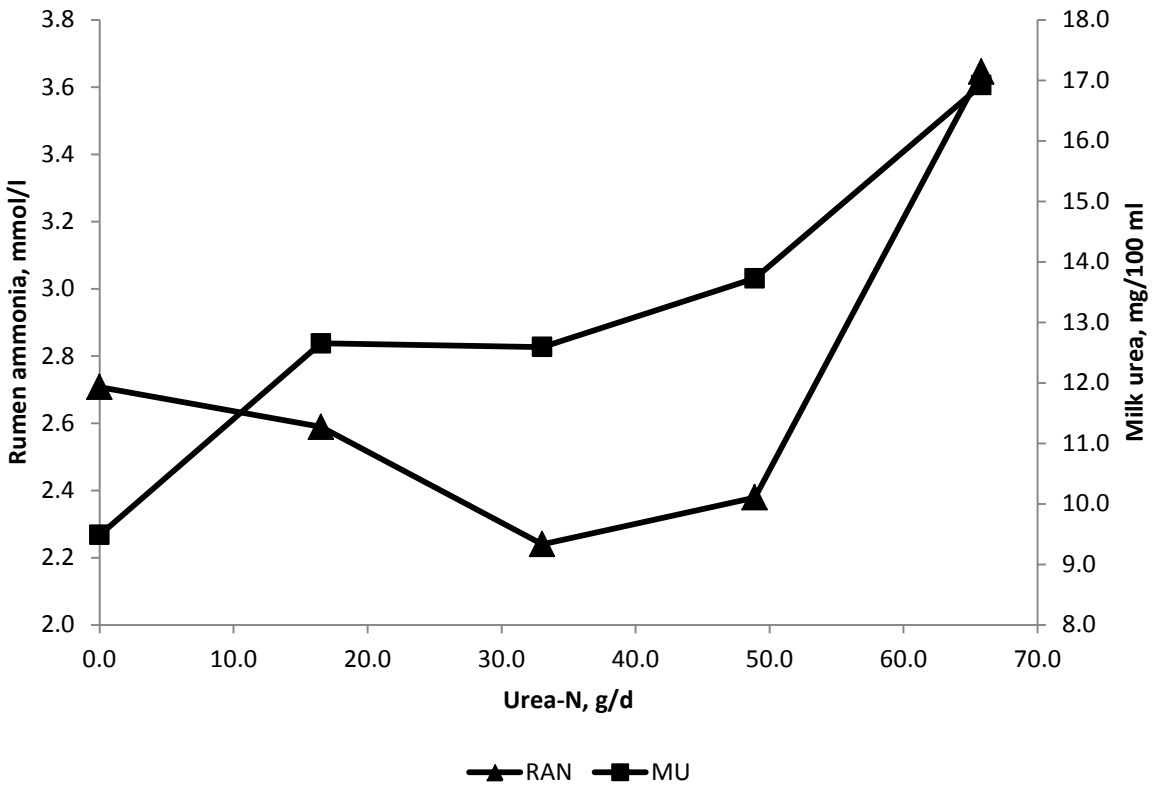

Figure 2 The effect of intraruminal urea-N infusion on rumen ammonia-N (RAN) and milk urea (MU) concentration.

Conclusions

Observations from our previous studies demonstrate that a lactating cow is capable of efficient utilization of both protein and non-protein N provided that dietary CP concentration doesn't exceed microbial requirements. However, more experimental evidence is needed to account for the contribution of urea recycling to ruminal N supply in the future models.

References

Broderick, G.A., 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci., 86, 1370-1381.

Hristov, A.N., Etter, R.P., Ropp, J.K. & Grandeen, K.L., 2004. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. J. Anim. Sci., 82, 3219-3229.

Hristov, A.N. & Ropp, J.K., 2003. Effect of dietary carbohydrate composition and availability on utilization of ruminal ammonia nitrogen for milk protein synthesis in dairy cows. J. Dairy Sci., 86, 2416-2427.

Huhtanen, P.H., 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.

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

Ipharraguerre, I.R. & Clark, J.H., 2005. Varying protein and starch in the diet of dairy cows. II. Effects on performance and nitrogen utilization for milk production. J. Dairy Sci., 88, 2556-2570.

Lapierre, H. & Lobley G.E., 2001. Nitrogen recycling in the ruminant: a review. J. Dairy Sci., 84, E223-E236.

Leng, L., Szanyiova, M. & Boda, K., 1985. The renal response of sheep to a low dietary nitrogen intake. Physiologia Bohemoslovaca, 34, 147-154.

Marini, J.C. & Van Amburgh, M.E., 2003. Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci., 81, 545-552.

NRC., 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Russell, J.B. & Strobel, H.J., 1987. Concentration of ammonia across cell membranes of mixed rumen bacteria. J. Dairy Sci., 70, 970-976.

Evaluation of protein supplementation for growing cattle: A meta-analysis

A. Huuskonen[1], P. Huhtanen[2] & E. Joki-Tokola[1]
[1] MTT Agrifood Research Finland, Animal Production Research, Tutkimusasemantie 15, FI-92400 Ruukki, Finland. [2] Swedish University of Agricultural Sciences (SLU), Department of Agricultural Research for Northern Sweden, S-90183 Umeå, Sweden
Correspondence: Arto.Huuskonen@mtt.fi

Introduction

Ruminants have two types of N requirements, the N requirements of ruminal fermentation and the amino acid (AA) requirements of the host animal. A shortage of rumen degradable feed protein (RDP) has been shown to reduce microbial digestion of carbohydrates, reduce synthesis of microbial protein, decrease feed intake and decrease weight gain of growing cattle (Schwab et al. 2005). A shortage of absorbed AA by cattle, either because of decreased synthesis of microbial protein or less than required intakes of rumen-undegraded protein (RUP), decrease weight gain of growing cattle (Lammers & Heinrichs 2000). Even though individual studies have examined the influence of protein supplementation on the intake and performance of growing cattle, limitations in the number of observations and basal diet composition do not allow for definitive conclusions. Therefore, the objective of this meta-analysis was to develop empirical equations predicting performance responses of growing cattle to protein intake.

Materials and Methods

A dataset was collected from published feeding experiments with growing cattle (bulls, steers, heifers) fed *ad libitum* total mixed ration (TMR) or *ad libitum* grass silage, or whole-crop silages (barley, oats or wheat), hay or straw partly or completely substituted for grass silage fed with fixed amounts of concentrates. The concentrate feeds consisted of cereal grains, fibrous by-products and various protein supplements [mainly rapeseed meal (RSM), soybean meal (SBM) and fish meal (FM)]. Approximately half of the studies were conducted with pure dairy breeds and the remainder with beef breeds or dairy × beef crossbred animals. Approximately half of the experiments were conducted in the UK and Ireland and the remainder in the Nordic countries. Variation in the design of experiments, animal performance, experimental diets, feeding routines, etc. between the experiments was substantial covering most practical on-farm feeding alternatives. The minimum prerequisite for an experiment to be included in the dataset was that forage and total dry matter (DM) intakes and initial and final body weights (BW) were available and adequate forage characterization [plant species, DM and crude protein (CP) concentration, *in vivo* or *in vitro* digestibility of organic matter and fermentation quality] and adequate concentrate characterization (proportion of ingredients, DM, CP concentration) were available. Overall, the dataset comprised 199 diets in 80 studies. More details and a complete reference list are provided in a paper by Huuskonen et al. (2014).

The dataset was analysed to evaluate the effects of different dietary variables on body weight gain (BWG) and carcass traits with a MIXED model regression analysis of SAS: $Y = B_0 + B_1X_{1ij} + b_0 + b_1X_{1ij} + e_{ij}$, where B_0 and B_1X_{1ij} are the fixed effects (intercept and effects of independent variables) and b_0, b_1, and e_{ij} are the random experiment effects (intercept and slope), where $i = 1 \ldots n$ studies and $j = 1 \ldots n_i$ values. Unstructured variance-covariance matrix for the intercepts and slopes (TYPE = UN option) was used in the random statement. The significance of the differences in the slope of dependent variable was tested with t-test.

Root mean squared errors (RMSE) presented in the tables are adjusted for random study effects as described by St-Pierre (2001). The effects of different animal and dietary variables on BWG responses to increased dietary CP concentration were evaluated by plotting the random slope effect ($B_1 + b_1$) in each study against observed animal and diet variables on the control diet in each study (lowest dietary CP concentration). The responses to increased dietary metabolisable protein (MP) concentration were also analysed but because the trends were similar as in CP the results were not presented.

Results and Discussion

Chemical composition of feeds and total diets as well as intake and performance data were reported by Huuskonen et al. (2014). The forage and concentrate components and the total diets displayed wide ranges in chemical composition and feeding values. On average, the silages were well fermented, but the dataset also included both extensively and poorly fermented silages. Total DM intake (DMI) ranged from 3.3 to 10.4 kg/d reflecting, for example, differences in BW of the animals and the intake potential of the diets. The large variation in BWG (from 521 to 1809 g/d) reflects differences in genetic potential of the animals and nutritive value and intake potential of the diets.

Increasing dietary CP concentration increased BWG ($P<0.01$; Table 1). However, the responses were quantitatively small (1.4 g BWG per 1 g/kg DM increase in dietary CP concentration, on average). The BWG responses were not different for bulls vs. steers and heifers (1.4 and 1.3 g per 1 g/kg DM increase in dietary CP concentration) and for dairy and beef breeds (1.2 vs. 1.7 g per 1 g/kg DM, respectively). The response showed diminishing responses with increased CP concentration (negative quadratic effect $P<0.01$). In terms of Akaike's information criteria (AIC) the MP model performed slightly better than the CP model (Table 1). When RUP and RDP were used in a bivariate model, only RUP had a positive effect on BWG and the slope was markedly greater than for CP. The BWG response to increased dietary CP concentration increased with decreased effective protein degradability (EPD). In terms of the smaller AIC the intake models performed better than the models based on protein concentrations. The model based on ME intake, CP concentration and EPD was the best on the basis of the smallest AIC.

The effect of increased diet CP concentration on BWG declined ($P<0.01$) with increasing mean BW of the animals and with improved BWG of the control animals (the lowest CP diet in each study) (Fig. 1). The BWG responses to increased protein supplementation were negatively related to DMI ($P<0.01$) and ME intake ($P<0.01$) (data not shown). This indicates that protein can be more limiting when diet characteristics are limiting intake and synthesis of microbial protein. Surprisingly, BWG responses to protein supplementation were not related to the CP concentration in the diet of animals fed the control diet (Fig. 1). The responses increased ($P<0.05$) with increased ammonia N concentration in silage N, and declined marginally ($P>0.10$) with increasing proportion of concentrate in the diet and increasing dietary concentrations of ME and MP. The BWG responses to protein supplementation were not related to the digestibility or total acids concentration of the forages (data not shown).

Table 1 Relationships between diet/intake parameters and body weight gain

X-variables			Inter-cept	SE[a]	*P*-value	X_1	SE	*P*-value	X_2	SE	*P*-value	X_3	SE	*P*-value	Adj. RMSE[b]	AIC[c]
Concentration[d]																
CP			921	60	<0.01	1.3	0.34	<0.01							32.3	2423
CP	CP^2		295	224	0.19	9.7	2.87	<0.01	-0.028	0.0093	<0.01				33.3	2423
CP	EPD		1310	225	<0.01	1.0	0.38	0.01	-416	233	0.08				32.8	2407
MP			663	114	<0.01	5.1	1.16	<0.01							37.7	2415
MP	MP^2		-891	1194	0.46	39	25.8	0.14	-0.18	0.140	0.20				36.6	2416
RUP	RDP		1007	56	<0.01	3.2	1.17	<0.01	0.18	0.538	0.74				35.3	2418
Intake[e]																
DMI			510	92	<0.01	84	12.2	<0.01							35.8	2362
MEI			527	88	<0.01	7.2	1.00	<0.01							37.0	2362
CPI			834	53	<0.01	284	46.3	<0.01							28.3	2378
MPI			616	74	<0.01	786	107	<0.01							32.3	2345
MEI	CPI		508	88	<0.01	6.0	1.09	<0.01	106	32.4	<0.01				34.8	2343
MEI	CP		359	94	<0.01	7.4	1.00	<0.01	1.01	0.229	<0.01				33.7	2345
MEI	CP	EPD	826	206	<0.01	7.2	0.98	<0.01	0.65	0.268	0.02	-496	199	0.02	33.7	2327

[a] SE = standard error; [b] Adj. RMSE = Residual mean squared error adjusted for random study effects; [c] Akaike's information criteria; [d] CP = crude protein (g/kg DM), EPD = effective protein degradability (kg/kg), MP = metabolisable protein (g/kg DM), RUP = rumen undegraded protein (g/kg DM), RDP = rumen degraded protein (g/kg DM); [e] DMI = dry matter intake (kg/d), MEI = metabolisable energy intake (MJ/d), CPI = CP intake (kg/d), MPI = metabolisable protein intake (kg/d).

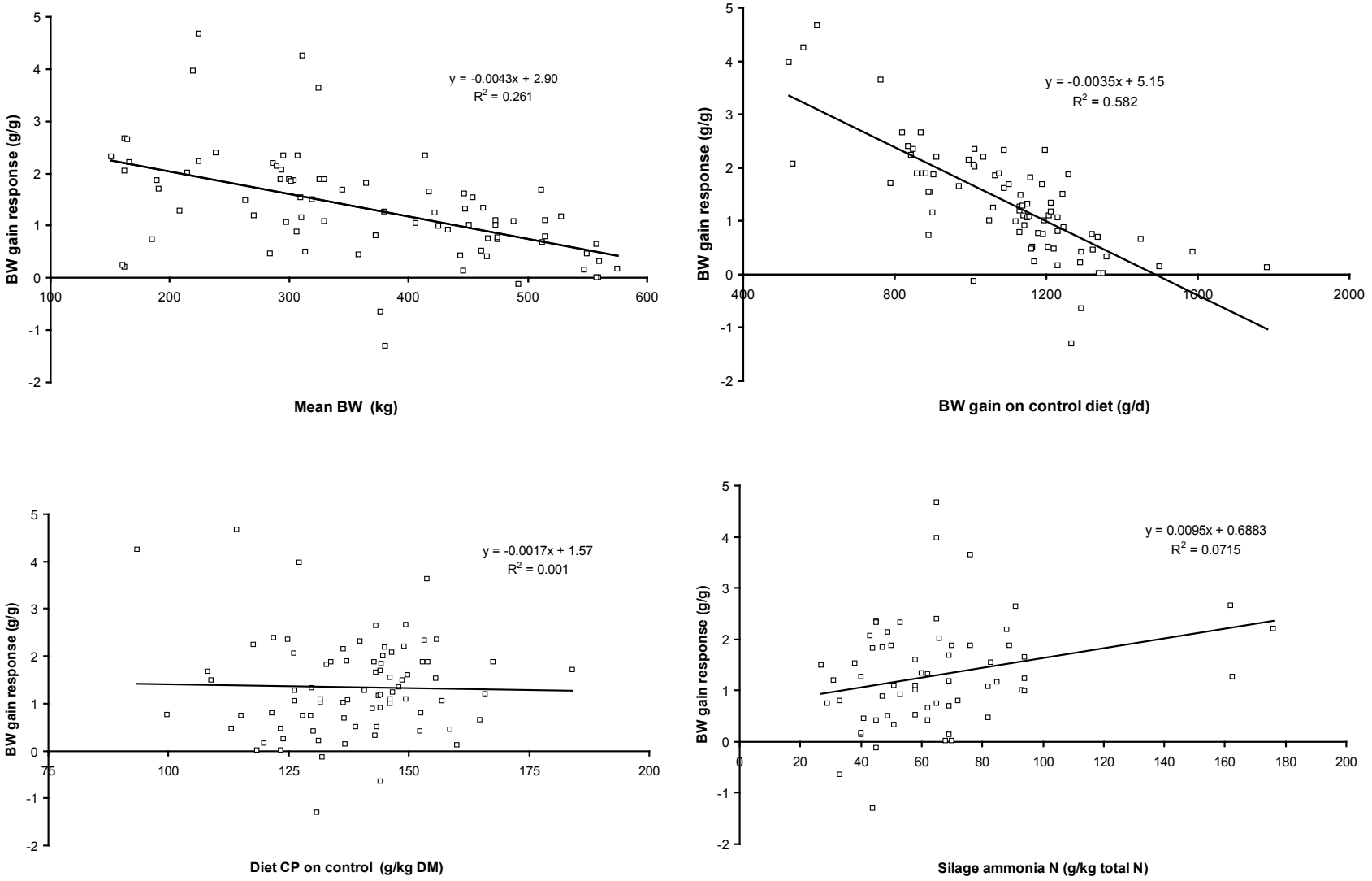

Figure 1 The effects of mean BW, BW gain of the control group (the lowest CP in each study), dietary CP concentration of the control group (the lowest CP in each trial) and silage ammonia N concentration on BW gain response (g/d per 1 g CP/kg DM) to increased CP concentration in the diet.

Increasing dietary CP concentration improved (P<0.01) feed efficiency expressed as BWG per kg DMI or MJ ME intake, whereas BWG per kg CP intake decreased markedly with increased dietary CP concentration (Table 2). Assuming CP concentration of 170 g/kg BW the marginal efficiency of the utilization of incremental CP intake was only 0.05 (SE = 0.008). Increased dietary CP concentration had no significant (P>0.10) effects on days in study, carcass weight, dressing proportion or carcass conformation score, but it increased (P<0.01) carcass fat score.

The differences between protein variables in predicting BW differences were generally small. Negative influence of EPD and greater positive effect of RUP compared with RDP suggest that the small positive responses to supplementary protein were associated with increased supply of RUP. There was no relationship between dietary CP concentration on the control diet (the lowest CP in the study) and BWG response to supplementary protein suggesting that the requirements of RDP were met by all diets or that recycling compensated for the limited supply from the control diet. The Finnish recommendation for growing cattle above 200 kg BW is that the protein balance in the rumen (PBV) is above -10 g/kg DM (MTT 2014). Deleting studies in which PBV of the control diet was below -20 g/kg DM had only a minimal influence on BWG response to increased CP (1.1 vs. 1.3 g per g CP/kg DM) suggesting that recommended PBV can even be reduced without adverse effects on BWG. The amounts of N recycled into the gastrointestinal tract was 27 g/kg DMI in cattle fed a low CP (80 g/kg DM) diet and approximately 40 g/kg DMI in cattle fed higher CP diets (Marini & Van Amburgh 2003). These values indicate that in growing cattle rumen PBV can be negative with minimal, if any, adverse effects on the BWG. Advantages of using MP in estimating protein supply and requirements are questionable at least for growing cattle above 200 kg BW; microbial protein and RUP from high quality forages and energy supplements (grain) can meet the requirements.

Table 2 The effects of protein supplementation on feed efficiency and carcass traits

	Intercept	SE[a]	P-value	X_1	SE	P-value	Adj. RMSE[b]	AIC[c]
Feed efficiency								
BWG[d] / DMI[e] (g/kg)	138	8.2	<0.01	0.18	0.046	<0.01	3.9	1706
BWG / MEI[f] (g/MJ)	11.6	0.70	<0.01	0.019	0.0041	<0.01	0.33	730
BWG / CPI[g] (g/g)	2.03	0.084	<0.01	-0.0061	0.00040	<0.01	0.026	-247
Carcass traits								
Days in trial	237	15.5	<0.01	-0.09	0.058	0.13	5.7	1875
Carcass weight (kg)	254	19.2	<0.01	0.15	0.113	0.20	3.0	1442
Dressing proportion (g/kg)	523	5.8	<0.01	0.05	0.037	0.15	2.7	1225
Conformation score[h]	6.7	0.67	<0.01	0.0009	0.00332	0.78	0.23	265
Fat score[i]	2.7	0.10	<0.01	0.0033	0.00064	<0.01	0.18	182

[a] SE = standard error; [b] Adj. RMSE = Residual mean squared error adjusted for random study effects; [c] Akaike's information criteria; [d] BWG = Body weight gain (kg/d); [e] DMI = dry matter intake (kg/d); [f] MEI = metabolisable energy intake (MJ/d); [g] CP = crude protein (g/kg DM); [h] Conformation score: 1 = poorest; 15 = excellent; [i] Fat score: 0 = leanest; 10 = fattest.

Assuming a CP concentration of 170 g/kg BW, marginal efficiency of CP utilisation was 0.05. Efficiency of N utilisation (N retention/N intake) decreased by 1.02 (SE=0.05; P<0.01) g/kg per 1 g/kg DM increase in dietary CP concentration. These numbers indicate a poor

utilisation of supplementary protein in growing cattle and consequently, increased N emissions to the environment. True CP digestibility of RSM and SBM estimated by the Lucas test was on average 0.95 in dairy cows (Huhtanen et al. 2011). These numbers suggest that about 90% of incremental N intake would be excreted in urine in growing cattle fed supplementary protein. Because urinary N is more susceptible to both leaching and evaporation the adverse effects to the environment increase proportionally more than N output in manure. Moreover, the concentration of P in protein supplements is usually high, especially in RSM (12–14 g/kg DM). Consequently, supplementary protein feeding also increases P emission, since the concentration P in the basal diet based on forages and grain typically contains 3.0–3.5 g P/kg DM in excess of the requirements of growing cattle (MTT 2014).

Conclusions

Due to quantitatively small production responses, increased feed costs and emissions of N and P, there is generally no benefit in using protein supplementation to growing cattle fed grass silage-based diets, provided that the supply of RDP is not limiting digestion in the rumen. The results suggest no benefits of feed protein systems based on metabolisable protein for cattle above 200 kg BW.

References

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.

Huuskonen, A., Huhtanen, P. & Joki-Tokola, E., 2014. Evaluation of protein supplementation for growing and finishing cattle fed grass silage-based diets: A meta-analysis. Animal. (in press).

Lammers, B.P. & Heinrichs, A.J., 2000. The response of altering the ratio of dietary protein to energy on growth, feed efficiency, and mammary development in rapidly growing prepubertal heifers. J. Dairy Sci., 83, 977–983.

Marini, J.C. & Van Amburgh, M.E., 2003. Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci., 81, 545–552.

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

Schwab, C.G., Huhtanen, P., Hunt, C.W. & Hvelplund, T., 2005. Nitrogen requirements of cattle. In: E. Pfeffer and A. Hristov (eds.). Nitrogen and Phosphorus Nutrition of Cattle. Cabi Publishing: Wallingford, UK. pp. 13–70.

St-Pierre, N.R., 2001. Invited review. Integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci., 84, 741–755.

A meta-analysis of milk production responses to increased supply of AAT

I. Schei[1], N.I. Nielsen[2], M. Åkerlind[3] & H. Volden[1,4]
[1]TINE Norwegian Dairies, 1431 Ås, Norway; [2]Knowledge Centre for Agriculture, Cattle, 8200 Aarhus N, Denmark; [3]Växa Sverige, 751 05 Uppsala, Sweden; [4]Norwegian University of Life Sciences, Department of Animal and Aquacultural Sciences, P.O. Box 5003, N-1432 Ås, Norway
Correspondence: ingunn.schei@tine.no

Introduction

Feed protein is the single most expensive nutritional factor in the feed ration of dairy cows. To obtain a high milk production, dairy cows are dependent on high quality feed protein often resulting in an overfeeding or imbalance between the requirement and utilization of feed protein leading to increased excretion of nitrogen in urine and feces. Increased focus on reducing the leakage of nutrients to the environment the last years has emphasized the importance of feed planning and nutrient optimization to dairy cows to reduce nitrogen excretion from urine and faces. Therefore, estimation of the expected marginal responses of increased supply of amino acids absorbed in the small intestine (AAT) is needed in order to improve the economic optimization of diets for dairy cows. The objectives of this work were to develop response curves for ECM and milk protein production to increased supply of AAT and to implement these models in the NorFor feed evaluation system (Volden, 2011).

Materials and Methods

Data were selected from international published protein production trials and Nordic protein production trials differing in protein sources and levels of rumen degradable protein. Protein sources were mainly rape seed meal, soybean meal and fish meal. Roughages were silages of grass, grass-clover, corn and alfalfa. Feeding regimes were either total mixed rations (TMR) or separate feeding and the rations were fed either *ad libitum* or restrictively. Concentrates in rations varied from 28.7 to 75.9 % on a dry matter (DM) basis (mean 45.8). All diets from the trials were calculated according to NorFor based on the information of the ingredients and composition of the diets in order to obtain energy and nutrient supplies.

In the NorFor system, there is a minimum recommendation of 10 g PBV/kg DM (protein balance in the rumen) when optimizing feed rations to dairy cows in order to secure high rumen microbial activity. Therefore, treatments with PBV less than this level were deleted. If this resulted in only one treatment left within a trial, the whole trial was deleted. Trials with less than 3 g AAT/kg DM between the lowest and the highest AAT level were not included in the final analysis. Breeds were Holstein, Norwegian Red and Swedish Red. All treatments included multiparous cows or a mix of primi- and multiparous, but all cows were classified as multiparous since splitting, based on lactation number, was not possible. The final dataset consisted of 87 treatments from 32 trials. Of these, 30 treatments included cows less than 100 days in milk (DIM), 50 treatments were between 100 and 200 DIM and 7 treatments were later than 200 DIM, with an overall average of 130 DIM.

Animal inputs to NorFor were breed, parity, body weight (BW; mean of first and last BW in the trial period), DIM (mean of trial period) and activity (loose housed or tied up). Animal characteristics, milk production including fat and protein content and feed intake of all feedstuffs were inputs to NorFor. Nutrient content of individual feedstuffs were used if reported and then supplemented with NorFor feed table values (Norfor.info). The ratio of

metabolisable protein/net energy for milk (AAT/NEL; g/MJ) was calculated as AAT available for milk production, i.e. total AAT intake minus requirement for maintenance, growth and mobilizing/deposition, and then divided by 3.14 MJ x ECM (energy corrected milk). Variation in feed values and milk production for the dataset is presented in Table 1.

Table 1 Variation of feed components and milk production in the data set.

	Mean	STD	Min	Max
AAT/NEL, g/MJ	15.5	2.66	7.5	23.8
AAT, g/kg DM	93	12.1	63	121
PBV, g//kg DM	32	16.2	10	81
NEL, MJ/ kg DM	6.64	0.65	5.01	8.38
Fatty acids, g/kg DM	28	5.9	18	55
Sugar + starch, g/kg DM	277	92	109	439
ECM, kg/day	29.0	5.7	12.6	39.9
Protein production, g/day	946	202	422	1371
DIM	130	54	49	273

Dependent response variables were the average daily yield of ECM or milk protein production calculated from milk yield and milk composition according to Sjaunja et al. (1991). The 32 trials are plotted in Figure 1 and 2 and the lines between treatments indicate the within study response of ECM and protein production on AAT/NEL. Data were analyzed using the linear mixed effects model in the SAS-package (SAS Institute, version 9.2). The independent continuous variables of AAT/NEL, and PBV, NEL, fatty acids, sugar+starch (per kg DM) and DIM and their quadratic effects were included in the full model. However, the quadratic effect of AAT/NEL was replaced by its logarithmic values. Breed was included as a fixed class effect. Trial within reference was treated as a random variable.

Results and Discussion

Estimates for ECM and milk protein responses to the parameters in the model are presented in Table 2. The model explained 98% of the variation in both ECM and protein production. However, the effect of AAT/NEL was not very clear. The linear effect of AAT/NEL was not significant for ECM or milk protein production. However, there was a logarithmic effect of AAT/NEL on ECM ($P<0.05$) and tendency on protein production ($P<0.1$). Even though the PBV level was set to a minimum of 10 g/kg DM, there was a significantly linearly and quadratic effect on ECM and protein production ($P<0.05$). This might suggest that microbial activity was still not optimal at a minimum level of 10 g/kg DM and could have been limited by the supply of soluble protein. Energy concentration (NEL/DM) and fatty acids (FA/DM) did not significantly affect ECM or protein production linearly, but there was some quadratic effect on protein production ($P<0.05$ and $P<0.1$, respectively).

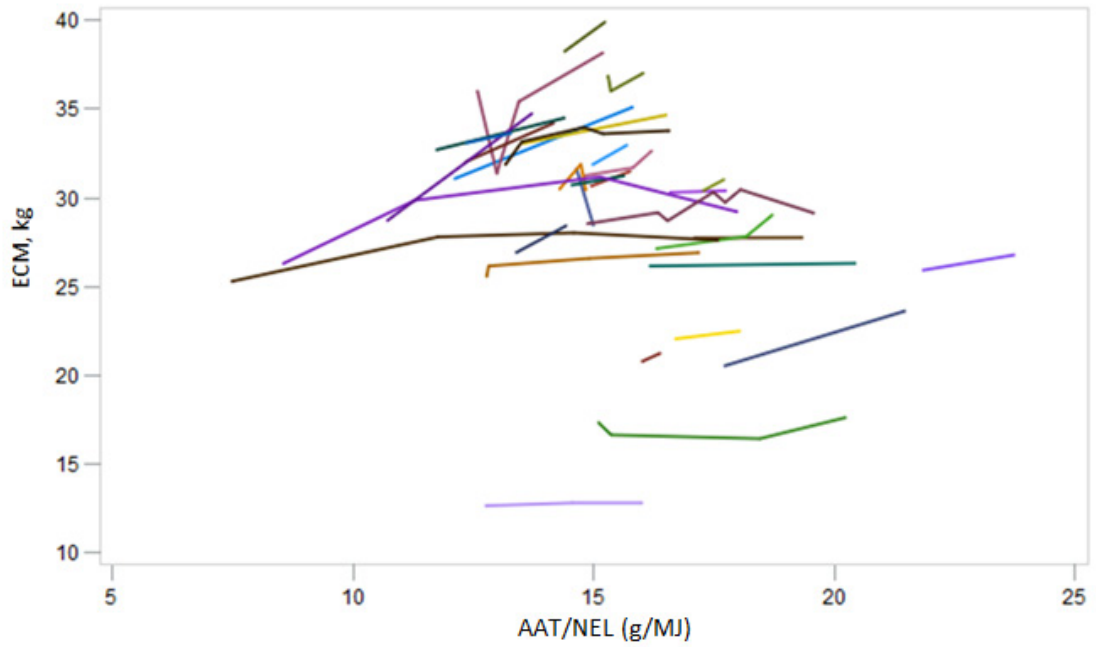

Figure 1 Plot of treatment-responses of AAT/NEL (g/MJ NEL) on ECM (kg/d). Lines indicate the within trial responses.

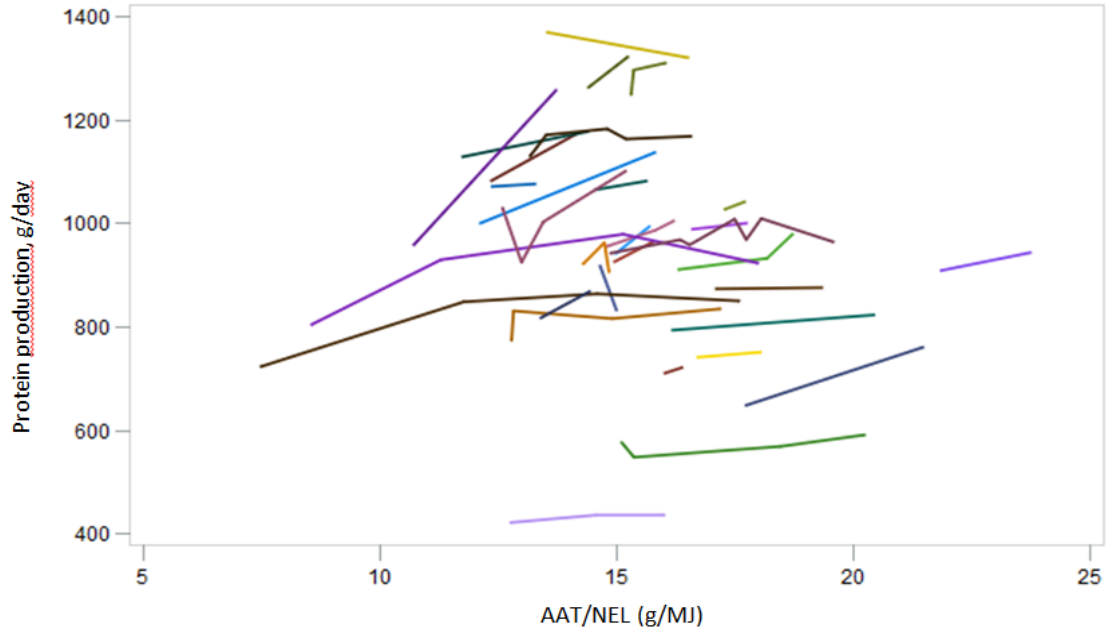

Figure 2 Plot of treatment-responses of AAT/NEL (g/MJ NEL) on ECM (kg/d). Lines indicate the within trial responses.

Response curves for ECM and milk protein production as a function of AAT/NEL for Swedish Red cows are presented in Figure 3. The curves for Holstein and Norwegian Red are parallel but at different levels. Increasing the supply of AAT/NEL from 10 to 19.5 g AAT/MJ lead to an ECM-response of 3.3 kg/d. Highest increase in ECM-responses was observed when increasing the AAT/NEL at the lowest levels. There was a diminishing return in the range of 18 to 19.5 g AAT/NEL, where ECM increased by only 0.2 kg/d. The response of

milk protein production to the linear effect of AAT/NEL supply was not significant, but tended to be significant for the quadratic effect. The response curve for protein production was not that steep in the beginning as for ECM and also showed a diminishing return with increasing supply. An increase in the supply of AAT/NEL from 10 to 25 g AAT/NEL increased milk protein production by 141 g/d. The marginal responses of AAT/NEL are presented in Figure 4.

Different random statements and covariance structure were tested, and the effect of AAT/NEL and the estimates of the response curves were quite sensitive to which covariance structure was used.

In the NorFor-system, the minimum-level of AAT/NEL for milk production recommended is 15.0 g/MJ NEL. By changing this level from 15 to 19.5 g AAT/NEL, the response curve suggests that ECM will increase by 0.8 kg and protein production by 38 g.

Table 2 Parameter estimates for ECM (kg/d) and milk protein production (g/d) responses based on AAT/NEL (g/MJ)

	ECM	Protein production
Intercept	5.32±9.98 n.s	-402.9±363.4 n.s
AAT/NEL	-0.65±0.42 n.s	-14.5±15.4 n.s
Ln(AAT/NEL)	14.14±5.53*	391.5±210 t
PBV	0.128±0.038**	3.11±1.41*
PBV^2	-0.0017±0.0005***	-0.053±0.016**
NEL	n.s	n.s
NEL^2	n.s	4.02±2.37 t
FA	n.s	n.s
FA^2	n.s	0.064±0.031*
DIM	0.123±0.049*	5.50±1.79**
DIM^2	-0.0006±0.00045***	-0.025±0.006***
RMSE[a]	0.81	29.0
AIC[b]	388.6	944.5
R^2	0.98	0.98

***P≤0.001, **P≤0.01, *P≤0.05, t=P≤0.1, ns=P>0.1; [a]Root mean square error; [b]Akaike's Information Criteria

Conclusions

ECM and protein production responded to increased supply of AAT according to the law of diminishing return with increasing supply. The maximum ECM-yield was observed at 19.5 g AAT/MJ NEL, but maximum could not be estimated for protein production within practical levels of AAT/NEL. This suggests that the minimum recommendation of 15.0 g/MJ in NorFor should be increased if high milk production is important. However, the response curves are very sensitive for the covariance structure chosen in the model, and this might explain why the maximum yield level is estimated so high. Moreover, the costs of increased AAT/NEL supply should also be considered.

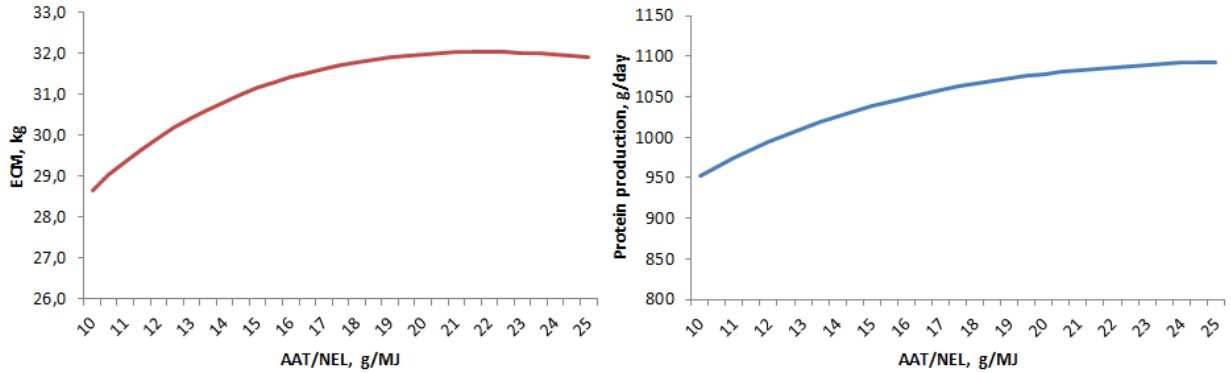

Figure 3 Response curves for ECM and protein production by AAT/NEL (g/MJ) for the breed SRB.

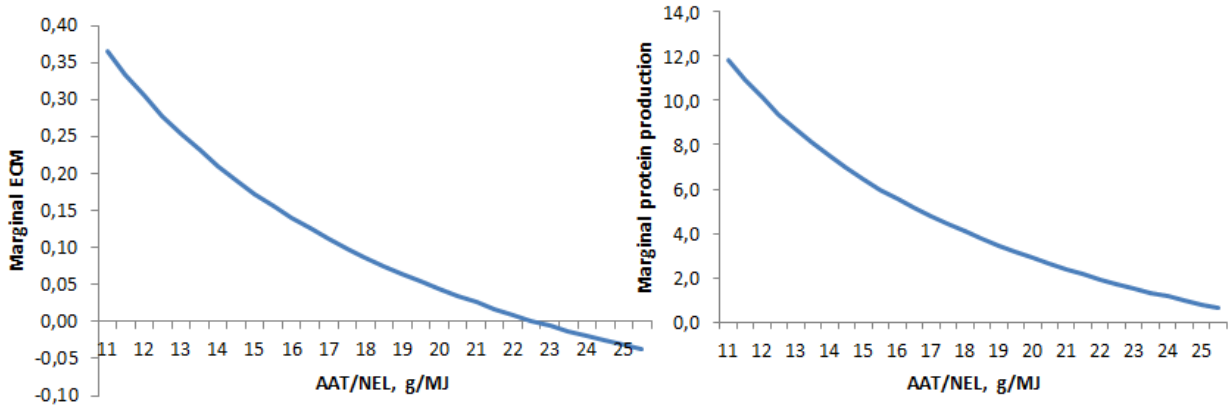

Figure 4 Marginal production responses of AAT/NEL (g/MJ) supply for SRB breed.

References

NorFor Feedtable, http://norfor.info/Feedtab.asp

Sjaunja L.O., Bævre, L., Junkkarinen,L., Pedersen, J., Setälä, J. 1990.A Nordic proposal for an energy corrected milk (ECM) formula. Paper presented at the 27th Session of the International Committee for Breeding and Productivity of Milk Animals, Paris, 2-6 July.

Volden, H., 2011. Overall model description. In: 428 Volden, H. (Ed.), NorFor - The Nordic Feed Evaluation System. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 23-26.

Dietary nutrient density and effects on intake and production

S. Rengman, B. Johansson & M. Murphy
Lantmännen Lantbruk, Feed development, 205 03 Malmö, Sweden
Correspondence: michael.murphy@lantmannen.com

Introduction

Dairy cow dry matter (DM) intake is influenced by both physical limits to rumen fill and fulfilment of metabolic requirements. Diet composition in different systems can vary, especially regarding the carbohydrate fraction, despite similar dietary goals, and may affect rumen fill and metabolic satiation differently. The Norfor system calculates digestibility and absorption in the entire digestive tract as well as calculating post absorption metabolism with a strong focus on least cost and maximum forage intake. The LFU system is mainly focused on digestion kinetics in the rumen and recommends a dietary nutrient composition to obtain a biological optimum for rumen metabolism to sustain the actual milk production. While both systems have similar approaches regarding rumen metabolism there are some significant differences, especially regarding fiber kinetics and interactions with non-structure fibers. The Norfor system models for interactions between substrates while the LFU system alters diet composition according to milk yield. The objective of this study was to compare rations composed according to Norfor and the LFU system

Materials and Methods

To compare the effects of diets composed after Lantmännen's LFU system and the Nordic program, Norfor, a trial was conducted at 'Nötcenter Viken', Falköping, Sweden with 48 cows in 3 groups in a continuous experiment. The groups were fed diets according to the Norfor system (Norfor), according to the LFU system (LFU) and a diminished LFU diet in which the fiber fraction was slightly improved in relation to LFU recommendations but with less starch (LFUdim). The Norfor diet was composed by an advisor from 'Växa'.

Table 1 Diet composition, kg dry matter, of the 3 diets offered in feeding trial

Feed	Norfor	LFU	LFUdim
Grass silage, 2nd cut	12.4	10.0	10.0
Maize silage	5.0	2,4	1,5
Barley, crushed		3.1	
Unik 52			1.0
Unik Prestige		3.5	
Solid 120			11,5
Solid 350	9.3		
Solid 520		5.0	
Mineral mix	0.1	0.1	0.1
Total DM	26.8	24.1	24.1

Cows (76 – 175 days in milk (DIM), first and second parity, were grouped and blocked after breed, DIM and milk yield. Following a 2 week pre-period, the diets were fed for 3 weeks. Average milk yield was 38 kg d^{-1} at blocking and diets were composed for a 40 kg production level. At the start of the trial (Dec 16) DIM for Norfor, LFU and LFUdim were; 121, 125 and

123, respectively. The trial was approved by the Ethics Committee for Animal Experiments, Göteborg.

All diets were fed ad lib as total mixed rations (TMR). New feed was offered once a day. Diet composition is presented in Table 1. All feeds on the farm were available for use as well as all standard feed products sold by Lantmännen. Silages were a second harvest mixed grass and clover ley and/or a corn silage. Both silages were grown and harvested at Nötcenter Viken, Sweden. The grass/clover silage had a DM content of 38% with 147 g crude protein (CP) and 446 g neutral detergent fibre (NDF) kg^{-1} DM. The corn silage had a DM content of 33% with 84 g CP, 415 g NDF and 258 g starch kg^{-1} DM. The rumen degradability of NDF (EFD) for the grass/clover and corn silages were 0.573 and 0.450, respectively, mean of 3 analyses.

Silages were sampled weekly and analyzed at the Kungsängen laboratory, SLU, Uppsala, for DM, protein, starch, neutral detergent fibre (NDF), fermentation acids and ash. In addition rumen protein and fiber degradation were analyzed by in sacco by the Norfor method and calculations for rumen degradability according to Kristensen et al. (1982).

Cows were housed in a free-stall barn and milked 3 x d^{-1} in a DeLaval carousel. Feed was distributed into bins resting on load cells and the decrease in mass was recorded automatically. Access to bins was governed by gates regulated by the cows' transponders (BioControl, Rakkestad, Norway) . Each group of 16 cows had access to 8 bins. Dry matter intake (DMI) and milk yield were monitored daily. Milk composition was monitored the last week of the trial.

Table 2 Nutrient content (g kg^{-1}DM) of the three diets offered in the feeding experiment and the LFU recommendations for a milk yields of 40 kg (LFU Rec)

Nutrient	Norfor	LFU	LFUdim	LFU Rec.
Crude Protein	174	186	189	180
RDP[a]	119	123	121	113
UDP[b]	56	63	67	67
Starch	136	190	151	190
NDF	381	326	350	340
Rumen degradable NDF	203	179	190	180
Rumen undegradable NDF	178	147	160	160
Ether extract (EE)	39	46	47	50
EFD[c]	0.533	0.549	0.543	
EPD[d]	0.681	0.661	0.643	

[a]Rumen degradable protein; [b]Rumen udegradable protein; [c]Fraction of NDF degraded in the rumen, estimated from individual feed values; [d]Fraction of crude protein degraded in the rumen, estimated from individual feed values.

Results were analysed using a one-way Anova with Minitab, version 16. Results are presented as group averages. Statistical significance was $P < 0.005$.

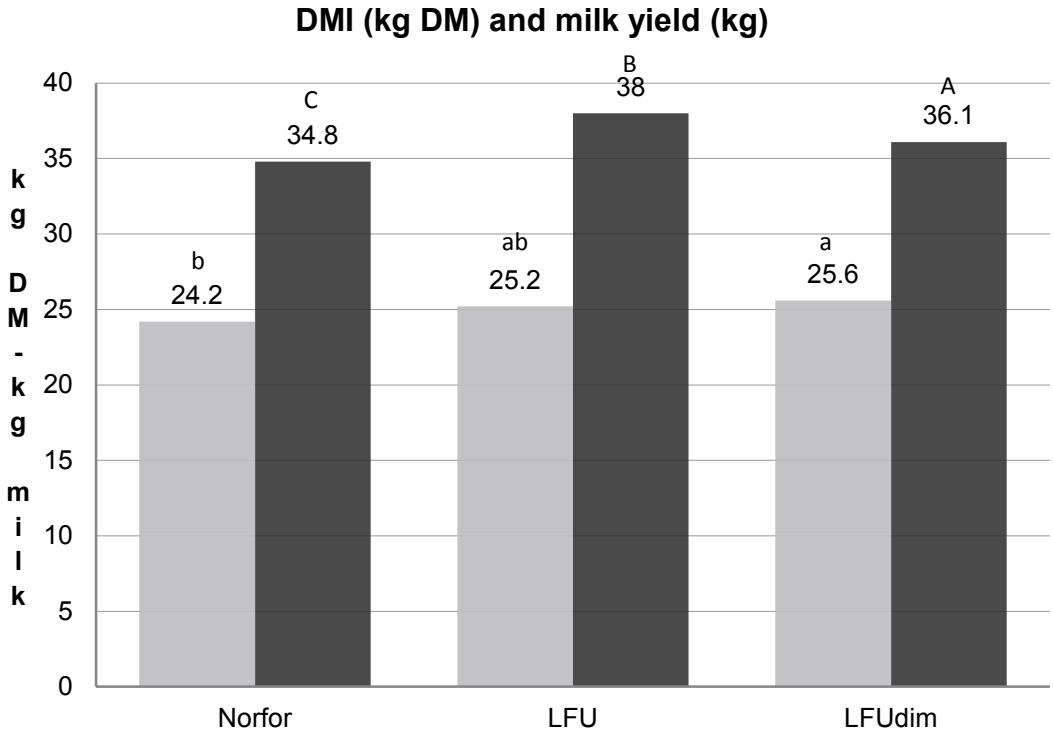

Figure1 DMI (kg DM d^{-1}) and milk yield (kg d^{-1}) for the 3 diets Norfor, LFU and LFUdim. Different letters denote significant differences, (0.005).

Results and Discussion

LFU recommendations for a production of 40 kg assume an intake of 22.9 kg and nutrient densities are based on that intake. DMI intake with the Norfor diet was only 24.2 kg, 2.6 kg less than predicted (Table 1). DMI with both LFU diets was 25 kg, 2.1 kg more than predicted (Table 1).

None of the diets had an EE content as high as recommended by the LFU system (5% of DM), but the LFU diet supplied the recommended amount due to a higher intake (Table 2). The LFU diet met the LFU recommended concentrations for CP and starch but was low in NDF and 0.4% units lower than the recommended rumen undegradable dietary protein (UDP). The Norfor diet had higher concentrations of NDF, rumen degradable NDF and rumen undegradable NDF but was lower in CP, UDP and starch than recommended by the LFU system (180, 67 and 190 g kg^{-1}DM, respectively). LFUdim had more CP, rumen degradable protein (RDP), NDF and rumen degradable NDF than recommended but less starch.

Due to an increased DMI with the LFU diet NDF intake (8.1 kg) was higher than recommended (7.7 kg). The NDF intake, despite a lower DMI, was even greater for the Norfor diet (9.1 kg). Intake of rumen undegradable NDF was 3.8 kg for the LFU diet which is only slightly higher than the recommended 3.7 kg, but was 4.5 kg for the Norfor diet, which may have limited DMI due to rumen fill. Intake of rumen undegradable NDF was 4.1 kg with LFUdim.

Despite a lower density than recommended, UDP intake with the LFU diet (1575 g) was greater than recommended (1534 g) due to a higher DMI. UDP intake with LFUdim (1675 g)

was much greater than recommended, which was also due to a higher DMI. Despite this, milk protein content with the LFUdim diet was numerically lower than with the LFU diet (Table 2). This may be associated with the lower starch content and a consequent diverting of amino acids to gluconeogenesis. UDP intake with the Norfor diet was only 1355 g, which was179 g less than recommended.

Table 2 Milk yield, kg d^{-1} and milk composition, group averages, for cows fed the 3 experimental diets; Norfor, LFU and LFUdim, in the last week of the trial

	Norfor	LFU	LFUdim
Milk yield	34.2[c]	37.5[b]	36.0[a]
Milk fat %	4.25	4.08	4.05
Milk protein %	3.36[b]	3.50[a]	3.42[ab]
Milk Urea mmols/l	4.96[b]	5.28[a]	5.37[a]

[a,b,c] Rows with different superscripts are significantly different.

Milk yield was significantly lowest with the Norfor diet. Milk fat content was numerically highest with the Norfor diet but the difference was not statistically significant. All diets had a higher fat content than the herd average, 3.9%. The increased fat content with Norfor is consistent with a larger roughage fraction. Fat yield was numerically higher with the LFU diets; 1.45, 1.53, and 1.46 kg for Norfor, LFU and LFUdim, respectively. The protein content with the Norfor diet was lower than the herd average, 3.4. The protein content with Norfor was numerically lowest and significantly lower than the LFU diet. Milk urea content was significantly lower with Norfor compared to the LFU diets. The lower milk yield and lower protein content with the Norfor diet may be associated with the lower UDP and starch intakes.

Cows on both LFU diets were able to maintain the production level and with LFU even increase their yields the first half of the trial (Fig. 2). Cows on the Norfor diet decreased milk production directly after start.

Grass silage and maize silage were estimated to cost 1.55 SEK kg^{-1} DM. These costs were based on a crop production and harvest cost of 1.35 SEK plus 0.20 SEK for labor and fuel in handling the silages for feeding. The concentrates and other feeds were assigned actual market prices. Per kg DM, the Norfor diet cost the least. Income from milk was calculated using prices from the major dairy cooperative, 'Arla räknesnurra' (10 March, 2014). Even though the feed costs were lowest for Norfor, milk minus feed costs for the entire period was highest for the LFU diets. Feed costs and milk prices will vary over time and, therefore, an exact relation cannot be established from a single trial. However, the results do not indicate that higher feed costs for rations supporting a higher milk production are negative for the producer's economy.

Norfor had a numerically lower feed efficiency than the LFU diets. However, variation among cows and over time was large and no differences were significant.

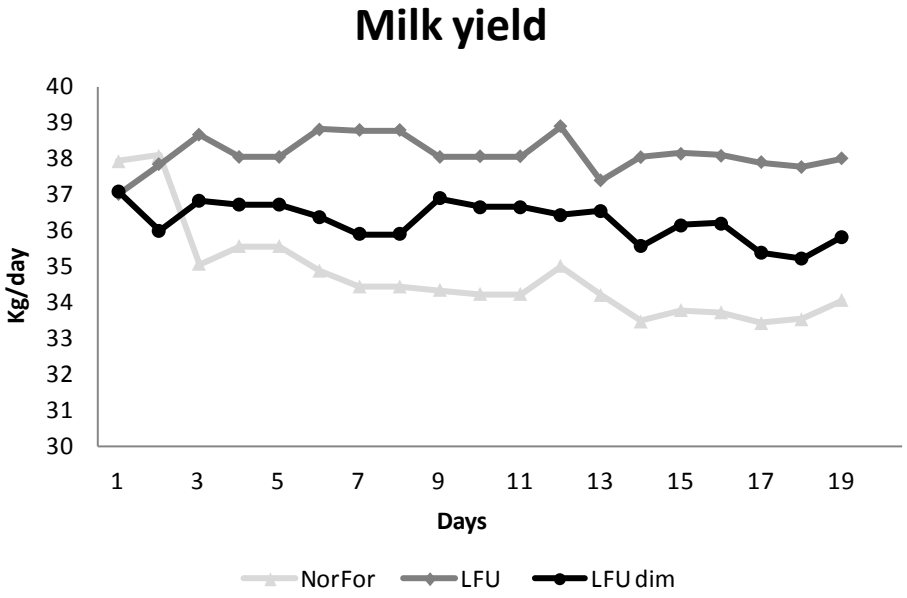

Figure 2 Milk yield, group averages, for cows fed the 3 experimental diets

Conclusions

Norfor overestimated the intake capacity of the cows while LFU underestimated DMI. The composition of the Norfor diet did not enable an increased DMI. Systems with the aim of maximizing roughage intake without a decreased milk yield must have a very good estimate of fiber digestion and passage kinetics, which apparently was not so in this trial. A high milk production level is dependent on gluconeogenic substances which are mainly originating from rumen propionate production and absorption of starch in the small intestines. Both of these are closely linked to the starch content of the diet, which was much lower on the Norfor diet, and this may also have influenced milk protein content negatively. Also the differences in milk production between LFU and LFUdim indicate the importance of starch in the diets.

References

Kristensen, E.S., Möller, P.D. & Hvelplund, T., 1982. Estimation of the effective protein degradability in the rumen of cows using the nylon bag technique combined with outflow rate. Acta Agric. Scand. Suppl. 32, 123-127.

Intra-ruminal mixing of concentrate pellets produced by conventional pellet press or extruder

M. Nordqvist, P. Lund, A. C. Storm, M. R. Weisbjerg, and M. Larsen
Department of Animal Science, Aarhus University, Blichers Allé D20, DK-8830 Tjele, Denmark
Correspondence: Mogens.Larsen@agrsci.dk

Introduction

Commercial compound concentrates used in dairy herds are for the vast majority pelleted using a conventional pellet press. Traditionally, low rumen pH have been attributed to high allowances of starch rich concentrates; however, recent observations have shown that pH in ventral rumen liquid decreased when calves were fed pelleted concentrates, irrespective of high or low starch content (Khafipour et al., 2009; Kristensen et al., 2009). This indicates that low pH in the ventral rumen could be more linked to physical characteristics of the pellets rather than starch content.

In the fish feed industry, extrusion has long been used to pelletize compound feeds in order to obtain physical functional properties needed to avoid rapid sinking and disintegration of pellets when fed in water. Extrusion is a processing technology which gives the opportunity to obtain a variety of functional properties of feed pellets. Depending on the setting, varying degrees of expansion of the feed can be achieved when it is forced through the matrix. This expansion affects feed density as well as floating ability. In addition, extrusion gives different levels of pellet durability in liquid. Pellet durability is important to avoid quick disintegration of feed pellets in water. The effect of extrusion on nutrient digestibility in cattle have been investigated for some feedstuffs (Benchaar et al., 1992); yet, to the authors knowledge there have been no reports of the effect of pelletizing by extrusion on postprandial intra-ruminal mixing of pellets.

Hypotheses: concentrate pellets fed separately during milking has a lower impact on ventral ruminal pH if the pellets are captured by the ruminal particle phase. The objective of the study was to compare conventional pellets with extruded pellets on their ability to be captured by the ruminal mat. The goal was to shift the fermentation of the concentrate pellets from the ventral rumen to the ruminal mat, thereby reducing the local acid exposure of the ruminal wall. This is a preliminary report, presenting some of the obtained data from a larger study.

Materials and methods

Three lactating Danish Holstein cows fitted with ruminal, duodenal and ileal cannulas were used in a 3 × 3 Latin square design. Cows were milked twice daily at 08.00 and at 15.30 h and were at the same time fed concentrates. The experimental concentrates were fed at the morning milking. The experimental concentrates were pellets produced from wheat meal (2-mm screen) using three different methods: conventional pelleting (sinking in liquid, high solubility), light extrusion (floating in liquid), or heavy extrusion (sinking in liquid, low solubility). The cows were fed a partial mixed ration (PMR) 30 min after each milking, based on a grass-clover silage, soy bean meal, and minerals. The PMR was low in starch; thus, starch from experimental concentrates could be used as a marker for the ability of concentrate pellet to be captured in the particle phase of the rumen. The PMR was fed restrictedly at 95% of *ad libitum* intake determined individually for the cows in a pre-experimental period. The

concentrates fed at 15.30 h also had a low starch concentration and was based on dried beet pulp, vegetable fat, and molasses.

Samples of medial rumen content as well as rumen fluid from the medial and ventral parts of the rumen, were obtained at -0.5, +0.5, +1.5, +2.5, +4.0, +5.5, +7.0, +8.0, +9.0, +10.0, +11.5 h relative to morning feeding. Medial rumen content and fluid was sampled by hand from the ruminal particle phase through the ruminal cannula. Two large handfuls were obtained 10 to 15 cm beneath the mat surface in the middle section of the rumen. The medial rumen particle associated fluid, was squeezed from the samples through a single layer of cheese cloth into a 45-mL test tube. The ventral rumen fluid was sampled with a suction strainer (#RT, Bar Diamond Inc., Parma, ID, USA) and a 60-mL syringe. Starch in medial rumen contents were determined by enzymatic liberation of glucose bound in starch, using an YSI analyser (D-glucose oxidase, YSI 2900, YSI Inc., Yellow Springs, OH, USA) to quantify liberated glucose.

Data was analysed using the Mixed procedure of SAS with period, processing method, time relative to feeding, and processing × time interaction as fixed effects. Cow was considered a random effect and time relative to feeding as repeated measurements.

Results and discussion

The starch concentration in medial rumen content increased from 0.8 ± 0.5 at -0.5 h to 4.6 ± 1.6 % of DM at +0.5 h, relative to morning feeding (Figure 1, $P < 0.001$). It was unaffected by processing method of pellets when all the 11 time points were included in the dataset. However, considering only samples obtained 5.5 h after morning feeding and later, there was a greater starch concentration in medial rumen content for the light extrusion pellets, as compared to conventional pellets ($P = 0.015$). On average across all the time points, starch concentration in medial rumen contents were numerically greater with heavy extrusion pellets as compared to conventional pellets ($P = 0.117$).

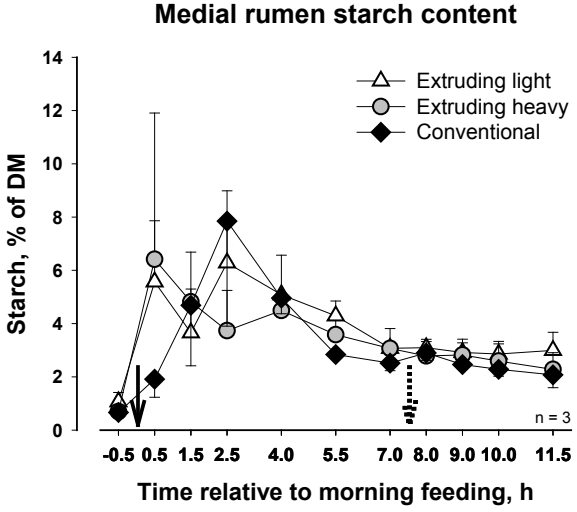

Figure 1 Starch concentration in medial rumen contents from 3 cows fed wheat meal pelletized conventionally or by extrusion giving either light or heavy (gravimetric properties) pellets.

The ventral rumen pH (Figure 2a) in samples obtained after the afternoon milking and feeding were not used in the analysis due to the effect on pH from feeding of concentrates.

The pH in ventral rumen decreased with a similar pattern for all treatments, after feeding the experimental concentrates, but after the nadir at +2.5 h, pH increased more rapidly with pellets produced by extrusion (interaction, P = 0.01).

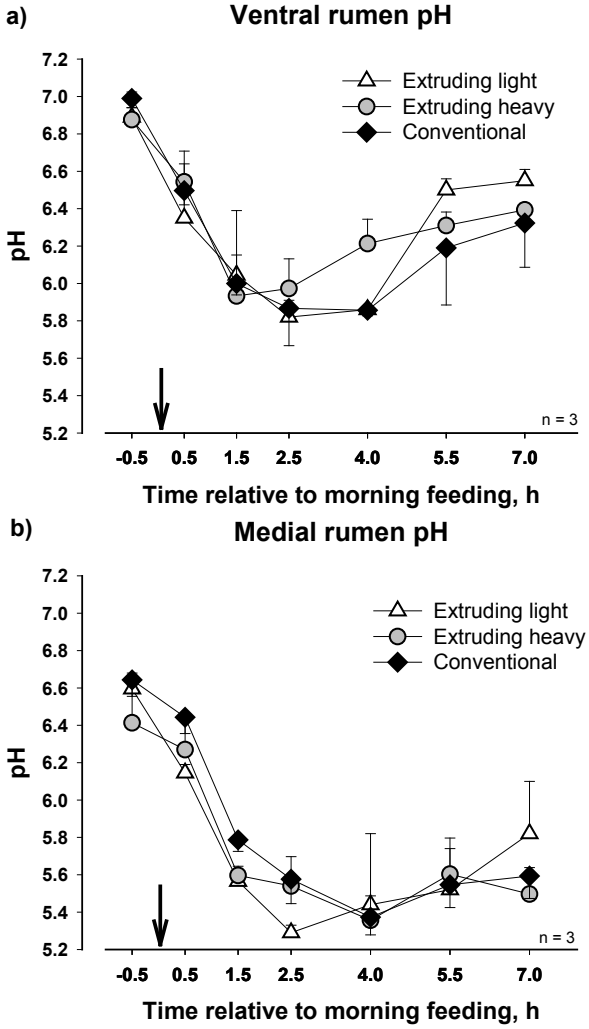

Figure 2 Ruminal pH in liquid obtained from ventral rumen (a) and squeezed from medial grab samples (b) in 3 cows fed wheat meal pelletized conventionally or by extrusion giving either light or heavy (gravimetric properties) pellets.

The pH in medial rumen content (Figure 2b) decreased more rapidly after feeding pellets produced by extrusion compared with conventional pellets, but after the nadir, between 2.5 and 4 h after feeding, pH did not differ among treatments (interaction, P = 0.02). The more rapid decrease in pH in medial rumen content with pellets produced by extrusion indicates a greater production of acids from fermentation; thus, these pellets may have been better captured by the particle phase. This is also supported by visual observation of intact extruded pellets in samples obtained at +0.5 h after feeding. Observation of intact extruded pellets in the medial mat also indicates limited crushing of pellets during eating.

Conclusion

The preliminary analysis of observations indicates that wheat concentrate pellets produced by extrusion may better mix with the rumen particle phase compared to conventional pellets. Yet, the effects were of relatively small magnitudes indicating that the rumen system is very robust in overcoming challenges with concentrate pellets differing greatly in physical functional properties.

References

Benchaar, C., Vernay, M., Bayourthe, C. & Moncoulon, R., 1992. Incidence of bean (Vicia-Faba) extrusion on starch and nitrogen intestinal flows in lactating cows. Repr. Nutr. Developm. 32, 265-275.

Khafipour, E., Krause, D. O. & Plaizier, J. C., 2009. Alfalfa pellet-induced subacute ruminal acidosis in dairy cows increases bacterial endotoxin in the rumen without causing inflammation. J. Dairy Sci. 92, 1712-1724.

Kristensen, N.B., Storm, A.C. & Vestergaard, M., 2009. Sund drøvtyggerfodring - ved vi hvad det er? Eksempler på fodringsmæssige muligheder for at ændre på vomfunktion hos småkalve og slagtekalve. I: Slagtekalveproduktion - Temamøde: Ernæring, Sundhed og Velfærd. red.: Mogens Vestergaard. Aarhus Universitet - Foulum, Det Jordbrugsvidenskabelige Fakultet. Intern Rapport Husdyrbrug 16, 14-19.

Prediction of methane emissions from dairy cows fed cold-pressed linseed cake

M. Kass[1,2], A. Olt[2], R. Leming[2] & M. Ots[1,2]
[1]Bio-Competence Centre of Healthy Dairy Products LLC, 1 Fr. R. Kreutzwaldi Street, 51014 Tartu, Estonia. [2] Estonian University of Life Sciences, Department of Animal Nutrition, 46 Fr. R. Kreutzwaldi Street, 51006, Tartu, Estonia
Correspondence: marko.kass@emu.ee

Introduction

Methane (CH_4) is a normal product of anaerobic fermentation, and is hazardous to the environment as a greenhouse gas. Methane emission is related with energy loss, 2-12% of gross energy intake (Johnson & Johnson, 1995). Thus, in order to reduce the emission of CH_4 various feed additives (e.g. oilseed crops, tannins, essential oils) have been supplemented to the diets of dairy cattle (Meale et al., 2013), to try to avoid the reduction of production efficiency related to CH_4 emission. Since direct measurements of CH_4 emission are expensive and time-consuming there exist equations based on animal factors or nutrient intake to determine the CH_4 output by an animal (Wilkerson et al., 1995; Benchaar et al., 1998; Mills et al., 2001; Mills et al., 2003). To reduce CH_4 emission through altering rumen fermentation parameters supplemental fat sources have been added to the diet of dairy cows (Dong et al., 1997; Giger-Reverdin et al., 2003). Feeding crushed linseed (Beauchemin et al., 2009), crude linseed and extruded linseed (Martin et al., 2008) to dairy cows have shown depressive effects on ruminal methanogenesis. In addition, feeding linseed cake, which is rich in α-linolenic acid, to dairy cattle, could improve the milk fatty acid (FA) profile, which would be beneficial for human health (Kennelly, 1996). Using mathematical models based on numerous experiments would provide a good tool to predict the effect of diet or feed supplement on CH_4 emissions by dairy cows. In addition to dry matter intake (DMI) and diet composition, correlations between CH_4 emission and rumen volatile fatty acids (VFA) (Ramin & Huhtanen 2013) and the milk FA composition (Chilliard et al., 2009; Dijkstra et al., 2011) have been found. The current study used a range of prediction equations to estimate CH_4 emissions from dairy cows when they were fed cold-pressed linseed cake.

Materials and Methods

The experiment was carried out on Eerika Experimental Farm (Estonian University of Life Sciences). Nine multiparous lactating Holstein cows were used in a 3 × 3 Latin square design, where within each group one cow had a rumen fistula. Each experimental period lasted 28 days; comprising an adaption period of 23 days and five days of data collection. The cows were housed individually, tethered in stalls, in which they were fed and milked. At the start of the experiment the cows in all groups had no significant differences between them regarding body mass, milk production or milk composition. The basal diet (on DM basis) contained grass silage (44%), concentrate mixture (54%), and a vitamin-mineral mixture (2%). Control group cows (no linseed) were given a concentrate mixture containing barley meal, maize meal and soybean meal; group 2 (LL – low linseed) barley meal, maize meal, soybean meal, heat-treated rapeseed cake and cold-pressed linseed cake (76 g/kg diet DM); and group 3 (HL – high linseed) was fed barley meal, heat-treated rapeseed cake and cold-pressed linseed cake (109 g/kg diet DM), respectively. All groups were fed a diet containing 11.3 MJ kg^{-1} DM metabolisable energy (ME) and 180 g kg^{-1} DM crude protein.

A list of the prediction equations based on scientific literature was chosen for CH_4 estimates (n=10, Table 1). Equations were based on either DMI, nutrient composition of the diet, ruminal VFA or milk fatty acid (FA) composition.

Table 1 Prediction equation for CH_4 emission estimates.

Reference	Equation	R^2
DM intake		
Ellis et al., 2007	CH_4 (MJ/d) = 3.23 + 0.809 × DMI (kg/d)	0.65
Eugène et al., 2008	CH_4 (MJ/d) = 3.0 + 0.9 × DMI (kg/d)	
Giger-Reverdin et al., 2003	CH_4 (l/kg DMI) = 544.9 – 0.0220 × DMI^2	0.58
Ramin, Huhtanen, 2013	CH_4 (l/d) = 20 + 35.8 × DMI − 0.50 × DMI^2	
Nutrient intake		
Yates et al., 2000	CH4 (MJ/day) = 1.36 + 1.21 × DM – 0.825 × DMI of concentrate mixture + 12.8 × NDF	
Giger-Reverdin et al., 2003	CH_4 l/kg DMI = 47.3 – 0.0212 × DMI^2 – 0.680 × %EE	0.76
Kirchgessner et al., 1995	CH_4 (g/d) = 63 + 79 × CF + 10 × NFA + 26 × CP – 212 × CF (kg/d)	0.69
Rumen volatile fatty acids		
Montoya et al., 2011	CH_4 (mmol/mol VFA) = 0.45 × acetate − 0.275 × propionate + 0.4×butyrate	
Milk fatty acids		
Chilliard et al., 2009	CH_4 (g/d) = 9.46 × milk 16:0 (% of total FA) – 97.6 × milk *trans*-16 +*cis*-14 18:1 (% of total FA) + 13.3 × forage intake (kg of DM/d) – 78.3 × milk *cis*-9 14:1 (% of total FA) + 77.4 × milk 18:2n-6 (% of total FA) – 21.2	0.95
Dijkstra et al., 2011	CH_4 (g/kg DM) = 24.6 + 8.74 × C17:0 anteiso − 1.97 ×trans-10 +11 C18:1 − 9.09×cis-11 C18:1 + 5.07×cis-13 C18:1	0.73

DMI – dry matter intake; EE – ether extract; CF – crude fat; CP – crude protein; NFE – nitrogen free extract; NDF – neutral-detergent fibre.

Feed samples were analysed for chemical contents using established methods (AOAC, 2005). Dry matter intakes were calculated based on daily intakes of diet and DM content of the feeds. Milk samples were collected in the last five days of each experimental period. The milk fat, protein and lactose contents were measured using infrared spectrometer MilkScan 4300 (FOSS Electric A/S, Denmark). Samples for milk FAs analyses were frozen (-18 °C) without preservative until analysis. Milk FA profile was analysed by an Agilent 6890 gas chromatograph equipped with a capillary column CP7420 (100 m × 0.25 mm (i.d.) with 0.25 µm film thickness). A sample of rumen fluid was collected three hours after morning feeding from the three parts of the rumen. Samples were mixed and drained through cheesecloth, and were taken to the laboratory for immediate VFA content analyses. The VFA content in rumen fluid were determined chromatographically using an Agilent Technologies 7890A GC system with a column packed with 80/120 Carbopack B-DA/4% Carbowax 20 M (Sigma-Aldrich Corp., USA) by the method described by Faithfull (2002). Statistical analyses were performed with MS Excel and SAS software (version 9.1; SAS Institute, 2003).

Results and Discussion

Dry matter intake and ME intake were not affected by the linseed cake supplementation, which is in agreement with a previous study (Gonthier et al., 2004). However, Martin et al. (2008) reported a reduced DMI and gross energy intake with inclusion of extruded linseed to the diet. Cows fed linseed cake had higher milk yields ($P<0.001$) and milk fat yields ($P<0.01$), but lower milk fat contents ($P<0.01$) compared to control cows. Martin et al. (2008) and Gonthier et al. (2005) found a decrease in milk yield when feeding linseed to dairy cows. The total ruminal VFA and individual VFA were not affected by the treatment, with the exception of propionate which was significantly increased ($P<0.01$) with linseed inclusion in the diet. The cold-pressed linseed cake supplementation of the diet had a significant effect ($P<0.05$) on the proportions of saturated FA, mono and polyunsaturated FA, and α-linolenic acid content in milk fat. This agrees with the findings of Caroprese et al. (2010), that linseed supplementation improves milk composition, thereby increasing polyunsaturated FA contents in milk fat.

Using prediction equations based on DM intakes showed that as DMI was not affected by the dietary treatment, there was no effect on CH_4 emission (Table 2). This confirms the results of Ellis et al. (2007) that methane emission is largely related to DMI. Calculation with equations based on the nutrient component (Giger-Reverdin et al., 2003; Kirchgessner et al., 1995), showed decreased CH_4 emissions with linseed supplementation, which is in agreement with Martin et al. (2008) that dietary supplementation based on linseed, will depress ruminal methanogenesis.

Table 2 Effect of linseed supplement of CH_4 emission predicted by different formulae

References, unit	Treatment			SE	P
	Control	LL	HL		
Ellis et al., 2007, MJ/day	21.8[a]	22.1[b]	22.4	0.49	NS
Eugene et al., 2008, MJ/day	23.7[a]	24.0[b]	24.3	0.54	NS
Giger-Reverdin et al., 2003, l/kg DMI	33.3[a]	32.7[b]	32.5	0.61	NS
Ramin, Huhtanen, 2013, l/day	578	576[a]	586[b]	8.0	NS
Yates et al., 2000, MJ/day	23.0	23.6	23.5	0.46	0.09
Giger-Reverdin et al., 2003, l/kg DMI	20.3[a]	17.8[b]	17.8[b]	1.80	<0.001
Kirchgessner et al., 1995, g/ day	445[c]	366[a]	393[b]	8.1	<0.001
Montoya et al., 2011, mmol/mol VFA	312	311	299	14.3	NS
Chilliard et al., 2009, g/day	535[a]	401[c]	450[b]	9.4	<0.001
Dijkstra et al., 2011, g/kg diet DM	26.6[a]	25.4[c]	26.1[b]	0.13	<0.001

[a,b,c] – within a row a means with different superscripts are different at $P<0.05$; LL – low linseed diet, HL – high linseed diet, NS – not significantly different ($P>0.05$).

As linseed feeding only altered rumen propionate concentration, the equation based on rumen VFA, by Montoya et al. (2011), showed that dietary manipulation had no significant effect on CH_4 emission. The effect of linseed feeding on milk composition has been studied previously (Glasser et al, 2008), therefore the CH_4 emission affected by the dietary fat source was also evaluated, using milk FA composition as a prediction tool. In the current study, calculations

using the equation based on milk FA (Chilliard et al., 2009; Dijkstra et al., 2011) showed that feeding linseed cake reduced CH_4 production.

Conclusions

The results of this study, using different equations to predict CH_4 emission in dairy cows, are generally in agreement with previous studies. Adding cold-pressed linseed cake to the diet of dairy cows showed that oilseed supplementation could potentially reduce CH_4 emission.

Acknowledgements

The research leading to these results was financed by the European Community's Regional Development Fund in the framework of the Competence Centre Programme of Enterprise Estonia under project no. EU30002 carried out by the Bio-Competence Centre of Healthy Dairy Products.

References

AOAC, 2005. Official Methods of Analysis of AOAC INTERNATIONAL, 18th ed. – Association of Official Analytical Chemists International, Gaithersburg, MD, USA.

Beauchemin, K.A., McGinn, S.M., Benchaar, C. & Holtshausen, L., 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. J. Dairy Sci., 92, 2118–2127.

Benchaar, C., Rivest, J., Pomar, C. & Chiquette, J., 1998. Prediction of methane production from dairy cows using existing mechanistic models and regression equations. J. Anim. Sci., 76, 617–627.

Caroprese, M., Marzano, A., Marino, R., Gliatta, G., Muscio, A. & Sevi, A., 2010. Flaxseed supplementation improves fatty acid profile of cow milk. J. Dairy Sci., 93, 2580–2588.

Chilliard, Y., Martin, C., Rouel, J. & Doreau, M., 2009. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. J. Dairy Sci., 92, 5199–5211.

Dijkstra, J., van Zijderveld, S.M., Apajalahti, J.A., Bannink, A., Gerrits, W.J.J., Newbold, J.R., Perdok, H.B. & Berends, H., 2011. Relationships between methane production and milk fatty acid profiles in dairy cattle. Anim. Feed Sci. Technol., 166–167, 590–595.

Dong, Y., Bae, H.D., McAllister, T.A., Mathison, G.W. & Cheng, K-J., 1997. Lipid-induced depression in methane production and digestibility in the artificial rumen system (RUSITEC). Can. J. Anim. Sci., 77, 269–278.

Ellis, J.L., Kebreab, E., Odongo, N.E., McBride, B.W., Okine, E.K. & France, J., 2007. Prediction of methane production from dairy and beef cattle. J. Dairy Sci., 90, 3456–3467.

Eugène, M., Massé, D., Chiquette, J. & Benchaar, C., 2008. Meta-analysis on the effects of lipid supplementation on methane production of lactating dairy cows. Can. J. Anim. Sci., 88, 331–337.

Faithfull, N.T., 2002. Methods in Agricultural Chemical Analysis: a Practical handbook, CABI Publishing, UK, 266 pp.

Giger-Reverdin, S., Morand-Fehr, P. & Tran, G., 2003. Literature survey of the influence of dietary fat composition on methane production in dairy cattle. Livest. Prod. Sci., 82, 73–79.

Glasser, F., Ferlay, A. & Chilliard, Y., 2008. Oilseed lipid supplements and fatty acid composition of cow milk: A meta-analysis. J. Dairy Sci., 91, 4687–4703.

Gonthier C., Mustafa, A.F., Berthiaume, R., Petit, H.V., Martineau, R. & Ouellet, D.R., 2004. Effects of feeding micronized and extruded flaxseed on ruminal fermentation and nutrient utilization by dairy cows. J. Dairy Sci., 87, 1854–1863.

Gonthier C., Mustafa, A.F., Ouellet, D.R., Chouinard, P.Y., R Berthiaume, R. & Petit, H.V., 2005. Feeding micronized and extruded flaxseed to dairy cows: Effects on blood parameters and milk fatty acid composition. J. Dairy Sci., 88, 748–756.

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

Kennelly, J.J., 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim. Feed Sci. Technol., 60, 137–152.

Kirchgessner M., Windisch W. & Müller H.L., 1995. Nutritional factors for the quantification of methane production. In: Ruminant physiology: Digestion, metabolism, growth and reproduction, Proceedings of the 8th International Symposium on Ruminant Physiology (eds. Engelhardt, Leonhard-Marek, Breves & Giesecke), Ferdinand Enke Verlag, Stuttgart, 1995, pp. 333–348.

Martin, C., Rouel, J., Jouany, J.P., Doreau, M. & Chilliard, Y., 2008. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. J. Dairy Sci., 86, 2642–2650.

Meale, S.J., McAllister, T.A., Beauchemin, K.A., Harstad, O.M. & Chaves, A.V., 2013. Strategies to reduce greenhouse gases from livestock. Acta Agric Scand., Section A – Anim. Sci., 62, 199–211.

Mills, J., Dijkstra, J., Bannink, A., Caunnell, S.B., Kebreab, E. & France, J., 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: Model development, evaluation, and application. J. Anim. Sci., 79, 1584–1597.

Mills, J., Kebreab, E., Yates, C.M., Crompton, L.A., Caunnell, S.B., Dhanoa, M.S., Agnew, R.E. & France, J., 2003. Alternative approaches to predicting methane emissions from dairy cows. J. Anim. Sci., 81, 3141–3150.

Montoya, J.C., Bhagwat, A., Peiren, N., De Campeneere, S., De Baets, B. & Fievez, V., 2011. Relationships between odd- and branched-chain fatty acid profiles in milk and calculated Enteri methane proportion for lactating dairy cattle. Anim. Feed Sci. Technol., 166, 596–602.

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

SAS Institute, 2003. SAS Online DOC, version 9.1. SAS Institute Inc, Cary, NC.

Wilkerson, V.A., Casper, D.P.& Mertens, D.R., 1995. The prediction of methane production of Holstein cows by several equations. J. Dairy Sci., 78:2402–2414.

Yates, C.M., Cammell, S.B., France, J. & Beever, D.E., 2000. Prediction of methane emissions from dairy cows using multiple regression analysis. Proc. Br. Soc. Anim. Sci., 94.

Effects of concentrate feeding strategies on performance of dairy bulls

K. Manni[1,2], M. Rinne[2] & A. Huuskonen[3]
[1]*HAMK University of Applied Sciences, 31310 Mustiala, Finland,* [2]*MTT Agrifood Research Finland, Animal Production Research, 31600 Jokioinen, Finland,* [3]*MTT Agrifood Research Finland, Animal Production Research, 71750 Maaninka, Finland,*
Correspondence: katariina.manni@hamk.fi

Introduction

The main factor that affects the profitability of beef production is feeding costs and animal performance. The concentrate feeding strategy may affect substantially animal performance (Keane et al., 2006; Huuskonen et al., 2007) and thus the efficiency of beef production. High levels of concentrates are typically used in intensive beef production to achieve high growth rate and carcass conformation. However, good quality silage alone can support high level of performance of growing cattle (Randby et al., 2010). There is also a possibility to divide the growth into periods by restricting feed intake during part of the growing period. In that case growth rate of animals can be manipulated, and compensatory growth can be induced after feed restriction with possible benefits in feed efficiency (Hornick, et al., 2000). In this work the effects of concentrate feeding strategies on performance of dairy bulls was studied.

Materials and methods

The feeding experiment comprised in total 36 dairy bulls, 20 Holstein-Friesian and 16 Nordic Red. The bulls were taken into the experiment at an average live weight (LW) of 230 (s.d. ±36.9) kg and an average age of 200 (±24.9) days. In the beginning of the experiment, the bulls were divided into nine blocks of four animals each by LW and breed so that there were five Holstein-Friesian blocks and four Nordic Red blocks. Within the block, the bulls were randomly allotted to one of the four treatments. During the experiment, three animals were eliminated due to reasons unrelated to the treatments.

During the feeding experiment the animals were housed in a tie stall barn. They were fed *ad libitum* (proportionate refusals of 5%) either grass silage alone or a total mixed ration (TMR), individually. The silages were prepared from primary growth of timothy stands, harvested at early heading stage of timothy using a mower conditioner, wilted for 5 h and harvested using a precision-chop forage harvester. Silages were ensiled in bunker silos and treated with a formic acid based additive applied at a rate of 5 l/tonne of fresh forage. The concentrate used was rolled barley. The feeds were analysed as described by Huuskonen (2013). Silage fermentation quality was analysed by electrometric titration as described by Moisio and Heikonen (1989). Concentrations of metabolizable energy (ME), amino acids absorbed from the small intestine (AAT) and protein balance in the rumen (PBV) values were calculated according to the Finnish feed evaluation system (MTT 2014). The whole experimental period, 12 months, was divided into two parts, early and late, both lasting for 6 months. The four dietary treatments were:

1. GS: Grass silage alone during the whole experimental period

2. SC: Steady concentrate allowance. TMR contained grass silage (700 g/kg dry matter (DM)) and barley (300 g/kg DM) during the whole experimental period.

3. IC: Increased concentrate allowance. Grass silage alone during the early part of the experiment and then TMR containing grass silage (400 g/kg DM) and barley (600 g/kg DM) during the late part of the experiment.

4. DC: Decreased concentrate allowance. TMR contained grass silage (400 g/kg DM) and barley (600 g/kg DM) during the early part of the experiment and then grass silage alone during the late part of the experiment.

The target average slaughter age was 560–570 days. After slaughter the carcasses were weighed hot and classified for conformation and fatness using the EUROP quality classification (EC 2006). 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. Carcass gain was calculated as the difference between the final cold carcass weight and the carcass weight in the beginning of the experiment divided by the number of growing days. Carcass weight in the beginning of the experiment was assumed to be 0.50 × initial LW.

The results are shown as least squares means. The normality of analysed variables was checked using graphical methods: box-plot and scatter plot of residuals and fitted values. The data was subjected to analysis of variance using the SAS MIXED procedure (version 9.1, SAS Institute Inc., Cary, NC). Differences between the dietary treatments were tested using three orthogonal contrasts: 1. GS vs. others, 2. SC vs. IC + DC, and 3. IC vs. DC. Used limit for statistical significant differences was $P<0.05$ and $P<0.1$ was limit for tendency.

Results

The grass silage quality was good both in terms of feed values and preservation quality (pH 3.97, DM 234 g/kg, 161 g crude protein (CP), 556 g neutral detergent fibre (NDF), 55 g lactic and formic acid, 12 g volatile fatty acids, 50 g water soluble carbohydrates and 11.3 MJ ME per kg DM, and 52 g ammonia N per kg N. The barley had a typical chemical composition and feed values of 13.2 MJ ME and 120 g CP per kg DM.

The results are presented in Tables 1 and 2. Including concentrate in the diet increased total DM intake (DMI) during the whole experimental period from 7.97 to 8.55 kg/d ($P<0.01$) when GS was compared to other feeding groups, and subsequently increased ME ($P<0.001$), AAT ($P<0.001$) and starch ($P<0.001$) intake. Concentrate intake decreased NDF intake ($P<0.001$) and PBV ($P<0.001$). Removing concentrate from the diet either in the early (IC) or late (DC) part of the growing period, decreased DMI ($P<0.001$). The DMI during the total experimental period, was greater in IC compared to DC ($P<0.05$).

Live weight gain (LWG) of the bulls consuming silage alone in the diet was as high as 1119 g/d when the whole growing period was observed. Including concentrate into the diet (SC, IC and DC), increased daily growth rate further to 1222 g/d ($P<0.1$). The average concentrate intake (SC, IC and DC) during the whole growing period was 2.70 kg DM/d and growth response to 1 kg DM increased concentrate intake was 38 g/d.

Concentrate intake during the early part of growing period (SC and DC) increased growth rate 223 g/d numerically compared to bulls with silage as the sole feed (GS and IC). The daily amount of concentrate, 300 vs. 600 g /kg DM, did not differ numerically in growth rates.

Concentrate intake during only the late part of growing period (IC) increased daily growth rate numerically 332 g compared to the growth rate in early part without concentrate. In the other feeding groups (GS, SC and DC) during the late part of growing period growth rates decreased numerically compared with the growth during the early part. When IC was

compared to DC, concentrate inclusion during the late part of growing period increased total daily growth rate by 157 g/d ($P<0.05$).

Concentrate inclusion increased carcass weight by 25 kg ($P<0.05$) and dressing proportion by 12 g/kg ($P<0.05$), comparing GS with other feeding groups. Conformation score tended to increase ($P<0.1$) by concentrate inclusion but fat score was not affected, comparing GS with others. Comparing IC to DC, concentrate inclusion in the late part of growing period increased carcass weight ($P<0.05$) and fat score ($P<0.01$).

Concentrate inclusion improved feed conversion rate. Both DMI ($P<0.1$, $P<0.05$), ME ($P<0.05$, $P<0.05$) and CP consumption ($P<0.1$, $P<0.05$) per kg LWG and kg carcass gain decreased, respectively.

Discussion

It is well established that increasing concentrate allowance increases DM and ME intakes and growth rates (Steen and Kilpatrick, 2000). However, when using good quality silage (high digestibility and preservation quality), the growth response to increased concentrate intake generally declines because the differences in intake remain marginal (Steen et al., 2002). In the present experiment the grass silage was of high quality and bulls achieved high growth rate, 1119 g/d, when the silage was given as a sole feed. It seems that the young animals could not make use of the higher level of concentrate probably due to the good quality silage because the highest level of concentrate did not seem to increase the growth rate.

The increase in growth per 1 kg DM increase in concentrate intake is consistent with results of Huuskonen et al. (2007; 27 g/d) but there are also experiments where responses have been greater (Manni et al., 2013; 73 g/d, Martinsson et al., 1990; 84 g/d). In IC during compensatory growth, increase in growth per 1 kg DM increased concentrate intake was 53 g. Increased feed conversion rate (kg DM/kg LW and MJ ME/kg LW) by concentrate inclusion is consistent with results reported by Huuskonen et al. (2007).

When concentrate was included to the diet during the late part of growing period (IC), growth rate increased substantially compared to the period without concentrate or to the other groups during the late part of growing period (Figure 1). The faster growth rate can be attributed partly to the compensatory growth potential and partly to the higher concentrate proportion in the diet at that time. The animals in IC had capacity to catch up the growth during the late part of growing period that their weight at the end of the experiment was even slightly higher compared to other groups. This indicates that the bulls made use of the concentrate intake during the late part of growing period.

Concentrate inclusion increased carcass weight, dressing proportion and tended to improve carcass conformation which is consistent with many previous experiments (Keane et al., 2006, Keane and Fallon, 2001) but in contrast to Manni et al. (2013) and Huuskonen et al. (2007). Concentrate intake did not affect the carcass fat score, consistent with Randby et al. (2010) but in contrary to many other experiments such as Huuskonen et al. (2007) and Keane and Fallon (2001). Concentrate intake during the late period increased carcass weight, but also carcass fatness, although the carcass fat scores were in general low.

Usefulness of periodic growth rate depends on the conditions of the farm, the targets of production and the prices of feeds used.

Table 1 Feed, energy and nutrient intake of growing dairy bulls using different concentrate feeding strategies

	GS	SC	IC	DC	SEM	Contrasts (*P*-value)		
						1	2	3
Number of observations	8	7	9	9				
Dry matter intake								
Early part								
Silage, kg/day	7.09	5.30	6.84	3.08	0.121	<0.001	0.024	<0.001
Kg/day	7.09	7.56	6.84	7.69	0.154	0.108	0.111	<0.001
g/kg$^{0.60}$ live weight	236	218	225	202	6.6	0.007	0.518	0.009
Late part								
Silage, kg/day	8.84	6.51	4.64	8.91	0.192	<0.001	0.534	<0.001
Kg/day	8.84	9.31	10.93	8.91	0.270	0.005	0.064	<0.001
g/kg$^{0.60}$ live weight	221	203	216	212	10.0	0.306	0.373	0.757
Total experimental period								
Silage, kg/day	7.97	5.91	5.74	5.88	0.141	<0.001	0.563	0.411
Kg/day	7.97	8.44	8.90	8.30	0.194	0.010	0.487	0.020
g/kg$^{0.60}$ live weight	227	213	213	211	6.2	0.039	0.956	0.810
Nutrient intake (g/d)								
Metabolizable energy, MJ/d	89.8	99.9	106.2	98.3	2.29	<0.001	0.396	0.010
Crude protein	1277	1249	1274	1247	28.5	0.514	0.734	0.465
Neutral detergent fibre	4425	3798	3854	3740	87.7	<0.001	0.990	0.305
Starch	69	1484	1838	1419	37.5	<0.001	0.003	<0.001
AAT (g/d)	681	750	792	741	17.1	<0.001	0.427	0.024
PBV (g/d)	263	130	90	143	3.6	<0.001	0.005	<0.001

GS = Grass silage alone; SC = Steady concentrate, 300 g/kg dry matter, allowance; IC = Increased concentrate allowance (first no concentrate, then concentrate 600 g/kg dry matter); DC = Decreased concentrate allowance (first concentrate 600 g/kg dry matter, then no concentrate); SEM = Standard error of the mean; Contrasts: 1 = GS vs. others; 2 = SC vs. IC + DC; 3 = IC vs. DC; AAT = Amino acids absorbed in the small intestine; PBV = Protein balance in the rumen.

Conclusions

Although good quality silage as the sole feed may support high levels of performance of growing cattle, including concentrate into the diet may improve performance of animals. Periodic concentrate allocation demonstrates the ability of growing bulls to adapt to different feeding regimes without affecting their performance.

Table 2 Growth performance, carcass characteristics and feed conversion of growing dairy bulls using different concentrate feeding strategies

	GS	SC	IC	DC	SEM	Contrasts (*P*-value)		
						1	2	3
Number of observations	8	7	9	9				
Live weight gain (LWG; g/d)								
Early part	1153	1378	1149	1370	71.3	0.068	0.172	0.019
Late part	1086	1009	1481	945	63.1	0.388	0.011	<0.001
Total experimental period	1119	1194	1315	1158	52.9	0.082	0.496	0.025
Carcass gain, g/d	580	642	697	621	29.3	0.028	0.618	0.048
Carcass weight, kg	324	341	366	339	8.6	0.013	0.281	0.018
Dressing proportion, g/kg	504	518	514	516	5.4	0.050	0.583	0.735
Carcass qualification								
Conformation score, EUROP	4.5	5.1	5.2	4.7	0.24	0.068	0.630	0.169
Fat score, EUROP	2.7	3.0	3.2	2.5	0.19	0.382	0.639	0.008
Feed conversion								
Kg DM/kg LWG	7.82	7.12	6.58	7.13	0.426	0.064	0.609	0.306
Kg DM/kg carcass gain	15.1	13.2	12.4	13.4	0.81	0.022	0.789	0.350
MJ ME/kg LWG	93	84	77	83	5.2	0.047	0.558	0.297
MJ ME/kg carcass gain	179	155	145	156	9.7	0.014	0.716	0.335
g crude protein / kg LWG	1161	1069	996	1069	61.5	0.090	0.622	0.349
g crude protein / kg carcass gain	2243	1977	1881	2010	122.2	0.038	0.831	0.404

GS = Grass silage alone; SC = Steady concentrate, 300 g/kg dry matter allowance; IC = Increased concentrate allowance (first no concentrate, then concentrate 600 g/kg dry matter); DC = Decreased concentrate allowance (first concentrate 600 g/kg dry matter, then no concentrate); SEM = Standard error of the mean; Contrasts: 1 = GS vs. others; 2 = SC vs. IC + DC; 3 = IC vs. DC; Carcass conformation score: 1 = poor, 15 = excellent; Carcass fat score: 1 = low, 5 = very high.

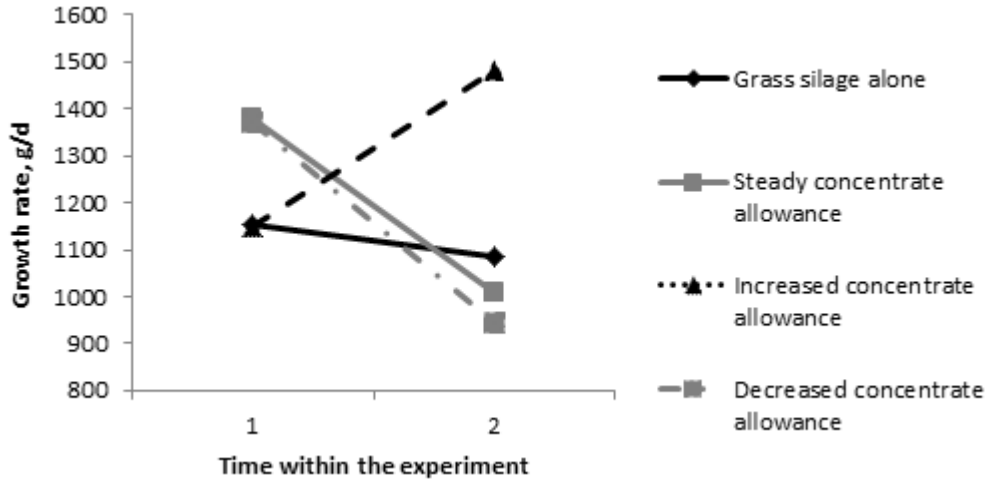

Figure 1 Growth rates of dairy bulls during early (1) and late (2) part of growing period given either silage alone or concentrate allowance being steady, increased or decreased.

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.

Hornick, J.L., Van Eenaeme, C., Gérard, O., Dufrasne, I. & Istasse, L., 2000. Mechanisms of reduced and compensatory growth. 2000. Domest. Anim. Endocrinol. 19, 121-132.

Huuskonen, A., 2013. Performance of growing and finishing dairy bulls offered diets based on whole-crop barley silage with or without protein supplementation relative to a grass silage-based diet. Agric Food Sci., 22, 424-434.

Huuskonen, A., Khalili, H., Joki-Tokola, E., 2007. Effects of three different concentrate proportions and rapeseed meal supplement to grass silage on animal performance of dairy-breed bulls with TMR feeding. Livest. Sci. 110, 154-165.

Keane, M.G., Drennan, M.J. & Moloney A.P., 2006. Comparison of supplementary concentrate levels with grass silage, separate or total mixed ration feeding, and duration of finishing in beef steers. Livest. Sci. 103, 169–180.

Keane, M.G. & Fallon, R.J., 2001. Effects of feeding level and duration on finishing performance and slaughter traits of Holstein-Friesian young bulls. Ir. J. Agric. Food Res. 40, 145-160.

Manni, K., Rinne, M. & Huhtanen, P., 2013. Comparison of concentrate feeding strategies for growing dairy bulls. Livest. Sci. 152, 21-30.

Martinsson, K., 1990. The effects of forage digestibility and concentrate supplementation on performance of finishing bulls. Swed. J. Agric. Res. 20, 161-167.

Moisio, T. & Heikonen, M., 1989. A titration method for silage assessment. Anim. Feed Sci. Technol. 22, 341–353.

Randby, Å.T., Nørgaard, P. & Weisbjerg, M.R., 2010. Effect of increasing plant maturity in timothy-dominated grass silage on the performance of growing/finishing Norwegian Red bulls. Grass Forage Sci. 65, 273-286.

Steen, R.W.J. & Kilpatrick, D.J., 2000. The effects of the ratio of grass silage to concentrates in the diet and restricted dry matter intake on the performance and carcass composition of beef cattle. Livest. Prod. Sci. 62, 181-192.

Steen, R.W.J., Kilpatrick, D.J. & Porter, M.G., 2002. Effects of the proportions of high or medium digestibility grass silage and concentrates in the diet of beef cattle on liveweight gain, carcass composition and fatty acid composition of muscle. Grass Forage Sci. 57, 279-291.

Effects of supplementary concentrate level and separate or total mixed ration feeding on performance of growing dairy bulls

M. Pesonen, E. Joki-Tokola & A. Huuskonen
MTT Agrifood Research Finland, Animal Production Research, Tutkimusasemantie 15, FI-92400 Ruukki, Finland.
Correspondence: Maiju.Pesonen@mtt.fi

Introduction

Beef production in Finland is based mainly on raising dairy bulls born on dairy farms. In the past, bulls fed on forage plus concentrates were generally offered their concentrate allowance once or twice daily separately from the forage. Recently, many beef producers have changed to using total mixed rations (TMR) to save labour and mechanise feeding. The rationale for TMR feeding is to achieve a relatively stable rumen pH and fermentation pattern throughout the day which would facilitate better cellulose digestion and a higher lipogenic to non-lipogenic volatile fatty acid ratio (Kaufmann 1976). Relative to dairy cows, there are only few reports in the literature comparing TMR and separate feedings in growing and finishing cattle. Nevertheless, Caplis et al. (2005) concluded that TMR-feeding had no effect on growth performance or carcass traits compared with separate feeding in finishing crossbred steers even though TMR-feeding increased silage and total dry matter (DM) intake. Keane et al. (2006) reported that feeding a TMR increased intake at the low (375 g/kg DM) but not at a high (750 g/kg DM) concentrate level compared to separate feeding in beef steers but feeding method had no effects on overall live weight gain (LWG) or slaughter traits. The interest of the present study is to obtain information concerning animal performance when growing dairy bulls are fed either TMR or separate diet with two different concentrate levels.

Materials and Methods

A feeding experiment was conducted in the experimental barn of MTT Agrifood Research Finland in Ruukki, Finland. A 2×2 factorial design was used to study the effects on animal performance of (1) the increasing concentrate level, and (2) feeding grass silage and concentrates separately or as a total mixed ration. A feeding experiment comprised in total 32 Nordic Red bulls. At the beginning of the feeding experiment the bulls with average live weight (LW) of 145 kg were divided into eight blocks of four animals by LW. Within the block, the bulls were randomly allotted to one of the four feeding treatments (8 bulls per treatment). The four feeding treatments were: (1) grass silage (660 g/kg dry matter) plus medium level of rolled barley (330) offered separately, (2) grass silage (660) plus medium level of rolled barley (330) offered as TMR, (3) grass silage (330) plus high level of rolled barley (660) offered separately, and (4) grass silage (330) plus high level of rolled barley (660) offered as TMR. During the feeding experiment (398 days) the bulls were fed *ad libitum* (proportionate refusals of 5%) either grass silage or TMR. The daily ration for all bulls included also 150 g of a mineral mixture. A vitamin mixture was given 50 g/animal weekly. The bulls were placed in an insulated barn in adjacent tie-stalls and were individually fed three times per day (at 0800, 1200 and 1800 hours). Refused feed was collected and weighed at 0700 daily. One bull (separate feeding, high level of rolled barley) was excluded from the study due to hoof problems.

The grass silage used in the experiment was from mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) stand and was cut using a mower conditioner, wilted for 5 h, and harvested using a precision-chop forage harvester. The grass silage was ensiled in

bunker silos and treated with a formic acid-based additive (760 g formic acid/kg, 55 g ammonium formate/kg) applied at a rate of 5 litres/tonne of fresh grass. 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), ether extracts, 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 Huuskonen (2013). Concentrate sub-samples were collected weekly, pooled over periods of 12 weeks and analysed for DM, ash, CP, NDF and ether extracts. The metabolisable energy (ME) concentration of the silages was calculated as 0.016 × D-value. The ME concentrations of the concentrate feeds were calculated based on concentrations of digestible crude fibre, CP, crude fat and nitrogen-free extract described by MTT (2014). The digestibility coefficients of the concentrates were taken from the Finnish Feed Tables (MTT 2014). Amino acids absorbed from small intestine (AAT) and protein balance in the rumen (PBV) values were calculated according to the Finnish feed protein evaluation system (MTT 2014).

The bulls were weighed on two consecutive days at the beginning of the experiment and thereafter single weightings were conducted at approximately every 28 days. Before slaughter, the bulls were weighed on two consecutive days. The target for average carcass weight was 300 kg. Slaughter time determined based on LW and assumed dressing proportion (0.52 g/kg) which was assessed based on earlier studies with Nordic Red bulls (Huuskonen et al., 2007; Manni et al., 2013). The bulls were slaughtered in the Atria Ltd. commercial slaughterhouse in Kuopio, Finland. 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). The study was set up according to a complete randomized block design with animal as an experimental unit. The results are shown as least squares means. The 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

$$y_{ijkl} = \mu + B_k + F_i + C_j + FC_{ij} + e_{ijkl}$$

where μ is the overall mean, B_k is blocking effect (k=1,…,8) and e_{ijkl} is the random error term. F_i (i=1,2), C_j (j=1,2) and FC_{ij} are the effects of feeding method (TMR, separate), concentrate level (330, 660 g/kg DM) and their interaction, respectively.

Results and Discussion

The chemical compositions and nutritional values of the experimental feeds and total mixed rations used in the present experiment are given in Table 1.The grass silage used was of reasonably good nutritional quality as indicated by the D value as well as the AAT and CP contents. The fermentation quality of the grass silage was also good as indicated by the pH value and the low concentration of ammonia N and total acids (Table 1). Barley grain used in the experiment had typical chemical composition and feed values. Because of the higher energy content of barley grain compared to the grass silage, increasing the concentrate proportion increased the calculated energy value of the ration (Table 2). Increasing the proportion of the concentrate also increased the DM content, but decreased the NDF content of the ration.

Table 1 Chemical composition and feeding values of the grass silage, barley grain and total mixed rations (TMR) used in the feeding experiment.

	Grass silage	Barley grain	TMR 330[a]	TMR 660[b]
Dry matter (DM), g/kg feed	242	917	321	476
Organic matter (OM), g/kg DM	919	975	937	955
Crude protein, g/kg DM	135	120	130	125
Neutral detergent fibre (NDF), g/kg DM	538	205	427	316
Ether extract, g/kg DM	46	22	38	30
Metabolisable energy, MJ/kg DM	10.7	12.9	11.4	12.2
AAT[c], g/kg DM	79	97	85	91
PBV[d], g/kg DM	16	-25	2	-11
Digestible OM in DM, g/kg DM	669	ND[e]		
Fermentation quality of the grass silage				
pH	3.72			
Volatile fatty acids, g/kg DM	18			
Lactic + formic acid, g/kg DM	47			
In total N, g/kg				
NH_4-N	39			
Soluble N	420			

[a] TMR 330 = concentrate level 330 g/kg dry matter. [b] TMR 660 = concentrate level 660 g/kg dry matter. [c] AAT = Amino acids absorbed from small intestine. [d] PBV = Protein balance in the rumen. [e] ND = Not determined.

There were no significant interactions for intake parameters between feeding method and concentrate level (Table 2). TMR-feeding increased total DM intake (DMI), energy intake and CP intake of the bulls by 10, 9 and 10%, respectively, compared to separate feeding (P<0.001). Concentrate level had no significant effects on DM or CP intake but the increasing concentrate allowance increased energy intake 11% (P<0.01) (Table 2).

A positive effect of TMR-feeding on DMI was also noted previously in finishing steers (Caplis et al., 2005; Keane et al., 2006) and heifers (Cooke et al., 2004). Earlier, Petchey and Broadbent (1980) compared separate or mixed feeding of silage and concentrates for finishing Friesian steers at silage:concentrate ratios ranging from 0 to 1.0. Mixing increased DMI 9% with no evidence of an interaction between feeding method and concentrate level (Petchey and Broadbent, 1980). It is suggested that some intake increases due to TMR feeding can be explained by the rejection of unpalatable feeds in unmixed rations, something which is not possible in TMR-feeding (Phipps et al., 1984). In another case, DMI increases could be due to the extra processing that occurs during the mixing of the TMR or to the fact that the whole diet is constantly available (Caplis et al., 2005).

It is well established that increasing concentrate allowance decreases silage intake and subsequently increases ME intake (e.g. Randby et al. 2010; Manni et al. 2013) as in the present study. In the present experiment the concentrate proportion had no significant effects on the total DMI which is in line with results by Huuskonen et al. (2007) with dairy bulls fed grass silage-barley-based rations. However, in many feeding experiments increasing concentrate level has also increased total DMI to some extent (Randby et al. 2010; Manni et al. 2013). The substitution rate (SR, decrease in silage DMI /kg increase of concentrate DMI) in the current experiment was 0.94 and 0.84 for TMR and separate feedings, respectively. This is in line with SR's observed at grass silage-based feedings reported by Keane (2010) with crossbred steers (0.82), Randby et al. (2010) with dairy bulls (0.75) and Manni et al. (2013) with dairy bulls (0.81).

Table 2 Intake, growth performance and carcass characteristics of the bulls fed either separate or total mixed ration (TMR) feeding with two different concentrate levels.

Feeding method (F)	TMR		Separate			*P*-value		
Concentrate level (C) (g/kg DM)	330	660	330	660	SEM[a]	F	C	F×C
Number of observations	8	8	8	7				
Duration of the experiment, d	398	398	398	398				
Initial live weight, kg	145	146	145	145	2.6	0.86	0.82	0.98
Intake								
Dry matter (DM), kg/d	7.78	7.95	6.93	7.33	0.178	<0.001	0.11	0.48
DM intake, g/kg$^{0.60}$ live weight	228	229	206	211	3.3	<0.001	0.30	0.64
Metabolisable energy (ME), MJ/d	89.0	97.3	80.2	90.5	2.12	<0.001	<0.001	0.62
Crude protein, g/d	1000	988	896	912	22.8	<0.001	0.94	0.52
Final live weight, kg	576	590	557	597	13.8	0.56	0.052	0.33
Live weight gain, g/d	1083	1117	1036	1136	33.1	0.56	0.045	0.30
Carcass gain, g/d	580	609	522	631	19.6	0.22	0.002	0.042
Feed conversion								
Kg dry matter/kg LWG	7.19	7.14	6.73	6.47	0.138	<0.001	0.27	0.43
Kg dry matter/kg carcass gain	13.44	13.09	13.46	11.64	0.494	0.19	0.034	0.13
MJ ME/kg LWG	82.3	87.5	77.9	79.9	1.63	0.048	0.026	0.31
MJ ME/kg carcass gain	153.8	160.3	155.9	143.7	5.85	0.24	0.66	0.10
Carcass characteristics								
Carcass weight, kg	303	315	280	324	8.1	0.22	0.002	0.048
Dressing proportion, g/kg	527	534	505	542	10.6	0.39	0.042	0.16
Conformation score, EUROP[b]	4.88	5.00	4.38	4.99	0.257	0.25	0.15	0.32
Fat score, EUROP[c]	2.50	2.63	2.38	2.26	0.180	0.17	0.94	0.49

[a] SEM = Standard error of mean. [b] Conformation: (1 = poorest, 15 = excellent). [c] Fat score: (1 = leanest, 5 = fattest).

There were no significant interactions for final LW or LWG between feeding method and concentrate level (Table 2). Feeding method had no effect on LWG but increasing concentrate level led to a 6% improvement of daily LWG ($P<0.05$). Carcass gain was not affected by feeding method but increasing concentrate allowance improved it 13%, on average ($P<0.01$). There was also a significant interaction ($P<0.05$) between feeding method and concentrate level for carcass gain. Carcass gain improved more with separate feeding than with TMR-feeding when concentrate level was increased (Table 2).

Consistent with the findings of Petchey and Broadbent (1980), Caplis et al. (2005) and Keane et al. (2006) there was no effect of mixing on LWG. On the contrary, Cooke et al. (2004) reported that mixing of maize silage, grass silage, straw and concentrates resulted in a LWG response of proportionately 0.15 for an intake increase of proportionately 0.04. A possible explanation for the difference between the other findings and those of Cooke et al. (2004) may be that the forage used by Cooke et al. (2004) included maize silage, straw and grass silage whereas in other studies only grass silage was used. Regarding dairy cows, Yan et al. (1998) have noted that when a benefit was obtained to TMR-feeding, forages other than grass silage were offered.

As in the present study, increasing concentrate allowance has improved growth in several feeding experiments (e.g. Huuskonen et al. 2007; Manni et al. 2013). In the present study observed increase in LWG was 13 and 39 g/d per kg increase in concentrate DMI for TMR and separate feedings, respectively. This is roughly consistent with Huuskonen *et al.* (2007) who reported an increase of 27 g/d in LWG per kg increase in concentrate DMI. There were no significant interactions for feed or energy conversion between feeding method and concentrate level (Table 2). However, due to differences in intake and growth parameters, there were differences between feeding treatments. Both feed (kg DM/kg LWG) and energy (MJ ME/kg LWG) conversion rates improved in separate feeding compared to TMR-feeding. However, there were no significant differences between feeding methods when calculated per carcass gain (Table 2). Furthermore, feed conversion (kg DM/kg carcass gain) improved and energy conversion (MJ ME/kg LWG) declined with increasing concentrate proportion ($P<0.05$).

The carcass weight of the bulls was 305 kg, on average, and close to pre-planned. Feeding method had no effect on carcass weight but increasing concentrate allowance increased it by 9% ($P<0.01$). There was also a significant interaction ($P<0.05$) between feeding method and concentrate level for carcass weight. There were no interactions for dressing proportion, carcass conformation score or carcass fat score between feeding method and concentrate level. Feeding method had no effect on dressing proportion, conformation score or fat score. Concentrate level had no significant effects on conformation or fat score but increasing concentrate allowance increased dressing proportion by 4% ($P<0.05$). As in the present experiment Caplis et al (2005) and Keane et al. (2006) observed that feeding method (separate vs. TMR) had no effects on slaughter traits of growing and finishing cattle. The increasing effect of concentrate level on dressing proportion agrees with previous reports (Caplis et al. 2005, Keane et al. 2006). In the present experiment, increasing concentrate proportion did not improve the carcass conformation, consistent with Huuskonen et al. (2007) and Randby et al. (2010) but contrary to Keane and Fallon (2001) and Caplis et al. (2005).

Conclusions

The present data showed that feeding a TMR increased feed intake of the bulls but had no effect on growth or carcass traits. Increasing concentrate proportion increased growth performance, carcass weight and dressing proportion of the bulls but had no effects on carcass conformation score or carcass fat score.

References

Caplis, J., Keane, M.G., Moloney, A.P. & O'Mara, F.P., 2005. Effects of supplementary concentrate level with grass silage, and separate or total mixed ration feeding, on performance and carcass traits of finishing steers. Ir. J. Agric. Food Res., 44, 27–43.

Cooke, D.W.I., Monahan, F.J., Brophy, P.O & Boland, M.P., 2004. Comparison of concentrates or concentrates plus forage in a total mixed ration or discrete ingredient format: effects on beef production parameters and on beef composition, colour, texture and fatty acid profile. Ir. J. Agric. Food Res., 43, 201–216.

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. The Official Journal of the European Union L, 214, 1–6.

Huuskonen, A., 2013. Performance of growing and finishing dairy bulls offered diets based on whole-crop barley silage with or without protein supplementation relative to a grass silage-based diet. Agric. Food Sci., 22, 424–434.

Huuskonen, A., Khalili, H. & Joki-Tokola, E., 2007. Effects of three different concentrate proportions and rapeseed meal supplement to grass silage on animal performance of dairy-breed bulls with TMR feeding. Livest. Sci., 110, 154–165.

Kaufmann, W., 1976. Influence of the composition of the ration and feeding frequency on pH regulation in the rumen and on feed intake in ruminants. Livest. Prod. Sci., 3, 103–114.

Keane, M.G., 2010 A comparison of finishing strategies to fixed slaughter weights for Holstein Friesian and Belgian Blue × Holstein Friesian steers. Ir. J. Agric. Food Res. 49, 41–54.

Keane, M.G., Drennan, M.J. & Moloney, A.P., 2006. Comparison of supplementary concentrate levels with grass silage, separate or total mixed ration feeding, and duration of finishing in beef steers. Livest. Prod. Sci., 103, 169–180.

Keane, M.G. & Fallon, R.J., 2001. Effects of feeding level and duration on finishing performance and slaughter traits of Holstein-Friesian young bulls. Ir. J. Agric. Food Res., 40, 145–160.

Manni, K., Rinne, M. & Huhtanen, P., 2013. Comparison of concentrate feeding strategies for growing dairy bulls. Livest. Sci. 152, 21–30.

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

Petchey, A.M. & Broadbent, P.J., 1980. The performance of fattening cattle offered barley and grass silage in various proportions either as discrete feeds or as a complete diet. Anim. Prod. 31, 251–257.

Phipps, R.H., Bines, J.A., Fulford, Rosemary J. & Weller, R.F., 1984. Complete diets for dairy cows: a comparison between complete diets and separate ingredients. J. Agric. Sci. Camb. 103, 171–180.

Randby, Å.T., Nørgaard, P. & Weisbjerg, M.R., 2010. Effect of increasing plant maturity in timothy-dominated grass silage on the performance of growing/finishing Norwegian Red bulls. Grass Forage Sci., 65, 273–286.

Yan, T., Patterson, D.C. & Gordon, F.J., 1998. The effect of two methods of feeding the concentrate supplement to dairy cows of high genetic merit. Anim. Sci. 67, 395–403.

Effect of salt addition to a barley concentrate on milking frequency, milk yield and feed intake in automatic milking systems

M. Johansen, M. Larsen, P. Lund & M.R. Weisbjerg
Aarhus University, AU Foulum, Department of Animal Science, P.O. Box 50, 8830 Tjele, Denmark
Correspondence: marianne.johansen@agrsci.dk

Introduction

In automatic milking systems (AMS) high amounts of concentrates are often offered in the milking robot to achieve a high number of voluntary visits. But high intake of concentrates in relative short time can affect the rumen environment negatively and increase the risk for production diseases as rumen acidosis (Krause and Oetzel, 2006). Therefore, it is interesting to investigate if some concentrates are so preferred that they increase the voluntary visits to the milking robot, which would open for a potential reduction in concentrate offer. A direct way to study this is by investigating cows' response to different concentrates offered in the milking robot on milking frequency, milk yield and feed intake. Most commercial compound concentrates have a content of salt, but little is known about the influence on feed preferences. Therefore, the objective of this study was to investigate how concentrates with different contents of salt offered in the milking robot affect milking frequency, milk yield and feed intake.

Materials and methods

The experiment was conducted at the Danish Cattle Research Centre (KFC), Foulum, Denmark, in a loose housing system with a DeLaval milking robot, to which the cows had free access. Forty-eight Jersey cows, 16 primiparous and 32 multiparous, were used for the experiment. The cows were 173 ± 128 (mean ± SD) days from calving at the beginning of the experiment. The cows were divided into 12 blocks according to parity and days from calving and randomly assigned to one of the four treatments in a 4x4 Latin square. Each period continued for one week without any adaptation, and in total the experiment lasted for four weeks. The four treatments were a standard pelletized robot concentrate with a content of 0.7 percent salt used as a control (CON) and pelletized barley with 0 (B0), 0.7 (B0.7) or 1.4 (B1.4) percent salt, respectively. Max concentrate offer was 3 kg daily, divided over daily robot visits according to time interval from last milking. Concentrate allowance was fed out at a rate of 0.4 kg/minute. At the feeding lane the cows had free access to a mixed ration (MR) composed of 31.5% grass silage, 25.5% maize silage, 11.2% rapeseed cake, 11.2% sugar beet pulp, 9.9% barley, 9.9% NaOH treated wheat, 0.7% minerals and vitamins and 0.1% salt on dry matter basis. Milking frequency, amounts of concentrates offered in the milking robot, orts of concentrates left, milk yield and feed intake of the MR were registered daily at cow level. Milk composition at each milking during 48 h was analyzed each week.

All statistical analyses were performed using repeated measures within the MIXED procedure of SAS (SAS® version 9.3; SAS, 2010). The model included cow nested in parity, treatment, parity (primi- or multiparous), day within period (day), and the two-way interactions between treatment and parity and between treatment and day. Measures within cow x treatment were handled as repeated measures with a compound symmetry (cs) covariance structure. The denominator degrees of freedom were estimated by the "KenwardRoger" method. Significance was determined by $P \leq 0.05$.

Results

One multiparous cow was removed from the data due to laminitis during the experiment. Weight of the cows was unaffected by the experiment, since the mean (±SD) weight at the beginning was 492.6 (±39.1) kg and at the end was 492.1 (±40.6) kg.

The milking frequency was unaffected by treatment, but multiparous cows had a higher milking frequency than primiparous cows (Table 1). The amount of concentrate offered in the robot was lower for the three barley treatments compared with the control treatment. There was an interaction between treatment and parity (P = 0.01) on orts of concentrates, as the primiparous cows had similar orts for B1.4 and the control treatment, whereas multiparous cows had 3.5 times higher orts for the B1.4 treatment compared with the control.

Table 1 Effect of treatments on milking frequency, amount of concentrate offered in the robot, amount of concentrate left in the robot, milk yield and intake of the mixed ration at the feeding lane

	Treatment[a]					P-value[b]				
	CON	B0	B0.7	B1.4	SEM[c]	Treat	Par	Treat x Par	Day	Day x Treat
Milking frequency (times/day)						0.97	<0.001	0.37	0.16	0.11
Primiparous	2.39	2.41	2.42	2.49	0.07					
Multiparous	3.10	3.15	3.08	3.03	0.05					
Amount offered (kg/day)						<0.001	0.20	0.56	0.27	0.006
Primiparous	2.83	2.71	2.61	2.66	0.05					
Multiparous	2.93	2.73	2.58	2.73	0.04					
Amount orts (kg/day)						0.05	0.01	0.01	0.36	0.48
Primiparous	0.09	0.18	0.13	0.09	0.05					
Multiparous	0.10	0.19	0.17	0.35	0.04					
Milk yield (L/day)						<0.001	<0.001	0.22	0.03	<0.001
Primiparous	19.3	17.9	18.2	18.4	0.27					
Multiparous	23.5	22.8	22.9	22.5	0.19					
ECM (L/day)						0.002	<0.001	0.18	0.03	<0.001
Primiparous	25.7	24.3	24.3	24.7	0.36					
Multiparous	31.4	30.8	30.9	30.2	0.26					
Intake of MR (kg DM/day)						0.12	<0.001	0.89	0.33	0.50
Primiparous	13.8	13.3	13.4	13.3	0.19					
Multiparous	17.7	17.3	17.5	17.4	0.14					

[a] CON: standard robot concentrate; B0: pelletized barley; B0.7: pelletized barley with 0.7% salt; B1.4: pelletized barley with 1.4% salt; [b] Treat.: treatments; Par.: parity; Day: day within period; [c] Standard error of the mean

The milk yield was influenced by treatment and parity (Table 1). Multiparous cows had a higher milk yield than primiparous cows and for both parities the milk yield was lower at the three barley treatments compared with the control treatment (P < 0.001). For energy corrected milk (ECM), based on the weekly milk analyses, the differences between the treatment means were as found for milk yield. Both for milk yield and ECM there was an interaction between

day within period and treatment (Table 1). At day one in the treatment period there was no difference in milk yield between treatments, but at day two and the following days, the milk yield was lower for the three barley treatments compared with the control treatment.

The feed intake of the MR at the feeding lane was unaffected by treatments (Table 1), but the multiparous cows had a higher feed intake than the primiparous cows.

Discussion

The content of Na in the MR was 3.1 g/kg DM (40% above the norm) to guarantee the requirement of Na to be fulfilled, by which possible effects would be due to taste and feed preference instead of unfulfilled requirements.

The milking frequency was unaffected by the treatments, which indicated that the cows' preferences for barley does not differ from their preference for the control concentrate, and moreover are not affected by salt addition. Barley is generally believed to be a feedstuff cows like to eat, and Klopfer et al. (1981) found barley to be preferred highest in a choice test comparing 20 different feedstuffs. In a choice test comparing pelletized barley with the same control concentrate as used in this experiment, the control concentrate was preferred highest (Primdal et al., 2014). This indicates that cows have high preferences for both barley and the control concentrate.

Addition of salt did not affect milking frequency, but the amount of orts left in the robot indicate that multiparous cows had lower preference for the pelletized barley with the high content of salt since they left more orts compared with the other treatments. In the choice test by Klopfer et al. (1981) salt blocks were preferred lowest, which indicate that cows do not prefer the taste of pure salt. Nombekela et al. (1994) found by testing preferences for the primary tastes sweet, salty, sour and bitter in total mixed rations, that the salty diet was ranked very low as the probability of the salty diet to be chosen first when all diets were presented was only 1%. Further, Nombekela et al. (1994) found that the daily dry matter intake was unaffected even though the most preferred diets one by one were removed. This shows that feedstuffs that have ranked low in a preference trial not necessarily influence the feed intake or the milking frequency, when the cows do not have a choice.

The amount of concentrate offered in the robot differed between treatments even though the milking frequency was unaffected. The average difference between the amounts offered was 0.2 kg/day, and Halachmi et al. (2005) found no effect at the milking frequency by reducing the amount of concentrates offered in the milking robot by 1.2-1.5 kg/day. The lower offer of concentrates could be a result of briefer visits in the robot on the test treatments compared with the control treatment, although not studied. The lower milk yield on the test concentrates compared with the control concentrate could partly be due to a lower intake of energy because of a lower offer of the test concentrates in the milking robot.

Conclusion

Milking frequency was not affected by the offer of different concentrates in the milking robot and no effects were found by salt addition to barley.

References

Halachmi, I., Ofir, S. & Miron, J., 2005. Comparing two concentrate allowances in an automatic milking system. Journal of Animal Science 80, 339-343.

Klopfer, F.D., Kilgour, R. & Matthews, L.R., 1981. Paired comparison analysis of palatabilities of twenty foods to dairy cows. Proceedings of the New Zealand Society of Animal Production 41, 242-247.

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

Nombekela, S.W., Murphy, M.R., Gonyou, H.W. & Marden, J.I., 1994. Dietary preferences in early lactation cows as affected by primary tastes and some common feed flavors. Journal of Animal Science 77, 2393-2399.

Primdal, L., Johansen, M. & Weisbjerg, M.R., 2014**.** Do dairy cows have preferences for different concentrate feeds? Animal Production Science [submitted, in process].

SAS, 2010. Statistical Analysis System. User's guide. SAS Institute Inc., Cary, NC.

The evaluation of efficiency of different mineral additives on milk yield and quality in dairy cows

V. Jokubauskienė, V. Špakauskas
Lithuanian University of Health Sciences, Tilzes str. 18, 47181 Kaunas, Lithuania
Correspondence: vaida.laukyte@gmail.com

Introduction

Scientific and technological progress in agriculture is of great importance to the performance of livestock. Higher milk yields, better quality of milk, higher gains and increased fertility rates are related with nutrition. A well balanced diet is one of the main factors affecting the health of livestock. A lot of trace elements supplements of various chemical compositions are created to improve performance of livestock. Various researchers (Griffiths et al., 2007; Karkoodi et al., 2012; Krys et al., 2009; Weiss et al., 2010) have tried to find out how trace minerals supplements impact on animal performance. The results have been diverse. This indicates that all factors, which are affecting the nutritional needs of organisms, are not fully understood. Therefore, the aim of our study was to evaluate the efficiency of different trace mineral additives on cows' milk yield and milk quality.

Materials and Methods

The experiment was carried out with cows in the dairy farm of Kaišiadoriai. Sixty clinically healthy cows were selected for the investigation taking into consideration time of calving, nutrition and state of health. The experimental cows were divided into three groups, of 20 cows each. Cows in the first groups were fed a ration with inorganic mineral additives (IMA), which was made from calcium, magnesium and potassium chlorides, sodium dihydrogen phosphate, cobalt carbonate, copper, zinc and manganese sulphates, potassium iodide, sodium selenite. The cows of the second group received a complexed mineral additive (CMA), which was made from inorganic (magnesium and potassium chlorides, sodium dihydrogencarbonate, manganese sulphate, potassium iodide and sodium selenite) and organic (zinc, copper and cobalt gluconates, and potassium lactate) salts. The last group – constituted the Control cows, which did not receive any mineral additives, only licks of sodium chloride. Tested minerals supplements were fed to the IMA and CMA cows every other day for 3 months at levels indicated in Table 1.

Samples of milk were taken once every month, during control milking. Amount of milk yield (kg/d), fats, proteins, lactose and somatic cell counts (SCC) in milk were determined once every month. Milk yield was measured on farm, and the indicators of composition and SCC were determined in the laboratory of 'Pieno tyrimai', by use of a Lacto Scope FTIR (FT1.0.2001) and Soma Scope (CA-3A4, 2004), respectively. Subclinical mastitis was detected by using the California Mastitis Test (CMT).

Results and Discussion

Treatment groups IMA and CMA produced more milk (4.84% and 6%, respectively) compared to the control cows. Cows, which received CMA, also produced more milk (1.25%), compared with cows in the IMA group (Fig. 1.).Milk fat contents of IMA, CMA and control cows ranged from 3.72 – 4.3%, 3.55 – 4.1% and 3.79 – 4.11%. Milk fat of IMA and CMA cows tended to

Table 1 The amount (g) of mineral additives fed to dairy cows once every day

The composition of IMA		**The composition of CMA**	
Calcium chloride	30.0 g	Calcium lactate, 19 proc.	28.00g
Magnesium chloride	5.00 g	Magnesium chloride	5.00g
Sodium dihydrogen phosphate	4.00 g	Sodium dihydrogen phosphate	4.00 g
Potassium chloride	0.50 g	Potassium chloride	0.50 g
Cobalt carbonate	0.03g	Cobalt gluconate, 12–13.5 proc.	0.03 g
Copper sulphate	0.48 g	Copper gluconate, 13 – 15 proc.	0.85 g
Zinc sulfate	1.66 g	Zinc gluconate, 12 – 14 proc.	3.86 g
Manganese sulphate	1.66 g	Manganese sulphate	1.66 g
Potassium iodide, stabilized	0.02 g	Potassium iodide, stabilized	0.02 g
Sodium selenite	0.01 g	Sodium selenite	0.01 g

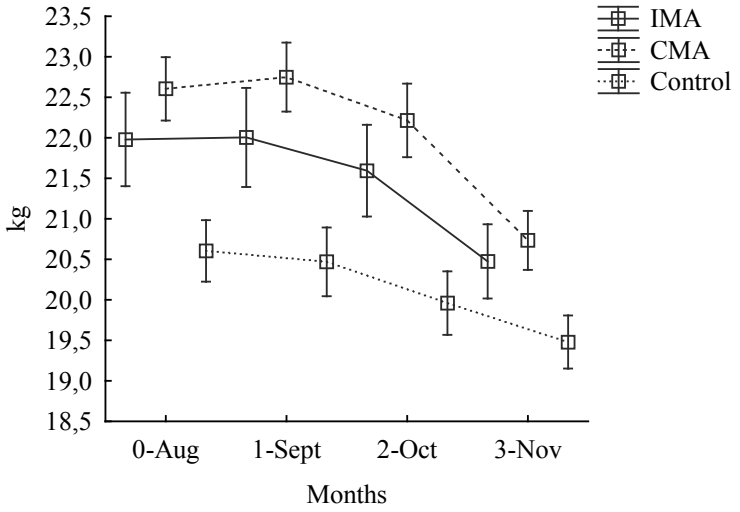

Figure1 The dynamic of milk yield of cows before and after supplementation. The milk yield of cows tended to decrease after 3 months following treatment. The cows in IMA and CMA group produced more milk compared to the control cows.

increase after two months following the feeding of mineral additives. Milk fat content of cows, which received IMA, was higher (4.09% ± 0.135; $P < 0.05$) compared with control cows (3.94 ± 0.072) and was 3.64% higher relative to cows, which received CMA ($P<0.05$) (Fig. 2).The amount of milk protein of treatment and control cows did not differ ($P>0,05$), but cows supplemented with CMA, produced more milk proteins (0,61%), compared with those receiving IMA (Fig. 3.). Milk lactose content of IMA, CMA and control cows ranged from 4.5 to 4.63, 4.3 – 4.68 and 4.43 – 4.60%, respectively. Milk lactose content tended to increase after 1, 2, 3 months following administration of CMA, and after 3 months, it was 1,08% higher compared with cows in the IMA group, $P > 0,05$.

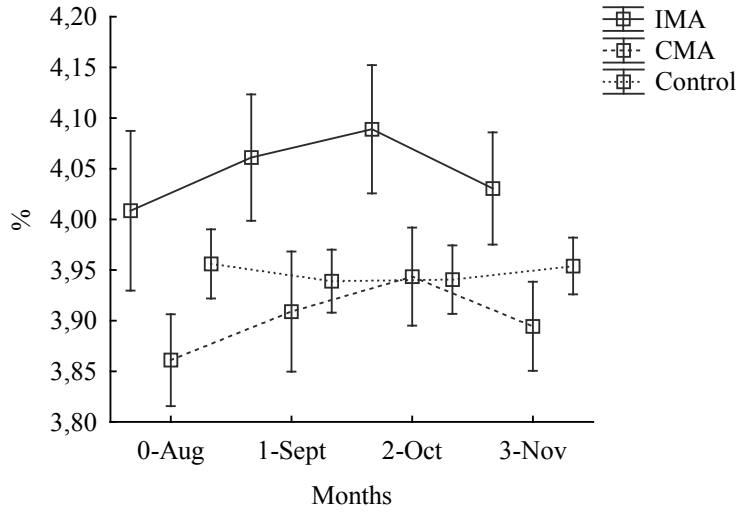

Figure 2 The dynamic of milk fats of cows before and after supplementation. Milk fat content of cows in IMA group was higher compared to the control and CMA cows.

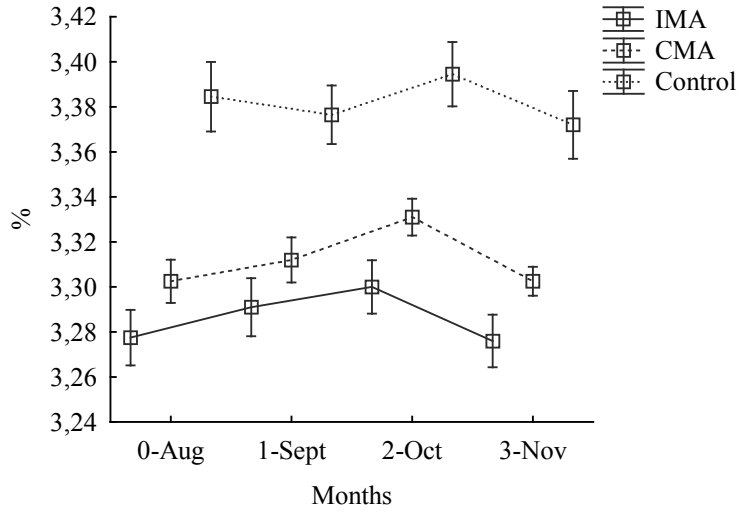

Figure 3 The dynamic of milk protein concentration of cows before and after supplementation. The amount of milk protein between treatment and control cows did not differ.

The least SCC in the milk of treatment cows was determined after 3 months, following administration of the mineral supplements. After 1 and 2 months, following administration of CMA, the SSC tended to decrease and was 8.7% and 10.7% lower than for the control cows and 13.8% and 23.29% lower than for cows on the IMA supplement.

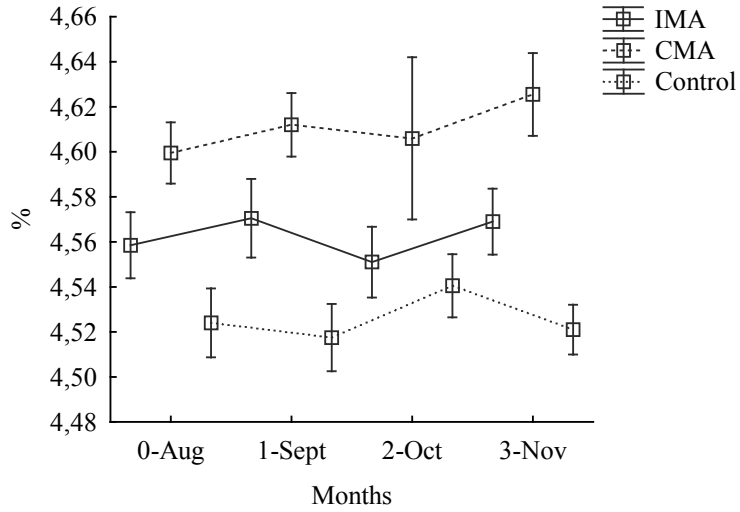

Figure 4 The dynamic of milk lactose of cows before and after supplementation. Milk lactose of cows of CMA and IMA groups tended to increase after 3 months following supplementation, compared with control cows.

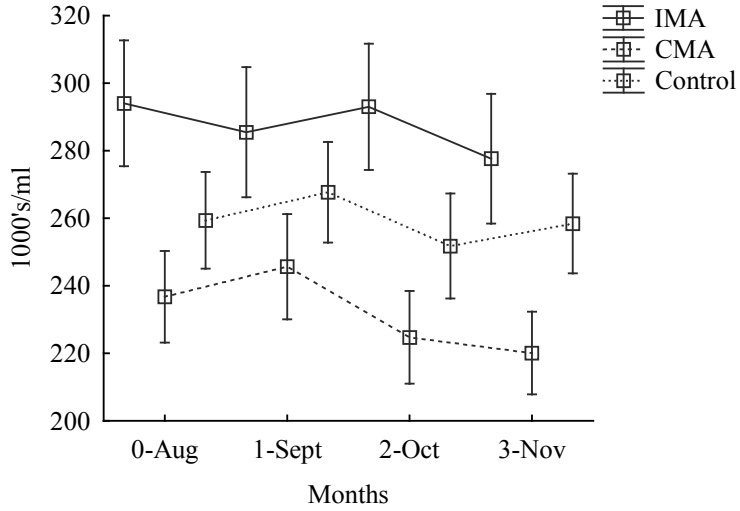

Figure 5 The dynamic of SCC in milk of cows before and after supplementation. The SCC in milk of treatment cows tended to decrease after 3 months following supplementation. Cows of CMA group had lower amount of SCC in milk compared to cows in IMA group.

Conclusions

Mineral additives have a positive effect on milk yield and milk quality in dairy cows. Complexed mineral additives seem to be more effective than inorganic mineral additives on productivity and milk quality of dairy cows.

References

Griffiths, L. M., Loeffler, S. H., Socha, M. T., Tomlinson, D. J. & Johnson, A. B., 2007. Effects of supplementing complexed zinc, manganese, copper and cobalt on lactation and reproductive performance of intensively grazed lactating dairy cattle on the South Island of New Zealand. Anim. Feed Sci. Technol., 137, 69–83.

Karkoodi, K. & Chamani, M., 2012. Effect of Organic Zinc, Manganese, Copper, and Selenium Chelates on Colostrum Production and Reproductive and Lameness Indices in Adequately Supplemented Holstein Cows. Biol. Trace Elem. Res., 146, 42 – 46.

Krys, S., Lokajová, E., Podhorský, A. & Pavlata, L., 2009. Microelement Supplementation in Dairy Cows by Mineral Lick. Acta Vet. Brno., 78, 29–36.

Weiss, W. P., Pinos-Rodríguez, J. M. & Socha, M. T., 2010. Effects of feeding supplemental organic iron to late gestation and early lactation dairy cows. J. Dairy. Sci., 93, 2153-2160.

Selenium supplementation by addition of sodium selenate with silage additive

A. Seppälä[1], Y. M. Albarran[2], H. Miettinen[3], M. P. Siguero[2], E. Juutinen[1,4] & M. Rinne[1]
[1]MTT Agrifood Research Finland, Animal Production Research, 31600 Jokioinen, Finland; [2]Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, Madrid Ciudad Universitaria s/n 28040 Madrid, Spain; [3]Kemira Oyj, P.O. Box 330, 00101 Helsinki, Finland, current: Nöykkiölaaksontie 27C6, 02330, Espoo [4]Current address: Atria Ltd, Finland
Correspondence: arja.seppala@mtt.fi

Introduction

Despite three decades of selenium enriched fertilizers in Finland, the problem with low selenium-bioavailability persists. Selenium content in agricultural soils in Finland has not changed significantly during those years and only 4% of the total selenium in the soil is available to plants (Eurola et al. 2011). Thus efforts should continue to prevent possible harmful consequences of low selenium bioavailability.

Although the most severe symptoms of selenium deficiency in cattle (white muscle disease) are quite rare nowadays (Eurola et al. 2011), the less-precise symptoms such as muscular weakness of the newborn, reduced weight gain, diarrhoea or decreased fertility (Koller and Exon 1986) may be linked to selenium deficiency. Further sufficient selenium is needed to ensure optimal udder health (Jukola et al. 1996). Smith et al. (1985, 1997) found over 30% reduction in the number of cases of clinical mastitis and nearly a 70% reduction in the number of high somatic-cell-count cows as a result of supplementation with Se and vitamin E. Flocks of dairy sheep having high incidence rates of clinical mastitis (> 10%) were found to have lower ($P<<0.001$) blood selenium and serum vitamin A concentrations than flocks with low rates of incidence of mastitis (<3%) (Giadinis et al. 2011). These authors suggested that the selenium status of ewes may possibly be used to indicate animals at risk of developing clinical mastitis (Giadinis et al. 2011). The low selenium content of cows' milk (less than 0.15 mg kg^{-1} DM) produced on organic farms compared to non-organic milk (average 0.23 mg kg^{-1} DM) reflects the low level of selenium in diets on those farms (Eurola et al. 2011). High cost of each case of clinical mastitis (270-670 € per case for dairy cows; Heikkilä et al. 2010) encourage to ensure sufficient selenium for lactating mammals.

Although selenium-enriched fertilizers exist they are not always used, as the benefit of selenium is coming indirectly via animal production. Furthermore, in organic farming, the use of compound fertilizers is not allowed. The selenium content of organic silages, or silages produced without compound fertilizers, was only one-tenth that found in conventionally produced silages from grassland fertilized with selenium: 0.02 vs. 0.2 mg Se kg^{-1} DM (Eurola et al. 2011). The selenium content of such silages is not sufficient to fulfill the selenium requirement of cattle (0.1 mg Se kg^{-1} DM according to MTT (2013) or 0.3 mg Se kg^{-1} DM for dairy cattle according to NRC (2001)). Organically produced cereal grains are also low in selenium, so additional selenium sources are needed to ensure sufficient selenium supply to livestock and humans.

Maintenance feeding of ruminants and horses is typically based on forage, typically supplemented with some minerals. Suckler cows and ewes may be fed forage as a sole energy source for more than half of the indoor-feeding period, typically until the last weeks of pregnancy when concentrates are normally added to the diet. The dosage of mineral feeds may be challenging if silage is fed *ad libitum* and minerals are offered separately without

individual control. It is difficult to ensure a sufficient, but not toxic, level of selenium in those situations if the forage itself is low in selenium. Supplementation of individual animals may also not be a practical solution in large herds, and therefore forage with sufficient selenium content would be needed, especially for these animal groups.

Silage additives are evenly sprayed on to the grass material prior to ensiling, and adding selenium to the silage additive might be a practical solution to ensure that the required dose of selenium is applied safely and evenly in the silage. An ensiling experiment was conducted to explore the effect of a sodium selenate-containing silage additive on the selenium content of silage and on the content of different forms of selenium.

Materials and Methods

The study was performed at MTT Agrifood Research Finland in Jokioinen in 2011. A timothy (Phleum pratense) -meadow fescue (Festuca pratensis) sward was fertilized using compound fertilizers without added selenate during summer 2011. The second cut was harvested on 2 August 2011 and prewilted for three hours before harvesting with a precision chopper.

Five batches of grass material were weighed and each batch was given the additive treatment according to the design of the experiment (Table 1). Each batch of grass was mixed manually during and after applying the additive. Three replicate silos (12 L, 7 kg grass per silo) were filled for each additive. The silos were opened and sampled after a 107-day ensiling period.

Table 1 Experimental treatments with additive dose level of 6 g kg^{-1}

Abbreviation	Composition	Added selenium to grass, mg kg^{-1}	
		Sodium selenate	Selenium
Control	Water	-	-
W50	Water with 50 mg kg^{-1} sodium selenate	0.30	0.125
A10	AIV Ässä* with 10 mg kg^{-1} sodium selenate	0.06	0.025
A50	AIV Ässä with 50 mg kg^{-1} sodium selenate	0.30	0.125
A500	AIV Ässä with 500 mg kg^{-1} sodium selenate	3.00	1.254

*Composition of AIV Ässä: 590 g formic acid, 200 g propionic acid, 45 g ammonium formate, 25 g benzoic acid/sorbate and 140 g water per kg.

The DM concentration was determined by drying at 105 °C for 16 h. Ash content was determined using a standard method of AOAC (1990; method 942.05). Nitrogen (N) content was determined by the Dumas method (AOAC method 968.06) using a Leco FP 428 nitrogen analyzer. Crude protein content was calculated as 6.25 × N content. Concentration of neutral detergent insoluble fibre (NDF) was determined according to Van Soest et al. (1991) using sodium-sulphite, without amylase and presented ash free.

Silages were analysed for volatile fatty acids (VFA) according to Huhtanen et al. (1998), lactic acid according to Haacker et al. (1983), water soluble carbohydrates (WSC) according to Somogyi (1945) and ammonia according to McCullough (1967). Oven DM concentration of silage was corrected for the loss of volatiles according to Huida et al. (1986).

The determination of the total selenium content was carried out by inductively coupled plasma mass spectrometry (ICP-MS) (HP 7700 Agilent Technologies, Santa Clara, California, United States) after a microwave acid digestion. Each dry sample (50 mg) was

weighed and 1 mL of nitric acid (650 g kg^{-1}) and 0.3 mL of hydrogen peroxide (350 g kg^{-1}) were added. The samples were subjected to acid digestion in a microwave oven (0-130°C for 15 min followed by 130°C for 10min). After digestion, the resulting extracts were diluted with Milli-Q water up to a final volume of 12 mL. Samples were analysed in triplicate. Identification of selenium species of silage samples was carried out by high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) (HP 7700 Agilent, Santa Clara, California, United States) after an enzymatic hydrolysis with protease. The recovery of selenium present in the samples after the enzymatic hydrolysis was more than 95%. The extraction of selenium species from 50 mg of dry sample was performed by 2 min sonication after the addition of 3 mL of buffer Tris-HCl (30mM, pH 7.5) and 0.020 mg of Protease type XIV isolated from Streptomyces griseus, ≥3.5 units/1mg solid (purchased from Sigma-Aldrich, St. Louis, USA). The extracts were centrifuged at 11000 rpm for 15 min and then filtered by means of using 0.20 µm nylon filters. The supernatants were analysed by anion exchange chromatography coupled to ICP-MS. The concentrations of selenium species were determined by monitoring 77Se, 78Se, and 82Se isotopes by comparison to known standards solutions. The identification was performed by comparing retention times with those of standard solutions. The standard included selenocysteine (SeCys2), seleno-methyl-selenocysteine (SeMetSeCys), selenite Se(IV), selenomethionine (SeMet) and selenate (Se(VI)).

Statistical analyses were performed using SAS GLM-procedure. Treatment effects on fermentation quality and selenium content of the silages were tested by analysis of variance and differences between treatments (lsmeans) were compared using the Tukey test.

Results and Discussion

The harvested grass prior to ensiling had a dry matter content of 283 g kg^{-1}. In the dry matter, the contents of ash, crude protein, WSC and NDF were 113, 175, 147 and 536 g kg^{-1}, respectively.

The formic acid-based silage additive 'AIV Ässä' restricted fermentation compared to the Control and W0 treatments, as AIV Ässä silages had more WSC, less fermentation products and less ammonium N than the silages without 'AIV Ässä' (Table 2). Selenium dosed within the additive was evenly sprayed on to the silage with the AIV Ässä product, as indicated by the total selenium content of the silages being close to the theoretical concentration (Table 3). The largest deviation (45%) was observed in the W50 treatment. Nearly all (95%) of the total selenium was detected as sodium selenate and no other forms were detected.

The Control silage had a total selenium content of 0.069 mg kg^{-1} DM (Table 3), which is clearly below the selenium requirement of cattle and sheep (0.1 mg Se kg^{-1} DM according to MTT (2013) or 0.3 mg Se kg^{-1} DM according to NRC for dairy cattle (2001)). The lowest level of addition of sodium selenate produced silage that had a content of selenium (0.159 mg kg^{-1}) that was sufficient to prevent deficiency symptoms in cattle. The intermediate level of addition resulted in silage that had selenium content 65% above the NRC (2001) recommendation for dairy cattle, but this was still lower than the highest permitted selenium content in feed. The selenium content should not exceed 0.5 mg kg^{-1} in a feed ration having 12% DM content (MMM 43/2005), which is equal to 0.568 mg kg^{-1} DM. The highest application level resulted in selenium content in silage that was 7.4-times higher than the maximum allowed level.

Table 2 Fermentation quality of the silages (units g kg^{-1} DM unless otherwise stated)

	Control	W50	A10	A50	A500	SEM	Treatment effect
Dry matter, g kg^{-1}	288	288	279	289	288	0.8	ns
pH	4.20^{b}	4.20^{b}	4.30^{a}	4.27^{ab}	4.32^{a}	0.018	<0.001
Lactic acid	99.7^{a}	97.8^{a}	61.2^{b}	66.5^{b}	41.2^{c}	2.29	<0.001
Acetic acid	21.18^{a}	21.07^{a}	9.65^{b}	9.51^{b}	9.29^{b}	0.458	<0.001
Propionic acid	0.21^{c}	0.15^{c}	2.94^{b}	2.89^{b}	4.14^{a}	0.211	<0.001
VFA4-VFA6[1]	0.28	0.33	0.16	0.13	0.4	0.087	ns
WSC[2]	14.0^{c}	14.1^{c}	62.4^{b}	60.5^{b}	80.1^{a}	2.38	<0.001
Ammonium N g kg^{-1} N	65.2^{a}	69.0^{a}	20.8^{b}	16.4^{b}	28.0^{b}	3.64	<0.001

[1]VFA4-VFA6 = the sum of butyric, isobutyric, valeric, isovaleric and caproic acids; [2]Water soluble carbohydrates; SEM=Standard error of the mean Treatment means within the same row but without the same superscript are statistically different (p<<0.05, Tukey test).

Table 3. Expected and analysed selenium content in the silages. Selenium content expressed as mg Se kg^{-1} DM. Treatments are explained in Table 1

	Control	W50	A10	A50	A500	SEM[4]	Treatment effect
Expected selenium content[1]	-	0.507	0.157	0.507	4.453		
Analysed total selenium[2]	0.069^{a}	0.920^{b}	0.159^{a}	0.496^{ab}	4.199^{c}	0.125	<0.001
Selenium detected as selenate[3]	0.000^{a}	0.667^{a}	0.156^{a}	0.486^{a}	4.457^{b}	0.193	<0.001

Treatment means within same row without same superscript are statistically different (p<<0.05, Tukey test); [1]Calculated as the sum of the selenium content of the Control silage and the amount of selenium theoretically provided with the additive treatment; [2]Determined by inductively coupled plasma mass spectrometry (ICP-MS); [3]Determined by high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry; [4]SEM=Standard error of the mean.

There are several aspects to be taken into account when optimizing the level of selenium inclusion in the silage additive. The inclusion should be sufficient for the selenium to have positive effects on animal health, but there is a need to ensure the amount is still safe, and takes account particularly of situations where the grass has also received selenium-enriched fertilizer. The optimization is complicated by the fact that the dosage recommendations of silage additives are typically expressed on fresh-matter basis and the DM content of the grass typically varies from 200 to 400 g kg^{-1}. Two possible scenarios were calculated assuming selenium content in grass either 0.03 or 0.37 mg kg^{-1} DM, DM in grass either 400 or 200 g kg^{-1} and additive dosage level 5 l/t. The lower level of inclusion (4.67 mg selenium kg^{-1} additive, 11 mg kg^{-1} sodium selenate) is sufficient to prevent selenium deficiency in cattle having silage as their sole feed, despite low inherent selenium content in the grass. The maximum allowed inclusion of selenium (6.61 mg selenium kg^{-1} additive, 15 mg kg^{-1} sodium selenate) was calculated by assuming low dry matter content of the grass and high inherent

selenium content. These two cases demonstrate that the selenium content of the silage additive can vary only within a relatively narrow range.

Conclusions

Addition of sodium selenate to silage additive provided a controlled way to add selenium to the diet of forage-fed animals. Silage additives were applied evenly to the material at ensilage so as to ensure successful ensiling; this would, at the same time, allow the possibility of distributing selenium evenly to animals consuming the silage without additional labour costs. The volume of added selenium was so small that it could be included in the acid-based additive without changing the dosage recommendations. The incremental addition of selenium into the additive could take place in the controlled environment of the factory, thus posing no danger to farmers or consumers. The level of selenium inclusion can be chosen so that selenium deficiency symptoms are prevented but without the risk of exceeding the highest allowed selenium content of the diet, taking account of grass material that has also received selenium enriched fertilizers.

References

AOAC, 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Inc., Arlington, VA. 1298 p.

Eurola, M., Alfthan, G., Ekholm, P., Root, T., Suoniitty, T., Venäläinen, E.-R. & Ylivainio, K., 2011. Seleenityöryhmän raportti 2011. MTT Raportti 35, 34 s.

Giadinis, N.D., Panousis, N., Petridou, E.J., Siarkou, V.I., Lafi, S.Q., Pourliotis, K., Hatzopoulou, E. & Fthenakis, G.C., 2011. Selenium, vitamin E and vitamin A blood concentrations in dairy sheep flocks with increased or low clinical mastitis incidence. Small Rumin. Res. 95, 193-196.

Haacker, K., Block, H.J. & Weissbach, F. 1983. Zur kolorimetrischen Milchsäurebestimmung in Silagen mit p-Hydroxydiphenyl. [On the colorimetric determination of lactic acid in silages with p-hydroxydiphenyl]. Arch.Tierernähr. 33, 505-512.

Heikkilä, A-M., Nousiainen, J. & Pyörälä, S. 2010. Kallis utaretulehdus. In: Toim. Anneli Hopponen. Maataloustieteen Päivät 2010, Suomen maataloustieteellisen seuran tiedote 26, 4 p. Available at: http://www.smts.fi/jul2010/esite2010/089.pdf (cited 18 December 2013).

Huhtanen, P.J., Blauwiekel, R. & Saastamoinen, I. 1998. Effects of intraruminal infusions of propionate and butyrate with two different protein supplements on milk production and blood metabolites in dairy cows receiving grass silage based diet. J. Sci. Food Agric. 77, 213-222.

Huida, L., Väätäinen, H. & Lampila, M. 1986. Comparison of dry matter contents in grass silages as determined by oven drying and gas chromatographic water analysis. Ann. Agric. Fenn. 25, 215-230.

Jukola, E., Hakkarainen, J., Saloniemi, H. & Sankari, S. 1996. Blood Selenium, Vitamin E, Vitamin A, and β-Carotene Concentrations and Udder Health, Fertility Treatments, and Fertility. J. Dairy Sci. 79, 838–845.

McCullough, H. 1967. The determination of ammonia in whole blood by direct colorimetric method. Clinica Chimica Acta 17, 297-304.

Koller, L.D. & Exon, J.H. 1986. The two faces of selenium – deficiency and toxicity – are similar in animals and man. Canad. J. Vet. Res. 50, 297-306.

MMM 43/2005. Maa- ja metsätalousministeriön asetus rehun lisäaineista ASETUS nro 43/05 Liite 1. Valmistajakohtaisesti hyväksyttävät sekä jakelu- ja käyttörajoituksien alaiset lisäaineet.

MTT 2013. Feed tables and nutrient requirements. Available at: https://portal.mtt.fi/portal/page/portal/Rehutaulukot/feed_tables_english (Cited 13 November 2013).

NRC 2001. Nutrient Requirements of Dairy Cattle. Seventh Revised Edition, National Research Counsil, Washington D.C.

Somogyi, M. 1945. A new reagent for the determination of sugars. J. Biol. Chem. 160, 61-68.

Smith, K.L., Hogan, J.S. & Weiss, W.P. 1997. Dietary vitamin E and selenium affect mastitis and milk quality. J. Anim. Sci. 75, 1659-1665.

Smith, K.L., Conrad, H.R., Amiet, B.A., & Todhunter, D.A. 1985. Incidence of environmental mastitis as influenced by dietary vitamin-e and selenium. Kieler Milchwirtschaftliche Forschungsberichte 37,482-486 (ref. Smith et al. 1997).

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

Factors influencing the production value of forage protein

P. Huhtanen
Swedish University of Agricultural Sciences (SLU), Department of Agricultural Research for Northern Sweden, S-901 83 Umeå, Sweden.

Introduction

Protein is the most expensive main nutrient in the diets of dairy cows. Increased prices of protein feeds, environmental concerns of increased N emissions with increased protein feeding and ethical concerns of using high quality proteins that could directly be used as human food or utilized by monogastric animals have increased interest of improving utilization of forage protein for ruminants. Development of new feed protein evaluation systems in 1980's that are based the supply of absorbed amino acids from microbial protein and feed protein form a more comprehensive basis for evaluation of feed protein value. Increasing the supply of amino acids from forages, especially of undegraded protein (RUP), has been a subject of intensive research. Considerable differences in the predicted supply of RUP from forages have been reported from studies based mainly on the in situ (nylon bag) technique or chemical analysis of N fractions. However, these observations have very seldom been validated in milk production studies or digesta flow studies in cannulated animals. The objective of this paper is to discuss different strategies in increasing the supply of absorbed amino acids (AAT) from forages.

Nitrogen fertilization

Increasing N fertilization of leys was a widely used strategy in 1970's to increase protein supply for dairy cows. However, this strategy was not challenged by comparing milk production responses to increased crude protein (CP) supply from increased N fertilization and high quality protein supplements. In a later study (Shingfield et al., 2001), increasing fertilization from 50 to 100 kg/ha increase silage CP concentration from 120 to 150 g/kg DM. Increased CP from silage had no influence on milk or protein yield when no protein supplements were fed despite very low dietary CP and milk urea concentrations (121 g/kg DM, 1.4 mM). However, when the low CP silage was supplemented with rapeseed expeller to increased dietary CP to the same level as the high CP silage without supplementary protein milk protein yield increased 120 g/d. Interestingly the response to rapeseed expeller was the same (115 g/d) with high CP silage. The results of this study indicate that the cows were responsive to increased protein supply, but the supply of utilizable protein could not be increased by N fertilization. Both silages were well-preserved and had similar DM concentration (340 g/kg). Consistently with production study increasing N fertilization of grass ley (40, 80 and 120 kg/ha) had no influence on duodenal NAN flow study in duodenally cannulated animals (Vanhatalo and Toivonen, 1993). It can be concluded that grass fertilization should be optimized according to yield with no extra benefits from increased N fertilization.

Grass maturity

It is well-known that harvesting grass silage at an earlier stage of maturity increases CP concentration and improve digestibility, and consequently increase intake and milk production. On average, in silage harvest studies (93 diets) marginal efficiency of incremental CP utilization (153 g milk protein/kg increase in CP intake) was greater than the corresponding responses to rapeseed feeds (136 and 133) and soybean meal (98)

supplementation in the meta-analysis by Huhtanen et al. (2011). However, increased ME supply is the most likely explanation to increased milk protein yield with earlier harvest. Intake of ME explained the variation in protein yield much better than CP intake, and in the bivariate model (ME intake + CP concentration) the effect of CP was non-significant (P = 0.43) and numerically even negative. Although early harvest of silages markedly increase milk protein yield, the diets based on high CP silages (>140 g/kg DM) were almost as responsive to increased supplementary protein (rapeseed feeds) as the diets based on low CP silages (129 vs. 144 g/kg increase in CP intake). It can be concluded that earlier harvest of silages increase milk yield mainly due to increased ME intake, and positive responses to supplementary protein can be expected even with highly digestible high CP silages.

Ruminal degradability of forage protein

Determination of ruminal degradability of forage CP has been intensively studied during the last three decades by in situ incubation technique. More recently, chemical fractionation of forage CP has been used to estimated ruminal degradability (Cornell system) and degradability is calculated using different fractional degradation rates for the fractions. The general conclusion of these studies is that effective ruminal protein degradability (EPD) increase with increasing buffer / water solubility and CP concentration and decrease with increasing NDF concentration (decree- sed digestibility) and increasing DM concentration. This usually increases the contribution of RUP to AAT with advancing maturity of forages. For example, according to NorFor feed tables AAT/NEL ratio decreases with increased NEL, i.e. early harvested silages are more limited in AAT relative to NEL than late harvested silages.

Rinne et al. (2009) calculated silage AAT values for 397 diets either using constant EPD for all silages or EPD estimated from empirical equations of Yan and Agnew (2004). Their equations were based on in situ incubations 136 silages. They used DM, CP, NDF, soluble N and lactic acid/VFA as predictors of silage EPD. The models explained about 80% of variation in EPD. Interestingly, AAT intake based on constant forage EPD predicted milk protein yield responses better than AAT intake calculated using EDP values estimated using Yan and Agnew equations. Interestingly, the coefficients of CP and NDF in the EPD-model were almost opposite to the coefficients used to correct EPD values for microbial contamination. Proportion of microbial N in undegraded residues increases with increased NDF (reduced digestibility) and declining forage CP concentration. It appears that differences in forage EPD determined by the in situ technique reflect more variation in microbial contamination than true differences. In addition to microbial contamination, the in situ technique has many inherent technical problems: escape of soluble NAN from the bags, loss of feed particles, and lower microbial activity within bags than in rumen digesta, inappropriate kinetic models. In addition, secondary particle loss can occur when the particles size decreases during fermentation. Considering the technical difficulties of the in situ method and the lack of evidence of improved predictions of production responses with feed specific EPD values using the in situ method for estimating forage EPD can hardly be justified.

Extent of in-silo fermentation

Both theoretical calculations and experimental evidence suggest that the efficiency of microbial protein synthesis decrease with increased extent of silage fermentation. This is because lactic acid and VFA provide less or no energy for rumen microbes. However,

discounting fermentable OM for silage acids in calculating forage AAT value did not improve the predictions of milk protein yield (Rinne et al., 2009), rather vice versa. This can be due to fermentation of silage lactic acid to propionic acid that the main glucose precursor. It is possible that increased supply of glucose per unit of intake was greater with extensively vs. restrictively fermented silages. This can thereby reduce the utilization of amino acids for glucose production and improve the efficiency of the utilization of absorbed amino acids for milk protein synthesis. As for earlier harvest of silage, greater milk protein yield in cows fed restrictively fermented silages is derived from increased feed and ME intake. It may be concluded that discounting for silage fermentation acids in calculating silage AAT values can result in greater errors than ignoring the discount for fermentation acids. If discounts are made then also the effects of increased glucose supply must be taken into account.

Hay vs. silage

In many feed protein evaluation systems, the AAT value of hay is higher than that of silage when harvested at the same stage of maturity. For example according to NorFor feed tables AAT/NEL is about 2.5 g/MJ greater for hay than silage at same NEL concentration. This is both due to discounts of silage fermentation acids in calculating microbial protein and lower EPD for hay compared to silage when determined by the in situ technique. However, experimental evidence from milk production studies conducted in SLU (Bertilsson, 1983) and MTT (e.g. Huhtanen, 1994; Shingfield, 2002) does not give any support for a greater protein value for hay compared to well-fermented silages from the same sward. In the flow study with duodenally cannulated cattle (Jaakkola and Huhtanen, 1993), there was no difference in duodenal NAN flow between diets based on silage or hay, each fed at three levels of contrite supplements.

N solubility

According to the original model calculating EPD from the in situ kinetic data it was assumed that immediately disappearing “a-fraction” is completely degraded in the rumen. However, several experimental techniques have demonstrated that a considerable fraction of soluble NAN can escape ruminal degradation. Although the degradation rate of soluble NAN (SNAN) fractions is much faster than insoluble N, much faster passage rate of fluid phase partly compensates for the difference in degradation rate between soluble and insoluble N fractions. In addition, feed particles are selectively retained in the rumen, which will increase ruminal degradability compared with models assuming a single compartment passage kinetic model. Further, all indigestible CP is included in the insoluble fraction of CP, which also decreases the difference in the supply of digestible RUP between soluble NAN and insoluble forage N fractions. In a meta- analysis, silage SNAN fraction had not significant effect on milk protein yield. SNAN fraction was included in the regression model together AAT intake calculated using a constant EPD for all silages irrespective of the proportion of soluble N (Huhtanen et al., 2009). If SNAN had had a specific negative effect on protein yield, the regression coefficient should have been significantly negative. This indicates that increased proportion of SNAN in the silage had no negative effect on milk protein yield compared with insoluble N. In contrast, the proportion of ammonia N had a significant negative effect on milk protein yield.

It is possible to take into account escape of SNAN in kinetic models, but the methodology to determine degradation rates of free amino acids, peptides and soluble protein from different feeds would be a demanding task. Benefits in practical feed evaluation can be questionable.

Red clover has much lower proportion of soluble N than grass silage, but the flow of protein in <38 μm fraction (soluble NAN, particles <<38 μm) was much higher for diets based on red clover silage compared with grass silage (Huhtanen et al., 2014). In this study in situ method predicted feed N flow in particle fraction reasonably well, but estimated in situ feed N flow was poorly correlated with in vivo feed NAN flow.

Red clover

Polyphenol oxidase system decreases proteolysis of red clover both during in-silo fermentation and in the rumen. Red clover protein is utilized efficiently in the rumen, and the recovery of incremental protein in the outflow from the rumen is usually high (Vanhatalo et al., 2009). However, although the NAN flow from the rumen was greater with red clover compared to grass silages (Dewhurst et al., 2003; Vanhatalo et al., 2009), milk protein yield did not increase indicating poor utilization of the incremental NAN flow. This can partly be due to lower intestinal digestibility of red clover protein than grass protein. In Finnish digestion trials in sheep fed at maintenance faecal CP output was 16 g/kg DM intake greater for red clover silages compared than for primary growth grass silages. Another reason for the small production responses to red clover silages can be that ME intake is the limiting factor. For example, in the study of Vanhatalo et al. (2009) milk protein yield responses were more closely related to intake of digestible OM than to NAN flow or methionine flow.

Prediction model of milk protein yield

A model of forage factors predicting milk protein yield is presented in Table 1. In addition to forage factors, the model included linear and quadratic effects of concentrate AAT intake.

Table 1 The best-fit mixed model regression equation of milk protein yield (g/d) to forage variables (RMSE adjusted for random study effect = 16.0). Adopted from Huhtanen et al. (2010)

Effect	Unit	Estimate	Error	P-value	Sd[a]	Response per SD unit
Intercept		-310	46.8	<0.001		
CAAT[b]	kg/d	790	66.7	<.0001		
CAAT × CAAT	kg/d	-192	38.7	<.0001		
Forage DMI[c]	kg/d	27.7	1.42	<0.001	1.53	42.4
D-Valued in DM[d]	g/kg	0.49	0.069	<0.001	40.9	20.1
Forage CP in DM	g/kg	0.417	0.114	<0.001	22.4	9.3
Ammonia N in total N	g/kg	-0.217	0.067	0.001	21.5	4.7

[a]SD = standard deviation; [b]CAAT = concentrate metabolisable protein; [c]DMI = dry matter intake; [d]D-value = digestible organic matter in DM.

Of forage factors intake and D-value were the most important emphasizing the importance of ME intake in regulating milk protein yield. The effect of forage CP concentration on milk protein yield was small (about 4 g per 10 g/kg DM increase in forage CP), although significant. The key role of energy intake was also demonstrated in meta-analysis by Huhtanen and Hristov (2009). Only 6–8% of increased supply of AAT (calculated according to NCR (2001) system) obtained by decreasing protein degradability (constant energy and CP intake) was recovered as milk protein. This indicates that potential to increase milk protein yield by manipulating forage CP degradability are minimal, provided that there are no associated increases in energy intake.

Conclusions

There are considerable differences in protein value of forages, but these differences are mainly associated to intake potential and digestibility of forages. It seems that forage AAT value is almost entirely related microbial protein (digestibility), whereas differences in protein fractions have much smaller influence. There is very little experimental evidence from milk production studies indicating that forage protein has any specific effects on milk protein yield beyond those related to differences in DM and ME intake. Therefore, accurate methods/models predicting forage intake potential and digestibility are more important than complicate models estimating the supply of AAT from undegraded forage protein. Prediction error of milk protein yield was small even when forage AAT was estimated by a simple model (microbial AAT from digestible OM – RUP and feed AAT using a constant EPD and digestibility of RUP) from large datasets (>1000 treatment means) (Huhtanen and Nousiainen, 2012). This suggests very limited potential for the improvements by more detailed protein models unless better experimental methods than in situ or chemical fractionation are developed.

Referenes

Bertilsson, J., 1983). Effects of conservation method and stage of maturity upon the feeding value .of forages to dairy cows. Swedish University of Agricultural Sciences. PhD thesis, Report 104.

Dewhurst, R.J., Fisher, W.J., Tweed, J.K.S., & Wilkins, R.J., 2003. Comparison of grass and legume silages for milk production. 1. Production response with different levels of concentrate. J. Dairy Sci. 86, 2598–2611.

Huhtanen, P. 1994. Forage influences on milk composition. In A. Fredeen (ed.) Proceedings of Nova Scotia Forage Conference, Forage: Seeding to Feeding, October 29–30, 1993, p. 144–162.

Huhtanen, P., Bayat, A.R., Krizsan, S.J. & Vanhatalo, A., 2014. Compartmental flux and in situ methods underestimate total feed nitrogen as judged by the omasal sampling method due to ignoring soluble feed nitrogen flow. Br. J. Nutr. 111, 535-546.

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

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

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

Huhtanen, P., Rinne, M. & Nousiainen, J., 2008. Effects of silage soluble N components on metabolizable protein concentration: a meta-analysis of dairy cow production experiments. J. Dairy Sci. 91, 1150–1158.

Huhtanen, P., Sudekum, K-H., Nousiainen, J. & Shingfield, K., 2010. In: Schnyder H. et al. (eds). Grassland in a Changing World. Grassl. Sci. Europe 15, 379–400.

Jaakkola, S. & Huhtanen, P., 1993. The effects of the forage preservation method and the proportion of concentrate on nitrogen digestion and rumen fermentation in cattle. Grass and Forage Sci. 48, 146–154.

Rinne M., Huhtanen, P. & Nousiainen, J., 2009. Effects of silage effective protein degradability and fermentation acids on metabolizable protein concentration: a meta-analysis of dairy cow production experiments. J. Dairy Sci. 92, 1633–1642.

Shingfield, K.J., Jaakkola, S. & Huhtanen, P., 2002. Effect of forage conservation method, concentrate level and propylene glycol on intake, feeding behavior and milk production of dairy cows. Anim. Sci. 74, 383–397.

Shingfield, K.J., Jaakkola, S. & Huhtanen, P., 2001. Effects of level of nitrogen fertilizer application and various nitrogenous supplements on milk production and nitrogen utilization of dairy cows given grass silage-based diets. Anim. Sci. 73, 541–554.

Vanhatalo, A., Kuoppala, K., Ahvenjärvi, S. & Rinne, M., 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. J. Dairy Sci. 92, 5620–5633.

Vanhatalo, A. & Toivonen, V., 1993. Influence of cutting time and N fertilization of grass silage and level of grass fertilized with different levels of N. Proceedings of the 10th International Conference on Silage Research, Dublin, Ireland, Sept. 6th–8th 1993. pp. 168–169.

Yan T., & Agnew, R. E., 2004. Prediction of nutritive value of grass silages: II. Degradability of nitrogen and dry matter using digestibility, chemical composition and fermentation data. J. Anim. Sci. 82, 1380–1391.

The new protein evaluation of horse feeds in Germany

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

Background

In equines, amino acids (AA) released through auto-enzymatic protein digestion can be absorbed from the small intestine, whereas AA from microbial protein production and break-down in the hindgut are not absorbed (Schubert, 1992) or absorbed in small amounts – from studies using labeled amino acids, up to 12% of the plasma amino acids originated from the large intestine (McMeniman et al., 1987). Woodward et al. (2010), based on AA transporter mRNA abundance in the large intestine, have concluded that "… results indicate that the large intestine might contribute to both cationic and neutral AA uptake and absorption". However, the potential contribution of the large intestine to AA supply of horses still needs to be quantified.

Therefore, a concept of protein evaluation that allows estimating the extent of auto-enzymatic digestion appears to be more appropriate and should result in more reliable data on the protein or AA supply to the horse than current systems that base on crude protein (CP) or digestible CP (DCP), the latter sometimes being estimated from CP (Pálson, 1973). In horses, the experimental data base on small intestinal digestibility (siD) of protein is too small for establishing a system based on small intestinally digestible CP (sidCP) or AA (sidAA), which would be in accordance with the 'ileal' or 'pre-caecal' digestibility variables used in other non-ruminant species such as pigs. While constructing a database for sidCP would have been possible, and this could have been extended by including data from studies using the mobile nylon bag technique (Martin-Rosset et al., 2012), only two (2!) studies have been published on measured sidAA (Almeida et al., 1998; Coleman et al., 2000), which has made it impossible to develop a consistent system of sidCP and sidAA similar to those in pigs that rely on measured data using strictly standardized methodology. As a consequence of this current status, the following idea was developed.

Based on the CP partitioning of the 'Cornell Net Carbohydrate and Protein System' for cattle using standardisation and recommendations published by Licitra et al. (1996), two CP fractions, namely (i) neutral detergent insoluble CP (NDICP; insoluble protein) and (ii) the corresponding ND-soluble CP (NDSCP; soluble protein; CP - NDICP) were used. The idea behind this fractionation is that CP bound to cell-wall material and, thus, recovered in the ND-insoluble residue cannot be broken down by auto-enzymatic digestion, whereas the soluble protein can be digested by body's own enzymes. As both cattle and horses are herbivores and may consume very similar diets and diet ingredients, data on NDICP in forage and concentrates available for cattle may also be used for constructing a horse database.

The aim of the present study was to investigate whether the suggested concept of protein evaluation on the basis of ND-soluble versus ND-insoluble CP is applicable to horse feeds. The following sections briefly summarizes work conducted by the Committee for Requirement Standards (AfBN, 'Ausschuss für Bedarfsnormen') of the Society of Nutrition Physiology (GfE, Gesellschaft für Ernährungsphysiologie) in Germany and is largely based on contributions of horse experts, namely (alphabetical order) Manfred Coenen, Ellen

Kienzle and Annette Zeyner. The outcome of this work will be published soon in a volume of the GfE series "Recommendations for the supply of energy and nutrients" (GfE, in press).

Materials and Methods

Neutral detergent insoluble CP and NDSCP were analysed and defined as given above, i.e., NDSCP = CP - NDICP. For silages, a correction was made for ammonia-N as follows:

DSCP = CP - NDICP - NH_3-N · 6.25. Data on NDICP of forages and concentrates were taken from published work on ruminant feedstuffs (for references, see GfE, in press). A literature survey was then conducted to investigate if the above fractionation scheme can be applied to estimate DCP and siD of CP and AA, respectively.

Results and Discussion

The survey included the following steps and major results (references are given in GfE, in press and can be obtained upon request from the author).

1. A correlation exists between NDSCP of feeds and siDCP in the horse and, likewise, between NDICP and the CP that is indigestible in the small intestine ($R = 0.719$, $p < 0.01$; $N = 24$).

2. A strong linear correlation exists between the daily intake of siDCP and NDSCP ($r = 0.832$, $p < 0.003$). Although based on only few experimental data, siDCP was measured on a range of rations reflecting typical horse diets. The slope of the regression between intakes of siDCP and NDSCP indicates that 0.9 of NDSCP intake is digested (and the AA are being absorbed) within the small intestine. Therefore, it is assumed that:

siDCP = 0.9 · NDSCP.

Moreover, the AA profiles of both, NDSCP and NDICP, appear to be similar within a given feed (Rebolé et al., 2001; Tedeschi et al., 2001), such that the NDSCP/NDICP schedule can be translated to a NDSAA/NDIAA schedule and the above equation can also be used to estimate siDAA as follows:

siDAA = 0.9 · NDSAA.

No data could be found on the fate of supplemented free AA in horses. It was assumed, therefore, that free AA are completely absorbed from the proximal small intestine and thus, siD of supplemented free AA is 100%.

Feedstuffs and mixed diets typically fed to horses are currently being evaluated in terms of supply of siDCP and siDAA. In a final step, the match between supply and requirements for CP and AA, also expressed as siDCP and siDAA, has to be assessed.

Conclusions

Neutral detergent-soluble crude protein can be easily calculated as the difference of analysed feed fractions, i.e. CP minus NDICP, and represents the CP fraction which can potentially be digested in the small intestine. Based on very limited evidence from horse studies, data on other non-ruminant species (i.e., pigs) and theoretical considerations, the siD of this fraction was estimated to be 90%. Because of the similar AA profiles of NDSCP and NDICP the concept can be translated to estimate siDAA from feed chemistry data without requiring animal experimenttation. The GfE (in press) recommends to include siD of lysine, (methionine + cysteine) and threonine in table values for horse feeds. The new system,

although based on very limited direct experimental evidence, more closely resembles the conditions in the gastro-intestinal tract of horses than the DCP systems currently used. Moreover, the new system can be easily extended and adopted to new data.

References

Almeida, F.C., Valadares Filho, S.C., Queiroz, A.C., Leão M.I., Silva, J.F.C. & Cecon, P.R., 1998. Digestibilidade aparente e verdadeira pré-cecal e total da proteína em dietas com diferentes níveis protéicos em eqüinos. R. Bras. Zootec. 27, 521-529.

Coleman R. J., Matthison G.W., Hardin R.T. & Milligan J.P., 2000. Effect of dietary forage and protein concentration on total tract, prececal and post-ileal protein and lysine digestibilities of forage based diets fed to mature ponies. 17th Equine Nutr. Physiol. Symp. Lexington, Kentucky, USA, pp. 461-463.

GfE (Gesellschaft für Ernährungsphysiologie), in press. Empfehlungen zur Energie- und Nährstoffversorgung der Pferde. DLG-Verlag, Frankfurt/Main (in German).

Licitra, G., Hernandez, T.M. & Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractions of ruminant feeds. Anim. Feed Sci. Technol. 57, 347-358.

Martin-Rosset, W., Macheboeuf, D., Poncet, C. & Jestin, M., 2012. Nitrogen digestion of a large range of hays by mobile nylon bag technique (MNBT) in horses. In: Saastamoinen, M., Fradinho, M.J., Santos, A.S., Miraglia, N. (Eds.), Forages and Grazing in Horse Nutrition. EAAP Publ. No. 132, Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 109-120.

McMeniman, N. P., Elliott, R., Groenendyk, S. & Dowsett, K. F., 1987, Synthesis and absorption of cysteine from the hindgut of the horse. Equine Vet. J. 19, 192-194.

Pálson, T., 1973. Bestämning av råproteinets smältbarhet i vallfoder (Determination of crude protein digestibility in forage). Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences. Uppsala, Sweden.

Rebolè, A., Treviño, J., Caballero, R. & Alzueta, C.,2001. Effect of maturity on the amino acid profiles of total and nitrogen fractions in common vetch forage. J. Sci. Food Agric. 81, 455-461.

Schubert, R., 1992. Verwertung von 15N-Harnstoff für die intestinale Synthese von Bakterienprotein und für die Milchbildung bei Ponystuten. Pferdeheilkde. 43 (Special Issue), 137-139 (in German).

Tedeschi, L.O., Pell, A.N., Fox, D.G. & Llames, C.R., 2001. The amino acid profiles of the whole plant and of four plant residues from temperate and tropical forages. J. Anim. Sci. 79, 525-532.

Woodward, A.D., Holcombe, S.J., Steibel, J.P., Staniar, W.B., Colvin, C. & Trottier, N.L., 2010. Cationic and neutral amino acid transporter transcript abundances are differentially expressed in the equine intestinal tract. J. Anim. Sci. 88, 1028-1033.

Effects of crude protein content in forage-only diets fed to horses

A. Jansson[1] & S. Ringmark[1]
[1] *Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Monogastric Division, Almas allé 8D , 750 07, Uppsala, Sweden*
Correspondence: Anna.Jansson@slu.se

Introduction

The advantages of feeding diets dominated by forage to horses are many, i.e. there appears to be a decreased risk of several health problems such as colic, rhabdomyolysis, gastric ulcers and stereotypic behaviors compared to traditional high concentrate, starch rich diets. Forages with a great variation in energy content can be produced by harvesting grasses at different stages of maturity. However, the content of crude protein (CP) may show an even bigger variation and both under and over feeding with CP is likely to be common. There are not many studies performed on effects of protein intake on the metabolism of horses, especially not in exercising horses (Jansson and Harris, 2013) and in horses fed forage-only diets. The aim of the present study is to highlight some of the recent studies performed on these subjects.

Effects of protein intake on digestion and hindgut environment

Being a grass eater, horses are adapted to a large variation in crude protein intake. The content of CP in forages and pastures varies with the maturity of the plants, plant species, dry periods/rainy periods, the number of animals grazing/ha, soil conditions and fertilization. The most common deficiency of the diet in free ranging herbivores is a protein/energy deficiency (Duncan, 1992) but in adult, non-productive (pleasure) horses, short pastures are likely to provide excessive amounts of CP.

Protein is mainly digested and absorbed in the small intestine. The digestibility of forage protein is affected by the maturity of the grasses. In mature, low CP forages (~8 % CP of dry matter (DM)) digestibility may be as low as 43 % and in forages with higher CP contents higher than 65 % (NRC, 1989). Excessive nitrogen is mainly excreted in the urine (Connysson et al., 2006). However, part of the surplus nitrogen enters the hind gut and affects not only the fecal nitrogen excretion (Connysson et al., 2006) but also the hind gut environment and microflora. We have observed small but significant drops in colon and fecal pH and DM content on high CP forage-only diets (Connysson et al., 2006; Muhonen et al., 2008a) and also an increase in total VFA concentrations in colon (Muhonen et al., 2008a). Excessive CP intake also seems to increase heat production and evaporative losses (Muhonen, 2008b), probably as a result of the increased production of urea. During hot and humid conditions this might be a limitation to exercise performance. The increased urea production may also affect acid base balance during exercise and the importance of this for performance remains to be proven, since results so far are not consistent (Graham-Thiers et al., 2001; Connysson et al., 2006).

The use of fecal samples to determine crude protein intake

Horses are selective grazers (Duncan, 1992) and to get a representative sample of what they consume at pasture may therefore be complicated. If protein intake could be monitored with reasonable precision in grazing horses the possibility to adjust deficiencies and make necessary supplementation would be improved. Fecal samples have earlier been used to estimate CP in diet of feral horses (Duncan, 1992; Salter and Hudson, 1979). In these studies,

seasonal variations in both CP and fiber content have been observed with highest fiber and lowest CP contents during wintertime. In a pilot study, we investigated the possibility to use fecal samples as a rough marker for CP intake in horses on forage-only diets. Forage and fecal samples were collected from stabled horses observed in three studies (A, B and C). Study A included 11 adult sedentary horses (11 samples) of various breeds from different stables (forage CP range: 7.8-10.6 % of DM). Study B included 16 Standardbred horses sampled once as 1-year olds and twice as 2- and 3-year olds respectively, while fed four different grass forage batches complemented daily with 250-1000 g of a lucerne product (65 samples, forage CP range (including lucerne): 9.8-16.4 % of DM). Study C included six adult trained horses on three grass forage batches (18 samples, forage CP range: 9.1-13.4 % of DM, Ringmark and Jansson, 2013). Feed and feces samples were collected in the same day except in study B where feed samples were collected for three consecutive days and mixed before analysis and the fecal samples used were from day 2 or 3.

CP in feces varied between 6.6 and 18.1 % of DM. There was a significant, but not very strong, correlation (Pearson´s correlation coefficient) between CP in feed and CP in feces ($R = 0.60$, $P < 0.0001$, figure 1). The correlation was not improved if data was separated into growing (< 3 years) and adult horses. The effect of CP intake on fecal CP content was also tested in a mixed model including effect of study and repeated measurements on individuals which showed an effect of CP in feed on CP in feces ($P < 0.001$). Additionally, observations were divided in two levels based on the CP content in feed DM (medium (n=52): < 13% CP and high (n=47): ≥ 13% CP) and the effect on fecal CP content was analyzed with a general linear model including effect of individual. This resulted in significantly higher content of CP in feces in the high level than in the medium level (11.3 ± 0.3 and 10.1 ± 0.2 % of DM, respectively, $P = 0.0001$). However, since there were no observations on forages with CP content lower than 7.8 % of DM, still no recommendations could be made for how this method could be used to assess CP intakes below requirements. The nutritive quality of pasture and the body condition of the horses should therefore always be considered primarily.

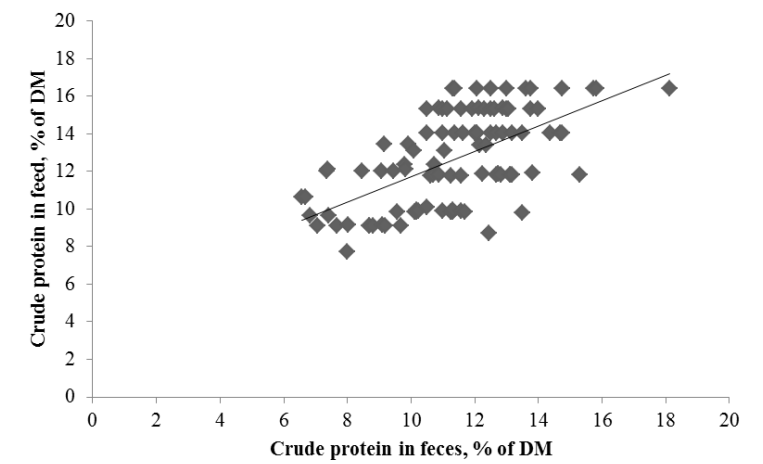

Figure 1 The relation between crude protein content in feed and in feces (% of dry matter). Y=0.6709x+5.0258.

Crude protein content may affect insulin response

Forage-only diets result in lower basal and post-prandial plasma insulin concentrations than starch-rich concentrate based diets (Connysson et al., 2010; Jansson and Lindberg, 2012). However, it has also been shown that the content of water soluble carbohydrates (WSC) in

forage affects the insulin response to feeding (Borgia et al., 2011; Ragnarsson and Jansson, 2011). In both horse and man it is also known that protein, and especially the amino acid leucine, may induce an increased insulin response (Cleator et al.,1975; Urschel et al., 2010) and in humans, an additional effect on insulin response with the simultaneous ingestion of amino acids and glucose compared with the ingestion of glucose alone have been observed (van Loon et al., 2000). The plasma insulin level is likely to affect both sedentary and athletic horses. In horses suffering from equine metabolic syndrome, high insulin levels might increase the risk for laminitis and in athletic horses insulin levels will affect the metabolic response to exercise and possible the performance. To assess the insulin response to forage-only diets with varying crude protein content both at rest and after exercise, a study on six adult conditioned Standardbred horses was recently conducted (Ringmark and Jansson, 2013). Two levels of CP intake was studied, ≤180 g CP/100 kg body weight (BW) and >180 g CP/100 kg BW. The intake of CP/100 kg BW was significantly higher ($P < 0.05$) in the high CP-intake group than in the low CP- intake group, but there was no difference in WSC (excluding fructans) intake. There was a tendency ($P = 0.08$) for higher plasma insulin response after feeding in the high CP group compared to the low group but no difference between groups was observed after feeding when horses had been exercised within one hour. An ANOVA model including horse, CP and WSC (excluding fructans) intake explained 95 % of the variation in plasma insulin whereas a model including horse and WSC intake only explained 87 % of the variation in plasma insulin. Although this study had a small number of observations, it indicates that insulin response to feeding forage can be elevated by a high CP content. This is also in accordance with previous observations in horses fed readily available carbohydrates together with protein or amino acids (Stull and Rodiek et al., 1966; Rérat et al., 1985; van Loon et al., 2000). However, the importance of CP intake for insulin response does not seem to be as central post exercise as before, at least not at the CP intakes level studied in the present experiment.

High glycogen levels on a high CP diet

Low plasma insulin levels could be an advantage for the athletic horse since low levels promotes increased plasma glucose levels and fat availability during exercise (Pagan and Harris, 1999), factors likely to improve performance. However, low levels could also be a limitation since insulin may stimulate replenishment of muscle glycogen and fat deposition. However, we have shown that horses fed a high CP forage-only diet will have very high muscle glycogen contents (Essén-Gustavsson et al. 2010), comparable to horses fed high concentrate diets, and also that replenishment may be improved compared to a more moderate CP intake (Figure 2). Together with the observations made by Ringmark and Jansson (2013), this implies that high insulin levels are not critical to the immediate post exercise glycogen replenishment. However, high muscle glycogen contents on protein rich diets are also in accordance with observations in rats, and it has been suggested that intake of branched amino acids stimulate glycogen synthesis through insulin-independent mechanisms (Nishitani et al., 2002; Morifuji et al., 2010).

Conclusions

Protein intake may show a great variation in horses and intake level will affect the digestive system and metabolism at rest as well in connection with exercise. However, further studies are needed to better understand the importance of the effects and how protein intake can be monitored in horses on pasture or mixed forage/pasture diets.

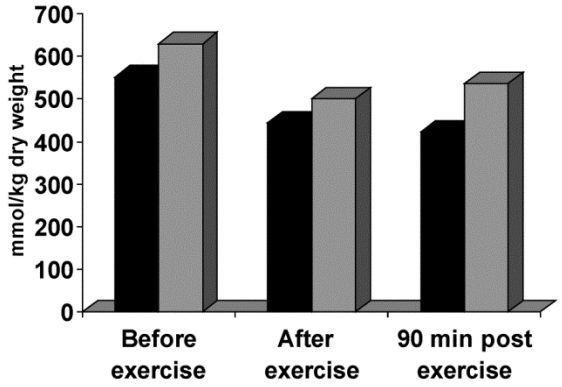

Figure 2 Muscle glycogen content (mmol/kg dry weight) before, immediately after and 90 min after an exercise test in horses fed a forage-only diet providing recommended (black bars, 12.5 % of DM) and high crude protein intakes (grey bars, 16.6 % of DM). The glycogen content was higher on the high CP-diet and the there was a significant ($P<0.05$) decrease after exercise in both diets and a tendency ($P=0.09$) to an increase after 90 min on the high CP-diet but not on the recommended diet. After Essén-Gustavsson et al. (2010).

References

Borgia, L., Valberg, S., McCue, M., Watts, K. & Pagan, J., 2011. Glycaemic and insulinaemic responses to feeding hay with different non-structural carbohydrate content in control and polysaccharide storage myopathy-affected horses. J. Anim. Physiol. Anim. Nutr. 95, 798-807.

Connysson, M., Muhonen, S., Lindberg, J. E., Essén-Gustavsson, B., Nyman, G., Nostell, K. & Jansson, A. 2006. Effects on exercise response, fluid and acid-base balance of protein intake from forage-only diets in Standardbred horses. Equine Vet. J. Suppl. 36, 648 - 653.

Connysson, M., Essen-Gustavsson, B. & Jansson, A., 2010. Effects of feed deprivation on Standardbred horses fed a forage-only diet and a 50:50 forage-oats diet. Equine Vet. J. 42, 335-340.

Duncan, P. 1992. Horses and Grasses. The Nutritional Ecology of Equids and Their Impact on the Camargue. Ecological studies 87, Springer-Verlag, New York.

Essén-Gustavsson B., Connysson, M. & Jansson, A., 2010. Effects of crude protein intake from forage-only diets on muscle amino acids and glycogen levels in horses in training. Equine Vet. J. 42, 341-346.

Graham-Thiers, P.M. , Kronfeld, D.S., Kline, K.A., Sklan, D.J. 2001. Dietary protein restriction and fat supplementation diminish the acidogenic effect of exercise during repeated sprints in horses. J. Nutr. 131(7), 1959-1964.

Jansson, A. & Harris, P.A. 2013. A bibliometric review on nutrition of the exercising horse from 1970 to 2010. Comp. Exercise. Physiol. Vol. 9, Number 3 - 4.

Jansson, A. & Lindberg, J.E., 2012. A forage-only diet alters the metabolic response of horses in training. Anim. 6, 1939-1946.

Muhonen, S.; Connysson, M.; Lindberg, J. E.; Julliand, V.; Bertilsson, J.; Jansson, A.2008a. Effects of crude protein intake from grass silage-only diets on the equine colon ecosystem after an abrupt feed change. J. Anim. Sci. 86, 3465-3472.

Muhonen, S. 2008b. Metabolism and hindgut ecosystem in forage fed sedentary and athletic horses. Acta Universitatis Agriculturae Sueciae. Doctoral Thesis No. 2008:68. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Morifuji, M., Kanda, A., Koga, J., Kawanaka, K. & Higuchi, M., 2010. Post-exercise carbohydrate plus whey protein hydrolysates supplementation increases skeletal muscle glycogen level in rats. Amino Acids 38, 1109-1115.

Nishitani, S., Matsumura, T., Fujitani, S., Sonaka, I., Miura, Y. & Yagasaki, K., 2002. Leucine promotes glucose uptake in skeletal muscles of rats. Biochem.Biophysical Res. Comm. 299, 693-696.

NRC.1989. Nutrient Requirements of Horses (5th Revised Ed.). Nutrient Requirements of Domestic Animals. National Research Council. National Academy Press, Washington, DC.

Pagan, J. & Harris, P., 1999. The effects of timing and amount of forage and grain on exercise response in thoroughbred horses. Equine Vet. J. Suppl. 30, 451-457.

Ragnarsson S & Jansson A. 2011. A comparison of grass haylage digestibility and metabolic plasma profile in Icelandic and Standardbred horses. J. Anim. Physiol. Anim. Nutr. 95, 3, 273-279.

Rérat, A., Chayvialle, J.A., Kande, J., Vaissade, P., Vaugelade, P. & Bourrier, T., 1985. Metabolic and hormonal effects of test meals with various protein contents in pigs. Canadian J. Physiol. Pharmacol. 63, 1547-1559.

Ringmark, S & Jansson, A., 2013. Insulin response to feeding forage with varying crude protein and amino acid content in horses at rest and after exercise. Comp. Exercise Physiol. Vol. 9, Number 3 - 4.

Salter, R.E. & Hudson, R. J., 1979. Feeding Ecology of feral horses in western Alberta. J. Range Managm., 32, 221-225.

Stull, C.L. & Rodiek, A.V., 1988. Response of blood glucose, insulin and cortisol concentrations to common equine diets. J. Nutr. 118, 206-213.

Urschel, K.L., Geor, R.J., Waterfall, H.L., Shoveller, A.K. & McCutcheon, L.J., 2010. Effects of leucine or whey protein addition to an oral glucose solution on serum insulin, plasma glucose and plasma amino acid responses in horses at rest and following exercise. Equine Vet. J. 42, 347-354.

van Loon, L.J.C., Saris, W.H.M., Verhagen, H. & Wagenmakers, A.J.M., 2000. Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. American J. Clinical Nutr. 72, 96-105.

Simple Excel VBA application for Monte Carlo simulation with ad hoc made spreadsheet models

T. Eriksson
Department of Animal Nutrition and Management, Swedish University Agricultural Sciences, Kungsängen Research Center, SE-753 23 Uppsala, Sweden.
Correspondence: torsten.eriksson@slu.se

Introduction

Researchers in animal science as well as in many other disciplines use spreadsheet models in a multitude of ways. There are large, well organized models created with considerable efforts. But, the majority of models are ad hoc made applications quickly put together when checking up some idea that struck the researcher examining the outcome of an experiment or reviewing a manuscript. These models may use only a handful of cells, but sometimes evolve into huge poorly organized "spaghetti models" as more factors are taken into account.

A typical issue when examining complex relationships involving multiple factors is what effect they may have on the variation of the response variables. This could be tested by Monte Carlo simulation, where, for instance, multiple model parameters are allowed to vary according to a chosen distribution. The outcome of thousands of simulations generates a distribution in response variables which could be regarded as an extended sensitivity analysis. This paper describes a simple spreadsheet application in Microsoft Excel that easily can be connected to existing ad hoc spreadsheet models for generating Monte Carlo simulations. The application is free for download and use.

The application

The application (APP) was made in Excel 2010 and consists of a worksheet and a short VBA code (macro) operated by a button on the worksheet. The VBA code allows simulations to be run automatically any chosen number of times (thousands). The actual value of each parameter in each simulation iteration as well as the corresponding values of response variables are logged and forms the outdataset from the simulation. The worksheet could either be inserted into the Excel file containing the existing model or, the model could be copied into the file with the APP. In both cases, the file must be saved as a macro enabled workbook.

The APP was made as transparent as possible, to facilitate understanding of the construction and make it easier for advanced users to modify it. The present worksheet has seven rows with constants or formulae (Figure 1), of which three require user activity for each new simulation setup. The other rows could be changed by expanding or diminishing the range of cells in use. The worksheet in the example is set up for a maximum of 30 model parameters and storage of these and a maximum of 20 model output variables.

Random coefficients are generated in Rows 1 and 2. Row 1 has a range of cells holding constants. In the example, there are 800 cells with values following a normal distribution with mean = 0 and s = 1, i. e., the values vary from approx. -3 to 3. The values were generated by Excel Analysis ToolPak but could also be imported from other sources or replaced by data following some other distribution if considered more suitable. Row 2 contains RANDBETWEEN and OFFSET formulas to retrieve a value from a randomly chosen cell in the range of normally distributed constants in Row 1. The value is replaced at each

recalculation of the worksheet, i.e. at any manual change to the worksheet, when F9 key is pressed or by 'Calculate' in a macro.

Row 3 is for user input of starting values for model parameters to be varied in the simulations. In the example, the average value for each parameter is used. The standard deviation for each parameter to be varied is entered in Row 4.

In Row 5, the random coefficients generated in the first step are multiplied with user entered standard deviations. This gives the absolute deviation for each parameter in the current simulation. In Row 6, all values are checked for non-negativity and added to the starting values to yield parameter values to be used in the simulation. If negative, a zero value is used.

Row 7 contains both current values of the parameters and model variable output data of interest, i.e. result of the simulation. Variable values are linked with Row 6 and with the existing model. Output data from the existing model are linked to cells in Row 7 to the right of the parameters. The row is logged below in a designated cell range after each simulation.

	Random coefficient generation		
Row 1	Cells populated with standardized distribution (In example normal distribution with x = 0; s = 1)	-2.7107	……..''
Row 2	Coefficients retrieved from randomly chosen cells containing the distribution	**-0.273**	……..''
	Input of values and known/assumed variation for model parameters of interest		
	Labels for model parameters	Ash, g/kg DM	……..''
Row 3	Original values for model parameters that should vary in the simulation	22.9	……..''
Row 4	Known/assumed standard deviation for each varying model parameter	2.0	
	Calculation of parameter value to be used in the simulation		
Row 5	Random coefficients are multiplied with the known/assumed parameter standard deviations	-0.5	……..''
Row 6	Results are added to the original values and checked for non-negativety	22.4	……..''
	Input in model of parameters with simulated variation and retrieval of results		
Row 7	Parameter values to be used are linked from Row 6 and further to the formulas in the model. Outdata cells of interest from the model are linked to the same row. The row is after each recalculation copied to a database.	22.4	……..''

Figure 1 Structure of the spreadsheet for generating Monte Carlo simulations from existing Excel models. Data continue to the right in as many columns as needed. User activity is required for entering parameter values and standard deviations in Rows 3 and 4, respectively and for linking cells to Row 7. Row 7 is logged after each simulation.

An example

The example chosen is the summative effect of intra-laboratory analytical variation of laboratory estimated metabolizable energy (ME) content of feeds and resulting ME intake of a dairy cow diet. Proximate analysis of concentrates with a set of tabulated coefficients for digestibilty and metabolizability has been used in Sweden since 1967. Together with forage ME estimation from in vitro organic matter digestibility, it has formed the Swedish system for ruminant ME calculation from the late 1970's and onwards and has only recently been partly replaced by the Norfor model.

A diet with barley, rapeseed cake and grass silage was used. Initially, variation in the ME concentration of barley was investigated and in the next, total ME intake from the diet and the ME allowable production of energy corrected milk (ECM) according to Spörndly (2003)

was simulated. Table 1 shows analytical values together with calculated ME concentration of the feeds and standard deviation for repeated analyses of control samples at the Kungsängen Research Centre laboratory (Verner et al., 2012).

Table 2 Metabolizable energy calculation from proximate analysis of concentrates and in vitro organic matter digestibility of forage for the feeds used in the simulation examples. Intra-laboratory standard deviations (reproducibility over time for the Kungsängen Research Centre laboratory) that were used in the simulations are included

Item	Barley	Rapeseed cake	Grass silage	Reproducibility (SD between repeated analyses)
Proximate analysis				
Ash, g/kg DM	28	66	60	2
Crude protein, g/kg DM	122	339		0.75
Crude fat, g/kg DM	27	169		1.10
Crude fiber, g/kg DM	62	137		4.0
Nitrogen free extractives, g/kg DM[a]	761	289		
Forage OMD and ME calculation				
In vitro OMD of forage, g/kg OM			873	9.2
MJ ME/ kg DM	13.16	15.46	11.34	

[a]Calculated as a residual: 1000-ash-crude protein-crude fiber

Table 2 Total ME intake and production potential of energy corrected milk (ECM) for the diet in the simulation example

Item	Barley	Rapeseed cake	Grass silage	Total diet
MJ ME/ kg DM	13.16	15.46	11.34	
Intake, kg DM/d	6	2	12	20
Total intake, MJ ME/d				246
ME allowable ECM, kg/d				34.5

Results

Simulations were run on a standard laptop computer with 2.1 GHz processor and 8 GB RAM memory, which required 150 seconds for 10000 simulations of barley ME concentration with four parameters. For the total diet, ten parameters were used and the simulation required 165 seconds for 10000 simulations. Correlations among parameters were in absolute values < 0.03. The range of ME values for barley was about 0.8 MJ/kg DM (Figure 2). The total diet ranged from 239 to 252 MJ ME/d with a standard deviation of 1.8 MJ. The estimated resulting ECM production supplied (Figure 3) varied over a range of 2.35 kg/d as an effect of laboratory analytical variation.

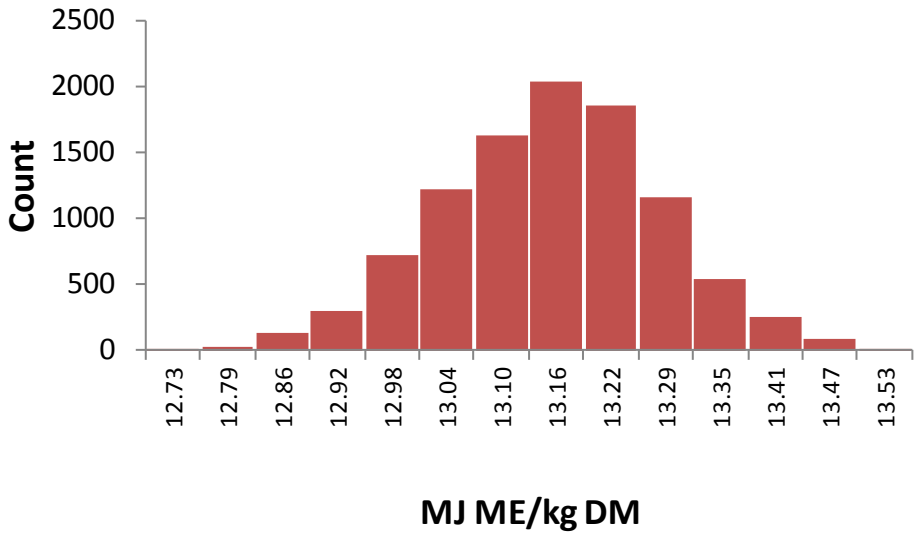

Figure 2 Metabolizable energy (ME) concentration from 10000 simulations with the example barley. All variation is for the result of known variation in laboratory analytical data. Range 12.72 – 13.54, s 0.123. Class width is 0.5 s.

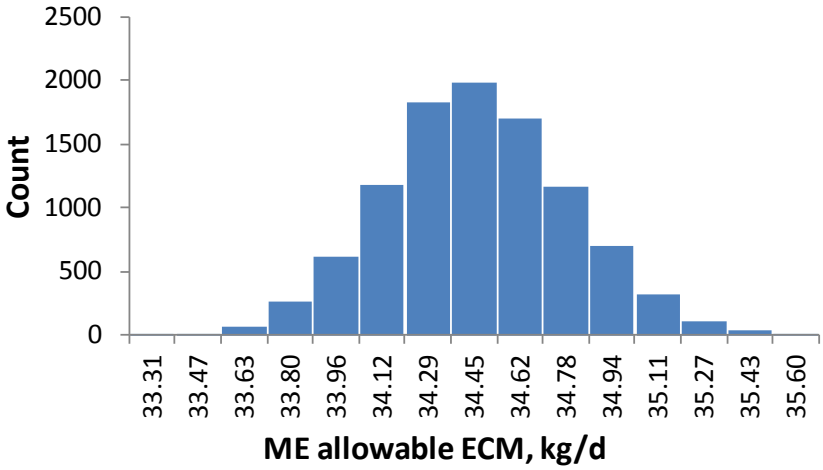

Figure 3 Energy corrected milk (ECM) production as allowed by metabolizable energy (ME) intake from 10000 simulations with the example diet. All variation is due to the known variation for laboratory analyses of the three feeds in the diet. Range 33.29 – 35.64, s 0.327. Class width is 0.5 s.

Discussion

The simulations displayed the possible variation in outcome when four or ten parameters are allowed to vary independently following normal distribution. It would have been possible to let a much larger number of parameters vary if feasible. In this specific case, the assumption of no covariance between variables is probably correct, because it deals with random analytical variation at a laboratory. However, when applying this approach to other models,

the possibility of covariance between variables should always be taken into account. Consider for instance a ruminant nutrition model, where there most likely exists covariance between ruminal digestibility and small intestinal digestibility of a feed component.

The random generator in Excel has occasionally been criticized (McCullough, 2008), but the absence of correlation between variables does not suggest systematic errors. It would be possible to extend the population of coefficients for sampling and also to perform repeated recalculations in each simulation step, although simulation time would increase.

The situation simulated here has likely occurred in many dairy cow experiments and would have had minor consequences for result interpretation if: 1) the experiment was of a change-over design and random analytical differences would just add to period effects or 2) feed samples from the entire experiment were analysed in the same batch. However, if none of these conditions are fulfilled, the repeatability variation at a research laboratory could cause differences of the magnitude, shown above.

Analytical variation of a control sample will not reflect what the variation will be in all sample types and it is likely that variation actually is larger in some cases. Reproducibility among research laboratories or commercial laboratories is generally greater and if such data is mixed within an experiment, variation would most likely increase considerably.

Conclusion

A relatively simple Excel APP can be used for investigating the outcome when multiple parameters are varied independently in simulations. They can easily be linked to existing Excel models by the user.

References

McCullough, B.D. 2008. Microsoft Excel's 'Not The Wichmann–Hill' random number generators. Comp. Stat. Data Anal. 52, 4587–4593.

Spörndly, R. 2003.(Ed) Fodertabeller för idisslare (in Swedish). Department of animal nutrition and management. Swedish Universtity of Agricultural Sciences. SLU/HUV Report 257, pp, 7-15.

Verner, E., Ericson, B. & Udén, P., 2012. The variation of control samples in the analyses of crude protein, neutral detergent fibre, in vitro organic matter digestibility and crude fat. 3rd Nordic Feed Science Conference, Uppsala, 28-29 of June 2012. SLU/HUV Report 280, p 27-30.

The Excel APP is downloadable at: www.slu.se/en/torsten-eriksson

Comparison between individual feeds and total diets on predicted methane production from *in vitro* simulations

M. Ramin[1], M. Vaga[1], E. H. Cabezas-Garcia[1] & E. Detmann[2]
[1]*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden*
[2]*Departamento de Zootecnia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil*
Correspondence: mohammad.ramin@slu.se

Introduction

Methane production is a major problem in ruminant production system as it represents a significant energy loss from the diet i.e., 2–15% of gross energy intake (Johnson and Johnson, 1995). Many factors influence methane production in ruminants such as digestibility, type of feed and dry matter intake (Ramin and Huhtanen, 2013). In production trials, total mixed ration feeding system is applied and occasionally methane production is measured. Measuring methane production from live animals is laborious and costly. An alternative method to estimate methane production from ruminants is by an *in vitro* technique (Cone *et al*., 1996). One main disadvantage of the *in vitro* technique is that it does not take into account the dynamic of rumen fermentation and digestion kinetics. On the other hand, interactive effects among feeds can influence the stoichiometry of rumen fermentation, which could modify the methane production per unit of dry matter. Recently, Ramin and Huhtanen (2012) developed an *in vitro* method to predict ruminant methane production *in vivo* by the use of modelling approaches. The objective of the current study was to compare predicted methane production from the *in vitro* simulation by incubating feeds either individually or as total mixed diets.

Materials and Methods

Eleven diets were evaluated, with forage-to-concentrate ratios from 90:10 to 45:55. The forages (n = 9) were tropical grass pasture plants (*Brachiaria sp.*) and maize silages (*Zea mays*). The concentrates (n = 7) were obtained by mixing whole soybeans (SBW) or soybean meal (SBM) with ground maize, wheat bran, urea and minerals in different proportions (Table 1). A fully automated gas *in vitro* system was used as described by Cone *et al*. (1996) with recording of gas production (GP) every 12 minutes. The recorded GP was corrected to normal air pressure (1013.5 h Pa). Feeds were incubated both individually and as mixed diets of forage and concentrates in varying ratios. Samples of 1 g were weighed directly into 250-ml serum bottles and incubated in 60 mL of buffered rumen fluid for 48 hours. The bottles were placed in water baths at 39°C and gently agitated continuously during the incubations. Rumen fluid for all three *in vitro* incubation runs was obtained from the same two ruminally cannulated Swedish Red cows about two hours after morning feeding. Cows were fed on a diet containing grass silage (60%) and commercial concentrates (40%). The rumen fluid was collected into pre-warmed thermos flasks previously flushed with carbon dioxide and afterwards filtered through four layers of cheese cloth into a buffered mineral solution (Menke and Steingass, 1988), with the ratio of rumen fluid to buffer of 1:4 (V/V). All 27 samples (forages, concentrates and total mixed diets) were incubated in three *in vitro* series (runs) and were randomly distributed within the runs, resulting in three *in vitro* observations per sample. In each run, a blank (buffered rumen fluid without a sample) was incubated in duplicates. Methane production was predicted as described by Ramin and Huhtanen (2012). Methane production was reported as weighted means for individual feeds and total diet

separately. The statistical comparison was performed by simple linear regression of values obtained from total diet incubations (Y) on values obtained from the weighted sum of the individual feeds (X) of respective diet. The following null hypotheses were tested:

$$H_0 : \beta_0 = 0 \quad (1)$$

$$H_0 : \beta_1 = 1 \quad (2)$$

where β_0 is the intercept, and β_1 is the slope. The methane production estimates obtained by diet or individual feeds incubations should be considered similar if both of the null hypotheses are not rejected. The model adjustment was performed by using the MIXED procedure of SAS ($\alpha = 0.05$). The random effect of the different runs was included in the model.

Results and Discussion

The descriptive statistics for methane production are given in Table 2. The mean of predicted *in vivo* methane production from the total diets was 30.1 mL/g DM and 30.8 mL/g DM from the weighted mean of individual feeds, respectively. There was a poor correlation (r = 0.15) between weighted methane production from individual feeds and total diets (Table 3 and Figure 1). Both complete diet and individual feed digestibility, providing energy for rumen fermentation, do not only depend on individual intrinsic feed characteristics. Interactive effects between dietary components on digestibility, especially on the neutral detergent fibre (NDF) fraction, have been reported for tropical diets (Detmann *et al*., 2008). This could be one reason for the differences in methane production between observed and predicted values from individual feeds observed in the current study. In this study, the digestibility of the NDF fraction showed a quadratic response in the complete diets based on *Brachiaria sp* to the replacement 0, 50 and 100% of the SBM with SBW in the concentrate (0.86:0.14 forage:concentrate ratio). Maximum digestible NDF was obtained at 50% SBM replacement with SBW (611.5 g/kg), compared with 0 (539.6 g/kg) and 100% (548.6 g kg) replacement (data not shown). On the other hand, the predicted weighted mean of methane production for the highest NDF digestibility (25.7 mL/g DM) was remarkably different from the observed value for the total diet (32.2 mL/g DM). The responses were completely opposite and are supported by the poor relationship shown in Table 3 and Figure 1.

Based on these findings, it is not possible to predict methane production of total diets based on individual ingredients because their sum is not equal to observed values from the *in vitro* incubation of the total diet. The associative effect of feeds on diet digestibility was also reported by Huhtanen (1991).

It is often assumed that energy values of feeds are additive and that there are no interactions when they are mixed. However, that might not always be true (Huhtanen, 1991). Associative effects have occurred when the apparent digestibility of a mixture does not equal the sum of the separately determined digestibilities of its components (Mould, 1988). Feed interactions could have been a reason for the differences between predicted methane production from individual feeds and predicted methane production from the total mixed rations.

Table 1 Proportions of ingredients of 7 concentrates used as substrates for the comparison of methane production from individual feeds and total diets in the *in vitro* gas production system.

	SBW relative to SBM (3 concentrates)[a]			4 concentrates[b]
	0%	50%	100%	
Ground maize	0	0	0	81.6
Wheat bran	43	42.5	41.5	0
Soybean meal	50	25	0	13.8
Soybean grain	0	25	50	0
Minerals	6	6	6	2.6
Urea	1	1.5	2.5	2
Total	100	100	100	100

[a] SBW = whole soybeans; SBM = soybean meal.
[b] The feed composition of concentrates are the same (the ratio of different feeds) but differed in their chemical composition.

Table 2 Descriptive statistics for the methane production (mL/g DM) obtained from incubation of total diet and from weighted information of individual feeds.

Statistic	Total diet	Weighted value
Mean	30.1	30.8
Minimum	21.3	22.9
Maximum	40.4	38.9
Standard deviation	5.64	4.31
n[a]	32	

[a] One total diet had 2 replicates in the *in vitro* runs.

Table 3 Estimates of linear regression parameters for the methane production (mL/g DM) obtained from incubation of total diet (Y) and from weighted information of individual feeds (X).

Parameter	Estimate	s.e.	P value
Intercept	22.1	7.28	0.005[a]
Slope	0.26	0.235	0.003[b]
r	0.15	-	0.431[c d]

[a] H_0: $\beta_0 = 0$. [b] H_0: $\beta_1 = 1$. [c] H_0: $\rho = 0$. [d] The correlation has been adjusted for the random variation among incubation runs.

Conclusions

It is concluded that the weighted sum of individual feeds cannot be used as a proxy for the estimation of methane production from total mixed diets.

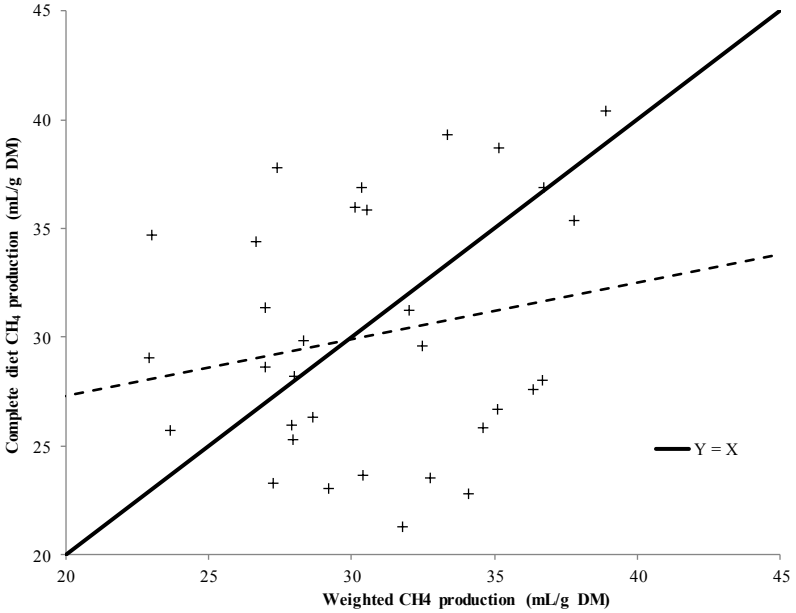

Figure 1 Descriptive relationship between the methane productions obtained from incubation of total diet and from weighted information of individual feeds (see Table 3 for details of the relationship).

References

Cone, J. W., Van Gelder, A. H., Visscher, G. J. W. & Oudshoom, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Technol. 61, 113-128.

Detmann, E., Paulino, M. F. & Valadares Filho, S. C. 2008. Nutritional evaluation of feeds or diets? A conceptual approach (in Portuguese). In: Proceedings of II International Symposium of Beef Cattle Production (II Simpósio Internacional de Produção de Gado de Corte and VI Simpósio de Produção de Gado de Corte – SIMCORTE). Universidade Federal de Viçosa. Viçosa, MG, Brazil. p.21-52.

Huhtanen, P., 1991. Associative effects of feeds in ruminants. Norw. J. Agric. Sci., Suppl. 5, 37-57.

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

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

Mould, F. L., 1988. Associative effects of feeds. In: E. R. Ørskov (ed), World Animal Science B4, Feed Science. Elsevier, Amsterdam, pp 279-292.

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

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

Relationship between chewing index and intake of metabolizable energy in pregnant ewes

M. Vestergaard Nielsen[1]*, E. Nadeau[2], B. Markussen[3], C. Helander[2], M. Eknæs[4], Å. Randby[4] & P. Nørgaard[1]

[1]Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 3, 1870 Frederiksberg, Denmark. mettevn@sund.ku.dk and pen@sund.ku.dk, [2]Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Gråbrödragatan 19, SE-532 31 Skara, Sweden. elisabet.nadeau@slu.se and carl.helander@slu.se, [3]Laboratory of Applied Statistics, Faculty of Sciences, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark. bomar@math.ku.dk, [4]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O.Box 5003, N-1432 Ås, Norway 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 optimizing ewe performance and, thereby, improve sheep productivity. Nørgaard and Mølbak (2001) have presented a linear model describing net energy (NE) intake in relation to metabolic BW as a linear function of the total ration chewing index (CI) for cattle of different types: lactating dairy cows, non-lactating non-pregnant cows, growing steers and bulls. This model was able to describe feed intake over three very different physiological stages, hence the model possibly could be used to describe feed intake also of other types of ruminants, e.g. pregnant ewes. Nørgaard and Mølbak (2001) hypothesized the biological explanation of the intercept of the model, to be the theoretical maximum energy intake capacity of the cattle. Nørgaard and Mølbak (2001) also hypothesized that the rate of decreasing energy intake with increasing CI is dependent on the theoretical maximum energy intake capacity, and further discovered a direct proportionality between slopes and squared intercept values from the individual experiments used in their model development. Increasing theoretical maximum energy intake capacity results in steeper slope. The greater potential an animal energy intake, the more sensitive that animal is to the filling effect of the feed.

The aim of this study was to investigate whether the linear relationship between metabolizable energy (ME) intake and the chewing index (CI) of the rations, also was present for pregnant ewes. A secondary objective was to test for direct proportionality between the slopes and squared intercepts when the model was developed for pregnant ewes.

Materials and Methods

The investigation was based on intake data from a total of 101 pregnant ewes, distributed on 11 different dietary treatments based on individual weekly means of energy intake over the last four weeks before parturition. One Norwegian and four Swedish experiments were included in the study. All ewes were fed *ad libitum* grass silage, supplemented with concentrate fed separately or as a total mixed ration. The ewes were adults of different breeds; the Swedish experiments included Finewool-Dorset crosses, and the Norwegian experiment included Norwegian White. Ages of the ewes ranged from two to seven years, with mean body weight (BW) of 99.6 kg (SD = 10.90), and the ewes were pregnant with 1 to 4 lambs. A summated representation of the data is presented in table 1.

Table 1 Summary characteristics[1] of the five experiments. The feed characteristics and BW are presented as mean of the experiment and minimum and maximum values of the individual animals; the concentrate (conc.) is listed as amount given or range of amounts given.

C	E	T	No	NDFf (g/kg DM)	CPf (g/kg DM)	iNDFf (g/kg NDF)	MEf (MJ/kg DM)	Conc. (kg/d)	CI corr (min/ MJ ME)	BW (kg)
S	1	2	6	541 (446-643)	150 (97-182)	121 (77-164)	11.4 (10.3-12.1)	0.2-0.8	27.8 (19.7-46.5)	103.0 (84.0-123)
S	2	3	7	553 (490-608)	156 (145-167)	71 (64-75)	11.0 (10.3-11.8)	0.8	30.5 (24.4-44.5)	98.2 (80.5-120)
S	3	3	7	453 (397-477)	202 (183-226)	34 (18-42)	11.5 (11.2-12.5)	0.8	24.7 (19.5-38.2)	99.6 (76.0-122)
S	4	3	8	473 (383-510)	152 (145-175)	35 (25-41)	11.5 (10.9-11.6)	0.5	23.9 (19.6-28.3)	98.2 (82.0-131)
N	5	3	4-12	506 (457-573)	132 (106-149)	79 (17-164)	11.2 (5.20-14.9)	0.0-0.4	32.5 (20.0-47.0)	102.8 (72.8-142)

[1] C= country, E= experiment, T= treatments, No=Number of animals per treatment. NDFf is content of NDF per kg DM of the forage, CPf is crude protein content of the forage, iNDFf is iNDF content of the forage per kg NDF, MEf is metabolizable energy content of the forage. CI corr respond to the CI values calculated in NorFor and corrected for NDF intake and BW.

The Nørgaard and Mølbak model considered intake of NE using Scandinavian Feed Units (SFU) with 7.89 MJ NE/SFU (Nørgaard and Mølbak 2001). Instead of predicting NE intake for ewes, this study utilized the more easily obtainable ME intake of the ewes. The ME intake was estimated based on dry matter intake and ME content of the feeds. The ME content of forages in the Swedish experiments was calculated from *in vitro* organic matter digestibility (VOS; Lindgren 1979, 1983, 1988). For concentrates in the Swedish experiments, the ME was calculated according to Axelsson (1941). In the Norwegian experiment *in vivo* digestibility trials were performed and ME was calculated for both concentrate and forage according to Van Es (1978). The CI was expressed per MJ of ME intake. The CI of the forages was estimated according to NorFor using neutral detergent fiber (NDF; g/kg dry matter), indigestible NDF (iNDF, g/kg NDF) and theoretical particle length (mm; Nørgaard et al., 2011). For some experiments the iNDF content was not available, and was therefore estimated from the NDF content and in vitro organic matter digestibility based on the work by Eriksson (2010) and Åkerlind et al. (2011) using iNDF (g/kg DM) = (-5.7462*VOS(%)+537.23)/(forage NDF (g/kg DM)*1000). The CI were corrected for BW and for the deviations of forage intake from 0.7% of the BW, which is the norm in NorFor (Nørgaard et al., 2011). All concentrates were given a CI value of 4 min/kg DM as described by Nørgaard et al. (2011). The data was standardized as weekly means of intake for each ewe, the corrected CI values were also calculated as weekly means for each ewe. Nørgaard and Mølbak (2001) described the NE intake in relation to metabolic BW due to the different types of cattle and type of production. However, ME intake estimates were

not corrected for metabolic BW in the present model, because only pregnant ewes of relatively similar size and production were considered.

The data was analysed by linear mixed effects modelling in R (ver. 2.15.2) with random intercept and slope dependent on experiment (Equation 1). Wald test was used to analyse the effect of model variables (Sørensen and Ekstrøm 2010), using an assumption of normal distribution to test a hypothesis about a single parameter, i.e. that intercept is different from zero. The random effect on slope and intercept was tested by a likelihood ratio test.

$$MEI_{i(,j)} = intercept_j + b_j * CI_{i(,j)} + \varepsilon_{i(,j)} \quad \text{(Equation 1)}$$

Where $MEI_{i(,j)}$ is the metabolizable energy intake (MJ ME/day) of the individual ewe in week *i* within experiment *j*, $intercept_j$ is the intercept (MJ ME/day) of experiment *j*, b_j is the slope ((MJ ME/day)^2/(min/day)) of experiment *j*, $CI_{i(,j)}$ is the chewing index (min/MJ ME) from the individual ewe in week *i* within experiment *j*, and $\varepsilon_{i,(j)}$ is the error of the regression in week *i* within experiment *j*. Note that the unit for the slope (MJ ME/day)^2/(min/day)) is to achieve unit balance of Equation 1. Linearity of the model was analysed by plots of standardized residuals versus fitted values and q-q plot, which compares the standardized residuals quantiles to those of the normal distribution (Sørensen and Ekstrøm 2010). To assess the fit of the model, R^2 was calculated, as the squared correlation between observed and predicted values based on the intercept and slopes including both fixed and random effect. As described by Nørgaard and Mølbak (2001), the proportionality between the slopes and squared intercepts values from each of the four weeks before parturition within the experiments was analyzed by linear modeling (Equation 2). Pearson's product-moment correlation between slopes from each experiment and squared intercepts from each experiment was calculated and was tested for significance by the Wald test (Sørensen and Ekstrøm 2010), the hypothesis being that the intercept, q (see Equation 2), is not significantly different from zero.

$$b_{i(,j)} = q + k * intercept_{i(,j)}^{\ 2} + \varepsilon_{i(,j)} \quad \text{(Equation 2)}$$

Where $b_{i(,j)}$ is the slope of week *i* within experiment *j*, $intercept_{i(,j)}^{\ 2}$ is the squared intercept of week *i* within experiment *j*, and $\varepsilon_{i(,j)}$ is the error of the regression in week *i* within experiment *j*.

Results and Discussion

A visualization of ME intake as a function of the CI of the rations showed that the data had large variation, but a pattern could be found suggesting that a linear relationship exists (Figure 1).

The likelihood ratio test showed significant random effect of both week and experiment on both slope and intercept values of the model for pregnant ewes, as shown in Table 2. The intercepts differed between experiments, and for all experiments the trend was for the intercepts to increase closer to parturition. The slopes did not show such a trend.

The standardized residual plot for Equation 1 showed no pattern of distribution and the q-q plot showed a linear relationship between quantiles of standard normal residuals versus standardized residuals, suggesting that the linear relationship between MEI and CI existed for the pregnant ewes. The R^2 for the model was 0.51 using fixed and random effects, suggesting that the model suffers from the large variation in the data and will have to be improved to predict satisfactory.

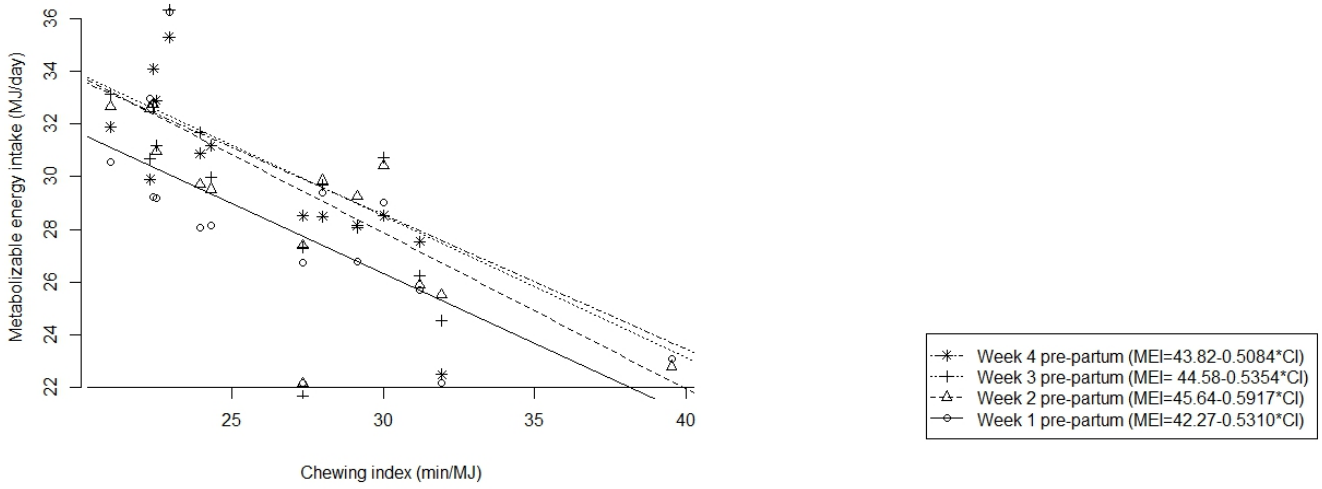

Figure 1 Treatment means with linear regression lines for metabolizable energy intake (MJ/day) of pregnant ewes in each of the last four weeks pre partum as a function of the rations' chewing index (min/MJ).

Table 2 Intercept and slope of the linear regression of metabolizable energy intake as a function of the dietary chewing index, MEI=intercept+b*CIcorr, expressed with fixed effects, standard error and significance for pregnant ewes.

Parameter	Estimate	SE	P-value
Intercept, MJ ME /day	43.40	3.99	<0.001
Slope (b), ((MJ ME/day)2/(min/day))	-0.517	0.120	<0.001

With regards to direct proportionality between the slope and the squared intercept in Equation 1, Figure 2 displays a scatterplot of the corresponding estimates from the five experiments. A linear regression was made between the slopes and the squared intercepts. Using Wald test for evaluation, the resulting linear regression, had an intercept not significantly different from zero (P=0.17) indicating that equation 2 is true. This suggests that the slope can be described as a function of the squared intercepts.

Nørgaard and Mølbak (2001) suggested that the intercept values from the individual experiments represent a corresponding theoretical capacity for energy intake, as it occurs when no dietary constraint on intake exists, for CI = 0. The theoretical capacity for energy intake of the ewes is considered to be exclusively related to animal characteristics (Elsen, 1988; Nørgaard and Mølbak, 2001). The described pattern of increasing intercepts of the model closer to parturition shows increased theoretical maximum intake capacity closer to parturition. Using animal characteristics to predict intercepts, and using forage characteristics to predict the slopes might improve predictability, and thereby improve the practical application of this linear relationship. Since direct proportionality is present between slopes and squared intercepts, any improvement in the prediction of the intercepts, would also impact the predicted slopes of the model, and might improve the model further.

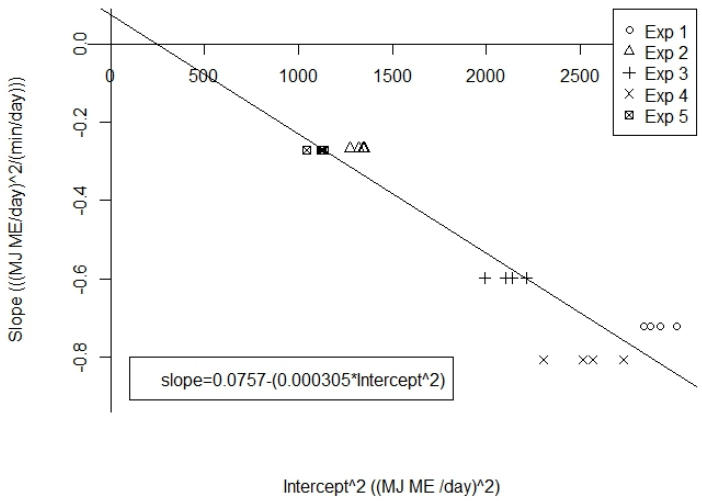

Figure 2 Plot of slopes and squared intercepts found with Equation 1 for pregnant ewes the last four weeks before parturition, the line represents the linear regression to illustrate linearity and intercept, which was not significantly different from zero (P=0.17).

Conclusions

This study supports the hypothesis that ME intake of pregnant ewes during the last four weeks before parturition decreases linearly at increasing dietary chewing index. The slope values appear to be proportional with the squared intercepts values. The intercept values are considered to reflect the metabolic capacity of the ME intake by pregnant ewes. Thus, the model proposed by Nørgaard and Mølbak (2001) could be relevant also for pregnant sheep. However, the potential prediction power of the model was unsatisfactory and the model needs further improvements.

References

Axelsson, J., 1941. Der Gehalt des Futters an umsetzbare Energie. Z. Züchtungsk. 16, 335–347.

Elsen, J. M., Wallach, D. & Charpenteau. J. L., 1988. The calculation of herbage intake of grazing sheep: A detailed comparison between models. Agric. Syst. 26, 123-160.

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

Lindgren, E., 1979. The nutritional value of roughages determined in vivo and by laboratory methods. Report 45. Dep. Anim. Nutr. Managem., Swed. Univ. Agric. Sci., Sweden (in Swedish with English summary), 66 p.

Lindgren, E., 1983. Nykalibrering av VOS-metoden för bestämning av energivärde hos vallfoder. Working paper. Dep. Anim. Nutr. Managem., Swed. Univ. Agric. Sci., Sweden (in Swedish), 4 p.

Lindgren, E., 1988. Fodrets energivärde. Course paper Feed Science HNU 3. Dep. Anim. Nutr. Managem., Swed. Univ. Agric. Sci., Uppsala, Sweden (in Swedish). 49 p.

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.

Sørensen, H. & Ekstrom, C.T., 2010. Introduction to Statistical Data analysis for the Life Sciences. CRC Press.

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

Van Es, A. J. H., 1978. Feed evaluation for ruminants. I. the systems in use from May 1977 onwards in the Netherlands. Livest. Prod. Sci. 5, 331-345.

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.

In-line oxygen monitoring in lab-scale silos

T.M. Pauly
Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Kungsängen Research Centre, 753 23 Uppsala, Sweden.
Correspondence: Thomas.Pauly@slu.se

Introduction

The principle of lab-scale ensiling experiments is to apply one or several specific treatments to homogenized forage or a group of silos while all important environmental factors, which might affect fermentation quality, are kept as equal as possible among silos. This way, a specific treatment effect can be studied with little interference from confounding factors. However, in many experiments, control over the ingress of air during the storage of silos (or wrapped bales) is either ignored or not reported despite the fact that the deteriorating influence of oxygen on fermentation quality of silage is well described (Honig, 1994; Ruxton et al., 1975; Tabacco et al., 2009; Woolford, 1990). This leads to unnecessary large response variations and clouds potential differences among treatments.

Figure 1 Glass jar silo with a water-filled siphon and 2 stoppers (top+bottom, see arrows), which were used to inject air into the silo.

At our research facility, we routinely use water-filled plastic siphons, which are attached to the lids of our mini-silos (Figure 1) and allow fermentation gases to escape (as bubbles) from the silos without the ingress of air. In addition, the level of the two water columns indicates if a silo was badly sealed (water columns in level) or if a silo was tight (water columns on different levels indicating an over- or underpressure in the silo). This has helped us to remedy badly sealed silos, increased accuracy of our studies and allowed us occasionally to reduce the number of silo replicates.

Our goal is not to exclude any oxygen from our mini-silos but to create the same degree of air leakage in all silos and, if possible, to quantify the amount of oxygen leaking into our silos. For this purpose, we recently bought oxygen sensor spots (from: www.pyro-science.com), which easily fit in our mini-silos and which can record oxygen concentrations over a wide range in both gas and liquid phases. Below, it is shown how this equipment is used in a small pilot study.

Materials and Methods

A single glass jar was filled with freshly mown loan grass (23% DM) to get an indication on how long time it took to deplete the oxygen concentration in the silo after sealing. At a later occasion (Oct. 24th), three glass jar silos (1.8 L volume) were filled with precision-chopped maize forage (41% DM) and injected them with either 4.25, 8.5 or 17.0 mL of air (equivalent to 0.2%, 0.5% and 0.9% of total silo volume). Air was injected by hand with a syringe and a thin hypodermic needle (0.7 x 30 mm) 3 times per week (Mon, Wed, Fri). Hypodermic needles were inserted through either the bottom or the top stopper of the silo and they were not removed during the study. Needle ends were plugged with silicone stoppers (except during injections) to avoid any air leakage (Figure 2). When air was injected through the top

stopper in the silo lid, the siphon was plugged with a stopper (to avoid loss of air) and the bottom stopper was removed from the needle (to release overpressure). Our intention was to study the decrease of oxygen concentration in the silo after air injections to get an indication on how fast oxygen might be consumed in silage. Air was assumed to contain 20.5% oxygen.

A vital part of the oxygen monitoring set is the oxygen sensor spot (Ø = 5 mm), which contains a green, oxygen-sensitive dye. Sensor spots were glued on the inside of each silo right below the lid, i.e. in the silo's head space.

On the opposite side of the glass wall, a fibre cable was attached with a special adapter. This fibre cable ended in a device (FireStingO_2) that emitted red light of a specific wave length and detected the IR light reflected from the sensor spot. A change in the silo's oxygen concentration changed the reflection properties of the dye, which was then translated by the FireStingO_2 device into a specific oxygen concentration. Before the onset of each recording, the whole setup was calibrated with <0.01% O_2 (in a tightly sealed silo) and air (20.5% O_2). A temperature probe corrected automatically for temperature changes during measurements. Measurement intervals and recorded values were saved and displayed graphically on a connected computer.

Figure 2 Glass jar silo (approx. 1.8 L volume) with hypodermic needle inserted through the bottom stopper. The needle was sealed by another stopper.

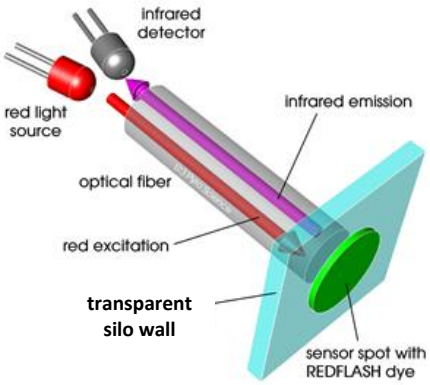

Figure 3 Detection principle of an oxygen sensor spot (green) containing an oxygen sensitive dye.

Results and Discussion

Decrease of oxygen concentration in a freshly sealed mini-silo with unwilted loan grass

In the unwilted grass, forage oxygen concentration fell from 20.5% to below 0.1% within 47 min. We assume that this decrease was mainly caused by plant respiration. The decrease was rather linear at approximately 4.4%-units for every 10 min. (Figure 4). Sprague (1974) reported that when he filled fresh alfalfa (24% DM) in mini-PVC-silos, about 0.5% O_2 remained 30 min. after sealing, which was a slightly faster O_2 decrease than in our silo.

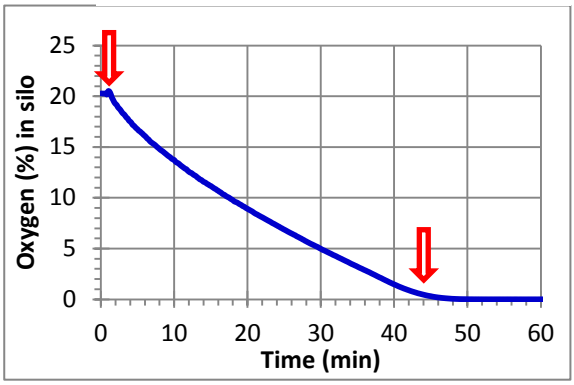

Figure 4 Decrease of oxygen concentration in freshly sealed grass forage (23% DM).

Decrease of oxygen concentration in 3 air-injected silos

We started to inject air through the bottom stopper but were unable to get any oxygen response from the sensor spots, not even after the injection of 17 mL of air (3 attempts and waiting for 30 min after injection). Therefore, injections through the top stoppers into the silo head space were done instead. By doing so, almost instantaneous and sharp oxygen peaks, proportional to the quantity of injected air, were seen (Figure 5).

After the injection peak, oxygen concentrations dropped at an exponential rate approaching zero (Figure 5). This change was different from the almost linear oxygen decrease after sealing (Figure 4) and was evident after each of the 3 air injection levels. The reasons for this might be that there are two underlying processes influencing oxygen concentration in the head space of the silo: a) the dispersion of oxygen in the head space and penetration into silage pores (rel. fast O_2 decrease by dilution) and b) the consumption of oxygen by micro-organisms (rel. slow O_2 decrease). The combination of these two processes might be responsible for the oxygen curves shown in Figure 5.

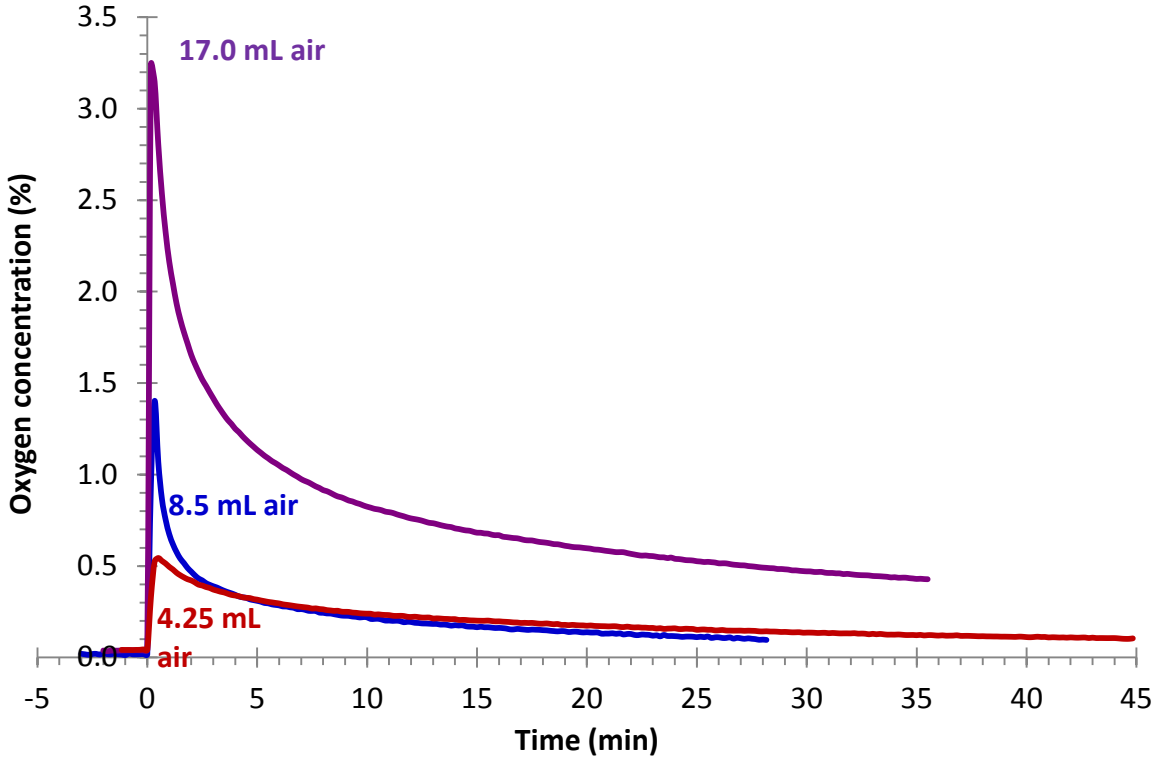

Figure 5 Decrease of oxygen concentration in the head space of maize silage (41% DM) 18 days after ensilage. 3 different quantities of air were injected.

Another explanation might be that oxygen-consuming microorganisms and oxygen are not evenly distributed within a silo. In case oxygen-consuming microorganisms have depleted oxygen at one spot, oxygen has to diffuse from surrounding areas, which takes time, particularly when the O_2 concentration gets low (i.e. low partial pressure difference).

Conclusions

- Oxygen disappears quickly (<1 h) from a freshly sealed silo with unwilted forage due to plant respiration.
- The reason why we didn't get any oxygen response from the sensor spots after injections through the bottom stopper is most likely that the oxygen was completely absorbed and/or metabolised on its way from the place of entry (bottom stopper) to the sensor spot (head space below silo lid; distance bottom stopper to sensor spot approx. 160 mm).
- Only when the oxygen sensor was placed in the head space above the silage (and not in the silage mass), a clear response of an oxygen leakage can be observed.
- Air injections into the silage head space lead to oxygen peaks proportional to the quantity of injected air. Oxygen levels decrease at an exponential rate as oxygen concentration approaches zero.

References

Honig, H., 1994. Amount and effects of gas exchange in silage stacks. Proc. of the 15th Gen. Meet. Europ. Grassl. Fed., 6-9 June 1994, Wageningen, The Netherlands. p.143-145.

Ruxton, I.B., Clark, B.J. & McDonald, P., 1975. A review of the effects of oxygen on silage. J. Brit. Grassl. Soc., 30, 23-30.

Sprague, M.A., 1974. Oxygen disappearance of alfalfa silage (*Medicago sativa* L.). Proc. of the 12th Internat. Grassl. Congr., Moskva, p.216-225.

Tabacco, E., Piano, S., Cavallarin, L., Bernardes, T.F. & Borreani, G., 2009. Clostridia spore formation during aerobic deterioration of maize and sorghum silages as influenced by *Lactobacillus buchneri* and *Lactobacillus plantarum* inoculants. J.Appl. Microbiol., 107, 1632–1641.

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

Effects of heat treatment on protein feeds evaluated *in vitro* utilizing the method utilizable protein

M. Vaga, M. Hetta & P. Huhtanen
Dept.of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden.
Correspondence: merko.vaga@slu.se

Introduction

There is an increasing interest in using domestic feeds in diets for dairy cattle in Sweden. Due to increasing energy prices as well as ethical considerations and growing demand for certified organic feeds it is not rational anymore to depend on imported protein supplements for dairy cattle. Seeds of field beans (*Vicia faba*), and lupines (*L. angustifolius)* have high crude protein (CP) and starch content (NRC, 2001) and therefore have potential to replace imported soybean meal (May et al. 1993; Tufarelli et a. 2012).

Meeting dietary protein requirements of high producing dairy cows rely largely on the proportion of ruminally undegraded protein which also has high total digestibility. Heat treating protein feeds have been suggested to decrease protein degradability and increase nitrogen efficiency in dairy production (Broderic & Graig, 1980; Cros et al. 1991; Canbolat et al. 2005). One of the challenges has been to evaluate the effects of heat treatments with relevant laboratory methods as production trials are expensive. Several new methods for evaluation of forage protein for ruminants have been presented, e.g. Karlsson *et al*. (2009). The technique described by Karlsson *et al*. (2009) originates from the method developed by Raab *et al*. (1983), which utilises the production of ammonia nitrogen (NH_3-N) for the estimation of protein degradation *in vitro*. The method has also been modified into another promising method for estimation of protein supply from forages by using utilisable crude protein (uCP) (Edmunds *et al*., 2012). It quantifies simultaneously the availability of protein as the sum of the microbial *de novo* synthesis of protein in combination with estimations of the degradation of feed protein resulting in a total estimate of supply of protein from the rumen to the small intestine. Using *in vitro* methods for protein evaluation remove the problems like escape of soluble non-ammonia-N and particle losses, which occur in *in situ* methods (Ørskov and McDonald, 1979). The aim of this experiment was to evaluate the effects of heat treatment on protein feeds utilising the *in vitro* method utilisable protein.

Materials and Methods

Feeds and Experimental design

The experiment had 2x2x4 factorial design plus one control, including two protein feeds, two heat treatment methods and three temperature levels + untreated and one control diet, resulting in a total of 17 treatments. The two protein feeds used in this experiment were grains of field beans and lupines. Each protein feed was mixed together with standard silage and barley in isonitrogenous total mixed rations (TMR) in order to simulate real diets and take into account effects on microbial protein synthesis which provides about two thirds of the of the protein supply to the ruminant. The TMR diets consisted of 500 mg of standard grass silage, barley and protein feed, combined to give a CP concentration of 180 g/kg DM (Table 1). The control diet consisted of only standard silage and barley (1:1, fresh weight) with total CP concentration of 132 g/kg DM. Each diet was randomly distributed within and between *in vitro* series (run) and replicated twice within two of total eight runs, resulting in

four observations per sample. In each run, a blank (buffered rumen fluid without a sample) and the control diet were incubated in duplicates.

Sample preparation and heat treatments

The feeds were dried at 60°C in force air oven for 48h and milled through a 1mm screen (cutter mill; SM300, Retsch GmbH, HAAN, Germany). Thereafter the samples of protein feeds, silage and barley were analysed for dry matter (DM) (105^{o}C for 16 h), ash (AOAC 1984) and CP (AOAC 1984). The chemical composition of the feeds and diets are presented in Table 1.

Table 1 Chemical composition of experimental feeds and diets

			Protein feeds		Diets		
	Silage	Barley	FB	LP	FBD	LPD	Control
DM, g/kg	939	866	905	882	914	907	903
OM, g/kg DM	941	969	960	959	956	956	960
CP, g/kg DM	143	120	299	348	180	180	132

FB – field beans; LP – lupines; FBD – field beans diet (field beans meal+silage+barley); LPD – lupines diet (lupines meal+silage+barley); Control - silage+barley diet.

The two protein feeds were heat-treated for 60 minutes at 120, 140 and 160°C in a ventilated oven (ULE 400, Memmert, Germany) with dry air or at 105, 120 and 135°C in an autoclave (PACS 50, Getinge Biofoe, Sweden) with steam pressure. The milled feed samples were placed on aluminium trays and evenly spread (2 cm deep layer). After the heat treatments, the samples were allowed to cool in open air for 24 h to regain original moisture content (avg. 92% DM) before packing and storing. A sample of each unheated feed was retained for use in the control treatments.

In vitro procedures

Rumen fluid was collected about two hours after morning feeding from two dry and ruminally cannulated Swedish red cows fed on a diet containing grass silage (600 g/kg DM) and commercial concentrates (400 g/kg DM). The rumen fluid was filtered through four layers of cheese cloth into a buffered mineral solution (Menke and Steingass, 1988), with a 1:4 volume ratio of rumen fluid to buffer. About 1 g of each TMR diet was incubated in 60 ml of buffered rumen fluid in 250-ml serum bottles (Schott, Mainz, Germany). The bottles were placed in water baths at 39°C for 48 h and continuously agitated. The *in vitro* gas production (GP) was recorded as described by Hetta et al. (2003). The *in vitro* procedures were performed with a fully automated system (Cone et al., 1996), recording GP every 12 minutes. Simultaneously with gas recordings, determinations of NH_3-N concentrations in the liquid phase were made at 8 h, 24 h and 48 h. The concentration of NH_3-N of the buffered rumen fluid in the incubation bottles was determined by sampling 0.4 ml of fluid with plastic syringes as described by Karlsson et al. (2009). The fluid samples were transferred into Eppendorf tubes kept on ice and thereafter, 0.016 ml of 96% H_2SO_4 was added for preservation. The samples were stored frozen (-20°C) until analysis. The sample tubes were thawed, centrifuged (12500 x g, 10 min) and 0.1 ml of supernatant was transferred to test tubes and diluted 1:20 with distilled water. The concentrations of NH_3-N was analysed using a continuous flow analyser (AutoAnalyzer 3 HR, SEAL Analytical Ltd).

Data handling and statistical analysis

For each treatment, we calculated the concentrations of uCP as described by Edmunds et al. (2012) (eqn. 1) as:

$$\text{uCP (g/kg DM)} = \frac{NH_3N_{blank} + N_{sample} - NH_3N_{sample}}{\text{weight (mg DM)}} \text{ x } 6.25 \text{ x } 1000 \text{ (eqn. 1)},$$

where NH_3N_{blank} is the average amount (g) NH_3N in the two blanks at the time of interest, N_{sample} is the amount (g) of N in the sample at the start of the incubation and NH_3N_{sample} is amount (g) of NH_3N in the incubation bottles at the time of interest for the treatment evaluated.

The effect of heat treatments on uCP and NH_3-N were analysed using the GLM procedure in SAS (SAS Institute 2008). The model included feed, run, treatment temperature and method. Treatment effects were considered to be significant at $P \leq 0.05$.

Results and discussion

The use of heat treatments effectively increased the uCP concentrations for both the field bean and lupine diets. The lowest temperature for both the oven- and autoclave-treatments actually reduced the uCP concentrations for the field bean diets after 8 h. This reduction was expected as it is generally known that modest heat treatments can improve protein degradability (Fennema, 1976). Increasing the temperatures for oven-treated field beans caused linear increase of uCP based on the 24 h incubations (Table 2). However for lupines, only the medium and high temperatures had positive effects, but low temperature treatments instead decreased the concentration of uCP.

The lupines were less affected by the heat treatments than the field beans. The high temperature oven-treatment increased uCP levels from 120 to 166 g/kg DM for the field bean diets and from 167 to 187 for the lupine diets at 24 h. For the autoclaved feeds the increases were from 120 to 173 g/kg DM for field beans and from 167 to 178 g/kg DM for lupines. Similar effects were reported by Martinussen et al. (2013) who found that Lupines required higher temperatures than field beans to achieve the same reducing effect on soluble protein. Despite the different temperatures, both oven and autoclave treatments seemed to have similar effect on uCP and NH_3-N concentrations in this study.

Changes in concentrations of NH_3-N 0, 8 and 24 h incubations are shown in Figure 1. The autoclave treatments seemed to decrease NH_3-N concentrations already after 8 h while for oven treated feeds, the effect of heat treatment was significant after 24 h of incubation. These results are supported by Tagari et al. (1986) who noted that autoclave treatment of feeds increased the NH_3-N concentrations at lower temperatures compared to dry heat treatments. Heat treatment with both oven and autoclave significantly ($P < .001$) decreased the NH_3-N concentrations as shown in Table 2.

Table 2 uCP and NH_3-N concentrations of untreated and treated field beans and lupines diets after 24 hour incubations

	Untreated	Oven treated			SE	P	Autoclave treated			SE	P
Temperature, °C		120°C	140°C	160°C			105°C	120°C	135°C		
					uCP 24h (g/kg DM)						
Field beans	121	141	158	167	18.7	***	168	158	173	18.7	***
Lupines	167	153	179	187	17.2	***	171	190	179	18.8	***
Control	166										
					NH_3-N 24h (mg/L)						
Field beans	451	405	357	340	42.6	***	317	363	320	42.6	***
Lupines	341	373	313	290	40.6	***	331	323	309	47.7	***
Control	226										
Blank	309										

*** P<0.001

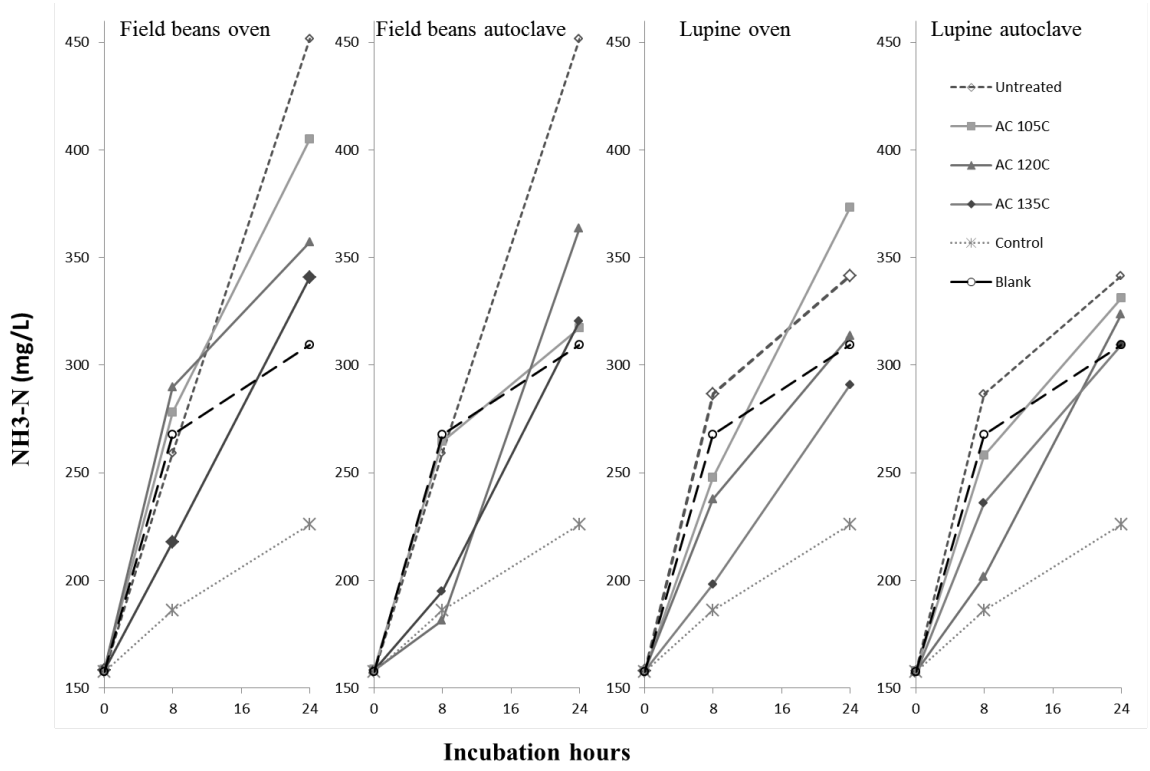

Figure 1 Effect of heat treatments of field beans and lupines on NH_3-N concentrations *in vitro.*

There were higher correlations (r = -0.97 and r = -0.80) between temperature and NH_3-N concentrations for oven-treated and autoclave-treated lupines than for field beans (r = -0.66 and r = -0.70).

The concentrations of NH_3-N (mg/L) had high variance among and within runs at 8 h (SE=24) and 24 h (SE=42), but very small variance at 0 h (SE=1). Small variance at the start

of the incubations when no interactions had occurred yet, suggest a potential for good precision of the analytical method.

Conclusions

This study shows that heat treatment was effective in increasing the concentrations of uCP in both field beans and lupines, but lupines needed higher temperatures to achieve the same effect as in field beans. The new analytical method provided by Edmunds et al. (2012) for estimating uCP seems to have good potential for evaluating protein quality *in vitro*. However more studies need to be carried out to evaluate the accuracy of this method. Especially, we need to evaluate the effects of heat treatments in production experiments with dairy cows in order to evaluate the relevance of the *in vitro* methods for potential use in feed evaluation systems.

References

AOAC, 1984. *Official methods of analysis of AOAC*. Washington DC, USA: Association of Official Analytical Chemists.

Broderick, G.A. & Craig W.M., 1980. Effect of heat treatment on ruminal degradation and escape, and intestinal digestibility of cottonseed meal protein. J. Nutr. 110, 2381.

Canbolat, O., Kamalak, A., Efe, E., Sahin, M. & Ozkan, C.O., 2005. Effect of heat treatment on in situ rumen degradability and in vitro gas production of full-fat soyabeans and soyabean meal. South African J. Anim. Sci. 3, 186-194.

Cone, J.W., van Gelder, A.H., Visscher, G.J.W. & Oudshoorn, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Technol. 61, 113–128.

Cros, P., Vernay, M. & Moncoulon, R., 1991. In situ evaluation of the ruminal and intestinal degradability of extruded whole horse beans. Reproduction Nutrition and Development, 31, 249–255.

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

Fennema, O.R., 1976. Principles of Food Science. Part I. Food Chemistry. Marcel Dekker, Inc., New York, NY.

Hetta, M., Cone, J.W., Gustavsson, A.-M. & Martinsson, K., 2003. The effect of additives in silages of pure timothy and timothy mixed with red clover on chemical composition and in vitro rumen fermentation characteristics. Grass Forage Sci. 58, 249–257.

Karlsson, L., Hetta, M., Udén, P. & Martinsson, K., 2009. New methodology for estimating rumen protein degradation using the in vitro gas production technique. Anim. Feed Sci. Technol. 153, 193-202.

Martinussen, H., Jørgensen, K.F., Strudsholm, F. & Weisbjerg, M.R., 2013. Heat treatment increases the protein value in Faba beans and Lupins. Proceedings of the 4th Nordic Feed Science Conference, 287, 34-38.

May, M.G., Otterby, D.E., Linn, J.G., Hansen, W.P., Johnson, D.G. & Putnam, D.H., 1993. Lupins (Lupinus albus) as a protein supplement for lactating Holstein dairy cows. J. Dairy Sci. 76, 2682–2691.

Menke, K.H. & Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal Research and Development, 28, 7-25.

NRC, 2001. Nutrient Requirements of Dairy Cattle, 7th ed. National Academy Press, Washington, DC, USA.

Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. J. Agr. Sci**.** 92, 499–503.

Raab, L., Cafantaris, B., Jilg, T. & Menke, K.H.,1983. Rumen protein degradation and biosynthesis. 1. A new method for determination of protein degradation in rumen fluid in vitro. Brit. J. Nutr. 50, 569–582.

SAS, 2002. User's Guide: Statistics. SAS Institute, Inc., Cary, NC, USA.

Tagari, H., Pena, F. & Satter, L.D., 1986. Protein degradation by rumen microbes of heat-treated whole cottonseed. J. Anim. Sci. 62, 1732.

Tufarelli, V., Khan, R.U. & Laudadio, V., 2012. Evaluating the suitability of field beans as a substitute for soybean meal in early-lactating dairy cow: production and metabolic responses. J. Anim. Sci. 83(2), 136-40.

Grass silage extract, feed component suitable for pigs – prospects for on farm biorefinery

A. Seppälä[1], S. Kyntäjä[1], L. Blasco[1], M. Siika-Aho[2], S. Hautala[3], O. Byman[3], H. Ilvesniemi[3], H. Ojamo[4], M. Rinne[1], M. Harju[5]
[1] MTT Agrifood Research Finland, 31600 Jokioinen, Finland; [2] VTT Technical Research Centre of Finland, Espoo, Finland; [3] Finnish Forrest Research Institute, Vantaa, Finland; [4] Aalto University, Espoo, Finland; [5] Valio Ltd, Helsinki, Finland
Correspondence: Arja.Seppala@mtt.fi

Introduction

There are approximately 470 000 hectares of grass in Finland that are not fully utilized (Seppälä et al., 2014). The potential of surplus grass or grass silage as raw material for green biorefineries has been evaluated in several research projects during last decades (e.g. Grass 2004; Kamm and Kamm 2004; Mandl 2010; Sieker et al., 2011), and demo or pilot plants have been built in Austria, Germany, Netherlands and Switzerland (Keijsers and Mandl, 2010). Due to different national interests, the explored process chain and the focused end products have varied including ethanol (Sieker et al., 2011), insulation boards (Grass, 2004), purified lactic acid and amino acids (Ecker et al., 2012) or biogas and insulation (O'Keeffe et al., 2011).

During ensiling, grass material undergoes proteolysis mainly caused by plant enzymes (McDonanld et al., 1991). Because of the proteolysis during the preservation, fresh grass is often regarded as a better raw material for protein separation than silage (Kammes et al., 2011). However under Nordic conditions, the short availability of fresh grass is a severe drawback. The extent of proteolysis during ensiling can be reduced by rapid drop of pH (McDonald et al., 1991) enhanced by the use of efficient additives. The solubilisation of protein during ensiling might actually alleviate separation of amino acids. Choi et al., (2003) measured in primary growth grass silage 427 g free amino acids, 188 g peptides and 53 g soluble true protein /kg total N meaning that 667 g/kg total N should be relatively easily separated from the fibrous part and being useful for monogastric animals.

Grass silage (Presto et al., 2013) or silage effluent (Patterson and Kilpatrick, 1991) can be fed to pigs as such although with some restrictions. However, the authors of this paper have no knowledge of scientific articles reporting on farm processing of grass silage to produce feeds for pigs, although the modern production systems (including liquid feeding and automated systems for dealing with silage, see e.g. www.Pellon.com) would enable automatic processing of silage before feeding. This paper focuses on the potential for on farm processing of silage for pigs. The argumentation for more research in this field is based on results of two preliminary experiments and literature.

Material and methods

Exp1. In the first experiment, an unfertilized timothy-red clover sward was harvested in a leafy growth stage (primary growth) with or without prewilting and ensiled using a formic acid based additive (AIV 2 Plus, Kemira Ltd., Helsinki, Finland), The amount of additive used was 0, 6, 12 or 18 l/t, but the combination "0 l/t on prewilted" was omitted due to restricted capacity. Three replicate silos were filled per treatment. After ensiling, the juice was separated from silages by a pneumatic press (7 bars). Silage quality was analysed using the ARTTURI® silage analysis (Valio Ltd., Helinki, Finland), where dry matter (DM) was

measured by oven drying, fermentation quality was analysed using titration method and silage composition using NIR method. The silage press juice was analysed for DM (freeze drying), ash (500°C, overnight) and nitrogen at MTT laboratory. From two samples representing the treatments "no prewilting-no additive" and "no prewilting - acid 6 l/t", three replicates were combined and amino acid profiles were analyzed (Eurofins, Finland).

Exp2. The second experiment was part of a larger project "Protein from grass" but only the first part of the work is described here. The project was funded by TEKES and Valio Ltd. Single batch of farm scale precision chopped silage was used as raw material for the process. The silage was a second cut of timothy-meadow fescue sward ensiled using a formic acid based additive (AIV 2 Plus 5 l/t, Kemira Ltd., Helsinki). The silage juice was separated using three different methods; either by the same pneumatic press as in the Exp1, or by combination of pressing and pressurized hot water extraction (PHWE, pilot scale, 60°C, 10 kg/min, 90 min), or by hot water extraction (HWE, pilot scale, 55°C, three batches of water, used 2805 kg water/750 kg silage). After the HWE, the extract was concentrated by evaporation.

Results

Exp1. Botanical composition of the silage was timothy 0.600, red clover 0.337, meadow fescue 0.055 and unsown species 0.008. On average, the silages had a crude protein (CP) concentration of 136 g/kg DM, neutral detergent fibre (NDF) 422 g/kg DM, digestible organic matter 735 g/kg DM and ash 56.6 g/kg DM. Ammonium N and soluble N concentrations of the formic acid treated silages were low (Table 1). The press juice represented 0.30 to 0.54 of the wet silage, equivalent to 0.15 to 0.32 of DM of the silage. The juice had a DM content of 100-150 g/kg and an ash content of 97–140 g/kg DM. The CP content in the juice (in which amino acids were determined) was 127 or 182 g/kg DM, having a lysine concentration of either 5.8 (not prewilted, acid 6 l/t) or 4.4 g/100 g CP (not prewilted, no additive) respectively.

Exp2. The silage had a DM-content of 241 g/kg, ash 115, crude protein 138, NDF 524 and digestible organic matter 646 g/kg DM, pH 4.19, lactic acid 69 g/kg DM, ethanol 29 g/kg DM and ammonium N 69 g/kg total N. Results (mass balances) of silage fractioning by different methods are presented in Table 2. The concentrated silage extract had 536 g DM/kg, 306 g ash/kg DM, 247 g CP/kg DM and a lysine content of 3.68 g/100 g CP.

Table 1 Silages used as raw material for juice separation by pressing in Exp1 and results of juice separation.

Prewilting	Not prewilted				Prewilted (PW)			SEM	Statistical effects		
	DM 220 g/kg				DM 283 g/kg						
Level of acid (L)	0	6	12	18	6	12	18		L	PW	LxPW
Silage quality											
pH	3.7	3.8	3.9	3.8	3.8	4.1	4.3	0.05	***	***	**
NH_3 N, g/kg total N	34.3	5.0	7.3	13.3	5.0	14.3	23.3	2.16	***	**	NS
Soluble N, g/kg total N	500	329	310	331	274	273	320	14.7	***	*	NS
Lactic acid, g/kg DM	104.0	71.7	64.3	72.7	63.3	47.0	49.0	3.80	***	***	NS
Volatile fatty acids, g/kg DM	33.0	16.3	18.7	16.0	24.3	34.0	29.0	2.67	***	***	NS
Sugars, g/kg DM	45.7	121.7	61.3	96.0	82.3	63.3	60.0	18.40	NS	NS	NS
Extraction rates (juice/silage)											
Fresh	0.519	0.542	0.497	0.453	0.347	0.313	0.297	0.0121	***	***	NS
Dry matter	0.254	0.283	0.231	0.221	0.179	0.134	0.136	0.0090	***	***	NS
Crude protein	0.291	0.225	0.195	0.178	0.142	0.118	0.119	0.0069	***	***	NS
Ash	0.526	0.505	0.509	0.454	0.378	0.287	0.292	0.0150	**	***	*

Table 2 Mass balances of silage fractioning by different methods. Values are percentages of the original amount.

	Pressing		Sequential combination of pressing and PHWE[2]		HWE[3] with mixing	
	Liquid	Solid	Liquid	Solid	Liquid	Solid
Mass	30	70	-	-	-	-
Dry matter[1]	9	91	25	75	23	77
Ash	23	77	60	40	-	-
Protein	17	83	30	70	42	58
Lactic acid	30	70				
Phosphorus	38	62	79	21	-	-

[1]Corrections for loss of volatiles not made; [2]PHWE = pressurized hot water extraction; [3]HWE = hot water extraction.

Discussion

Towards optimal process to fractionate grass silage

Cell solubles (DM minus NDF) represent nearly half of the DM of high digestibility grass silage. Results of Exp1 suggest that more than half of cell solubles could be separated by simple pressing when the leafy silage was not prewilted but ensiled with a formic acid based additive using 6 l/t. Further, the results of Exp2 suggest that the gain would be improved by using hot water extraction instead of pressing. Over 80% of organic matter of leafy grass silage can be solubilised by the combined use of pepsin and cellulases in a laboratory process (Huhtanen et al., 2006). The fractioning could possibly also be facilitated by the use of enzymes.

Protein value of silage extract

Both silages in Exp1 and Exp2 had relatively low CP contents. In Exp1, low proportion of clover was not able to compensate for the shortage of nitrogen fertilization. When targeting to optimize raw material species, fertilization and growth stage should be considered. Further, proteolysis during ensiling should be balanced so that enough solubilisation takes place while breakdown of amino acids is restricted. Highest proportion of protein was separated when using no additive, but this resulted in protein having less lysine than in the additive treated silage juice. This is in line with the results of Winters et al. (2001), who suggested that additives aid to achieve better amino acid profile in silage than without additives.

Potential to reduce phosphorus load

High amount of phosphorus is silage effluent has given it a bad reputation in relation to environmental load. When targeting to produce silage for pigs, high solubility of phosphorus in silage could be exploited to reduce nutrient runoff to the waterways. The effect would be achieved in three ways:

1) In Finland, typically more phosphorus is removed from soil by harvested grass silage than by harvested grain yield.

2) Nowadays, feeds typically bring more phosphorus to the farm than will be sold as animal products. On farm cultivated grass extract could replace part of the monocalcium phosphate and soybean meal in pig diets, thus reducing phosphorus accumulation.

3) Grass cultivation is encouraged (by subsidies), especially on riversides and lakesides, to avoid erosion. If there is no use for the grass and it is not harvested, phosphorus within the biomass is in great danger to leak into the water. An efficient usage for grass biomass would alleviate that problem.

Economic feasibility

In Exp2, the value of concentrated silage extract was calculated to be 381 €/t DM, when included in diets (10 % DM) to fattening pigs (Table 3). To achieve economic feasibility, the gain of the separation process should be improved (e.g. from 25 to 40 % for DM and from 20 to 40 % for CP) while keeping the process simple. Further, the economic value of reduced phosphorus load should be included into the calculations. While the calculated price of silage extract already covers the production cost of grass silage for biogas production (80 €/t DM, not including the silos (Kässi and Aro-Heinilä, 2014), economic feasibility might be achievable in near future.

The fibrous residue of processing could be used e.g. as stimulus feed for pregnant sows, high quality feed for horses or as a source of energy. For horse owners, a feed with positive health effects could be marketed at a higher price. The reduction in protein and sugar as a consequence of fractioning would be beneficial for animals with low energy requirements.

Table 3 Recipes for liquid feeding with and without silage extract.

		Two recipes for liquid pig feeding (% of feed DM)	
Feed components	Price, €/t	Without silage extract	Silage extract included (10 % DM)
Barley	140	73	65
Wheat	165	10	10
Soybean meal (GMO-free)	500	13.3	11
Silage extract	204		10
Rapeseed oil	1250	0.57	1.5
L-Lysin	1400	0.34	0.37
L-Threonin	2000	0.08	0.08
DL-Methionine	2750	0.03	0.07
Monocalcium phosphate	500	1.04	0.78
Calcium carbonate	75	1.59	1.47
Added acid	2000	Dosage 2 l/liquid feed	-
Price of the mixture, €/t		211	211

Conclusions

Processing grass silage for pig feeding could improve protein self sufficiency and reduce phosphorus leakage into watercourses. Economic sufficiency could be achieved by combining all positive effects. More research is however needed on several aspects of the process and further on the effects on pig production and environment.

References

Choi, C.-W., Vanhatalo, A. & Huhtanen, P., 2003. Effects of type of grass silage and level of concentrate on the flow of soluble non-ammonia nitrogen entering the omasum of dairy cows. J. of Anim. feed Sci. 12, 3-22.

Ecker, J., Schaffenberger, M, Koschuh, W., Mandl, M., Böchzelt, H.B., Schnitzer, H., Harasek, M. & Steinmüller, H., 2012. Green Biorefinery Upper Austria – Pilot Plant operation. Sep. Purif. Technol. 96, 237–247.

Grass, S., 2004. Utilisation of grass for production of fibres, Protein and energy. In: Biomass and Agriculture: Sustainability, Markets and Policies. OECD Publication services, Paris. Available at: http://www.bpsag.ch/pdf/Publication%20OECD.pdf. (Accessed May 1, 2014).

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

Kamm, B. & Kamm, M., 2004. Principles of biorefineries. Appl. Microbiol. Biotechnol. 64, 137–145.

Kammes, K.L., Bals, B.D., Daleb,B.E. & Allena, M.S., 2011. Grass leaf protein, a coproduct of cellulosic ethanol production, as a source of protein for livestock. Anim. Feed Sci. Technol. 164, 79–88.

Keijsers, E. & Mandl, M., 2010. Green Biorefinery, IEA Biorefinery Course. Available at: http://www.bioref-integ.eu/fileadmin/bioref-integ/user/documents/8._Keijsers-Green_Biorefinery_130910.pdf (cited May 1, 2014).

Kässi, P., Aro-Heinilä, E. 2014. Nurmibiokaasulaitoksen talous. In: Seppälä, A., Kässi, P., Aro-Heinilä, E., & Lehtonen, H. 2014. Nurmesta biokaasua liikennepolttoaineeksi. Bionurmi-hankkeen loppuraportti. Available at www.mtt.fi/bionurmi.

Mandl, M.G., 2010. Status of green biorefining in Europe. Biofuels, Bioproducts and Biorefining 4, 268–274.

McDonald, P., Henderson A.R. & Heron S.J.E., 1991. The Biochemistry of Silage. 2nd edition, Chalcombe Publications, Marlow, UK. 340 p.

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

Patterson, DC. & Kilpatrick, D.J., 1991. Effluent from grass-silage for finishing pigs. J. Agric. Sci., 116, 119-124.

Presto, M., Rundgren, M. & Wallenbeck, A. 2013. Inclusion of grass/clover silage in the diet of growing/finishing pigs – Influence on pig time budgets and social behaviour. Acta Agric. Scand., Section A - Anim. Sci. 63, 84-92

Seppälä, A., Kässi, P., Aro-Heinilä, E. & Lehtonen, H. 2014. Nurmesta biokaasua liikennepolttoaineeksi. Bionurmi-hankkeen loppuraportti. Available at www.mtt.fi/bionurmi.

Sieker, T., Neuner, A., Dimitrova, D., Tippkötter, N., Muffler, K., Bart, H.-J., Heinzle, E. & Ulber, R., 2011. Ethanol production from grass silage by simultaneous pretreatment, saccharification and fermentation: First steps in the process development. Engineering in Life Sciences 11, 436-442.

Winters, A.L., Fychan, R. & Jones, R., 2001. Effect of formic acid and a bacterial inoculanton the amino acid composition of grass silage and on animal performance. Grass Forage Sci. 56, 181–192.

Dry matter yields and feed values of faba bean and pea as bi-crops with wheat or oats in Northern Finland

K. Kuoppala[1], A. Huuskonen[2], E. Saarinen[3] & M. Rinne[1]
[1]MTT Agrifood Research Finland, Animal Production Research, Animale, FI-31600 Jokioinen, Finland; [2]MTT Agrifood Research Finland, Animal Production Research, Halolantie 31 A, FI-71750 Maaninka; [3]MTT Agrifood Research Finland, Animal Production Research, Tutkimusasemantie 15, FI-92400 Ruukki
Correspondence: kaisa.kuoppala@mtt.fi

Introduction

Producing whole crop small grain cereal silages (WCS) provides an opportunity to improve the efficiency of forage production for ruminants under Nordic conditions. Harvesting and storage of WCS is possible with the same methods as used for grass silage so that only one machinery chain is needed on the farm. Using on-farm grown cereals as silages rather than as grains results in significant economic benefits, when combine harvesting and drying of the grains are not needed (Turunen, 2003). The nutritional characteristics of WCS are similar to those of maize silage, and the Nordic growing conditions are much better suited for it.

The WCS can be produced from pure stands of typically wheat or barley, but inclusion of grain legumes (faba bean, pea) or vetches is of interest particularly in organic production. Inclusion of legumes allows reductions in nitrogen fertilization, increase biodiversity and improve soil structure.

Inclusion of legumes into WSC may also improve the nutritional quality of the feed. The CP concentration of cereal based WCS is typically around 100 g/kg DM or even lower (Huuskonen & Joki-Tokola, 2010; MTT 2014). Finnish protein feeding recommendations for growing cattle (MTT, 2014) are not usually fulfilled if whole-crop silage based rations are fed without protein supplementations. By including legumes into the mixture, the CP concentrations may rise to approximately 130-150 g/kg DM. Such concentration is already high enough to be used for growing cattle without separate protein supplementation (Huuskonen et al., 2007; Huuskonen, 2009). According to Finnish feeding recommendations the protein intake for growing cattle over 200 kg live weight is considered adequate if the protein balance in the rumen (PBV) of the total diet is not lower than -10 g/kg DM (MTT, 2014).

In this paper the composition, digestibility and dry matter (DM) yield of legume and cereal bi-crops are discussed in order to better understand the basis of the feed value of whole crop silages made of legumes and cereals grown together, and their relevance to cattle feeding under Nordic conditions.

Materials and Methods

Three cultivars of faba bean (*Vicia faba*) and four cultivars of pea (*Pisum sativum*) were cultivated in a plot experiment at MTT Ruukki (64°N) in Northern Finland. Both legumes were cultivated as bi-crops with spring wheat (*Triticum aestivum* cv. Wappu) and with oats (*Avena sativa* cv. Wilhelmiina) as four replicates. The cultivars of faba bean were Kontu, Fuego and Tangenta and of pea Arvika, Dolores, Florida and Jermu. The sowing dates were 29 and 30 May for faba bean and pea, respectively. Seeding rates were: faba bean 170, pea 156, wheat 80 and oats 70 kg/ha. The bi-crop plots were harvested with a plot harvester (Haldrup) three times: 16 August, 30 August and 13 September in 2012 for faba bean and 15

August, 27 August and 10 September for pea. The yield was measured by weighing the whole yield harvested from each plot.

Samples of one replicate of each bi-crop and also from the samples after manual separation of different plant species were analysed in the laboratory of MTT. The DM concentration was determined by drying at 105 °C for 20 h and of organic matter (OM) by ashing at 600 °C for 2 h. Crude protein (CP) concentration was analysed by the Dumas method using Leco FP 428 nitrogen analyser (Leco Corp., St Joseph; USA). Starch was analysed according to Salo & Salmi (1968). The concentration of neutral detergent fibre (NDF) was determined according to Van Soest et al. (1991) using Na sulphite, without amylase and with ash excluded. The concentration of indigestible NDF (iNDF) was determined by a 12 d ruminal incubation in the rumen of dairy cows fed a forage-based diet and using nylon bags with a pore size of 17 µm (Huhtanen et al., 1994) and expressed ash-free.

Digestibility of OM (OMD) was determined using *in vitro* pepsin-cellulase method and general correction equation was used to convert pepsin-cellulase solubility values into *in vivo* digestibility according Huhtanen et al. (2006). D-value (the concentration of digestible OM, g/kg DM) was calculated using the analysed ash concentration. The results are presented for both legume bi-crops as means of the cultivars and the two cereal grain species and for pure plant species separately. Statistical analyses were conducted with ANOVA with legume bi-crop (pea or faba bean) and harvesting date in the model (Table 1) and with plant species (pea, faba bean, oats and wheat) and harvesting date in the model (Table 2). Pairwise comparisons were made with Tukey t-test.

Results and Discussion

Harvesting later increased the DM concentration of the bi-crops of both legumes (Table 1). The DM yield was higher for faba bean than for pea bi-crops and it increased for both legume cereal bi-crops when harvested later. Highest DM yield was recorded for pea cv. Dolores and faba bean cv. Fuego (data not shown). The average proportion of legume in the bi-crop was higher for pea (mean 0.62) than for faba bean (mean 0.49).

The CP concentration was higher for pea bi-crops than for faba bean which may originate from the higher proportion of pea in the bi-crop compared with faba bean. The CP concentration of pea bi-crops did not change significantly with postponed harvest but that of faba bean increased from first to second harvest. When analysed separately the pure legume whole crops had the average CP concentrations of 167 and 163 g/kg DM for pea and faba bean, respectively (Table 2). The CP concentration increased for faba bean but did not change for pea whole crops. The average CP concentration of pure whole crop cereals was low, 109 and 106 g/kg DM for oats and wheat, respectively. Crude protein yield was on the average 1194 and 1274 kg/ha for pea and faba bean bi-crops, respectively and it increased for both legumes when the harvest was postponed.

Table 1 The effect of harvesting date for performance and chemical composition of pea cereal and faba bean cereal bi-crops

	Pea cereal bi-crop (n=8)				Faba bean cereal bi-crop (n=6)			
Harvest date in 2012	1	2	3	SEM	1	2	3	SEM
DM concentration	189[a]	187[a]	239[b]	6.4	182[a]	206[ab]	218[b]	4.2
DM yield, kg/ha	7193[a]	7437[ab]	8867[b]	254.1	7717[a]	9713[b]	9881[b]	262.4
Proportion of legume	0.54	0.65	0.68	0.032	0.46	0.50	0.50	0.016
Crude protein yield, kg/ha	1053[a]	1173[ab]	1356[b]	37.5	992[a]	1402[ab]	1429[b]	61.7
Chemical composition, g/kg DM								
Ash	73.6[a]	72.6[ab]	65.1[b]	2.1	60.1[a]	59.7[a]	57.1[a]	1.41
Crude protein	147[a]	160[a]	154[a]	7.2	128[a]	144[b]	144[b]	4.3
Starch	31.6[a]	62.1[ab]	94.8[b]	11.17	86.1[a]	105.1[a]	151.1[b]	6.2
NDF	503[a]	458[b]	471[ab]	11.9	499[a]	475[ab]	472[b]	7.9
iNDF	188[a]	185[a]	184[a]	4.7	200[a]	170[b]	172[b]	4.1
iNDF/NDF	0.376[a]	0.404[a]	0.390[a]	0.0109	0.400[a]	0.358[b]	0.364[b]	0.0078
OMD, g/g	0.662[a]	0.690[b]	0.699[b]	0.0074	0.657[a]	0.691[b]	0.696[b]	0.0055
D-value, g/kg DM	613[a]	640[b]	653[c]	7.7	618[a]	650[b]	656[b]	5.6

Harvesting dates for pea bi-crops: 1 =August, 15; 2 = August, 27; 3 = September, 3; and for faba bean bi-crops: 1 = August, 16; 2 = August, 30; 3 = September, 13. DM=dry matter, NDF=neutral detergent fibre, iNDF=indigestible NDF, OMD= digestibility of organic matter, D-value=concentration of digestible organic matter in dry matter. Same superscript on the same row within the plant means no statistical significance between harvesting dates, P>0.05.

The starch concentration was lower for pea than for faba bean bi-crops and it increased with postponed harvest for both. According to Salawu et al. (2001) the increase in starch concentration means that the growth is changing from vegetative phase to generative phase and preparing the seeds.

The NDF and iNDF concentrations were on average 477 and 482 g/kg DM and 185 and 181 g/kg DM for pea and faba bean bi-crops, respectively. The NDF concentrations of these legume bi-crops were on the same level or lower than for early cut grass herbages but higher than for red clover whereas the concentration of iNDF was much higher than in grass or red clover (Kuoppala et al., 2009). Red clover typically has lower NDF and higher iNDF concentration compared to grasses both in primary growth and regrowth (see Kuoppala, 2010 for review). In that review the average proportion of totally indigestible part in NDF, i.e. iNDF, was calculated to be 0.35 for red clover and only 0.18 for grasses. In the present data it was higher, 0.39 for pea bi-crops and 0.37 for faba bean bi-crops. For pea bi-crops it did not change whereas for faba bean bi-crops it decreased when harvest was postponed. When the plant species were analysed separately (Table 2) the concentration of NDF was higher for the cereals than for the legumes whereas that of iNDF was slightly higher or at the same level. High proportion of iNDF in NDF seems to be common to the grain legumes pea and faba bean as well as forage legumes (Kuoppala, 2010).

Table 2 The effect of harvesting time for chemical composition (g/kg DM) of pea, faba bean, oats and wheat separated from the whole bi-crop samples.

Plant	Pea (n=4)				Faba bean (n=3)				Oats (n=2)				Wheat (n=2)			
Harvest	1	2	3	SEM	1	2	3	SEM	1	2	3	SEM	1	2	3	SEM
Ash	61.9	61.6	56.4	3.66	55.7[a]	50.4[b]	55.5[a]	0.96	81.2	86.6	81.6	8.62	69.4	69.0	75.8	4.98
CP	172	165	164	11.1	147[a]	159[a b]	182[b]	8.0	115	112	100	7.8	103	107	107	7.6
Starch	40.1	73.5	86.8	13.5	60.4	94.3	90.4	9.1	46.4	86.5	124.9	20.82	22.3[a]	86.2[b]	118.3[b]	8.07
NDF	403	398	411	15.9	420	425	398	8.39	559	562	541	14.7	563	551	584	11.9
iNDF	184	163	162	10.7	181	165	146	11.9	190	189	186	3.22	187	190	189	1.8
iNDF/NDF	0.455	0.409	0.393	0.0165	0.430	0.438	0.367	0.0191	0.336	0.344	0.332	0.023	0.332	0.345	0.324	0.023
OMD, g/g	0.706	0.730	0.732	0.0128	0.704	0.722	0.740	0.0107	0.639	0.639	0.635	0.0491	0.623	0.635	0.628	0.039
D value	662	685	691	13.4	665	686	699	9.74	587	584	584	9.31	579	591	581	4.7

Harvesting dates for pea bi-crops: 1 =August, 15; 2 = August, 27; 3 = September, 3; and for faba bean bi-crops: 1 = August, 16; 2 = August, 30; 3 = September, 13. DM=dry matter, CP=crude protein, NDF=neutral detergent fibre, iNDF=indigestible NDF, OMD= digestibility of organic matter, D-value=concentration of digestible organic matter in dry matter. Same superscript on the same row within the plant means no statistical significance between harvesting dates, P>0.05.

The digestibility expressed as OMD or D-value was rather low for both legume bi-crops (Table 1). Opposite to grasses it increased with the postponed harvest. The D-values corresponded to the values of very late cut grass but were on the same level as the average values for red clover (Kuoppala, 2010). When the plant species were analysed separately (Table 2) it seems that the pure legume whole crops' digestibility was higher than that of the cereals, being on the same level as good quality grass silage at least in the two later harvest dates.

Inclusion of forage legumes or WCS into the diet of dairy cows has been shown to increase the voluntary feed intake of dairy cows (Huhtanen et al., 2007). Cows eat more silage when red clover is mixed with grasses in the herbage in spite of the higher concentration of iNDF. It could be assumed that the situation will be same for pea and faba bean also. There are also positive experiences of using ensiled cereal + legume mixtures as feeds for dairy cows (Haag, 2007; Juutinen, 2011).

It is suggested that the starch concentration of whole-crop silages, together with high starch digestibility could compensate for the high concentration of indigestible fibre (Wallsten, 2008). Both dairy cows (Ahvenjärvi et al., 2006) and finishing beef cattle (Keady et al., 2013) have often been able to maintain or even increase silage dry matter intake (DMI) after inclusion of whole-crop silage into the diet, although the digestibility of whole-crop silage has been lower than that of the grass silage. According to Huhtanen et al. (2007), the dairy cows responses to replacing grass silages partially or totally with whole-crop silages on DMI could not be accurately predicted from differences in silage D-value, DM concentration or fermentation characteristics. The maximum silage DMI increase was obtained when the proportion of the whole-crop silage was 0.48 of total silage DM, and the effect was quadratic (Huhtanen et al., 2007).

Conclusions

Postponing the harvest increased dry matter and crude protein yields and digestibility of the faba bean cereal and pea cereal bi-crops. Inclusion of legumes has a potential to increase energy and protein values of whole crop cereal silages, because they were higher in the legume fractions compared to the cereals.

References

Ahvenjärvi, 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.

Haag, T., 2007. Åkerböna i samodling med vårvete som helgrödesensilage till mjölkkor. [Field beans cultivated together with spring wheat as whole-crop silage to dairy cows]. SLU Institutionen för norrländsk jordbruksvetenskap. Rapport 3, 2007 (In Swedish).

Huhtanen, P., Kaustell, K. & Jaakkola, S., 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Anim. Feed Sci. Technol. 48, 211–227.

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

Huuskonen, A., 2009. The effect of cereal type (barley versus oats) and rapeseed meal supplementation on the performance of growing and finishing dairy bulls offered grass silage-based diets. Livest. Sci. 122, 53–62.

Huuskonen, A. & Joki-Tokola, E., 2010. Performance of growing dairy bulls offered diets based on silages made of whole-crop barley, whole-crop wheat, hairy vetch and grass. Agric. Food Sci. 9, 116-126.

Huuskonen, A., Khalili, H. & Joki-Tokola, E., 2007. Effects of three different concentrate proportions and rapeseed meal supplement to grass silage on animal performance of dairy-breed bulls with TMR feeding. Livest. Sci. 110, 154-165.

Juutinen, E. 2011. Säilörehua herneestä ja härkäpavusta. Nauta 4/11, pp. 34-35. (In Finnish).

Keady, T.W.J., Hanrahan, J.P., Marley, C.L. & Scollan, N.D., 2013. Production and utilization of ensiled forages by beef cattle, dairy cows, pregnant ewes and finishing lambs: a review. Agric. Food Sci 22, 70–92.

Kuoppala, K. 2010. Influence of harvesting strategy on nutrient supply and production of dairy cows consuming diets based on grass and red clover silage. MTT Science 11: 50 s. Doctoral Dissertation. Available at: www.mtt.fi/mtttiede/pdf/mtttiede11.pdf

Kuoppala, K., Ahvenjärvi, S., Rinne, M. & Vanhatalo, A., 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 2. Dry matter intake and cell wall digestion kinetics. J. Dairy Sci. 92, 5634-5644.

MTT. 2013. Finnish Feed Tables and Feeding Recommendations – web service. Available at: www.mtt.fi/rehutaulukot/english.

Salawu, M.B., Adesogan, A.T., Weston & C.N., Williams, S.P., 2001. Dry matter yield and nutritive value of pea-wheat bi-crops differing in maturity at harvest, pea to wheat ratio and pea variety. Anim. Feed Sci Technol. 94, 77-87.

Turunen, H., 2003. Kokoviljasäilörehun taloudellisuus nautakarjatilalla. In: Lampinen, K., Harmoinen, T. & Teräväinen H. (Eds.). Kokoviljasäilörehun tuotanto ja käyttö. Jyväskylä, Finland: Gummerus Kirjapaino Oy. pp. 5-16. (In Finnish).

Wallsten, J., 2008. Whole-crop cereals in dairy production. Digestibility, feed intake and milk production. Doctoral Thesis. Swedish University of Agricultural Sciences, Umeå, Sweden. 45 p.

DISTRIBUTION:
Sveriges Lantbruksuniversitet
Institutionen för husdjurens utfodring och vård
Box 7024
750 07 UPPSALA
Tel.018/672817

Margareta.Norinder@slu.se