Proceedings of the 8th Nordic Feed Science Conference, Uppsala, Sweden

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

Rapport 296
Report

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Foreword

The aim of the Nordic Feed Science Conference is to create an arena for Nordic feed scientists to meet and discuss ruminant feeds and feeding.

After 8 years of coming together in Uppsala, are we ready for a change or happy with the way things are? Approximately 50% wanted the conference to remain in Sweden in last year's questionnaire, maybe because Swedish participants were in majority. Results also showed that 80% wanted the conference to be held in June, 60% wanted it every year, 76% were satisfied with 2-3 keynote speakers and 80% wanted Proficiency testing and analytical methods to be part of, or satellite to the conference. Results and comments are shown below. Also this year, we have some funding to include Proficiency testing and feed data bases as a separate session and to invite Dr Arngrimur Thorlacius from Iceland and Dr Harinder Makkar as keynote speakers. After the conference, there will be an additional closed session for participants involved in the North European Proficiency Testing (NEPT) scheme. Two very renowned scientists have accepted this year to speak at the conference on issues related to Precision Livestock Farming. Professor Ilan Halachmi is travelling all the way from the Volcani Centre, Israel and Professor Jeffrey Bewley from University of Kentucky, USA. In addition, we can look forward to cross-pollination from listening to Dr Martin Mak from Linköping University who is extremely knowledgeable in the area of Biosensor Technology. Last but not the least, Emeritus Professor and SLU Honorary and great friend Doctor Glen Broderick is for the 5th time honoring us by participating in the conference. We wish to welcome all these eminent scientists and thank them for coming all the way to join us in this year's conference.

A total of 27 papers have been received, covering topics, apart from those previously mentioned, related to feed conservation, laboratory and feed evaluations, animal responses to variation in feed composition, etc.

You are all most welcome to the conference! For downloading proceedings of earlier conferences, please go to our homepage (http://www.slu.se/nordicfeedscienceconference Menu item: Contributions) where you also find a list of all titles.

Uppsala 2017-06-01

Peter Udén

2016 Survey answers (% of 25 to 26 answers)

When?		How often?		Keynote speakers?		Where?		Proficiency testing & analytical methods?	
Feb.	4	Every year	60	2 - 3	76	Uppsala	54	In NFSC or satellite	84
April	4	Every other year	40	More	16	Nordic rotation	46	Separate conference	16
May	4			Fewer	8				
June	81								
Aug.	4								
Nov	4								

Program suggestions (work in progress, reviews, etc.)

- It was very interesting as it was!
- Error propagation in ultra-low gas flow measures
- New research. Ruminant nutrition. More ruminant behavior and grazing related research.
- Questions related to analyses and near-market topics
- Maybe to expand the organizing committee to the Nordic countries, including the Baltic states.
- Work in progress, status and reviews regarding forage, concentrate feed, feed processing, analysis and feeding practice, feeding strategies, NorFor
- Focusing on effects of different raw materials (and rations) on effect for milk and meat production.
- Exciting results from young scientists. Good to see what is going on at the Nordic universities.
- In each conference version it will be nice to organize one satellite event.
- Focus on feed, feeding and results in research. Less presentations on tests of commercial products.
- Work in progress

Your comments to individual questions above

- First time to join the conference, and a very interesting and pleasant experience! Thank you for organizing!
- It is important to get more people outside Sweden and more people in total.
- none
- For my schedule this years' conference was held at perfect time. Also I think that Uppsala is most convenient place for conference; easy to get to and everything is at nice reachable distance. So the best place is Uppsala and the best time is in the middle of June.
- I think it is a good and nice conference. Good work!
- The NFSC is extremely cozy event with very good keynote speakers and it should be continue in future too. I guess the Uppsala is best place as it is in the center of Nordic countries. Perhaps there should best poster award for PhD student to attract them to participate.
- Place for conference does not matter, but it is important with a short every year conference and it seems to me, that only you Swedish people so far have given it the higher priority.
- "It would be nice to include some organized social event with the purpose of getting people to get to know some more (new) people. Maybe as a workshop session, or some kind of group activity/game during the dinner.
- It would also be nice to offer coffee during registration hours just before the start of the conference. That would make people more relaxed, alert and focused during the first session, so it would be an even better start of a good conference. "
- Will the key note speakers attract participants? It was very ambitious with the SARA speakers. Did that give what you wanted? The laboratory presentations were a disappointment. Were north European laboratories invited?
- Regarding satellite event: such as workshops in statistics (i.e. mixed models), analytical methods (i.e. fiber determinations), feed processing (i.e. pelleting) etc.
- The proposal for also include topics related with monogastric animals is also interesting.
- Patrik N: I could see a point where NorFor could be interested to take a more active organization part in the Conference. However that might harm the original intentions and objectives. I would like to suggest that speaker should be somehow quality tested before entering.

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Miscellaneous II

Posters

Monitoring or modelling the individual feed efficiency - quantifying the efficient and inefficient dairy cows in commercial farms

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Introduction

Feed cost is the greatest single expense in intensive dairy farm (Buza et al., 2014). Feed efficiency heritability level is rather high, 0.27, therefore, knowledge of cow individual feed efficiency could contribute to: (a) selection of farm-level replacement cows and (b) national breeding program. However, animal individual feed efficiency cannot, yet, be simply measured in commercial dairy farms on an individual basis (Halachmi et al., 2016).

Measures of feed efficiency (FE), in this presentation, are (a) FE= milk yield (kg) divided by feed intake (kg dry matter), and (b) residual feed intake (RFI; NRC, 2007). Milk yield (often together with milk composition) is on-line electronically monitored by the milking parlor's milk meters but individual feed intake is, as yet, unknown on commercial farms. Therefore, in order to estimate FE, individual feed intake must be known.

Several nutrition models have been developed to predict feed intake (Halachmi et al., 2004; Halachmi et al., 2016; NRC, 1989, 2001, 2007; Volden, 2011), but even the best models have been unable to account for more than 70% of the variation in intake (Shelley, 2013; Vandehaar, 1998), i.e. existing models may only fit group-wise (Arnerdal, 2005).

Camera based feed intake evaluation (Shelley, 2013) is an option that has not yet appeared on commercial farms. Load cell based systems for individual cow's feed intake evaluation (Halachmi et al., 1996; Calan, 1997; Halachmi et al., 1998a; Schwartzkopf-Genswein et al., 1999; Grant and Albright, 2001; Huisma, 2002; DeVries et al., 2003; Bach et al., 2004; Ferris et al., 2006; Wang et al., 2006; Chapinal et al., 2007; Mendes et al., 2011; Krawczel et al., 2012; Halachmi and Maltz, 2013) are currently too expensive for commercial farms, in other words - feasible only under research conditions.

Therefore, the aim of the project was to develop an individual cow feed intake evaluation system operating under commercial farm conditions.

Materials and Methods

The feed intake evaluation project developed two parallel systems (Figure 1). Applying one system or another is determined by the nature of the decision to be taken at the farm. The "old" Agricultural Research Organization (ARO) – the Volcani research centre's 'feed intake monitoring system' (Halachmi et al., 1998b), based on weighing troughs, was used for delivering reference values for calibration of the new systems.

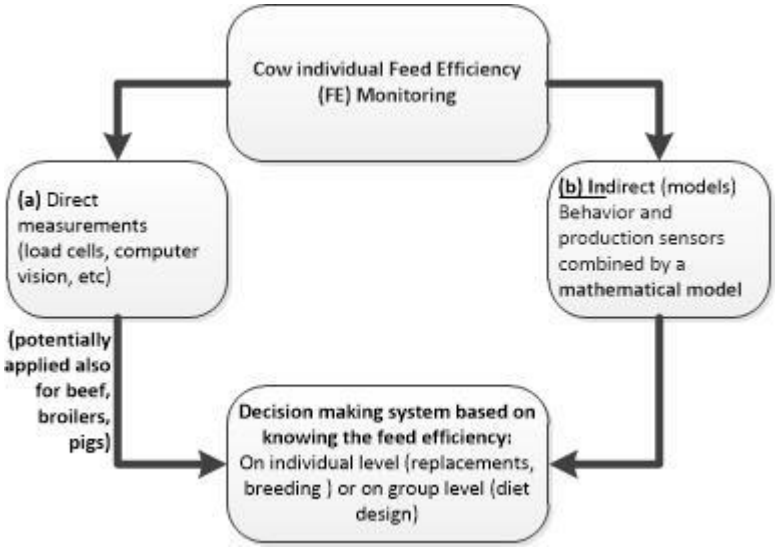

Figure 1 Two parallel paths for evaluating feed intake – mechanical design (a- left side) or via mathematical models (b – right side). Selecting 'path a' or 'path b' depends on type of decision to be taken.

Due to patents issues, the exact engineering solutions cannot be provided in this manuscript.

Results and Discussion

Out of the 152 cows who recently went through the ARO feed intake monitoring system, we classified 30 cows as efficient, FE>1.55 and 30 cows as inefficient, FE<1.40. The FE classification appeared to be 80% in agreement with the RFI classification (RFI<0: defined "efficient" and RFI>3.0 stands for "inefficient" cows). It can be seen in Table 1 that milk production did not reflect feed efficiency as it did not differ between the two classified groups of cows. Instead feed intake differed considerably between groups (24.7 vs. 30.5 kg DM/day) at approximately the same level of production (44.6, 44.2 kg/day) for efficient and inefficient cows, respectively.

Table 1 Feed efficiency vs. milk production

Measure of performance[a]	Efficient cows	Inefficient cows	Remaining cows
Efficiency:			
RFI (kg DMI)	-1.2	4.85	1.41
FE (ECM/DMI)	1.64	1.34	1.52
Milk yield kg/day	44.6	44.2	46.2
Fat (%)	3.37	3.37	3.38
Protein	3	3.16	3.1
Feed intake (kg DM/d)	24.7	30.5	27.7

[a]RFI = residual feed intake; FE = feed efficiency ratio; DM = dry matter; DMI = DM intake.

In a large farm with long feeding lanes where direct monitoring (Figure 1, path a) is expensive, models can instead be applied. Table 2 explores 7 models. It can be seen here that, in general, model accuracy is about 70-80% (R^2) on daily basis. The PLF model listed in Table 2 (R^2 = 0.93) was superior and used information from sensors that are not yet existing on many commercial farm.

Table 2 Model tested for prediction of intake by dairy cows

Model[a]	Daily R^2	Single Meal R^2
1	0.79	
2	0.73	
3	0.64	
4	0.36	
Non-PLF model[b]	0.74	0.78
PLF based model[b]	0.93	0.88

[a]1: NRC (2001); 2: Halachmi et al. (2004); 3: Covariate model; 4: Simple linear regression model; [b]Richter et al. (2016).

Figure 2 shows a load cell based system and Figure 3 presents output of a camera based system with an accuracy (R^2) in the laboratory of 0.93.

When writing this manuscript, load cells based systems (Figure 2) and camera based systems (Figure 3) have not yet appeared in the scientific literature. In further research, these systems should be validated under commercial conditions.

Figure 2 Load cell based systems on the ARO research farm. It is an early prototype that has not been commercialized.

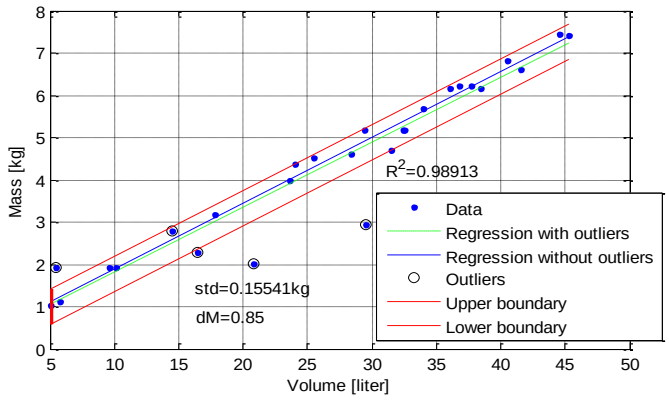

Figure 3 A camera based system for feed intake evaluation. Y-axis is the real, measured reference value, x-axis is the output from the new, camera-based system.

There are several important issues to think about when you begin monitoring (direct measurement) or evaluating (well calibrated mathematical models) individual cow feed efficiency. Model application is problematic as milk yield means appear both in the numerator and the denominator of equations. Therefore, application of a mathematical model on a cow individual basis are, in some cases, less advised. On the other hand, direct measurements in large farms is costly and, typically, requires cleaning and maintenance that is impractical under these conditions.

Conclusions

In modern intensive farming, knowing individual cow feed efficiency is of importance. Currently, there is research going on at the ARO. There are quite a few options to cope with the challenges, something to be discussed at the conference.

Acknowledgment

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Exploring the potential of precision dairy tools

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Introduction

Across the globe, the trend toward fewer, larger dairy operations continues. Dairy operations today are characterized by narrower profit margins than in the past, largely because of reduced governmental involvement in regulating agricultural commodity prices. Consequently, small changes in production or efficiency can have a major impact on profitability. The resulting competition growth has intensified the drive for efficiency resulting in increased emphasis on business and financial management. Furthermore, the decision making landscape for a dairy manager has changed dramatically with increased emphasis on consumer protection, continuous quality assurance, natural foods, pathogen-free food, zoonotic disease transmission, reduction of the use of medical treatments, and increased concern for the care of animals. These changing demographics reflect a continuing change in the way in which dairy operations are managed. In large part, many of these changes can be attributed to tremendous technological progress in all facets of dairy farming, including genetics, nutrition, reproduction, disease control, and management. W. Nelson Philpot (2003) captured this change effectively in describing modern dairy farms as "technological marvels." Conceivably, the next "technological marvel" in the dairy industry may be in Precision Dairy Farming.

What is Precision Dairy Farming?

Precision Dairy Farming is the use of technologies to measure physiological, behavioral, and production indicators on individual animals to improve management strategies and farm performance. Many Precision Dairy Farming technologies, including daily milk yield recording, milk component monitoring (e.g. fat, protein, and SCC), pedometers, automatic temperature recording devices, milk conductivity indicators, automatic estrus detection monitors, and daily body weight measurements, are already being utilized by dairy producers. Eastwood et al. (unpubl.) defined Precision Dairy Farming as "the use of information technologies for assessment of fine-scale animal and physical resource variability aimed at improved management strategies for optimizing economic, social, and environmental farm performance." Spilke and Fahr (2003) stated that Precision Dairy Farming, with specific emphasis on technologies for individual animal monitoring, "aims for an ecologically and economically sustainable production of milk with secured quality, as well as a high degree of consumer and animal protection." With Precision Dairy Farming, the trend toward group management may be reversed with focus returning to individual cows through the use of technologies (Schulze et al., 2007). Technologies included within Precision Dairy Farming range in complexity from daily milk yield recording to measurement of specific attributes (e.g. fat content or progesterone) within milk at each milking. The main objectives of Precision Dairy Farming are maximizing individual animal potential, early detection of disease, and minimizing the use of medication through preventive health measures. Precision Dairy Farming is inherently an interdisciplinary field incorporating concepts of informatics, biostatistics, ethology, economics, animal breeding, animal husbandry, animal nutrition, and engineering (Spilke and Fahr, 2003).

Potential Benefits of Precision Dairy Farming

Perceived benefits of Precision Dairy Farming technologies include increased efficiency, reduced costs, improved product quality, minimized adverse environmental impacts, and improved animal health and well-being. These technologies are likely to have the greatest impact in the areas of health, reproduction, and quality control (de Mol, 2000). Realized benefits from data summarization and exception reporting are anticipated to be higher for larger herds, where individual animal observation is more challenging and less likely to occur (Lazarus et al., 1990). As dairy operations continue to increase in size, Precision Dairy Farming technologies become more feasible because of increased reliance on less skilled labor and the ability to take advantage of economies of size related to technology adoption.

A Precision Dairy Farming technology allows dairy producers to make more timely and informed decisions, resulting in better productivity and profitability (van Asseldonk et al., 1999b). Real time data can be used for monitoring animals and creating exception reports to identify meaningful deviations. In many cases, dairy management and control activities can be automated (Delorenzo and Thomas, 1996). Alternatively, output from the system may provide a recommendation for the manager to interpret (Pietersma et al., 1998). Information obtained from Precision Dairy Farming technologies is only useful if it is interpreted and utilized effectively in decision making. Integrated, computerized information systems are essential for interpreting the mass quantities of data obtained from Precision Dairy Farming technologies. This information may be incorporated into decision support systems designed to facilitate decision making for issues that require compilation of multiple sources of data.

Historically, dairy producers have used experience and judgment to identify outlying animals. While this skill is invaluable and can never be fully replaced with automated technologies, it is inherently flawed by limitations of human perception of a cow's condition. Often, by the time an animal exhibits clinical signs of stress or illness, it is too late to intervene. These easily observable clinical symptoms are typically preceded by physiological responses evasive to the human eye (e.g. changes in temperature or heart rate). Thus, by identifying changes in physiological parameters, a dairy manager may be able to intervene sooner. Technologies for physiological monitoring of dairy cows have great potential to supplement the observational activities of skilled herdspersons, which is especially critical as more cows are managed by fewer skilled workers.

Precision Dairy Farming Examples

The list of Precision Dairy Farming technologies used for animal status monitoring and management continues to grow. Because of rapid development of new technologies and supporting applications, Precision Dairy Farming technologies are becoming more feasible. Many Precision Dairy Farming technologies including daily milk yield recording, milk component monitoring (e.g. fat, protein, and SCC), pedometers, automatic temperature recording devices, milk conductivity indicators, automatic estrus detection monitors, and daily body weight measurements are already being utilized by dairy producers. Despite its seemingly simplistic nature, the power of accurate milk weights should not be discounted in monitoring cows, as it is typically the first factor that changes when a problem develops (Philpot, 2003). Other theoretical Precision Dairy Farming technologies have been proposed to measure jaw movements, ruminal pH, reticular contractions, heart rate, animal positioning and activity, vaginal mucus electrical resistance, feeding behavior, lying behavior, odor, glucose, acoustics, progesterone, individual milk components, color (as an indicator of

cleanliness), infrared udder surface temperatures, and respiration rates. Unfortunately, the development of technologies tends to be driven by availability of a technology, transferred from other industries in market expansion efforts, rather than by need. Relative to some industries, the dairy industry is relatively small, limiting corporate willingness to invest extensively in development of technologies exclusive to dairy farms. Many Precision Dairy Farming technologies measure variables that could be measured manually, while others measure variables that could not have been obtained previously.

Investment Analysis of Precision Dairy Farming Technologies

Today's dairy manager is presented with a constant stream of new technologies to consider including new Precision Dairy Farming technologies. Galligan and Groenendaal (2001) suggested that "the modern dairy producer can be viewed as a manager of an investment portfolio, where various investment opportunities (products, management interventions) must be selected and combined in a manner to provide a profit at a competitive risk to alternative opportunities." Further, dairy managers must consider both biological and economic considerations simultaneously in their decisions. Traditionally, investment decisions have been made using standard recommendations, rules of thumb, consultant advice, or intuition. Thus, more objective methods of investment analysis are needed (Verstegen et al., 1995).

Adoption of sophisticated on-farm decision-making tools has been scant in the dairy industry to this point. Yet, the dairy industry remains a perfect application of decision science because: (1) it is characterized by considerable price, weather, and biological variation and uncertainty, (2) technologies, such as those characteristic of Precision Dairy Farming, designed to collect data for decision making abound, and (3) the primary output, fluid milk, is difficult to differentiate, increasing the need for alternative means of business differentiation. In "Competing on Analytics: The New Science of Winning," Davenport and Harris (2007) pose that in industries with similar technologies and products, "high performance business processes" are one of the only ways that businesses can differentiate themselves.

Investment analyses of information systems and technologies are common within the general business literature (Streeter and Hornbaker, 1993; Ryan and Harrison, 2000; Bannister and Remenyi, 2000; Lee and Bose, 2002). However, dairy-specific tools examining investment of Precision Dairy Farming technologies are limited (Carmi, 1992; Gelb, 1996; van Asseldonk, 1999), though investment analyses of other dairy technologies abound (Hyde and Engel, 2002). Empirical comparisons of technology before or after adoption or between herds that have adopted a technology and control herds that have not adopted are expensive and biased by other, possibly herd-related differences. As a result, the normative approach, using simulation modeling, predominates in decision support models in animal agriculture (Dijkhuizen et al., 1991). Investing in new agricultural technologies is all too often a daunting and complex task. First, the standard approach using the Net Present Value is often misleading because it does not adequately account for the underlying uncertainties. Second, the incremental costs and benefits of new technologies require complex interactions of multiple variables that are often non-linear and not intuitive. The complexities surrounding investment in Precision Dairy Farming technologies is one example of this type of complex decision.

Ward (1990) listed three benefits to investment in technology: 1) substitutive, replacing human power with machine power, 2) complementary, improving productivity and employee effectiveness through new ways of accomplishing tasks, and 3) innovative, obtaining a

competitive edge. In addition to impacts on production, many technologies may also change milk composition, reproductive efficiency, and disease incidences (Galligan and Groenendaal, 2001). In an analysis of an investment opportunity at the dairy level, cash flows are generally uncertain because of biological variability or incomplete knowledge of the system (Galligan and Groenendaal, 2001). The impact that a Precision Dairy Farming technology has on productive and economic performance is difficult to examine because of the changing nature of the decision environment where investments are often one-time investments but returns accrue over a longer period of time (van Asseldonk, 1999; van Asseldonk et al., 1999a,b; Verstegen et al., 1995; Ward, 1990). Further, benefit streams resulting from investment in a Precision Dairy Farming technology are highly dependent upon the user's ability to understand and utilize the information provided by the new technology (Bannister and Remenyi, 2000). An economic analysis of the value of Precision Dairy Farming technologies requires consideration of the effect of adoption on both quality and timeliness of decisions (Verstegen et al., 1995). Improvements associated with adoption of new Precision Dairy Farming technologies may increase profits directly through improved utilization of data provided by the technology or indirectly through recommendations of consultants utilizing the new information (Tomaszewski et al., 1997). It is difficult, if not impossible to quantify the economic value of personal welfare associated with a proposed change (e.g. free time or prestige) (Otte and Chilonda, 2000). For example, it is nearly impossible to quantify the satisfaction of having a healthy herd, reduction of animal suffering, reduced human health risks, and environmental improvements (Huirne et al., 2003). Despite efforts to formalize the rational decision making analysis of investment in information technologies, many business executives ultimately make their investment decision based on "gut feel" or "acts of faith" (Bannister and Remenyi, 2000; Passam et al., 2003). Ultimately, decision making is and should be dependent upon both rational analysis and instinct (Bannister and Remenyi, 2000).

Simulation of dairy farms

Mayer et al. (1998) proposed that with the variety of management issues a dairy manager faces in an ever-changing environment (e.g. environmental, financial, and biological), best management strategies cannot be verified and validated with field experiments. As a result, simulation is the only method of "integrating and estimating" these effects (Mayer et al., 1998). Simulations are mathematical models designed to represent a system, such as a dairy farm, for use in decision-making. Simulation models are useful and cost-effective in research that requires complex scenarios involving a large number of variables with large groups of animals over a long period of time under a large range of conditions (Bethard, 1997; Shalloo et al., 2004). The primary advantages of using mathematical computer simulation models in evaluating dairy production issues are the ability to control more variables within the model than with a field trial and the reduced costs associated with this kind of effort (Shalloo et al., 2004; Skidmore, 1990). These economic models can also be useful in evaluating alternatives where very little real data is available yet (Dijkhuizen et al., 1995). Simulating a system is particularly useful when uncertain, complex feedback loops exist (e.g. disease affects production which then impacts other variables further back in the system) (Dijkhuizen et al., 1995). Models that represent system uncertainty, while effectively using available information, provide more realistic insight than models that do not consider a range of responses (Bennett, 1992; Passam et al., 2003).

Simulation or other systemic methods are preferred to capture the complexity of a dairy system as they can evaluate multiple biological and economic factors affecting performance, including management, feeding, breeding, culling, and disease (Skidmore, 1990, Sorensen et al., 1992). Because the dairy system includes environmental, economic, and physical components, accounting for interactions among components and tracing the effects of an intervention through the entire system are essential (Cabrera et al., 2005). Simulation models are ideal for analyzing investment strategies because they can effectively examine improvement in biological parameters based on farm-specific data rather than simple industry averages (Jalvingh, 1992; Dijkhuizen et al., 1995; Delorenzo and Thomas, 1996; van Asseldonk et al., 1999b; Gabler et al., 2000). Simulation of a farm can be accomplished by conducting two simulations, one with and one without a proposed change or intervention and then comparing these simulations to examine the impact on biological or economic parameters of interest (van Asseldonk, 1999). The output of a series of simulations provides a range of results, more realistically depicting biological variability than simple models (Marsh et al., 1987).

Risk and uncertainty are major considerations within a dairy production system because of the random nature of milk production, biology, disease, weather, input costs, and milk prices (Delorenzo and Thomas, 1996). This risk and uncertainty represents a major portion of the difficulty and complexity of managing a dairy operation (Huirne, 1990). Uncertainty must be considered in decision-making to avoid biased estimates and erroneous decisions (Kristensen and Jorgensen, 1998). Future costs and returns are always uncertain (Lien, 2003). Within precision agriculture, accurate representation of risk associated with technology adoption is critical in the decision making process (Marra et al., 2003).

When managers do not have sufficient information to assess the risk outcomes of decisions, they use subjective probabilities based on past experiences and their own judgment (Huirne, 1990). In most situations, decision makers are primarily concerned with the chances of the realized returns from an investment being less than predicted (Galligan et al., 1987). The ability of a model to reflect real world conditions increases with consideration of more variables (Jalvingh, 1992). Nevertheless, to ensure that the model remains practical and reasonable, only variables with the most influence on the final desired outcome should be entered into the model as random (Jalvingh, 1992; Lien, 2003).

Purdue/Kentucky research model

Bewley et al. (2010b) developed a simulation model of a dairy farm to evaluate investments in precision dairy farming technologies by examining a series of random processes over a ten-year period. The model was designed to characterize the biological and economical complexities of a dairy system within a partial budgeting framework by examining the cost and benefit streams coinciding with investment in a Precision Dairy Farming technology. Although the model currently exists only in a research form, a secondary aim was to develop the model in a manner conducive to future utility as a flexible, farm-specific decision making tool. The basic model was constructed in Microsoft Excel 2007 (Microsoft, Seattle, WA). The @Risk 5.0 (Palisade Corporation, Ithaca, NY) add-in for Excel was utilized to account for the random nature of key variables in a Monte Carlo simulation. In Monte Carlo simulation, random drawings are extracted from distributions of multiple random variables over repeated iterations of a model to represent the impact of different combinations of these variables on financial or production metrics (Kristensen and Jorgensen, 1998).

The basic structure of the model is depicted in Figure 1. The underlying behavior of the dairy system was represented using current knowledge of herd and cow management with relationships defined from existing literature. Historical prices for critical sources of revenues and expenses within the system were also incorporated as model inputs. The flexibility of this model lies in the ability to change inputs describing the initial herd characteristics and the potential impact of the technology. Individual users may change these inputs to match the conditions observed on a specific farm.

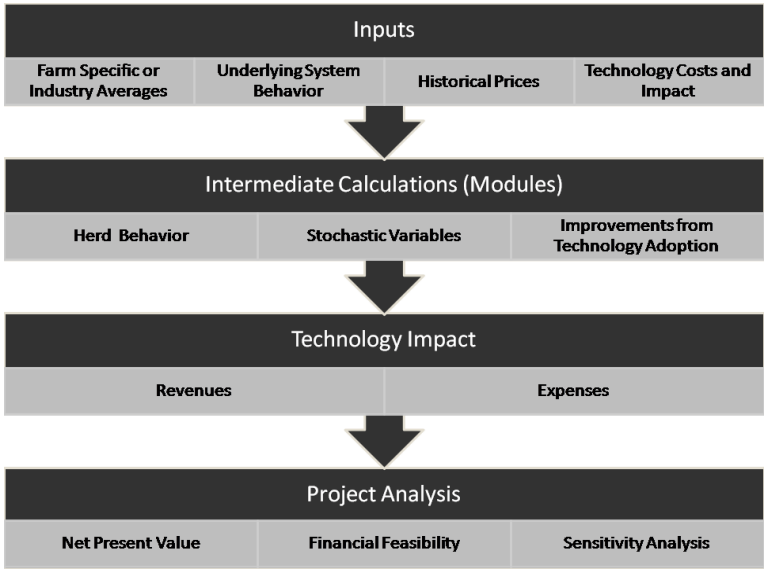

Figure 1 Diagram depicting general flow of information within the model

After inputs are entered into the model, an extensive series of intermediate calculations are computed within 13 modules, each existing as a separate worksheet within the main Excel spreadsheet. Each module tracks changes over a 10-year period for its respective variables. Within these inter-connected modules (Figure 2), the impact of inputs, random variables, and technology-induced improvements are estimated over time using the underlying system behavior within the model. Results of calculations within 1 module often affect calculations in other modules with multiple feed-forward and feed-backward interdependencies. Each of these modules eventually results in a calculation that will influence the cost and revenue flows necessary for the partial budget analysis. Finally, the costs and revenues are utilized for the project analysis examining the net present value (NPV) and financial feasibility of the project along with associated sensitivity analyses.

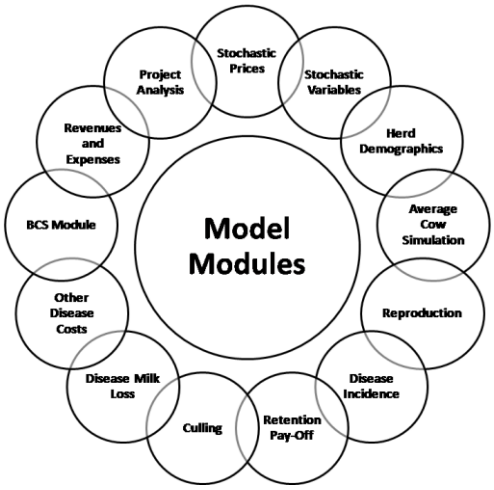

Figure 2 Diagram of model modules

Agricultural commodity markets are characterized by tremendous volatility and, in many countries, this volatility is increasing with reduced governmental price regulation. As a result, economic conditions and the profitability of investments can vary considerably depending on the prices paid for inputs and the prices received for outputs. Producers are often critical of economic analyses that fail to account for this volatility, by using a single value for critical prices, recognizing that the results of the analysis may be different with higher or lower milk prices, for example. In a simulation model, variability in prices can be accounted for by considering the random variation of these variables. In this model, historical U.S. prices from 1971 to 2006 for milk, replacement heifers, alfalfa, corn, and soybeans were collected from the "Understanding Dairy Markets" website (Gould, 2007). Historical cull cow prices were defined using the USDA-National Agricultural Statistics Service values for "beef cows and cull dairy cows sold for slaughter" (USDA-NASS, 2007). Base values for future prices (2007 to 2016) of milk, corn, soybeans, alfalfa, and cull cows were set using estimates from the Food and Agricultural Policy Research Institute's (FAPRI) U.S. and World Agricultural Outlook Report (FAPRI, 2007). Variation in prices was considered within the simulation based on historical variation. In this manner, the volatility in key prices can be considered within a profitability analysis.

Although there is probably no direct way to account for the many decisions that ultimately impact the actual profitability of an investment in a Precision Dairy Farming technology, this model includes a Best Management Practice Adherence Factor (BMPAF) to represent the potential for observing the maximum benefits from adopting a technology. The BMPAF is a crude scale from 1 to 100% designed to represent the level of the farm management. At a value of 100%, the assumption is that the farm management is capable and likely to utilize the technology to its full potential. Consequently, they would observe the maximum benefit from the technology. On the other end of the spectrum, a value of 0% represents a scenario where farm management installs a technology without changing management to integrate the newly available data in efforts to improve herd performance. In this case, the farm would not recognize any of the benefits of the technology. Perhaps most importantly, sensitivity analyses allow the end user to evaluate the decision with knowledge of the role they play in its success.

Investment Analysis of Automated Body Condition Scoring

To show how it can be used practically, this model was used for an investment analysis of automatic body condition scores on dairy farms (Bewley et al., 2010a). Automated body condition scoring (BCS) through extraction of information from digital images has been demonstrated to be feasible; and commercial technologies are being developed (Bewley et al., 2008). The primary objective of this research was to identify the factors that influence the potential profitability of investing in an automated BCS system. An expert opinion survey was conducted to provide estimates for potential improvements associated with technology adoption. Benefits of technology adoption were estimated through assessment of the impact of BCS on the incidence of ketosis, milk fever, and metritis, conception rate at first service, and energy efficiency. For this research example, industry averages for production and financial parameters, selected to represent conditions for a U.S. dairy farm milking 1000 cows in 2007 were used. Further details of model inputs and assumptions may be obtained from the author.

Net present value (NPV) was the metric used to assess the profitability of the investment. The default discount rate of 8% was adjusted to 10% because this technology has not been marketed commercially; thus, the risk for early adopters of the technology is higher. The discount rate partially accounts for this increased risk by requiring higher returns from the investment. The general rule of thumb is that a decision with a NPV greater than 0 is a "go" decision and a worthwhile investment for the business. The investment at the beginning of the project includes the purchase costs of the equipment needed to run the system in addition to purchasing any other setup costs or purchases required to start the system. Recognizing that a simpler model ignores the uncertainty inherent in a dairy system, Monte Carlo simulation was conducted using the @Risk add-in. This type of simulation provides infinite opportunities for sensitivity analyses. Simulations were run using 1000 iterations in each simulation. Simulations were run, using estimates provided by experts, for scenarios with little to no improvement in the distribution of BCS and with definite improvement.

Profitability analysis

For the small likelihood of improvement simulation, 13.1% of simulation iterations resulted in a positive NPV whereas this same number was 87.8% for the scenario with a definite improvement. In other words, using the model assumptions for an average 1000 cow U.S. dairy in 2007, investing in an automated BCS system was the right decision 13.1% or 87.8% of the time depending on the assumption of what would happen with BCS distribution after technology adoption. The individual decision maker's level of risk aversion would then determine whether they should make the investment. Although this serves as an example of how this model could be used for an individual decision maker, this profitability analysis should not be taken literally. In reality, an individual dairy producer would need to look at this decision using herd-specific variables to assess the investment potential of the technology. The main take home message was that because results from the investment analysis were highly variable, this technology is certainly not a "one size fits all" technology that would prove beneficial for all dairy producers.

Sensitivity Analyses

The primary objective of this research was to gain a better understanding of the factors that would influence the profitability of investing in an automated BCS system through sensitivity

analysis. Sensitivity analysis, designed to evaluate the range of potential responses, provides further insight into an investment analysis (van Asseldonk et al., 1999b). In sensitivity analyses, tornado diagrams visually portray the effect of either inputs or random variables on an output of interest. In a tornado diagram, the lengths of the bars are representative of the sensitivity of the output to each input. The tornado diagram is arranged with the most sensitive input at the top progressing toward the least sensitive input at the bottom. In this manner, it is easy to visualize and compare the relative importance of inputs to the final results of the model.

Improvements in reproductive performance had the largest influence on revenues followed by energy efficiency and then by disease reduction. Random variables that had the most influence on NPV were as follows: variable cost increases after technology adoption; the odds ratios for ketosis and milk fever incidence and conception rates at first service associated with varying BCS ranges; uncertainty of the impact of ketosis, milk fever, and metritis on days open, unrealized milk, veterinary costs, labor, and discarded milk; and the change in the percent of cows with BCS at calving ≤ 3.25 before and after technology adoption. Scatter plots of the most sensitive random variables plotted against NPV along with correlation coefficients demonstrate how random variables impact profitability. In both simulations, the random variable that had the strongest relationship with NPV was the variable cost increase. Not surprisingly, as the variable costs per cow increased the NPV decreased in both simulations (Figure 3). Thus, the value of an automated BCS system was highly dependent on the costs incurred to utilize the information provided by the system to alter nutritional management for improved BCS profiles.

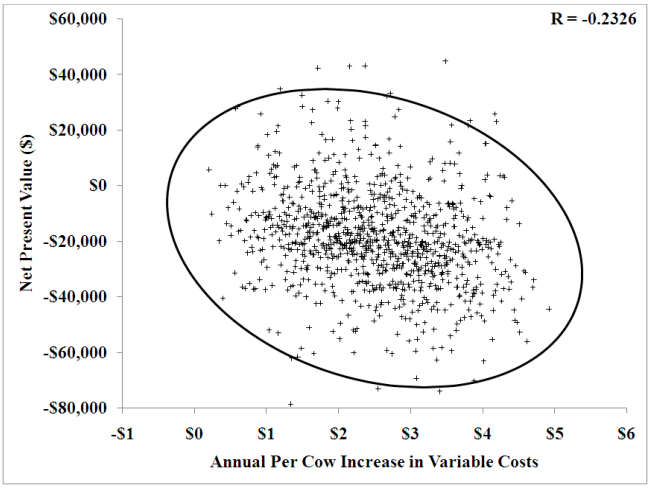

Figure 3 Scatter plot of Net Present Value versus annual percentage increase in variable costs (for simulation using all expert opinions provided).

Finally, the results of any simulation model are highly dependent on the assumptions within the model. A one-way sensitivity analysis tornado diagram compares multiple variables on the same graph. Essentially, each input is varied (1 at a time) between feasible high and low values and the model is evaluated for the output at those levels holding all other inputs at their default levels. On the tornado diagram, for each input, the lower value is plotted at the left end of the bar and the higher value at the right end of the bar (Clemen, 1996). Simulations were run for high and low feasible values for 6 key inputs that may affect NPV. The tornado diagram for the 95th percentile NPV from the simulation with a small likelihood of improvement in BCS distribution is presented in Figure 4. Herd size had the most

influence on NPV. The NPV was higher for the larger herd because the investment costs and benefits were spread among more cows.

The next most important variable was the BMPAF. Again, this result was not surprising and reiterates that one of the most important determinants of project success was what the producer actually does to manage the information provided by the technology. There are many nutritional, health, reproductive and environmental decisions made by the dairy producer that have a major impact on changes in body reserves for both individual cows and groups of cows. Management level plays a critical role in determining returns from investing in a Precision Dairy Farming technology. The level of management in day-to-day handling of individual cows may also influence the impact of Precision Dairy Farming technologies. Van Asseldonk (1999) defined management capacity as "having the appropriate personal characteristics and skills to deal with the right problems and opportunities in the right moment and in the right way." Effective use of an information system requires an investment in human capital in addition to investment in the technology (Streeter and Hornbaker, 1993). Then, the level of milk production was the next most sensitive input. As the level of milk production increased, the benefits of reducing disease incidence and calving intervals increased. As would be expected, the NPV increased with an increased base incidence of ketosis because the effects of BCS on ketosis would be exaggerated. The purchase price of the technology had a relatively small impact on the NPV as did the base culling rate.

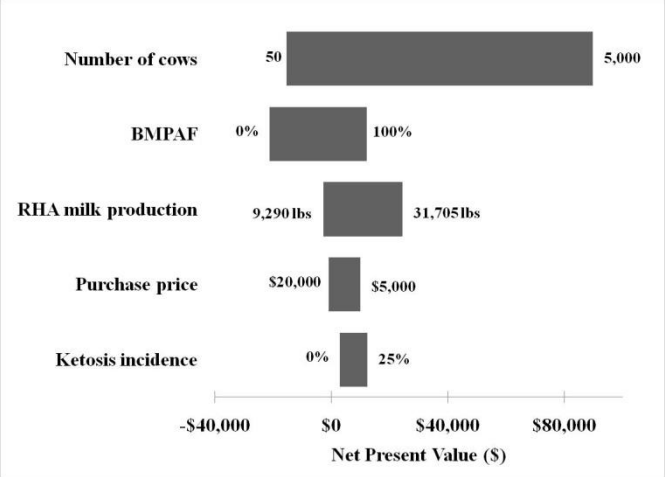

Figure 4 Tornado diagrams for inputs affecting 95th percentile of Net Present Value for simulations using the estimates of all survey respondents[1]

Adoption considerations

The list of Precision Dairy Farming technologies used for animal status monitoring and management continues to grow. Despite widespread availability, adoption of these technologies in the dairy industry has been relatively sparse thus far (Huirne et al., 1997; Gelb et al., 2001). Perceived economic returns from investing in a new technology are always a factor influencing technology adoption. Additional factors impacting technology adoption include degree of impact on resources used in the production process, level of management needed to implement the technology, risk associated with the technology, institutional constraints, producer goals and motivations, and having an interest in a specific technology

[1] 1 BMPAF is the Best Management Practice Adherence Factor, RHA milk production is rolling herd average milk production in lbs.

(Dijkhuizen et al., 1997, van Asseldonk, 1999). Characteristics of the primary decision maker that influence technology adoption include age, level of formal education, learning style, goals, farm size, business complexity, increased tenancy, perceptions of risk, type of production, ownership of a non-farm business, innovativeness in production, average expenditure on information, and use of the technology by peers and other family members. Research regarding adoption of Precision Dairy Farming technologies is limited, particularly within North America.

To remedy this, a five-page survey was distributed to all licensed milk producers in Kentucky (N=1074) on July 1, 2008. Two weeks after the first mailing, a follow-up postcard was mailed to remind producers to return the survey. On August 1, 2008, the survey was resent to producers who had not returned the survey. A total of 236 surveys were returned; 7 were omitted due to incompletion leaving 229 for subsequent analyses (21%). The survey consisted of questions covering general farm descriptive demographics, extension programming, and decision making behavior. With regard to Precision Dairy Farming the following question was presented to survey participants: "Adoption of automated monitoring technologies (examples: pedometers, electrical conductivity for mastitis detection) in the dairy industry has been slow thus far. Which of the following factors do you feel have impacted these modest adoption rates? (check ALL that apply)." Data were entered into an online survey tool (KeySurvey, Braintree, MA). Statistical analyses were conducted using SAS® (Cary, NC). Surveys were categorized by herd size, production system, operator age, and production level. Least squares means among categories were calculated for quantitative variables using the GLM procedure of SAS®. Statistical differences were considered significant using a 0.05 significance level using Tukey's test for multiple comparisons. For qualitative variables, $\chi 2$ analyses were conducted using the FREQ procedure of SAS®. Statistical differences were considered significant at a 0.05 significance level.

Among the 229 respondents, mean herd size was 83.0 ± 101.8 cows and mean producer age was 50.9 ± 12.9. Reasons for modest adoption rates of Precision Dairy Farming technologies and dairy systems software are presented in Table 1. The reasons selected by the highest percentage respondents were (1) not being familiar with technologies that are available (55%), (2) undesirable cost to benefit ratios (42%) and (3) too much information provided without knowing what to do with it (36%%). The high percentage of producers who indicated they were unfamiliar with available technologies indicates that marketing efforts may improve technology adoption. Actual or perceived economic benefits appear to influence adoption rates demonstrating the need for economic models to assess technology benefits and re-examination of retail product prices. As herd size increased, the percentage of producers selecting "poor technical support/training" and "compatibility issues" increased ($P < 0.05$), which may be reflective of past negative experiences. In developing technologies, manufacturers should work with end-users during development and after product adoption to alleviate these customer frustrations. Few significant differences were observed among age groups, though the youngest producers were more likely to select "better alternatives/easier to accomplish manually." Prior to technology development, market research should be conducted to ensure that new technologies address a real need. Utilizing this insight should help industry Precision Dairy Farming technology manufacturers and industry advisors develop strategies for improving technology adoption. Moreover, this information may help focus product development strategies for both existing and future technologies.

Table 1 Factors influencing slow adoption rates of Precision Dairy Farming technologies

Factor	N	Percent
Not familiar with technologies that are available	101	55%
Undesirable cost to benefit ratio	77	42%
Too much information provided without knowing what to do with it	66	36%
Not enough time to spend on technology	56	31%
Lack of perceived economic value	55	30%
Too difficult or complex to use	53	29%
Poor technical support/training	52	28%
Better alternatives/easier to accomplish manually	43	23%
Failure in fitting with farmer patterns of work	40	22%
Fear of technology/computer illiteracy	39	21%
Not reliable or flexible enough	33	18%
Not useful/does not address a real need	27	15%
Immature technology/waiting for improvements	18	10%
Lack of standardization	17	9%
Poor integration with other farm systems/software	12	7%
Compatibility issues	12	7%

Conclusions and outlook

Though Precision Dairy Farming is in its infancy, new Precision Dairy Farming technologies are introduced to the market each year. As new technologies are developed in other industries, engineers and animal scientists find applications within the dairy industry. More importantly, as these technologies are widely adopted in larger industries, such as the automobile or personal computing industries, the costs of the base technologies decrease making them more economically feasible for dairy farms. Because the bulk of research focused on Precision Dairy Farming technologies is conducted in research environments, care must be taken in trying to transfer these results directly to commercial settings. Field experiments or simulations may need to be conducted to alleviate this issue. Because of the gap between impact of Precision Dairy Farming technologies in research versus commercial settings, additional effort needs to be directed toward implementation of management practices needed to fully utilize information provided by these technologies. To gain a better understanding of technology adoption shortcomings, additional research needs to be undertaken to examine the adoption process for not only successful adoption of technology but also technology adoption failures.

Before investing in a new technology, a formal investment analysis should be conducted to make sure that the technology is right for your farm's needs. Examining decisions with a simulation model accounts for more of the risk and uncertainty characteristic of the dairy system. Given this risk and uncertainty, a stochastic simulation investment analysis will represent that there is uncertainty in the profitability of some projects. Ultimately, the dairy manager's level of risk aversion will determine whether or not he or she invests in a technology using the results from this type of analysis. Perhaps the most interesting conclusion from our model case study was that the factors that had the most influence on the profitability investment in an automated BCS system were those related to what happens with the technology after it has been purchased as indicated by the increase in variable costs needed for management changes and the management capacity of the farm. Decision support tools, such as this one, that are designed to investigate dairy herd decisions at a systems level may help dairy producers make better decisions. Precision dairy farming technologies

provide tremendous opportunities for improvements in individual animal management on dairy farms. In the future, Precision Dairy Farming technologies may change the way dairy herds are managed.

Take home messages are:

- Precision Dairy Farming is the use of technologies to measure physiological, behavioral, and production indicators on individual animals to improve management strategies and farm performance.
- Many Precision Dairy Farming technologies, including daily milk yield recording, milk component monitoring, pedometers, automatic temperature recording devices, milk conductivity indicators, automatic estrus detection monitors, and daily body weight measurements, are already being utilized by dairy producers.
- Other theoretical Precision Dairy Farming technologies have been proposed to measure jaw movements, ruminal pH, reticular contractions, heart rate, animal positioning and activity, vaginal mucus electrical resistance, feeding behavior, lying behavior, odor, glucose, acoustics, progesterone, individual milk components, color (as an indicator of cleanliness), infrared udder surface temperatures, and respiration rates.
- The main objectives of Precision Dairy Farming are maximizing individual animal potential, early detection of disease, and minimizing the use of medication through preventive health measures.
- Perceived benefits of Precision Dairy Farming technologies include increased efficiency, reduced costs, improved product quality, minimized adverse environmental impacts, and improved animal health and well-being.
- Real time data used for monitoring animals may be incorporated into decision support systems designed to facilitate decision making for issues that require compilation of multiple sources of data.
- Technologies for physiological monitoring of dairy cows have great potential to supplement the observational activities of skilled herdspersons, which is especially critical as more cows are managed by fewer skilled workers.
- The economic implications of technology adoption must be explored further to increase adoption rates of Precision Dairy Farming technologies.

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Relative value of lucerne and red clover silages for lactating cows

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Introduction

In many areas of the world, hay-crops such as lucerne (Medicago sativa) are harvested as silages rather than hay because of greater speed of harvest and reduced risk of weather damage. However, during ensiling, typically more than half of the crude protein (CP) in lucerne is broken down to small peptides, amino acids and ammonia by enzymes released from cell rupture in the foliage. Red clover (Trifolium pratense) has a polyphenol oxidase enzyme system (PPO) that forms *o*-quinones from endogenous plant *o*-diphenols; the *o*-quinones react with foliage proteins to substantially reduce their breakdown both in the silo (Lee *et al.*, 2004) and in the rumen (Broderick and Albrecht, 1997). Lower yields, poorer persistency, and slower field drying rates of red clover have limited its widespread use in North America. However, red clover appears to be well adapted to Northern Europe. We have conducted a number of lactation trials to determine the relative feeding value of lucerne silage (LS) and red clover silage (RCS) for dairy cows. A summary from 5 trials showed similar yield of milk and protein, reduced MUN and 3.5 percentage units greater N efficiency when lactating cows were fed RCS versus LS (Broderick, 2002). However, dry matter (DM) intake and milk fat yield were lower on RCS.

Lee et al. (2009) speculated that the PPO reaction might result in greater reduction in bioavailability of Met than Lys in rumen-undegraded protein (RUP) in RCS. Relative response to Met or Lys fed as rumen-protected AA (RP-AA) in cows fed RCS versus LS may indicate which of essential AA is most affected by PPO action. Therefore, the objective of this trial was to compare the effects of supplementing rumen-protected Met (RP-Met) and rumen-protected Lys (RP-Lys) in lactating cows fed diets based on either LS or RCS.

Materials and Methods

Thirty-two multiparous Holstein cows with mean (SD) 2.4 (0.71) parity, 111 (35.4) days in milk (DIM), 50 (4.9) kg milk/day and 582 (55) kg body weight (BW), plus 16 primiparous Holstein cows with mean (SD) 126 (29.7) DIM, 46 (2.0) kg milk/day and 512 (34) kg BW were used in the trial. Cows were blocked by parity and DIM and randomly assigned within squares to treatment sequences in 6 replicated, incomplete 8 x 8 Latin squares with 4 experimental periods. Periods lasted 28 d and consisted of 14 d for diet adaptation and 14 d for data and sample collection. All cows were injected with rBST (500 mg of Posilac; Elanco Animal Health, Greenfield, IN, USA) beginning on d 1 of the trial and at 14-d intervals thereafter. Cows were housed in tie stalls and had free access to water throughout the experiment. Care and handling of the animals was conducted as outlined by the guidelines of the University of Wisconsin institutional animal care and use committee. The 8 basic diets were fed as total mixed rations (TMR): 4 diets contained (DM basis) 47% LS plus 13% grass silage and 4 diets contained 60% RCS. Except for RP-AA, the balance of dietary ingredients was similar across the 8 diets. The 4 diets containing LS-grass silage, or the 4 with RCS, were supplemented with no RP-AA, RP-Met, RP-Lys, or RP-Met plus RP-Lys. The RP-Met was fed as SmartamineM® (Adisseo Corp., Alpharetta, GA, USA) to provide 15 g/d of chemical DL-Met. The RP-Lys was fed as AminoShure-L® (Balchem Corp., New Hampton,

NY, USA) to provide 27 g/d of chemical L-Lys. Assuming 80% bioavailability of Met in the RP-Met (Zhou et al., 2017) and 64% bioavailability of Lys in the RP-Lys (Lee et al., 2012), this corresponded to 9 g/d of absorbed Met and 17 g/d of absorbed Lys. Mean composition of the forages fed during the trial is in Table 1. Compositions of the 8 experimental diets actually consumed during the trial are in Table 2. Also, 4 multiparous cows, previously fitted with permanent 10-cm rumen cannulas (Bar Diamond, Inc., Parma, ID, USA), were randomly assigned to a 4 x 4 Latin square with 2 forage sources and treatment sequences to assess effect of dietary LS and RCS on rumen traits.

Diets were offered once daily at 10:00 h; orts were collected and weights recorded at 09:00 h. Feeding rate was adjusted daily to yield refusals equivalent to about 5-10% of intake. Weekly composites of wet feeds (LS, RCS, grass silage, corn silage, high-moisture shelled corn, the 8 TMR, and the 8 orts) were obtained from daily subsamples of about 0.5 kg of each material that were stored at -20ºC. Weekly samples also were collected of dry feeds (soybean meal, dry ground corn, and control and RP-AA containing premixes). Dry matter was determined in weekly composites of wet feeds and samples of dry feeds at 60ºC for 48 h. These DM contents were used to adjust DM composition of TMR every week over the trial. Intake of DM was computed based on 60ºC DM determinations of weekly composites of TMR and orts. Dried samples from all feeds were ground to pass a 1-mm screen (Wiley mill) and analyzed for total N by elemental analysis (Leco FP-2000 N Analyzer), DM at 105ºC, ash and OM by combustion (overnight at 550°C), sequentially for neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent insoluble N (ADIN) using heat stable α-amylase and Na_2SO_3 for the NDF analysis (Van Soest et al., 1991), and for NDIN omitting α-amylase and Na2SO3 during extraction (Licitra et al., 1996). The TMR samples were also analyzed for total lipid ("Ether Extract"; Dairyland Laboratories, Arcadia, WI) and for indigestible ADF (ADF remaining after 288 h in situ) to use as a digestibility marker. Frozen composites of LS and RCS were thawed and analyzed for non-protein N (NPN) (Muck, 1987).

Cows were milked twice daily at 05:00 and 17:00 and milk yield recorded at each milking in all experimental periods. Milk samples from a.m. and p.m. milkings were collected from 4 milkings mid-week in weeks 3 and 4 of each period and analyzed for fat, true protein, lactose, solids-not fat (SNF) and milk urea N (MUN) by infrared analysis (AgSource, Verona, WI, USA) with a Foss FT6000 (Foss North America Inc., Eden Prairie, MN, USA). Concentration and yield of fat, true protein, lactose and SNF, and MUN concentration, were computed as weighted means based on individual milk yields on each test day. Yields of ECM also were computed (Krause and Combs, 2003). Efficiency of conversion of feed DM was calculated for each cow for weeks 3 and 4 of each period by dividing mean yield of actual milk and ECM by mean DM intake. Apparent N efficiency (assuming no retention or mobilization of body N) was computed for each cow by dividing period mean milk N secretion (milk true protein/6.38) by mean N intake. For computation of BW change, BW was measured on 3 consecutive days at the beginning of the experiment and at the end of each period.

Spot urine and fecal grab samples were collected on d-27 of each period at 6 h before and 6 h after feeding. Urine was immediately diluted by mixing 15 mL of each sample with 60 mL of 0.072 N H_2SO_4 and storing at –20°C until analysis. Fecal samples were dried for 72 h at 60°C, ground through a 1-mm screen and composited on an equal DM basis to obtain 1 fecal sample/cow per period. All fecal samples were analyzed for DM, ash, OM, NDF, ADF, total N, and indigestible ADF using the assays described above for TMR. Indigestible ADF was

used as an internal marker to estimate apparent nutrient digestibility and fecal N output (Cochran et al., 1986). Metabolic fecal N excretion, estimated assuming N = 4.8 g/kg DM intake (NRC, 2001), was used to estimate true N digestibility from mean apparent N digestibility. Urine samples were thawed and analyzed for total N by elemental analysis, for urea using an automated colorimetric assay (Broderick and Clayton, 1997) adapted to flow-injection (Lachat Quik-Chem 8000 FIA, Lachat Instruments, Loveland, CO, USA), and for creatinine (Valadares et al., 1999). Urine volume and excretion of urea N and total N were estimated from mean urinary concentrations in each period assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

Table 1 Composition of dietary forage ingredients

	Lucerne silage		Red clover silage		Grass silage		Corn silage	
Components	Mean	SEM[1]	Mean	SEM	Mean	SEM	Mean	SEM
DM, %	42.8	1.20	38.9	2.42	32.8	1.46	37.7	0.75
CP, % of DM	20.9	0.25	17.8	0.29	11.8	0.19	6.7	0.08
Ash, % of DM	10.8	0.22	12.4	0.17	8.7	0.08	4.3	0.13
NDF, % of DM	39.9	0.75	38.5	0.52	57.0	0.53	42.6	1.31
ADF, % of DM	29.9	0.61	24.6	0.33	34.5	0.32	26.6	0.82
Hemicellulose, % of DM[2]	10.0	. . .	13.9	. . .	22.5	. . .	16.0	. . .
NDIN, % of total N	7.9	0.26	25.5	1.83	. . .	. . .	. . .	. . .
ADIN, % of total N	3.4	0.11	6.0	0.43	. . .	. . .	. . .	. . .
B3[3], % of total N	4.5	. . .	19.5	. . .	. . .	. . .	. . .	. . .
NPN, % of total N	51.6	1.95	35.0	2.23	. . .	. . .	. . .	. . .
NH_3, % of total N	6.0	1.00	4.7	0.42	. . .	. . .	. . .	. . .
Total AA-N[4], % of total N	28.8	1.41	15.7	0.92	. . .	. . .	. . .	. . .
pH	4.63	0.054	4.59	0.126	. . .	. . .	. . .	. . .

[1]Standard error of the mean; [2]Hemicellulose = NDF – ADF; [3]N fraction 3 = NDIN – ADIN; [4]Computed assuming 40.3 µmol of total free AA/mg N in alfalfa and red clover silages (Broderick, 1987).

On d 27-28 of each period, about 100 mL of fluid digesta was collected from 4 locations in the ventral rumen at 0 (just before feeding), 2, 4, 6, 8, 12, 18 and 24 h after feeding from the 4 lactating cows fitted with rumen cannulas. At each sampling, mixed digesta was strained through 2 layers of cheesecloth and pH measured immediately in strained fluid using a glass electrode. Two, 10-mL aliquots of rumen fluid were then preserved by addition of 0.2 mL of 50% H_2SO_4 and stored at -20°C. The remaining fluid and digesta were returned to the rumen. Just prior to analysis, one set of samples was thawed and centrifuged (15300 x *g* for 20 min at 4°C) and flow-injection analyses applied to supernatants to determine ammonia, using a phenol-hypochlorite method (Lachat Method 18-107-06-1-A), and total AA using a fluorimetric procedure based on the reaction with o-phthaldialdehyde (Roth, 1971). Leu was the standard for this assay and total AA values are reported in Leu equivalents. The second set of samples was thawed and centrifuged (28000 x *g* for 30 min at 4°C) prior to VFA analysis using a modification of the GLC method for free fatty acids described in Supelco Bulletin 855B (Supelco Inc., Supelco Park, Bellefonte, PA) with flame-ionization detection.

Statistical Analysis

The basic design of the lactation trial was an incomplete 8 x 8 Latin square, replicated 6 times. Results were analyzed using the mixed procedures of SAS (2013). The following model was used to assess effects of treatments on yield, N excretion and digestibility in the lactation trial:

$$Y_{ijklmn} = \mu + S_i + P_j + F_k + M_l + L_m + F*M_{kl} + F*L_{km} + M*L_{lm} + F*M*L_{klm} + C_{n(j)} + E_{ijklmn},$$

where Y_{ijklmn} = dependent variable, μ = overall mean, S_i = effect of square i (i = 1 to 6), P_j = effect of period j (j = 1 to 4), F_k = effect of forage k (k = 1 to 2), M_l = effect of RP-Met (l = 0 or 1), L_m = effect of RP-Lys (m = 0 or 1), $F*M_{kl}$ = interaction of forage and RP-Met, $F*L_{km}$ = interaction of forage and RP-Lys, $M*L_{lm}$ = interaction of RP-Met and RP-Lys, $F*M*L_{klm}$ = interaction of forage, RP-Met and RP-Lys, $C_{n(j)}$ = effect of cow n within square j, and E_{ijklmn} = residual error. All terms were considered fixed, except for $C_{n(j)}$ and E_{ijklmn}, which were considered random. Time-weighted means were computed for all rumen traits and the effect of forage source was statistically analyzed using the following model:

$Y_{ijkl} = \mu + Seq_i + P_j + F_k + C_{l(i)} + E_{ijkl}$,

where Y_{ijkl} = dependent variable, μ = overall mean, Seq_i = effect of sequence i (i = 1 to 2), P_j = effect of period j (j = 1 to 4), F_k = effect of forage k (k = 1 to 2), and $C_{l(i)}$ = effect of cow l within sequence i, and E_{ijkl} = residual error. All terms were considered fixed, except for $C_{l(i)}$ and E_{ijkl}, which were considered random. For both models, least squares means are reported and significance was declared at $P \leq 0.05$ and trends were declared at $0.05 < P \leq 0.10$.

Results and Discussion

Feed Quality and Diet Composition

As was observed in previous trials (Broderick, 2002), LS contained about 3-percentage units more CP than RCS when harvested at similar NDF contents (Table 1). Also as found in earlier comparisons of these 2 silages, RCS had greater hemicellulose content and, appropriate for this trial comparing forage effects on CP utilization, RCS had typically greater NDIN and N fraction B3 (NDIN – ADIN) plus about one-third less NPN as a proportion of total CP. Greater content of N fraction B3 is related to improved N efficiency (Licitra et al., 1996). Reduced proportions of silage NPN are related to improved N utilization (Nagel and Broderick, 1992; Broderick, 2002).

Because of its greater CP content, the LS was diluted with grass silage such that the blend of LS plus grass silage was comparable in CP to the RCS (Table 2). An additional 1-percentage unit of soybean meal DM was added to the 4 RCS diets in an attempt to equalize CP; although generally similar in chemical composition, the LS diets averaged 15.9% CP and 31% NDF versus 15.6% CP and 29% NDF for the RCS diets. The greater N fraction B3 in RCS carried over into trial diets; LS diets averaged 10% and RCS diets 19% N fraction B3 in total CP. The NRC (2001) model predicted somewhat greater milk yield based on dietary NEL and MP content. Without any RP-AA and with supplementation of RP-Lys alone, estimated Lys: Met ratio ranged from 3.5 to 4.0; supplementation with RP-Met reduced Lys: Met ratio to 2.8 to 3.2. The NRC (2001) model gives 3.0 as the optimum Lys: Met ratio; thus, the NRC model predicts that cows should be responsive to RP-Met as first-limiting AA.

Production responses to legume silages and RP-AA

Production, N excretion and digestibility data are in Table 3. Many of the observations reported in the 5-trial summary (Broderick, 2002) were also observed in the current study: cows consumed more feed on LS but did not produce more milk; thus milk/DMI was greater on RCS. Because of greater milk fat content and yield on LS than RCS, ECM yield was also greater on LS. Moorby et al. (2009) reported that feeding RCS increased intake of unsaturated fatty acids. Greater content of these fatty acids in RCS versus LS may have resulted in elevated rumen formation of conjugated linoleic acid on the RCS diets, thus depressing milk fat secretion (Bauman and Griinari, 2003). Milk true protein content was also

greater, and there was trend for greater protein yield on LS versus RCS. This indicated that the lower NPN and greater N fraction B3 in RCS did not result in all of the cow's requirement for metabolizable protein being met. However, substantially lower MUN and excretion of urea N and total urinary N, and higher milk N/N-intake, document greater N efficiency when dietary RCS rather than LS is the principal dietary forage. Additionally, milk lactose content was elevated on RCS, a surprising response that apparently has not been reported earlier. There were large increases in apparent digestibility of DM, organic matter, NDF, ADF and hemicellulose on RCS versus LS; the magnitude of these effects was comparable to that observed in the 5-trial summary (Broderick, 2002). Relative to the LS diet, apparent digestibility, and true digestibility (based on metabolic fecal N estimated from DMI), of N were reduced, respectively, 1.8 and 1.2 percentage units. Although significant, that this reduction was < 2 units suggested that most of the additional RUP provided by RCS would be digested and absorbed as AA in the intestine.

Supplementation of both diets with RP-Met increased DMI and concentration of milk true protein and SNF (most of the SNF was contributed by true protein; Table 3); however, true protein yield was not altered (P = 0.11). The NRC (2001) reported that the response to both Met and Lys supplementation of milk protein concentration was much less variable than that of milk protein yield. These results are also consistent with the NRC (2001) assessment of Met limitation on these experimental diets (Table 2). The other effects detected with feeding of RP-Met were reduced milk/DMI and a trend for reduced ECM/DMI; this occurred because milk and ECM volumes were not affected despite increased DMI. The only affect detected with RP-Lys supplementation was increased urinary N excretion, which was elevated 8 g/d (Table 3). Because there was no change in milk protein secretion, one would expect all of the N fed as RP-Lys to be excreted; on average, about 6 g/d of N was consumed as RP-Lys in the trial. As anticipated, no effects of RP-AA supplementation were detected on apparent nutrient digestibility.

Specific interactions were omitted from Table 3 and no forage x Met interactions were detected, indicating the effects of Met were the same on both LS and RCS. However, a curious forage x Lys interaction was detected: feeding RP-Lys on LS appeared to reduce DMI by 0.5 kg/d, while feeding RP-Lys on RCS appeared to increase DMI by 0.5 kg/d; this resulted in milk/DMI and ECM/DMI interactions that were the inverse of those of DMI. One Met x Lys interaction was observed: RP-Lys supplementation alone had little effect on true protein yield while adding RP-Lys to RP-Met appeared to reduce true protein yield. Although this affect is difficult to explain biologically, it suggests that Lys was not second limiting essential AA in this trial. No significant 3-way interactions of forage source, RP-Met and RP-Lys were detected ($P \geq 0.15$).

These results suggest that PPO action in RCS does not result in Lys becoming more limiting. Moreover, Met was first-limiting on both LS and RCS diets, and no inference can be made on whether Met bioavailability was reduced by PPO. Despite apparently large reduction in MUN and urinary N excretion, and increased N efficiency on RCS, there was no improvement in milk protein yield. The small reduction in true N digestibility indicated that the greater RUP would supply proportionately more MP, but with no detectable effect on milk protein secretion. Greater DM as well as N efficiencies are likely driven by greater digestibility of organic matter and fiber on the RCS diet. Purwin et al. (2015) determined AA contents of lucerne and red clover before and after ensiling in large round bales. Because NPN contents of their silages were much lower (23 and 14% of total N in, respectively, LS and RCS), extent of fermentation was likely much reduced compared to that in silages fed in the present

trial. Although no direct effect of ensiling on silage Met content was detected, Purwin et al. (2015) reported substantially greater Met concentration in LS than in RCS in their study. Thus, it seems possible that MP provided by RCS is more limiting in this essential AA than is RUP from LS.

Table 2 Diet composition (% of DM unless otherwise stated)

		Lucerne silage				Red clover silage			
Item	RP-AA[1]	Ctrl	Met	Lys	M+L	Ctrl	Met	Lys	M+L
Lucerne silage		47.4	47.4	47.4	47.4	0.0	0.0	0.0	0.0
Red clover silage		0.0	0.0	0.0	0.0	59.8	59.9	59.9	59.8
Grass silage		12.9	12.9	12.9	12.9	0.0	0.0	0.0	0.0
Corn silage		5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
Total forage		65.4	65.4	65.4	65.4	65.0	65.0	65.0	65.0
High moisture shelled corn		18.4	18.4	18.4	18.4	18.5	18.6	18.6	18.5
Ground shelled corn		9.8	9.8	9.5	9.4	9.1	8.9	8.7	8.7
Solvent soybean meal		4.0	3.9	4.0	4.0	4.9	5.0	5.0	5.0
Smartamine-M®[2]		0.00	0.06	0.00	0.06	0.00	0.06	0.00	0.06
AminoShure-L®[3]		0.00	0.00	0.30	0.30	0.00	0.00	0.30	0.30
Calcium sulfate		1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36
Biophos		0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Sodium chloride		0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Magnesium oxide/sulfate		0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamins-trace minerals[4]		0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Composition									
Crude protein		16.0	15.9	15.9	15.9	15.6	15.6	15.6	15.6
Ash		7.1	7.1	7.1	7.1	8.4	8.4	8.4	8.4
NDF		30.8	30.7	30.7	30.7	28.6	28.6	28.5	28.5
ADF		20.9	20.9	20.9	20.9	17.7	17.7	17.7	17.7
Hemicellulose		9.8	9.8	9.8	9.8	10.8	10.8	10.8	10.8
Ether extract		2.8	2.8	2.8	2.7	2.7	2.7	2.7	2.7
NFC[5]		45.0	45.0	45.1	45.1	47.7	47.7	47.8	47.8
NDIN, % of total N		10.1	10.1	10.1	10.1	19.1	19.1	19.1	19.1
ADIN, % of total N		3.9	3.9	3.9	3.9	5.0	5.0	5.0	5.0
B3, % of total N		6.2	6.2	6.2	6.2	14.1	14.1	14.1	14.1
NE_L,[6] Mcal/kg DM		1.51	1.51	1.51	1.51	1.54	1.54	1.54	1.54
Metabolizable protein,[6] g/d		2035	2035	2035	2035	2114	2114	2114	2114
NE_L-allowable milk,[6] kg/d		34.8	34.8	34.8	34.8	36.8	36.8	36.8	36.8
MP-allowable milk,[6] kg/d		26.1	26.1	26.1	26.1	27.4	27.4	27.4	27.4
Lys: Met ratio in MP[6]		3.5	2.9	3.9	3.2	3.6	2.8	4.0	3.1

[1]RP-AA = rumen-protected Met and Lys. Ctrl (control) = no RP-AA supplement; Met = supplementation with RP-Met; Lys = supplementation with RP-Lys; M+L = supplementation with RP-Met and RP-Lys; [2]Rumen-protected Met product from Adisseo Co., Alpharetta, GA; [3]Rumen-protected Lys product from Balchem Corp., New Hampton, NY; [4]Provided (per kilogram of DM): 56 mg of Zn, 46 mg of Mn, 22 mg of Fe, 12 mg of Cu, 0.9 mg of I, 0.4 mg of Co, 0.3 mg of Se, 6440 IU of vitamin A, 2000 IU of vitamin D, and 16 IU of vitamin E; and 12 mg Monensin; [5]NFC = 100 - %NDF – [%CP x (100 - %NDIN)/100] - %ether extract - %ash, using NDIN; [6]Computed according to NRC (2001) model using mean DMI (23.3 kg/d). Lys: Met ratios estimated assuming 80% bioavailability of Met and 64% bioavailability of Lys in rumen-protected AA.

Rumen metabolite concentrations

Concentrations of rumen metabolites are reported in Table 4. Relative to LS, there were large reductions in concentrations of ammonia, total free AA, and the branched-chain VFA, all of which are products of protein degradation in the rumen (Van Soest, 1994). There was also a small reduction in rumen propionate concentration on RCS. It is possible that RDP supply could have limited microbial protein formation on RCS versus LS diets. Brito et al. (2007) quantified omasal

flow on two sources each of LS and RCS in lactating dairy cows and found similar microbial protein synthesis and greater RUP outflow on RCS; however, reduced microbial protein yield per unit organic matter truly digested in the rumen was detected on RCS diets.

Table 3 Effect of source of legume silage and rumen-protected AA on least squares means for production, digestibility and nitrogen excretion in lactating dairy cows[1]

	Forage		RP-AA					Probability[3]		
Trait	LS	RCS	- Met	+ Met	- Lys	+ Lys	SEM[2]	Silage	Met	Lys
					Production					
DMI, kg/d	23.9	22.7	23.1	23.6	23.3	23.3	0.30	< 0.01	0.01	0.98
BW gain, kg/d	0.39	0.37	0.36	0.39	0.34	0.41	0.059	0.79	0.70	0.39
Milk, kg/d	35.0	34.8	34.9	34.9	35.1	34.7	0.59	0.47	0.92	0.23
Milk/DMI	1.47	1.54	1.53	1.48	1.51	1.49	0.022	< 0.01	0.01	0.25
ECM, kg/d	34.0	33.2	33.5	33.7	33.7	33.5	0.66	0.03	0.48	0.64
ECM/DMI	1.43	1.47	1.46	1.43	1.45	1.44	0.023	0.02	0.07	0.64
Fat, %	4.06	3.93	3.96	4.04	3.98	4.01	0.055	< 0.01	0.06	0.43
Fat, kg/d	1.40	1.34	1.37	1.38	1.38	1.37	0.031	< 0.01	0.48	0.81
True protein, %	3.04	3.01	2.99	3.06	3.01	3.04	0.028	0.03	< 0.01	0.12
True protein, kg/d	1.04	1.03	1.03	1.04	1.04	1.03	0.019	0.09	0.11	0.67
Lactose, %	4.79	4.86	4.82	4.83	4.83	4.83	0.023	< 0.01	0.40	0.87
Lactose, kg/d	1.66	1.67	1.67	1.66	1.67	1.66	0.032	0.64	0.53	0.32
SNF, %	8.74	8.76	8.71	8.80	8.74	8.77	0.037	0.26	< 0.01	0.22
SNF, kg/d	3.02	3.00	3.01	3.01	3.02	3.00	0.054	0.51	0.91	0.39
MUN, mg/dL	15.1	12.9	14.0	14.0	13.9	14.1	0.19	< 0.01	0.64	0.12
Milk-N/NI, %	26.9	28.5	27.8	27.5	27.7	27.6	0.36	< 0.01	0.33	0.85
					Nitrogen excretion[4]					
Urea-N, g/d	147	114	132	130	128	133	2.8	< 0.01	0.52	0.17
Urinary-N, g/d	182	148	166	163	161	169	3.4	< 0.01	0.48	0.05
Urea-N/total-N, %	81.8	78.9	80.3	80.5	80.9	79.8	1.34	0.14	0.91	0.56
Urinary-N, % NI	29.9	26.2	28.5	27.7	27.2	28.9	0.56	< 0.01	0.27	0.02
Fecal N, g/d	227	222	223	226	226	223	4.0	0.19	0.50	0.39
Fecal N, % of NI	37.2	38.9	38.1	38.0	38.3	37.8	0.42	< 0.01	0.73	0.37
N excretion, g/d	410	369	389	390	387	392	6.0	< 0.01	0.93	0.43
N excretion, % NI	67.1	65.2	66.7	65.6	65.5	66.7	0.70	0.04	0.26	0.22
					Apparent digestibility,[5] %					
Dry matter	61.3	65.2	63.1	63.3	63.1	63.3	0.33	< 0.01	0.66	0.62
Organic matter	62.7	66.5	64.5	64.7	64.5	64.7	0.32	< 0.01	0.73	0.64
Nitrogen	62.8	61.1	61.9	62.1	61.7	62.2	0.42	< 0.01	0.68	0.33
NDF	43.9	49.5	46.4	47.0	46.5	46.9	0.50	< 0.01	0.38	0.57
ADF	45.8	52.3	48.7	49.4	48.9	49.2	0.51	< 0.01	0.32	0.65
Hemicellulose	39.8	43.3	41.3	41.7	41.2	41.8	0.55	< 0.01	0.56	0.45
"True" Nitrogen	81.6	80.4	80.9	81.1	80.7	81.3	0.42	0.02	0.63	0.30

[1]RP-AA = rumen-protected Met and Lys (- = no RP-Met or RP-Lys; + = added RP-Met or RP-Lys); [2]Standard error of the least squares means; [3]Probability of effects of legume silage source, RP-Met or RP-Lys; [4]Urinary excretion estimated from creatinine concentration (Valadares et al., 1999) and fecal nitrogen excretion estimated using indigestible ADF as an internal marker (Cochran et al., 1986); [5]Apparent digestibility estimated using indigestible ADF as an internal marker (Cochran et al., 1986); "True" N digestibility estimated from metabolic fecal nitrogen = 4.8 g N/kg DMI/d (NRC, 2001).

Conclusions

Replacing LS with RCS reduces extent of rumen protein degradation, MUN and urinary N excretion and improves N efficiency. Although DMI is reduced on RCS, both organic matter and fiber digestion are substantially greater, resulting in improved milk and ECM secretion per unit DMI relative to LS. However, compared to LS, there were small but significant

Table 4 Effects of source of dietary legume silage on rumen pH and metabolite concentrations

	Silage			
Trait	Lucerne	Red clover	SEM[1]	Probabiliy[2]
pH	6.67	6.75	0.021	0.06
Ammonia-N, mg/dL	7.47	4.42	0.480	0.01
Total AA, m*M*	1.66	1.18	0.323	0.01
Total VFA, m*M*	79.8	75.6	1.85	0.18
Acetate, m*M*	52.2	50.4	1.09	0.30
Propionate, m*M*	15.4	14.2	0.84	0.03
Acetate:Propionate ratio	3.41	3.55	0.162	0.06
Butyrate, m*M*	8.4	7.8	0.32	0.26
Isobutyrate, m*M*	0.99	0.81	0.023	0.01
Isovalerate + 2-methyl butyrate, m*M*	1.49	1.23	0.057	< 0.01
BCVFA,[3] m*M*	2.48	2.04	0.053	< 0.01
Valerate, m*M*	1.31	1.18	0.045	0.12

[1]Standard error of the least squares means; [2]Probability of dietary lucerne silage versus red clover silage; [3]Branched-chain VFA (isobutyrate plus isovalerate + 2-methyl butyrate).

reductions in milk fat, probably related to greater unsaturated fatty acid content of RCS, and milk protein content and yield. Supplementation with RP-Met, but not RP-Lys, increases milk protein concentration on both LS and RCS, suggesting it is the limiting AA in both silage sources. Utilization of the RUP contributed by RCS appears not to be limited by intestinal digestibility but may be limited by its relatively low Met content.

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Biosensor technologies for agriculture and environment – opportunities and challenges

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Introduction

Ensuring agricultural and environmental safety has been a major global concern throughout the human history. Traditionally, agriculture was mainly operated within an individual family or village and quality control was given by knowledge gained from past observations. With increased demand of food supply for the fast growing global population, modernization of the agriculture industry became important. Farm productivity is largely dependent on surrounding environmental conditions, various pollutants, crop diseases and animal diseases outbreak. Therefore, sensing technologies which allow rapid monitoring of environment conditions, crop and animal health, as well as food products safety are necessary. Conventional off-site monitoring require samples to be sent to centralized laboratories for analysis. However, traditional laboratory detection methods with large-scale instruments cannot meet requirements of simple, portable and fast analyses, which are useful for field/farm applications, because of their higher cost and complex operational procedures. Biosensors are relatively new analytical tools that have attracted great attention for reasons of simplicity, low-cost, high sensitivity, selectivity and rapidness. The blood glucose biosensor is a well-known example of this. Here, we will present general principles of various biosensor systems and review current biosensor technologies for agricultural and environmental monitoring and discuss their opportunities and challenges.

Biosensors

A biosensor is an integrated analytic device composed of two key elements: a bio-recognition element and a transducer. The bio-recognition element convenes biochemical events and transmit signals to the transducer (e.g. electrochemical or optical transducers) into a rapid readout, with the resulting signal being proportional to the concentration of the tested analyte (Figure 1).

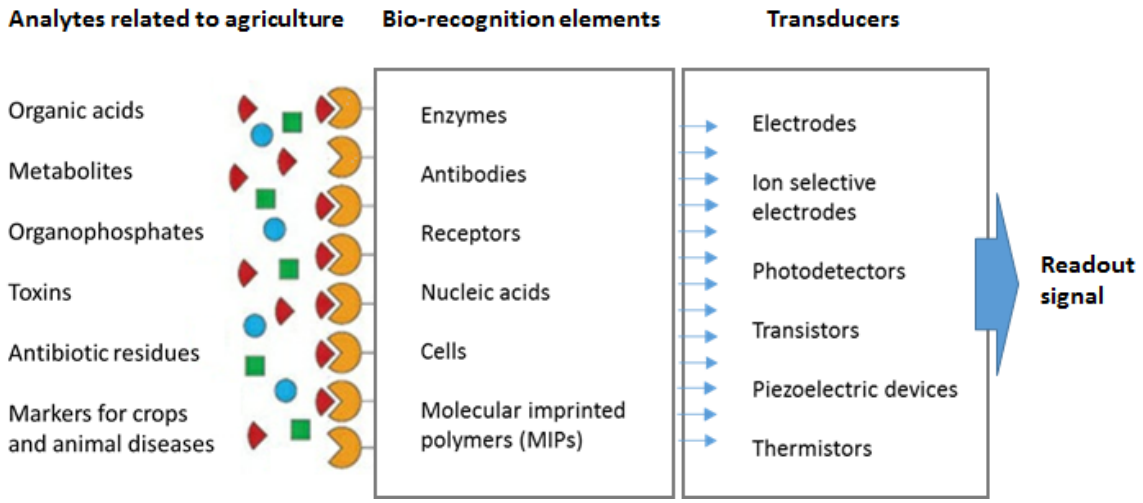

Figure 1 Schematic diagram illustrating components and signal transduction principle of biosensors.

The most commonly used bio-recognition elements include enzymes, antibodies, receptor proteins, nucleic acids; as well as the more advanced synthetic bio-recognition elements such as molecular imprinted polymers (MIPs). Choice of bio-recognition molecule mainly depends on analytes of interest. For examples, enzyme-based biosensors for detection of metabolites (e.g. organic acids and urea), antibody-based biosensors for detection of immuno-related molecules (e.g. biomarkers for animals and crop diseases and antibiotics), nucleic acid-based biosensors for detection of genetic biomarkers. Moreover, there are new classes of nucleic acids such as "aptamers" (i.e. nucleic acids with specific protein binding affinity) and "deoxyribozymes" (i.e. nucleic acids with catalytic activities), which have been used to construct biosensors. Natural bio-recognition molecules have many advantages, one of the limitation is instability of the biomolecules, which limits operational life-time for on-line continuous monitoring. Molecular imprinted polymers have, for that reason, been developed as highly stable synthetic bio-recognition elements for biosensors applications. In brief, MIPs use polymer matrices to create molecular cavities that mimic biological functions of the catalytic sites of enzymes or the binding site of antibodies. The MIPs are more resisted to temperature, pH, ionic strengthen and biodegradation and are promising candidates for development of biosensors for long-term continuous monitoring in automatic farming systems.

Biosensor applications

Sugars are important indicators for growth and maturation of fruits and are also indicators of shelf life of fruits as related to growth of microorganism (Cano et al., 1994). Various electrochemical enzyme-based biosensors have been developed for detection of organic acids in fruits. Glucose is one of the most common sugars presence in fruits and vegetables as the primary product of photosynthesis. There are many glucose biosensors based on glucose oxidase or glucose dehydrogenase reported in the literature for determination of glucose levels in fruit juices and a summary on the glucose biosensor for food analysis was published by Mello et al. (2002). Sucrose concentration in fruits is important for determining maturity levels and is a ripening parameter of fruits. Sucrose biosensors are commonly constructed by co-immobilization a combination of 3 enzymes (i.e. invertase, mutarotase and glucose oxidase) with a detection limit of approximately 1 mM sucrose (Maestre et al., 2010). However, sucrose biosensors also response to glucose, and therefore a compensating glucose biosensor is needed for correcting sucrose levels. Abayomi et al. (2006) reported development of a pyruvic acid biosensors based on a mediated Meldolas Blue pyruvate dehydrogenase electrode for determining pungency in onions. This biosensor could detect mild and pungent bulbs with pyruvic acid concentrations between 4 to 8 mM (Abayomi et al., 2006). Concentrations of dissolved L-lactate can also be used as a measure of freshness in food industry. Electrochemical lactate biosensors have been fabricated using a lactate oxidase immobilized electrode with detection limit of 50 mM lactate (Wei et al., 2003). While an alternative approach, based on immobilization of a NADH-dependent lactate dehydrogenase biosensor has improved detection limits down to 5 nM lactate (Jena and Rai, 2006). There are also several other example of sugars important for agriculture food monitoring. More detailed information about sugar biosensors is found in a comprehensive review article by Rana et al. (2010).

Certain pathogenic microorganism, such as fungi, attack crops and cause crop diseases. One important indicators of *Aspergillus flavus* infection is the production of aflatoxin. Fungal attack is not only causing agricultural problems, but aflatoxin endangers human health. Low

levels of aflatoxin are sufficient to cause serious life threatening medical conditions. Therefore, aflatoxin detection in the agriculture industry is highly important. Carlson et al. (2000) developed an automated handheld biosensor for aflatoxin detection. The biosensor is operated on the principles of immune-affinity and fluorescence detection. This handheld aflatoxin biosensor also has a relatively good analytical performance that allows approximately 100 measurements before refurbishment is required. Detection of aflatoxin concentrations range from 0.1 to 50 ppb in less than 2 minutes. To monitor crop health in a large field and without sample extraction is highly desired. Schutz et al. (2000) reported development of a gas biosensor for detection of volatiles released by diseased potato tubers. This biosensor showed good selectivity to distinguish diseased potatoes, while not being disturbed by odours emitted by healthy and mechanically damaged ones. This gas biosensor also had good sensitivity and could detect one single diseased potato within up to 100 kg healthy potatoes.

Monitoring and control of animal diseases is another important topic in agriculture industry. Mastitis in dairy cow is one of the major animal disease problems in agriculture with a large financial impact on dairy farmers. Mastitis could be treated at an early stage and, therefore, biosensor for early diagnosis of mastitis are important. Mottram et al. (2000) reported development of an electrochemical mastitis biosensor for detection of an enzyme biomarker (N-acetyl glucosaminidase - NAGase), which is related to tissue damage when the cow is resisting intra-mammary infection. The mastitis biosensor could detect NAGase concentration ranging between 12-120 mU/mL of milk. Another important example of animal diseases is the avian influenza viruses (AIVs) infection hosted by aquatic birds and causing global threat to animal health and to the international poultry industry, as well as possible transmission of AIVs to humans. Based on the difference in sialic acid linkages on the surface of the AIVs, biosensors were developed for detecting and differentiating between avian and human influenza viruses. A glycan-immobilized field effect transistor biosensor was able to detect and discriminate between human (H1) and avian (H5) influenza viruses based on surface protein marker hemagglutinin (HA) with concentrations ranging between 50 aM−5 nM (Hideshima et al., 2013). Moreover, portable and low-cost membrane based lateral flow strip tests have been developed for detection of various specific influenza biomarkers in complex media (Sajid et al., 2015). In the presence of a specific influenza biomarker, an immune-complex is formed on the test strip membrane indicated by an eye-visible label (typically nanogold particle label), offering a portable and cost-effective biosensor platform for rapid semi-quantitative field tests on farms.

Conclusions

Advances in biosensor technologies could provide a useful analytical tools for agricultural monitoring, particularly due to their rapid response, relatively low operational cost and portability for field/farm application. The promise, demonstrated by various examples of biosensor technologies, is very appealing. However, there are still many hurdles to bring commercial agricultural biosensors into real practice. Sampling size, sample extraction and pre-treatment, especially of solid sample such as crops and feeds, are important factors for the implementation of biosensor technologies for agricultural monitoring. Biosensors integrated with automatic systems enabling sampling and continuous monitoring is essential for the future of modern agricultural industry.

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Effects of supplementation of glycerol on feed intake, milk production and methane emissions in dairy cows

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Introduction

Methane is a potent greenhouse gas that contributes to global warming and is generated during fermentation in ruminants. Cattle production is responsible for approximately 9% of global anthropogenic greenhouse gas emissions (FAO, 2013). However, nutrition and management factors influence methane emission and it is possible to reduce emissions by dietary and management means.

Glycerol, a by-product of biodiesel production, can be absorbed across the rumen epithelium of cattle (Werner Omazic *et al.*, 2015) which in turn reduces the amount of glycerol which is available for the microbes in the rumen. Feeding glycerol increases propionate concentration in the rumen (Rémond *et al*., 1993), and has been used as a glucogenic substance for the postpartum period in dairy cows (Kass *et al*., 2013). Propionate production in the rumen acts as a hydrogen sink and leads to a reduction of CH_4 emissions (Boadi *et al*., 2004). Glycerol has a potential to reduce CH_4 emissions since some of it is absorbed or escapes the rumen and, thereby, microbial fermentation but also because the fraction, which actually is subjected to microbial fermentation, will increase propionate production. Starch is known to lower CH_4 emissions by increasing propionate production but also by rumen escape providing less substrate for the rumen microbes (Millis *et al*., 2001). In addition, with more starch in the diet, the digestible organic matter will increase (Oba & Allen, 2003), which leads to decreased CH_4 emissions (Ramin & Huhtanen, 2015). Diets with more fibre on the other hand, leads to higher proportions of acetate in the rumen (Sutton *et al*., 2003), which in turn increase CH_4 emissions (Boardi *et al*., 2004).

It is unclear how glycerol effects CH_4 emissions in dairy cows *in vivo*. Some *in vitro* studies have shown either no effect (Avila *et al*., 2011; Castagnino *et al.*, 2015), a decrease (Lee *et al*., 2011) or an increase (Danielsson *et al*., 2014; van Cleef *et al*., 2015) in CH_4 emissions when adding glycerol to the diet. The objective of the present experiment was to study the effect of replacing starch with glycerol on CH_4 emissions in dairy cows fed grass silage and barley based diets.

Materials and Methods

Twenty-two Swedish Red dairy cows in mid-lactation (104 ± 22; mean ± SD) days in milk at the start of the study) were housed in an insulated loose housing system and milked twice a day in a milking parlour (SAC, S.A. Christensen and Co Ltd., Kolding, Denmark). The cows were blocked by milk yield and parity before randomly assigned into two groups. The experiment used a switch-back change-over design with two different diets as treatments and three periods of each 21 days. The first two weeks of each period were used for adaptation while sampling and data collection were performed during the last week of each period. Milk

was sampled during four consecutive milkings in the end of each period. The cows were fed total mixed rations (TMR) *ad libitum* in Roughage Intake Control feeders (Insentec B. V., Marknesse, the Netherlands) which recorded individual intake. Treatments consisted of two different TMR; one with 20% of dry matter (DM) wheat starch (STA) and one with 20% of DM pure glycerol (GLY) (Table 1). Both diets were balanced for content of net energy for lactation (NEL) and crude protein (CP) (Table 2). The silage was a first cut grass sward (80% timothy and 20% red clover) stored in a bunker silo.

Table 1. Ingredients (g/kg dry matter; DM) of the two experimental total mixed rations (STA = 20% of DM starch, GLY = 20% of DM glycerol)

	STA	GLY
Grass silage	605	605
Barley[1]	70	70
Rape seed meal[2]	120	120
Glycerol[3]	0	200
Wheat starch[4]	200	0
Minerals[5]	5	5

[1]Crimped barley preserved with propionic acid and stored in air tight bags; [2]Solvent-extracted and heat-moist treated rape seed meal with low levels of glucosinolates and erucic acid (ExPro, AahusKarlshamn AB, Malmö, Sweden); [3]Pure glycerol (99,5% glycerol, AahusKarlshamn AB, Malmö, Sweden); [4]Food-grade wheat starch (Foodstar, Kröner-Stärke, Ibbenbüren, Germany); [5]Contained 15% Ca, 12% Mg, 8% Na and 1% P (Effekt Intensiv, Lantmännen Lantbruk, Malmö, Sweden).

Table 2. Chemical composition of the two experimental total mixed rations, calculations based on analyzed or tabulated values for each feed ingredient (STA = 20% of DM starch, GLY = 20% of DM glycerol)

	STA	GLY
Dry matter (DM), g/kg	332	320
Ash, g/kg DM	52	51
Crude protein, g/kg DM	145	144
NDF[1], g/kg DM	324	323
Ether extract, g/kg DM	33	33
Starch, g/kg DM	239	51
Glycerol, g/kg DM	0	199
NEL[2], MJ/kg DM	6.7	6.5

[1]Neutral detergent fibre; [2] "Net energy lactation 20 kg DM" according to NorFor feed table.

Individual emissions of CH_4 was automatically measured by mass flux from the breath of the cows every time they visited the GreenFeed (C-Lock Inc., Rapid City, SD, US; Zimmerman, 2011) where they were offered in average 1.4 kg/day of a pelleted concentrate (Komplett Fiber 170, Lantmännen Lantbruk, Malmö, Sweden).

Feed analyses of ash, CP, neutral detergent fibre (NDF) and starch were performed as described by Bertilsson and Murphy (2003). Dry matter was determined according to Åkerlind *et al.* (2011) and ether extract according to the European Economic Community (1998). Tabulated values were used for wheat starch, glycerol and NEL (NorFor, 2017). Milk samples were analysed for composition of fat, protein and lactose in an infrared Fourier

transform spectroscopy (CombiScope FTIR 300 HP, Delta Instruments B.V., Drachten, The Netherlands).

Data was analysed by SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) Proc Mixed using a change-over model with the effects of treatment, period, order and cow as random variables.

Results and Discussion

Dry matter intake (DMI) was higher for cows fed the GLY diet (Table 3). The higher DMI was probably caused by the palatability of the glycerol. Since glycerol is a liquid with high viscosity it might also help against sorting and separation of different feed components in a TMR (Drouillard, 2008). A higher DMI is in accordance with Kass *et al*. (2012) who fed up to 3 kg of crude glycerol per cow and day, while others did not find an effect of glycerol on DMI (Donkin *et al*., 2009; Boyd *et al*., 2011). In spite of a higher DMI, milk yield, both in terms of kg milk and energy corrected milk (ECM), were lower compared with the STA diet and body weights were not improved ($P = 0.16$). Previous studies did not find any effect of glycerol on milk production (Donkin *et al*., 2009; Boyd *et al*., 2011; Kass *et al*., 2012). Other studies (both *in vitro* and *in vivo*) show that glycerol increases the proportion of both propionate and butyrate in the rumen (Rémond *et al*., 1993; Kass *et al*., 2012; Danielsson *et al*., 2014), while starch only increases the proportion of butyrate in the rumen (McDonald *et al*., 2002). Kass *et al.* (2012) suggested that a glycerol diet cause no increase in milk yield even though DMI increases due to an increase in the proportion of butyrate in the rumen. Earlier studies by Huhtanen *et al*. (1993) have suggested that an increasing proportion of butyrate could reduce glucose production in the liver. However, they did not observe any reduction in milk yield.

Our assumption was that glycerol would be rapidly absorbed across the rumen epithelium and that the microbes would have a limited amount of glycerol to ferment into CH_4 according to findings by Werner Omazic *et al.* (2015). However, the cows in the present study emitted more CH_4 compared with cows given STA (Table 3). Since they also produced less ECM, they had a higher CH_4/ECM ratio (Table 3).

The glycerol in the present study was mixed into the TMR, which can affect its fate when entering the rumen. Linke (2004) found that when drenching dairy cows with 1 kg of glycerol per day, the blood plasma concentrations of both glucose and insulin increased while no effect was found when mixing glycerol with the feed. Therefore, it can be assumed that while drenched glycerol is readily absorbed across the rumen epithelium, glycerol mixed with the feed is, to a lesser extent, available for absorption. The results indicate that when feeding glycerol in a TMR, the proportion of glycerol is fermented in the rumen to a greater extent than suggested by Werner Omazic *et al.* (2015).

Wheat starch is rapidly degradable (Reynolds, 2006) and at least 90% is fermented in the rumen to VFA which, in turn, are absorbed over the rumen epithelium (Ørskov, 1986). It is thus reasonable to assume that virtually all wheat starch consumed was fermented and absorbed from the gastrointestinal tract as VFA.

Table 3 Effects of replacing starch with glycerol in the diet on dry matter intake (DMI), milk production, feed efficiency, CH_4 and relationships between CH_4 and production parameters (STA = 20% of DM starch, GLY = 20% of DM glycerol)

	STA	GLY	SEM	P-value
Total DMI, kg/d	20.4	21.2	0.46	0.008
Milk yield, kg/d	27.0	25.7	0.96	0.033
ECM yield, kg/d	30.0	28.7	1.05	0.034
Fat, g/kg	47.8	48.1	1.01	0.781
Protein, g/kg	36.7	37.3	0.46	0.046
Lactose, g/kg	46.7	46.1	0.29	0.003
ECM/DMI	1.47	1.36	0.038	<0.001
CH_4, g/d	430	473	12.9	<0.001
CH_4/DMI (g/kg)	21.3	22.5	0.69	0.008
CH_4/ECM (g/kg)	14.8	16.9	0.73	<0.001

Conclusions

Dairy cows, which were in mid lactation and fed a grass silage based TMR containing glycerol at levels of 20% of DM, emitted more CH_4, had higher DMI but produced less milk compared with cows fed a grass silage based TMR containing wheat starch at 20% of DM.

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Effect of a grain or by-product based concentrate with early or late harvested first cut grass-clover silage on dairy cow performance

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Introduction

In traditional Swedish farming systems, a large proportion of feed resources are fed to dairy cows that could rather be used directly as human foods or be utilized with a higher efficiency in poultry and pig production. Restrictions in use of animal sources of protein and genetically modified crops in EU has renewed the use of agro-industrial by-products as alternative dietary ingredients for dairy cows. Feeding of such products is a viable option to improve sustainability of dairy production systems by decreasing the dietary proportion of human edible products (Bocquier and González-García, 2010). Inclusion of by-products in dairy cow diets have given variable results in the literature and likely reflect that different ingredients have been replaced (e.g. grains or pulses or both) and that the basal diet has varied. Recently, Ertl *et al*. (2015b) showed that a concentrate mixture completely composed of by-products combined with grass and alfalfa forage increased the human-edible feed conversion ratio (eFCR) without impairing milk production performance. In this study, a by-product concentrate was used as a complete supplement with grass-clover silages of different quality to evaluate milk production performance, feed conversion efficiency and eFCR of lactating cows representing typical Northern European dairy production feeding.

Materials and Methods

Twenty lactating Swedish Red cows were used in a replicated 4 × 4 Latin square design in 5 blocks during four 21-d periods with the last 7 days for registrations and sampling. The cows were at a mean of 81±29.9 days in milk, an average body weight of 595±77.6 kg and yielding 31.9±4.50 kg milk per day at the beginning of the experiment, and were divided in blocks according to milk yield and parity. The cows were randomly assigned to a grain based or by-product based concentrate fed with either early or late harvested first cut grass-clover silage within the blocks. An early first cut silage was prepared on June 17 and a late cut on July 1, 2015. An acid-based additive (Promyr™ XR 630, Perstorp, Sweden) was used to preserve the silages, which were ensiled and stored in bunker silos. The grain-based concentrate was made of oats, barley, wheat and soybean meal and the by-product based concentrate was a mixture of sugar beet pulp, distillers grains, heat-treated rapeseed meal, wheat bran and palm kernel cake. Grain based and by-product based mixtures were composed to be isonitrogenous. Experimental diet composition was formulated to contain 670 g/kg of first cut silage and 330 g/kg of concentrates on dry matter (DM) basis. All diets were formulated to support a milk production of 35 kg energy corrected milk (ECM) yield (Luke, 2015). The diets were fed ad libitum as a total mixed ration. Daily feed intake was recorded by a roughage intake control system (Insentec B. V., Marknesse, The Netherlands). The cows were milked at 06:00 and 15:00 h and individual milk yields were recorded daily using gravimetric milk recorders. Milk samples were collected at four subsequent milking time points from the afternoon of day 19 until the morning of day 21 in each period. All samples were analysed for fat, protein, lactose and urea. Faeces were collected from 12 cows in connection to morning and afternoon

milking on day 15, 16 and 17, and pooled within cow and period. Dry matter and ash concentrations of feeds and faeces were determined by drying at 105℃ for 16 h and incinerating at 500℃ for 4 h. Concentrations of crude protein (CP) in feed and fecal samples were determined from Kjeldahl digestion in a Block Digestion 28 system (SEAL Analytical Ltd., Mequon, WI, USA) with determination of total N by continuous flow analysis using an Auto Analyzer 3 (SEAL Analytical Ltd., Mequon, WI, USA). Neutral detergent fibre (NDF) analysis was conducted using an ANKOM^{200} Fibre analyser with heat stable α-amylase and sodium sulphite. The indigestible NDF (iNDF) concentration was determined by a 12-d *in situ* ruminal incubation according to the procedure of Krizsan *et al*. (2015). Values of NDF and iNDF were expressed on ash-free basis. Total tract digestibility of the diets was determined using iNDF as an internal marker (Huhtanen *et al*., 1994). Mass flux of CH_4 was measured by a portable open-circuit head chamber system (GreenFeed; C-Lock Inc., Rapid City, SD, USA), as described by Huhtanen *et al*. (2015). Blood samples from the coccygeal vein were collected on the last day of each period from the same cows that were sampled for faeces. The blood samples were collected, prepared and analysed for parameters of energy metabolism and inflammation as described by Bionaz *et al*. (2007). Gross energy analysis of feed ingredients was conducted according to Gordon *et al*. (1995) by using 2 Parr 6400 Oxygen Bomb Calorimeters (Parr Instrucment Co. Moline, IL 61265, USA) with benzoic acid (CAS 65-85-0, Cat No 3415, Parr Instrument Company) as a standard. Energy concentration of the milk was calculated according to the equation given by Tyrrell & Reid (1964). The eFCRs for protein and energy were calculated from proportions of human-edible output of animal product per human-edible feed input in the diets according to Wilkinson (2011) and Ertl *et al*. (2015b). Experimental data was analysed by applying a model correcting for effect of block, period, cow within block and diet using the General Linear Model of SAS (Release 9.3; SAS Inst., Inc., Cary, NC). Least square means were reported and mean separation was done by least significant difference to test differences among treatments.

Results and Discussion

Chemical composition of the experimental diets are in Table 1. Differences in chemical composition between diets arose from quality differences of the concentrate ingredients as well as from effects of developmental stage of the silages at harvest. Generally, by-products are lower in starch and CP, but richer in NDF and fat compared to traditional grains and soybean meal. Differences in the present diet chemical composition reflected plant maturity at harvest with the later harvest having higher NDF and iNDF values and lower CP concentration in the DM.

Table 1 Chemical compositions of diets (g/kg DM unless otherwise stated)

Diet[a]	EG	EB	LG	LB
DM, g/kg	390	389	457	457
OM	940	932	942	935
CP	174	173	135	134
NDF	339	397	438	496
Indigestible NDF	38	42	75	79
pdNDF	301	355	363	417

[a] EG: early cut silage-grain concentrate diet; EB: early cut silage-byproduct concentrate diet; LG: late cut silage-grain concentrate diet; LB: late cut silage-byproduct concentrate diet.

Intake, production and methane emission data for cows fed the experimental diets are in Table 2. Feeding the by-product concentrate increased NDF intake (P<0.01), but had no effect on DM intake (DMI; P=0.06), which is in line with Ertl *et al.* (2015b). Crude protein intake (P=0.01) decreased for cows fed by-product vs. grain based concentrate. Postponing the first cut harvest decreased total DMI, silage DMI and CP intake (P<0.01) of the diets, but increased NDF intake (P<0.01). Similar observations of silage maturity effects on intake were also reported by Kuoppala *et al*. (2008) and Randby *et al*. (2012).

Table 2 Intake, production and methane emission data for cows fed the experimental diets

	Diet[a]					*P*-value		
Items	EG	EB	LG	LB	SEM	Concentrate	Silage	Interaction
Intake, kg/d								
DM	23.7	22.6	21.3	20.9	0.38	0.06	<0.01	0.34
Silage DM	14.8	14.1	13.1	12.9	0.25	0.07	<0.01	0.33
CP	4.1	3.9	2.9	2.8	0.06	0.01	<0.01	0.20
NDF	8.0	9.0	9.3	10.4	0.17	<0.01	<0.01	0.72
Digestibility, g/kg								
OM	808	793	722	718	6.1	0.11	<0.01	0.37
CP	775	729	672	631	10.4	<0.01	<0.01	0.77
NDF	689	740	623	680	11.8	<0.01	<0.01	0.79
Yield, kg/d								
Milk	28.9	28.5	25.5	24.9	0.40	0.34	<0.01	0.99
ECM	32.4	32.0	29.3	27.5	0.53	0.08	<0.01	0.33
Fat, g/d	1357	1354	1262	1167	38.2	0.28	<0.01	0.31
Protein, g/d	1042	1014	891	867	15.6	0.11	<0.01	0.84
Composition, g/kg								
Fat	48.2	48.6	50.2	48.0	0.92	0.40	0.46	0.19
Protein	36.9	36.1	36.0	35.4	0.29	<0.01	<0.01	0.91
MUN[b], mg/dL	12.0	10.8	11.5	10.7	0.18	<0.01	0.13	0.54
Production efficiency								
N[c], g/kg	249	255	308	306	7.4	0.09	<0.01	0.32
ECM/DMI	1.37	1.42	1.37	1.34	0.033	0.83	<0.01	0.17
CH_4, g/kg ECM	13.1	13.0	14.5	14.0	0.28	0.14	<0.01	0.29
CH_4, g/kg DMI	17.8	18.5	19.6	18.3	0.41	0.48	0.60	0.02
eFCR for protein[d]	0.94	4.15	1.00	3.61	0.161	<0.01	0.14	0.05
eFCR for energy[d]	0.92	4.56	0.92	3.69	0.099	<0.01	<0.01	<0.01

ECM, energy corrected milk; [a]EG: early cut silage-grain concentrate diet; EB: early cut silage-byproduct concentrate diet; LG: late cut silage-grain concentrate diet; LB: late cut silage-byproduct concentrate diet; [b]Milk urea N; [c]Calculated as N in milk/N intake; [d]eFCR, edible feed conversion ratio, calculated as human-edible output in animal product/potentially human-edible feed input.

The by-products concentrate diets also gave a lower CP digestibility (P<0.01) and higher NDF digestibility (P<0.01) than the grain based concentrate diets, which was supported by results of the *in vitro* study by Ertl *et al*. (2015a). The higher NDF digestibility was most likely the result of a higher proportion of more degradable fibre (e.g., hemicellulose) in the by-products concentrate with sugar beet pulp as a major source of NDF known to contain

highly digestible fibre (Getachew *et al*., 2004). Milk protein ($P<0.01$) and urea ($P<0.01$) concentrations were lower when cows were consuming concentrate composed of by-products compared to conventional ingredients, which can be explained by the effects on digestibility of the corresponding diets. Feeding more digestible early harvested silage increased yields of milk and milk components, and milk protein content ($P<0.01$), which is in accordance with results of Kuoppala *et al*. (2008) and Randby *et al*. (2012).

Type of concentrate had no effect on N efficiency in accordance with Ertl *et al*. (2015b). The lower CP concentration in late cut silage diets promoted a higher N efficiency ($P<0.01$), which is in accordance with Kuoppala *et al*. (2008) and Randby *et al*. (2012). However, the lower feed conversion rate (ECM/DMI) when feeding late cut silage diets ($P<0.01$) was not observed by Kuoppala *et al*. (2008) or Randby et al. (2012). . Methane production (CH_4, g/kg ECM) was lower with early cut silage diets ($P<0.01$), consistent with result of Bannink *et al*. (2010). There was also an interaction between concentrate mixture and silage quality on CH_4 yield (CH_4, g/kg DMI, $P=0.02$), which suggested that the effect of concentrate source on CH_4 yield was greater with late-cut silage than early cut silage. There were also interactions on eFCRs for protein ($P=0.05$) and energy ($P<0.01$), which indicated that replacing the grain based concentrate with by-products improved eFCRs and that improvements were greater with early f cut than late first cut silage.

Table 3 Plasma energy metabolites and inflammation parameters for cows fed different experimental diets,

	Diet[a]					*P*-value	
Items	EG	EB	LG	LB	SEM	Concentrate	Silage
Cholesterol, mmol/L	7.15	7.95	7.68	8.45	0.211	<0.01	0.02
Glucose, mmol/L	3.95	4.07	3.86	3.98	0.083	0.05	0.16
NEFA, mmol/L	0.140	0.119	0.213	0.152	0.0254	0.12	0.05
BOHB, mmol/L	1.009	0.983	0.966	0.859	0.0846	0.40	0.37
Albumin, g/L	37.3	37.9	38.0	38.3	0.38	0.22	0.17
Globulin, g/L	45.2	43.6	42.6	43.9	0.85	0.82	0.17
Haptoglobin, g/L	0.194	0.169	0.119	0.144	0.0219	0.98	0.03
Paraoxonase, U/mL	103	111	113	119	3.6	0.06	0.02

NEFA, non-esterified fatty acids; BOHB, β-hydroxybutyrate; [a]EG: early cut silage-grain concentrate diet; EB: early cut silage-byproduct concentrate diet; LG: late cut silage-grain concentrate diet; LB: late cut silage-byproduct concentrate diet.

Energy metabolites and inflammation parameters in plasma are in Table 3. There were no interactions between the effects of concentrate and silage source on any trait ($P\geq0.11$). Results were all within the normal ranges which indicated that the cows were in good health during the whole experiment. Feeding by-products increased concentrations of cholesterol ($P<0.01$) and glucose ($P<0.05$). A higher cholesterol concentration was also reported by Ertl *et al*. (2015b), which reflect a greater fat mobilisation in cows and subsequently a higher potential risk of liver metabolic disorder. Feeding early first cut silage diets decreased concentrations of cholesterol ($P=0.02$), NEFA ($P<0.05$) and paraoxonase ($P=0.02$), but increased concentration of haptoglobin ($P=0.03$). This suggested that feeding early first cut silage could decrease fat mobilisation and subsequently reduce the risk of cows suffering fatty liver disease and ketosis (Whitaker, 2004). The higher haptoglobin concentration suggested an increased acute phase response in the liver of cows fed early cut silage, which indicated a higher risk of cows suffering from inflammatory event (Humblet *et al*., 2006).

Conclusions

A complete replacement of conventional concentrate ingredients with agro-industrial by-products as a supplement of a grass-clover silage did not affect milk yield, but slightly decreased milk protein concentration. Replacing cereals and soybean meal reduced human-edible inputs and increased eFCR for both protein and energy without affecting feed efficiency or health status. There were few benefits of improved silage quality in relation to concentrate source on milk production performance. However, silage quality had a stronger effect on production than type of concentrate supplement to lactating dairy cows in this study.

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In vitro evaluation of agro-industrial by-products replacing soybean meal in two different basal diets for ruminants

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Introduction

Large number of by-products from the agricultural industry can potentially be used as protein sources in diets to dairy cows. The increasing demand for alternative dietary protein supplements in ruminant production systems is due to a growing requirement for a more sustainable food production from the livestock industry. However, use of agro-industrial by-products in diets to dairy cows and beef cattle have to be efficient in terms of nutrient utilization, be complementary to basal feed ingredients and not impair production. Since *in vivo* studies are very expensive and laborious, using an *in vitro* gas production technique enables identification of by-products which can efficiently replace conventional ingredients. Recently, there has been great progress in the development of the automated gas *in vitro* technique, which enables treatment evaluation of ruminal fermentation profiles, diet digestion rates (Huhtanen *et al*., 2008), methane (CH_4) production (Ramin and Huhtanen, 2012) and estimation of utilizable crude protein (uCP; Edmunds *et al*., 2012). The aim of this study was to evaluate effects of levels of agro-industrial by-products replacing soybean meal in diets based on silage and barley or beet fibre on neutral detergent fibre (NDF) digestibility, true organic matter (OM) digestibility, uCP, fermentation parameters and CH_4 production *in vitro*.

Materials and Methods

The two basal diets used as controls for the *in vitro* incubations were grass silage:barley and grass silage:beet fibre in a ratio 600:400 g/kg of dietary dry matter (DM). Soybean meal (SBM) was used as a conventional crude protein source and was replaced by heat treated rapeseed meal (Expro®), dried distillers grain with solubles (Agrodrank™90) (DG), rapeseed cake (RSC) and rapeseed meal (RSM). Inclusions were made at two levels of crude protein (CP) concentration in the diets, differing by 2%-units. Basal, first and second levels of by-product inclusion resulted in 14.6, 16.6, and 18.6% dietary CP, respectively, for diets based on silage and barley. In diets based on silage and beet fibre, dietary CP was 12.6, 14.6 and 16.6%, respectively. All treatments had the same silage:barley or silage:beet fibre ratio across experimental diets.

Two lactating Swedish Red cows fed a diet of 600 g/kg grass silage and 400 g/kg concentrate on DM basis *ad libitum* were used for *in situ* incubation for iNDF analysis, and for collection of rumen fluid for the *in vitro* incubations (Cone *et al*., 1996). Rumen fluid was collected from the same cows for all three *in vitro* incubations. The collected rumen fluid from each cow was strained separately through a double layer of cheesecloth into pre-heated (39°C) steel thermoses that had previously been flushed with carbon dioxide and immediately taken to the laboratory. In the laboratory, rumen fluid was homogenized and filtered through four layers of cheesecloth and kept in a water bath at 39°C under CO_2 saturation. Prior to incubation, the rumen fluid was pre-incubated during 3 h with a carbohydrate mixture. In this procedure, a mixture of maltose, starch, xylose, pectin, and $NaHCO_3$ was added to the rumen fluid, which was stirred for 10 minutes. After 30 minutes, the top layer of foam was removed

with a vacuum pump and the stirrer was turned on again. The rumen fluid was then incubated at 39ºC under a constant flush of CO_2 for an additional 2.5 h. After pre-incubation, the rumen fluid was mixed with a low-N bicarbonate buffer, micro and macro minerals and resazurin.

Diets of 500 mg were previously weighed directly in 250-mL serum bottles (Schott, Mainz, Germany), which were flushed with CO_2. Diets were then incubated in 60 mL of the buffered rumen fluid for 48 h. Incubations were conducted at 39ºC and the bottles were continually agitated. All diets were incubated in 3 consecutive runs, resulting in 4 replicates per diet, including one blank per bath. Diets were randomized within baths and among baths in subsequent runs. Gas production was automatically recorded and corrected to normal atmospheric pressure (101.3 kPa; Cone *et al*., 1996). Mean blank gas production within run was subtracted from the sample gas production.

Gas samples were drawn from each bottle by a gas tight syringe (Hamilton, Bonaduz, Switzerland) at 24 and 48 h of incubation. Methane production was calculated as described by Ramin and Huhtanen (2012). Samples of 0.6 mL were taken and preserved with 0.024 mL of 18 M H_2SO_4 at 8, 16, 24, and 30 h after incubation for ammonia nitrogen (NH_3-N) analysis and estimation of uCP at 16 h as described by Edmunds *et al*. (2012):

$$\text{uCP (g/kg)} = \frac{\text{NH3N}_{\text{blank}} + \text{N}_{\text{sample}} - \text{NH3N}_{\text{sample}}}{\text{weight (mg DM)}} \text{ x } 6.25 \text{ x } 1000$$

Another sample of 0.6 mL of rumen fluid was collected at 48 h of incubation from the bottles and immediately stored at -20°C until processed for VFA determination. Discrete and total VFA production was calculated after subtracting mean blank VFA concentration from sample concentration. After 48 hours incubation, all flasks were removed from the baths and placed on ice to stop fermentation. Residues were quantitatively transferred to 11-µm bags (Saatifil PES; Saatitech S.p.A., Veniano, Como, Italy) and analysed for NDF, according to Mertens (2002). *In vitro* true OM digestibility was also determined for the diets, considering OM of individual feeds and residue after incubation.

Residual moisture of all feed samples was determined by oven drying for 16 h at 105°C. Ash concentration was determined by ignition of the dried sample at 500°C for 4 h. The indigestible NDF (iNDF) concentration was determined by a 12-d *in situ* ruminal incubation according to Krizsan *et al*. (2015). The samples were analyzed for NDF using a heat stable α-amylase (Mertens, 2002) in an ANKOM200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA). Values of NDF and iNDF were expressed on an ash-free basis. Concentrations of N were determined by Kjeldahl digestion of 1.0 g sample in 12 M sulfuric acid using Foss Tecator Kjeltabs Cu (Höganäs, Sweden) in a Block Digestion 28 system (SEAL Analytical Ltd., Mequon, WI, USA) with determination of total N by continuous flow analysis using an Auto Analyzer 3 (SEAL Analytical Ltd., Mequon, WI, USA). Individual VFA concentrations in rumen fluid samples were determined using a Waters Alliance 2795 HPLC system with Waters 2414 RI detector (Waters Corporation, Milford, MA, USA) as described by Ericson and André (2010), and NH_3, according to the method provided by the SEAL Analytical (Method nr G-102-93 multitest MT7) using the AutoAnalyzer 3.

The data was analysed using the GLM procedure (SAS Inc. 2002-2003, Release 9.2; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability. The sum of squares was further partitioned into orthogonal polynomial contrasts, where SBM was contrasted against by-products, and linear and quadratic responses to level of by-products.

Results and Discussion

The chemical composition of the silage, barley, beet fibre, soybean meal, and by-products are in Table 1. Levels of CP in the by-products ranged between 315 and 392 g/kg DM, while in the soybean meal, it was 496 g/kg DM.

Table 1 Chemical composition of silage, barley, beet fibre, soybean meal and by-products (g/kg DM unless otherwise stated)

					By-products			
Item	Silage	Barley	Beet fibre	SBM	Expro	DG	RSC	RSM
DM, g/kg	255	953	917	854	906	877	921	911
OM	842	926	848	925	837	827	859	840
CP	157	129	78	496	387	315	378	392
NDF	611	239	339	237	322	288	251	270
NSC	286	714	578	688	584	589	670	641
iNDF	102	42	30	6	129	63	109	118

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; NSC: non-structural carbohydrate; iNDF: indigestible neutral detergent fibre; SBM: soybean meal; Expro: heat treated RSM, DG: distillers grain; RSC: rapeseed cake; RSM: rapeseed meal.

In Table 2, results indicate that Expro, RSC and RSM decreased ($P<0.05$) NDF and true OM digestibility when replacing soybean meal in diets based on silage and barley. However digestibilities were not affected ($P>0.05$) by DG inclusion, which may be explained by its low proportion of iNDF, providing more digestible matter compared to the other by-products. Utilizable CP increased ($P<0.05$) for all by-products replacing soybean meal. A high uCP level, defined as the sum of microbial crude protein (MCP) and rumen undegraded protein (RUP) (Edmunds *et al*., 2012), indicates a higher proportion of utilisable protein substrate available in the duodenum. In the *in vitro* uCP estimation, RUP and MCP are simultaneously estimated and cannot be differentiated. According to Edmunds *et al*. (2012), validation using *in vivo* data is recommended. Even though it was not possible to differentiate the two sources, it is likely that Expro, RSC and RSM have a relatively high proportion of RUP as NDF and true OM digestibilities decreased ($P<0.05$) when those by-products replaced soybean meal.

Distillers grain replacing soybean meal in diets based on silage and barley decreased ($P<0.05$) acetate and increased ($P<0.05$) propionate proportions (Table 2), while they were not affected ($P>0.05$) by the other by-products. Furthermore, when soybean meal was replaced by DG or Expro, CH_4 production decreased ($P<0.05$). None of the by-products affected ($P>0.05$) total VFA and digestion rate in these diets.

Incremental levels of by-products in the diets based on silage and barley linearly increased ($P>0.05$) (Table 2) true OM digestibility, uCP, isobutyrate and valerate, and linearly decreased ($P<0.05$) acetate and butyrate proportions. The increase in dietary crude protein concentration increased ($P<0.05$) uCP, which suggests that all by-products tested are potential protein feed sources without detrimental effect on uCP. Total VFA, NDF digestibility, digestion rate and CH_4 production were not affected ($P>0.05$) by the inclusion level of by-product.

Table 2 Effect of increasing level of agro-industrial by-products replacing soybean meal on digestibility, estimated utilizable crude protein, fermentation parameters and methane production in diets based on silage and barley

	Basal 14.6%	Diets 16.6% CP					Diets 18.6% CP						P-value[a]				
Item	CP	SBM	Expro	DG	RSC	RSM	SBM	Expro	DG	RSC	RSM	SEM	C1	C2	C3	C4	Lin
NDFD, g/kg	781	790	776	804	776	789	811	769	784	769	769	7.2	<0.01	0.37	<0.01	<0.01	0.68
TOMD, g/kg	849	856	849	864	852	857	869	849	859	855	853	3.3	<0.01	0.60	0.01	0.02	0.03
uCP, g/kg DM	150	158	164	165	161	163	167	179	182	172	171	0.8	<0.01	<0.01	<0.01	<0.01	<0.01
Total VFA, mmol/l	91.9	91.9	87.6	90.8	92.6	93.2	92.5	92.5	90.0	87.4	88.6	1.96	0.27	0.36	0.26	0.51	0.37
Molar proportions, mmol/mol																	
Acetate	591	590	586	585	594	591	589	587	579	584	590	1.9	0.15	<0.01	0.89	0.66	0.01
Propionate	228	226	229	233	221	228	227	227	241	227	224	1.9	0.47	<0.01	0.18	0.71	0.41
Butyrate	111	111	107	105	110	109	108	109	102	107	108	1.1	0.20	<0.01	0.42	0.30	<0.01
Isobutyrate	19	20	20	19	20	19	20	20	20	21	20	0.4	0.94	0.12	0.33	0.91	<0.01
Valerate	27	27	29	31	29	28	28	31	31	32	30	0.5	<0.01	<0.01	<0.01	0.03	<0.01
Isovalerate	25	26	28	27	26	25	27	26	27	29	28	1.1	0.60	0.90	0.50	0.91	0.10
k_d, 1/h	0.073	0.073	0.072	0.074	0.077	0.077	0.077	0.068	0.077	0.077	0.082	0.0028	0.09	0.80	0.42	0.10	0.26
CH_4, ml/g DM	53.5	53.3	52.1	50.9	49.6	51.8	53.9	51.4	49.8	51.9	52.9	1.06	0.09	<0.01	0.01	0.24	0.33

CP = crude protein; SBM = soybean meal; DG = distillers grain; RSC = rapeseed cake; RSM = rapeseed meal; SEM = standard error of mean; NDFD = neutral detergent fibre digestibility; TOMD = true organic matter digestibility; uCP = utilizable crude protein; Total VFA = volatile fatty acids (sum of all individual acids); kd = diet digestion rate. [a]C1 = SBM vs. Expro; C2 = SBM vs. DG; C3 = SBM vs. RSC; C4 = SBM vs. RSM; Lin = linear effect of supplementary inclusion level; Quad = quadratic effect of supplementary inclusion level.

Table 3 Effect of increasing level of agro-industrial by-products replacing soybean meal on digestibility, estimated utilizable crude protein, fermentation parameters and methane production in diets based on silage and beet fibre

	Basal 12.6%	Diets 14.6% CP					Diets 16.6% CP						P-value[a]				
Item	CP	SBM	Expro	DG	RSC	RSM	SBM	Expro	DG	RSC	RSM	SEM	C1	C2	C3	C4	Lin
NDFD, g/kg	799	819	794	830	791	795	822	801	798	793	790	11.7	0.05	0.56	0.01	0.02	0.99
TOMD, g/kg	841	850	837	856	838	839	855	845	846	846	842	6.0	0.05	0.85	0.09	0.05	0.33
uCP, g/kg DM	140	151	152	155	152	151	158	167	171	162	162	1.0	<0.01	<0.01	<0.01	0.08	<0.01
Total VFA, mmol/l	90.1	94.9	87.8	91.1	95.4	90.3	97.7	94.4	92.4	92.8	95.4	2.66	0.06	0.09	0.41	0.20	0.08
Molar proportions, mmol/mol																	
Acetate	633	628	622	623	627	623	623	619	613	620	622	2.6	0.07	<0.01	0.45	0.28	<0.01
Propionate	230	222	228	231	222	228	224	225	238	228	226	1.6	0.03	<0.01	0.17	0.02	0.70
Butyrate	81	85	84	80	85	83	86	86	81	83	86	1.3	0.50	<0.01	0.20	0.34	0.05
Isobutyrate	17	18	19	18	18	18	18	19	17	18	18	0.7	0.24	0.28	0.86	0.70	0.32
Valerate	22	24	26	26	25	24	25	28	27	26	26	0.6	<0.01	0.01	0.30	0.54	<0.01
Isovalerate	24	23	26	23	23	25	24	23	24	25	22	1.1	0.30	0.94	0.58	0.95	0.73
k_d, 1/h	0.076	0.077	0.074	0.081	0.079	0.077	0.078	0.077	0.082	0.088	0.083	0.0030	0.54	0.24	0.06	0.43	0.05
CH_4, ml/g DM	52.8	52.1	52.0	50.9	49.8	51.9	55.6	51.6	51.8	54.9	51.1	1.10	0.04	0.01	0.14	0.02	0.36

CP = crude protein; SBM = soybean meal; DG = distillers grain; RSC = rapeseed cake; RSM = rapeseed meal; SEM = standard error of mean; NDFD = neutral detergent fibre digestibility; TOMD = true organic matter digestibility; uCP = utilizable crude protein; Total VFA = volatile fatty acids (sum of all individual acids); kd = diet digestion rate. [a]C1 = SBM vs. Expro; C2 = SBM vs. DG; C3 = SBM vs. RSC; C4 = SBM vs. RSM; Lin = linear effect of supplementary inclusion level; Quad = quadratic effect of supplementary inclusion level.

For diets based on silage and beet fibre (Table 3), replacement of soybean meal by RSC or RSM decreased (P<0.05) NDF digestibility, while true OM digestibility was not affected (P>0.05). None of the digestibilities were affected (P>0.05) by Expro or DG replacing soybean meal. Similar to the diets based on silage and barley, diets based on silage and beet fibre, uCP increased (P<0.05) for all by-products replacing soybean meal, except for RSM, where there was only a tendency (P<0.09). This indicates that the by-products used in this *in vitro* experiment are good feed protein sources. However, intestinal digestibility of uCP of the different diets can vary and data on that is needed in order to fully evaluate diet protein values.

Distillers grain decreased (P<0.05) (Table 3) acetate and butyrate, and increased (P<0.05) propionate when replacing soybean meal in diets based on silage and beet fibre. Moreover, propionate also increased (P<0.05) when Expro or RSM replaced soybean meal. Except for RSC, increasing level of by-product decreased (P<0.05) CH_4 production. A reason for this could be that propionate and CH_4 production requires H_2, and since propionate production increased, less hydrogen was available for CH_4 production. The high protein concentration of the by-products could also act in formation of bicarbonate from CO_2 and, thereby, reducing CO_2 production (Cieslak *et al*., 2013). According to Menke *et al*. (1979) and Ramin and Huhtanen (2013) there is a high correlation between CH_4 production and digestibility. This was obvious in the RSM diet, where NDF digestibility decreased (P<0.05), true OM digestibility tended to decrease (P<0.06) and also CH_4 production decreased (P<0.05). The same pattern was also observed when soybean meal was replaced by RSC in diets based on silage and barley, in accordance with Jentsch *et al*. (2007). There is a negative correlation between uCP and gas production (Vaga *et al*., 2016), which was also seen in this study when Expro, DG and RSM replaced soybean meal in diets based on silage and beet fibre. Moreover, the same pattern was evident for diets based on silage and barley, with DG and RSC replacing soybean meal. None of the by-products replacing soybean meal affected (P>0.05) total VFA and digestion rate in diets based on silage and beet fibre.

There was a positive linear effect (P<0.05) (Table 3) of by-product level on uCP and valerate, and a negative linear effect (P<0.05) for acetate in diets based on silage and beet fibre. The increase in uCP concentrations was expected due to its correlation with dietary CP. There was a quadratic effect (P<0.05) of supplementary inclusion level of by-product on propionate and CH_4 production, where the first inclusion level decreased, followed by an increase for the highest level for both parameters.

In general, detrimental effects (e.g., digestibility) were more prominent in the diets based on silage and barley than in the diets based on silage and beet fibre. It may have been due to its lower CP and higher NDF concentration (Table 1) in comparison with barley.

Conclusions

Caution should be taken when by-products replace soybean meal in diets based on silage and barley with respect to detrimental effects on digestibility in spite of an increase in uCP. However, this does not seem to be a problem in a diet based on silage and beet fibre, with a potential benefit in the form of reduced methane production increased uCP production. It should be noted that intestinal digestibility of uCP is not known and requires evaluation in order to assess the true protein value of the diet.

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The effect of nitrate content in forage on quality of silage fermentation

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Introduction

Nitrate content in fresh herbage is one of the factors affecting fermentation in silage. Hein (1970) observed that ensiling of forages with low nitrate content often results in silages with high butyric acid contents. Butyric acid is an undesirable product of clostridia in silages indicating low silage nutritional quality (Pahlow et al., 2003). The effect of nitrate on butyric acid formation is derived from its degradation products. Nitrate undergoes reduction to nitrite which can be further converted to nitric oxide which is considered to be toxic for clostridia (Spoelstra, 1983). Therefore, crops high in nitrate decreases clostridial activity and, hence, butyric acid formation. The effect of nitrate content in fresh crops on butyric acid formation was summarized by Weissbach (1996). The summary shows high occurrence (78%) of butyric acid in silages made from crops low ($<10^5$) in epiphytic lactic acid bacteria (LAB) while containing <0.5 g NO_3 per kg dry matter (DM). In contrast, incidence of butyric acid in silages from crops with similarly low LAB count but containing >1 g NO_3 per kg DM was only 26%. Since it is common to use silage additives to improve or secure a proper ensiling process, it is interesting to study how different nitrate contents in fresh crops influence efficiency of silage additives. The objective of the study was, therefore, to study the effect of nitrite containing silage additives on silage quality with crops differing in nitrate content.

Material and Methods

Two types of crops were used representing high (Crop 1) and low (Crop 2) nitrate levels. Crop 1 which represented a mixture of perennial ryegrass (50%, vegetative stage), and red clover (vegetative stage, 50%) was fertilized with a manure slurry and harvested as a third cut on 16th of October. Crop 2 consisted of timothy (15%, head visible), perennial ryegrass (30%, vegetative stage), meadow fescue (16%, head visible), and red clover (vegetative stage, 39%). Crop 2 was cultivated without fertilizer and harvested as a first cut on 10th of June. Both crops were directly chopped in a stationary cutter to approx. 2 cm particle length. After chopping, both forages were mixed with a suspension of *Clostridium tyrobutyricum* spores at the rate of 10^5 per g fresh matter (FM) and partitioned into fractions. One forage fraction was left untreated and served as control and another fraction was treated with an additive mixture of 20% sodium benzoate, 10% potassium sorbate and 5% sodium nitrite at the rate of 3 L/t (fresh matter). The silage additive was applied by hand with a spray bottle on the forage which was spread out on a sheet of plastic film and mixed thoroughly. Forages from each fraction were then ensiled in lab-silos (1.7 L volume with water locks). Crops were ensiled according to the DLG design for testing efficiency of silage additives WR1 (DLG, 2009) with a compaction density of 100 kg DM per m^3. Each treatment consisted of 3 replicates. Silos were stored for 98 days in room temperature of 20˚C. Two samples of fresh crop prior to additive application were collected. Each sample was mixed and divided into 3 sub-samples; microbiological sample, chemical sample and reserve sample. Microbiological samples were analyzed for homofermentative and heterofermentative lactic acid bacteria (LAB), yeasts, moulds, enterobacteria and clostridia spores. Chemical analyses

determined DM, ash, total N, water soluble carbohydrates (WSC), metabolizable energy (ME), nitrate+nitrite, and buffering capacity. In addition, botanical composition of harvested crop and growing stage of plant were assessed.

At the end of storage, silo contents were emptied into separate plastic bags and mixed thoroughly. Extracted silage samples were analyzed for DM, volatile fatty acids, lactic acid, ethanol, pH, WSC, LAB, clostridia spores, yeasts and for aerobic stability by standard methods described by Knicky & Spörndly (2009).

Results and Discussion

Chemical and microbiological composition of the forages, prior to ensiling, are in Table 1. The application of slurry (Crop 1) resulted in high nitrate and CP contents, whereas absence of fertilization caused low nitrate and CP contents in Crop (2). The calculated fermentation coefficient (FC) of 26 indicates that the Crop (1) should be difficult to successfully ensile whereas the FC of Crop (2) of 38 characterized it as intermediate for ensiling purposes (Weissbach et al., 1974).

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

Analyses	Unit	Crop (1)	Crop (2)
DM	%	18.5	19.9
Ash	%	11.8	9.5
CP	%	24.4	11.6
WSC	%	7.3	15.7
NDF	%	41.1	44.8
Nitrate-N	mg/kg DM	1467.6	2.1
Nitrite-N	mg/kg DM	1.9	2.1
ME	MJ/kg DM	10.9	11.1
Ammonia-N	% TN	-	1.2
Buffering capacity	g LA/100 g DM	7.5	7.1
LAB-homofermentative	log cfu/g FM	5.8	6.2
LAB-heterofermentative	log cfu/g FM	5.6	3.9
Clostridia spores	log cfu/g FM	3.8	3.8
pH		6.0	5.8
Fermentation coefficient		26	38

DM-dry matter; FM-fresh matter; CP-crude protein; WSC-water soluble carbohydrates; NDF-neutral detergent fiber; ME-metabolizable energy; TN-total nitrogen; LAB-lactic acid bacteria; cfu-colony-forming unit.

Table 2. Chemical composition of silages after 98 days of storage (n=3)

Treatment	DM	pH	NH_3-N*	NO_3-N	Lactic acid	Acetic acid	Butyric acid	2.3-butanediol	Ethanol	WSC
	%		% of TN	mg/kg DM	% of DM					
Crop (1)										
Control	18.1	4.2	6.3	868.4	9.8	2.6	0.1	0.05	0.7	0.10
Additive	18.6	4.1	5.4	1224.8	10.1	2.1	0.0	0.04	0.5	0.03
$LSD_{0.05}$		**0.05**	**0.91**	**170.0**	**1.54**	**0.31**	**0.09**	**0.01**	**0.03**	**0.17**
P-value		0.02	0.05	0.004	0.7	0.01	0.6	0.1	0.001	0.4
Crop (2)										
Control	18.1	4.5	10.9	1.0	9.3	2.6	1.7	2.9	2.0	0.7
Additive	19.4	4.1	4.9	18.8	11.6	1.4	0.0	0.1	0.4	6.4
$LSD_{0.05}$		**0.07**	**0.38**	**6.11**	**1.28**	**0.47**	**0.29**	**0.53**	**0.35**	**0.20**
P-value		0.001	0.001	0.001	0.01	0.002	0.001	0.001	0.001	0.001

* N.S. – Not significant. DM-dry matter; FM-fresh matter; TN-total nitrogen; WSC-water-soluble carbohydrates.

Table 3. Microbiological composition and aerobic stability of silages after 98 days of storage (n=3)

Treatment	Yeasts	Clostr. spores	LAB Homoferm.	Heteroferm.	Weight loss	Time (hours) until temp. aerated silages increased 3˚C	Max-temp	Max. temp-increase	pH after stability
	log cfu/g				% DM		(˚C)	(˚C)	
Crop (1)									
Control	-	2.4	5.1	7.9	2.8	210.5	31.6	11.0	5.0
Additive	-	2.5	5.3	7.7	2.0	262.0	21.9	0.9	4.5
$LSD_{0.05}$		**0.72**	**0.68**	**0.53**	**0.36**	**13.6**			**0.56**
P-value		0.9	0.6	0.4	0.01	0.001			0.6
Crop (2)									
Control	<1.7	4.6	<4.7	7.4	14.7	216.0	20.5	0.0	4.5
Additive	<1.7	1.7	<4.7	6.2	2.4	216.0	20.7	0.2	4.1
$LSD_{0.05}$	-	**0.19**	-	**0.45**	**0.76**	-			**0.07**
P-value	n.s.	0.001	n.s.	0.002	0.001	n.s.			0.001

* N.S. – Not significant. DM-dry matter; LAB-lactic acid bacteria.

Results from chemical and microbiological analyses of the silages are in Tables 2 and 3. As expected, low DM contents of the crops caused extensive fermentation. This was evidenced by low silage pH and high levels of fermentation products associated with a high depletion of WSC.

Additive treated silages had lower pH, lower concentration of acetic acid, ethanol and ammonia than control silages. Concentration of butyric acid was near the detection limit in all additive treated silages which confirms the efficiency of the present additive composition to eliminate clostridial activity in silage shown in previous studies (Knicky & Spörndly, 2009, 2011). Reduced formation of undesirable ensiling products such as butyric and acetic acid, ethanol and 2.3-butanediol were probably the reasons for lower silage losses in the additive treatments as compared to the control.

However, differences between additive and control treatments were not obvious in Crop (1). According to the fermentation coefficient, Crop (1) should be more difficult to ensile successfully and would, therefore, be expected, at least in the untreated control silage, to show signs of undesirable processes in comparison with Crop (2). However, results of the control silage from Crop (1) were not different for several silage parameters in comparison with the additive treated silage. This situation was likely associated with an abundance of nitrate in the fresh crop, resulting in nitric oxide production which eliminated clostridial activity and, hence, butyric acid formation (Spoelstra, 1983). In contrast, lack of nitrate in Crop (2) was reflected in a high clostridial activity in the control silage and a pronounced butyric acid and ammonia formation, and consequently high silage losses. Presence of butyric acid stabilized the control silage in Crop (2), whereas lack of butyric acid reduced aerobic stability of the control silage in Crop (1), compared to the additive treatment. A minor increase in concentration of nitrate in additive treated silages was probably the consequence of $NaNO_2$ addition, a component of the silage additive. It is assumed that the nitrate concentration increase was caused by conversion of added $NaNO_2$ to nitrate (McDonald et al., 1991).

Conclusions

Ensiling of the nitrate rich forage resulted in a good fermentation process, similar to treatment with the additive. In contrast, the quality of fermentation in the low nitrate forage was poor and lower ($P<0.001$) than the additive treated silage. Results verify earlier observations about the importance of appropriate nitrate content in forages for successful ensiling.

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Effect of extracted seaweed protein fractions on estimated utilizable crude protein, methane emission and fermentation parameters – an in vitro evaluation

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Introduction

There are many species of seaweeds but only a few of them are of interest for animal feeding (Makkar *et al*., 2016). Seaweeds have been used traditionally in livestock feeds for thousands of years with reports originating from Ancient Greece (Makkar *et al*., 2016). There are reports in the early 1900s that seaweed has been preserved as silage and used in winter time for feeding sheep (Evans and Critchley, 2014). In the early 20th century, numerous reports revealed use of seaweeds to feed livestock in France (Brittany), and Scandinavia (Gotland, Norway, Finland), mostly ruminants (Chapman and Chapman, 1980). However, the nutritional value of seaweeds as a ruminant feed varies widely, depending on components such as protein, minerals and polysaccharides (Makkar *et al*., 2016). The potential of harvesting seaweeds from the sea has once again renewed the attention of using seaweeds as animal feedstock (Tayyab *et al*., 2016). Seaweeds have a highly variable nutritional composition, with large differences in protein, lipid and fibre contents (Makkar *et al*., 2016). The increased demand for food has increased search for novel protein sources. Some seaweeds are rich in protein and could be used as alternatives to traditional protein feeds in livestock such as soybeans. The Norwegian seaweed industry has been successful in harvesting of different species of seaweeds for alginate production, and for animal food as well (Meland and Rebours, 2012). Since there is demand for seaweed in animal feeds, more studies are needed to evaluate the effect of seaweeds on nutritional and fermentation parameters. Previous experiments have focused on feeding single or a mixtures of different seaweeds, especially to small ruminants (Ventura and Castañón, 1998). To our knowledge, the use of extracted protein fractions from seaweeds as supplements in ruminant diets are scarce. Since in vivo studies are very expensive and laborious to conduct, many in vitro techniques have been developed to study ruminant nutrition, fermentation processes as well as estimating utilizable crude protein and CH_4 production (Ramin and Huhtanen, 2012; Edmunds *et al*., 2012). In vitro techniques are a useful tools for screening purposes and feed evaluation. Our in vitro study aimed to evaluate replacement levels of seaweed protein fractions in silage on utilisable crude protein (uCP), methane production, true organic matter (OM) digestibility and volatile fatty acids.

Materials and Methods

Three different seaweed species were used in the current study. Wild *Palmaria palmata* biomass was harvested in Bodø, Norway. Cultivated *Saccharina latissima* and *Alaria esculenta* biomass were harvested at the coast of Trøndelag, Norway. Biomass was cleaned of epiphytes and associated species, both flora and fauna. Thereafter, surface salt was briefly rinsed with freshwater and drained. Damp biomass was packed and frozen at -20°C until extraction of protein fractions. Protein-enriched fractions of *Saccharina latissima* (fraction

S2), *Alaria esculenta* (fraction A2), and *Palmaria palmata* (fractions P2 and P5) were produced by treating the seaweeds with different enzymes and removal of soluble compounds, in particular salts. Three different levels of each seaweed protein fractions i.e. 15, 30 and 45 % were incorporated into high quality grass silage. Since the nitrogen concentration in fraction P2 was high, the levels incorporated were 7.5, 15 and 22.5%. The in vitro study was performed at the Swedish University of Agricultural Sciences in Umeå, Sweden. Three dairy cows of the Swedish Red breed, fed a total mixed ration (grass silage/concentrate ratio 600/400 g/kg on DM basis) were used as donor animals of rumen inoculum. All handling of animals was approved by the Umeå Ethical Committee for Animal Research, Sweden. Rumen fluid was collected 2 h after the morning feeding. Rumen fluid from each cow was strained separately through a double layer of cheesecloth into pre-warmed thermos flasks that had previously been flushed with carbon dioxide. Prior to incubation of diets, the rumen fluid was pre-incubated during 3 h with a carbohydrate mixture. In this procedure, a mixture of maltose, starch, xylose, pectin, and $NaHCO_3$ was added to the rumen fluid, which was stirred for 10 minutes. After 30 minutes, the top layer of foam was removed with a vacuum pump and the stirrer was turned on again. The rumen fluid was then incubated at 39 ºC with constant CO_2 steam for another 2.5 h. After the pre-incubation, rumen fluid was mixed with a buffered mineral solution supplemented with peptone (pancreatic digested casein) at 39°C under constant stirring and continuous flushing with CO_2. The buffer used had a low nitrogen concentration. Prior to the in vitro incubation, 500 mg of substrate (organic matter incubated) was weighed into serum bottles. All bottles were filled with 60 mL of buffered rumen fluid and placed in a water bath at 39°C for 48 h. The bottles were continuously agitated. Incubations were performed in three consecutive runs. Methane (CH_4) production was measured as described by Ramin & Huhtanen (2012). Liquid samples were taken during the incubation at 8, 16, 24 and 30 h in order to measure ammonia and later to estimate uCP at 16 h. Utilizable crude protein was estimated at 16 h as described by Edmunds *et al*. (2012):

$$\text{uCP (g/kg DM)} = \text{NH}_3\text{N blank} + \text{N sample} - \text{NH}_3\text{N sample} / \text{weight (mg DM)} \times 6.25 \times 1000$$

At the end of incubation (48 h), liquid samples were taken for volatile fatty acids (VFA) analysis. Residues were used to measure digestibility. Data for in vitro measurements (uCP, CH_4 production, VFA production and digestibility parameters) were analysed statistically using the GLM procedure of SAS. The sum of squares of each increased level fraction was further partitioned into control vs. other treatments, linear and quadratic effects of the substrate level using orthogonal polynomial contrasts.

Results and Discussion

Composition of the grass silage and protein fractions extracted from each seaweed is given in Table 1. The P2 fraction had a greater protein content and lower ash content compared to other fractions.

Table 1 Composition of the grass silage and protein fractions extracted from each seaweed

	Dry matter, g/kg	Crude protein, g/kg DM	Organic matter, g/kg DM
Grass silage	897	157	841
Saccharina latissima S2	973	287	714
Alaria esculenta A2	937	194	789
Palmaria palmata P2	956	481	911
Palmaria palmata P5	963	256	829

The results indicate a linear increase of estimated uCP for all extracted protein fractions of seaweeds when grass silage was replaced by each seaweed protein fraction (Table 2, 3, 4, 5). For all fractions, except for P2, the increase in uCP was stronger than the increase in dietary crude protein concentration. This indicates that the tested fractions may be good feed protein sources. However, we have no information about the intestinal digestibility of uCP, which is needed in order to fully evaluate the protein value. The increase in uCP with increasing dietary crude protein level was relatively stronger with A2 than with the other seaweed fractions. The uCP is an estimate of the sum of microbial crude protein (MCP) and rumen undegraded feed protein (RUP) entering the duodenum. We cannot differentiate the two sources but in A2, a relatively high proportion of RUP is likely as the digestibility of the OM decreased with increasing levels of A2 in the diet. The fractions of the brown alga *Saccharina latissima* (S2) and the red alga *Palmaria palmata* (P5) increased OM digestibility with increasing levels. This may have contributed to increasing uCP by providing fermentable OM for MCP synthesis. Increased levels of the *Alaria*-fraction decreased CH_4 production linearly.

The resurgence in the use of raw seaweeds in animal diet has been studied in vitro and in situ (Tayyab et al., 2016; Molina-Alcaide *et al*., 2017). Intact seaweeds differ in protein degradability, in which some species have relatively high proportion of rumen un-degradable protein and high digestibility.

Table 2 Effect of level of *Saccharina latissima* protein fraction (S2) on estimated utilizable crude protein, methane production and fermentation parameters

Item	Level					Contrast		
	Control	15	30	45	SEM	Control vs. Other	Linear	Quadratic
uCP, g/kg DM	159	164	204	243	12.0	<0.01	<0.01	0.13
OMD, %	78.6	78.1	80.0	81.9	0.97	0.096	<0.01	0.21
CH_4, ml/g OM	44.8	43.1	43.4	42.1	3.06	0.44	0.50	0.94
Total VFA, mmol/l	69.5	69.6	68.7	65.2	0.96	0.23	0.042	0.25
Acet, mmol/mol	684	689	700	703	2.2	<0.01	<0.01	0.73
Prop, mmol/mol	211	208	199	195	0.67	<0.01	<0.01	0.61
But, mmol/mol	105	103	101	102	2.45	0.48	0.48	0.86
pH	6.56	6.57	6.59	6.63	0.029	0.36	0.24	0.76

uCP: utilizable crude protein; DM: dry matter; OMD: organic matter digestibility; CH_4: methane; VFA: volatile fatty acids.

Table 3 Effect of level of *Alaria esculenta* protein fraction (A2) on estimated utilizable crude protein, methane production and fermentation parameters

Item	Level					Contrast		
	Control	15	30	45	SEM	Control vs. Other	Linear	Quadratic
uCP, g/kg DM	159	149	180	212	6.86	0.049	<0.01	0.07
OMD, %	78.5	76.0	74.6	72.6	0.62	<0.01	<0.01	0.74
CH_4, ml/g OM	44.8	43.5	40.5	36.0	1.46	0.036	<0.01	0.5
Total VFA, mmol/l	69.5	67.8	65.4	61.7	1.10	0.011	<0.01	0.58
Acet, mmol/mol	684	689	693	697	2.09	<0.01	<0.01	0.87
Prop, mmol/mol	211	207	201	195	0.94	<0.01	<0.01	0.77
But, mmol/mol	105	104	106	108	2.52	0.72	0.53	0.81
pH	6.56	6.59	6.58	6.64	0.031	0.32	0.25	0.80

uCP: utilizable crude protein; DM: dry matter; OMD: organic matter digestibility;CH_4: methane; VFA: volatile fatty acids.

Table 4 Effect of level of *Palmaria palmata* protein fraction (P2) on estimated utilizable crude protein, methane production and fermentation parameters

Item	Level					Contrast		
	Control	7.5	15	22.5	SEM	Control vs. Other	Linear	Quadratic
uCP, g/kg DM	159	160	202	245	7.0	<0.01	<0.01	0.08
OMD, %	78.5	78.3	77.6	77.9	0.46	0.39	0.4	0.74
CH_4, ml/g OM	44.8	46.5	46.4	46.8	1.58	0.43	0.57	0.79
Total VFA, mmol/l	69.5	69.9	67.6	66.7	1.31	0.43	0.22	0.77
Acet, mmol/mol	684	687	688	690	1.85	0.09	0.09	0.88
Prop, mmol/mol	211	208	205	202	1.01	<0.01	<0.01	0.76
But, mmol/mol	105	105	107	107	2.41	0.61	0.55	0.98
pH	6.56	6.57	6.58	6.63	0.021	0.37	0.25	0.72

uCP: utilizable crude protein; DM: dry matter; OMD: organic matter digestibility; CH_4: methane; VFA: volatile fatty acids.

Protein digestibility of raw seaweeds measured *in situ* in the rumen of dairy cows showed that *Acrosiphonia* sp., *Alaria esculenta*, *Laminaria digitata*, *Mastocarpus stellatus* and *Palmaria palmata* can supply the rumen with high amounts of rumen degradable protein, while *Porphyra* spp. and *Ulva* spp. can be used as a source of digestible RUP. Conversely, *Pelvetia canaliculata* had a very low degradability and should not be used to feed dairy cows (Tayyab *et al*., 2016).

Table 5 Effect of level of *Palmaria palmata* protein fraction (P5) on estimated utilizable crude protein, methane production and fermentation parameters

Item	Level					Contrast		
	Control	15	30	45	SEM	Control vs. Other	Linear	Quadratic
uCP, g/kg DM	159	157	193	227	6.9	<0.01	<0.01	0.11
OMD, %	78.5	81.0	81.7	82.8	0.57	<0.01	<0.01	0.54
CH_4, ml/g OM	44.8	45.6	49.6	46.2	1.47	0.31	0.46	0.44
Total VFA, mmol/l	69.5	72.2	72.7	69.3	0.83	0.13	0.99	0.04
Acet, mmol/mol	684	683	678	675	2.43	0.16	0.07	0.80
Prop, mmol/mol	211	212	215	214	1.11	0.21	0.20	0.67
But, mmol/mol	105	105	107	111	2.48	0.40	0.21	0.66
pH	6.56	6.56	6.55	6.60	0.031	0.78	0.53	0.59

uCP: utilizable crude protein; DM: dry matter; OMD: organic matter digestibility; CH_4: methane; VFA: volatile fatty acids.

Total polyphenols content, gas production kinetics and in vitro rumen fermentation in batch cultures of ruminal microorganisms have also been investigated in raw seaweed (Molina-Alcaide *et al*., 2017). Total polyphenol values varied among species and between seasons with values ranging from 1.46 to 50.3 mg/g dry matter (DM). The DM effective degradability, ranged from 424 to 652 g/kg, with highest levels in *Mastocarpus stellatus* and *Porphyra* spp. and lowest in *Pelvetia canaliculata* and *Acrosiphonia* spp. On the other hand, volatile fatty acids (VFA) and CH_4 production were highest in *Palmaria palmata*. In contrast to our findings, increased level of *Palmaria palmata* did not have any effect on CH_4 production or on VFA production. These results indicate that using raw seaweed biomass, species differ markedly in their in vitro rumen degradability, and that samples collected in autumn had lower rumen degradability than those collected in spring (Molina-Alcaide *et al*., 2017). However, these results are not directly comparable to the present results with processed material.

Conclusions

This study concludes that there are positive seaweed species specific effects on estimates of utilizable crude protein content and fermentation parameters when extracted protein fractions replace grass silage in vitro.

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Potential of fibrolytic enzymes in ensiling grass for a biorefinery process

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Introduction

The potential of surplus grass biomass as raw material for green biorefineries has been reviewed during last decades (e.g. Grass 2004; Kamm and Kamm, 2004; Mandl, 2010; Sieker et al., 2011). Grass is effective in converting solar radiation into chemical forms of energy and grows well in humid temperate areas with a capacity for high biomass production compared to annual crops. Further, existing technology is available for its cultivation, harvesting and ensiling. Due to its low lignin content, it is easier to process than wood or straw and offers a versatile raw material for feed and other purposes.

When preserved as silage, grass biomass can be refined all year round. During the ensiling process, sugars are partly converted into lactic acid, ethanol and volatile fatty acids and protein is degraded into peptides, free amino acids and ammonia (McDonald et al., 1991). Also structural carbohydrates are partly decomposed. Hemicelluloses are degraded mainly through hydrolysis by organic acids produced during ensiling and to smaller extent through endogenous enzymes present in fresh grass (Dewar et al., 1963).

Separating silage juice from fibre has been suggested as the first step of silage processing for various biorefinery purposes (Ecker, 2012; Sieker et al., 2011; Kamm et al., 2010). In this study, we wanted to evaluate if fibrolytic enzyme application prior to ensiling could be used as a pretreatment for a biorefinery process to improve the press-juice yield as well as content of soluble nutrients in press-juice. The ultimate aim of the processing was to create suitable grass based feed for monogastrics (Seppälä et al., 2014).

Material and methods

The experimental grass silages were produced at Jokioinen, Finland (60°48′N, 23°29′E) during late summer of 2014 from timothy meadow fescue swards cultivated for farm scale silage production for dairy cattle. The first regrowth (RG1) grass was harvested on 4 August, while the second regrowth (RG2) was harvested on 11 September. Both swards were mown with a mower conditioner, wilted in the field and harvested with a precision chopper. A formic acid based additive (AIV2 Plus, Eastman Chemical Company, Helsinki, Finland) was applied at the chopper. The enzyme used was a liquid product Flashzyme Plus (kindly provided by Roal Ltd., Rajamäki, Finland) with cellulase and hemicellulase activities. In the laboratory, the grass was divided into 4 batches, which received the following treatments: Control, no enzyme addition; Low, 0.10; Medium, 0.50 and High, 2.50 mL enzyme solution per kg grass DM.

Two replicate silos (cylinder shape, 12 l effective volume, diameter 14.2 cm) were used for each treatment in RG1, while three replicates were used in RG2. The silos were stored, protected from light, in room temperature and opened on 18 November 2015 after an ensiling period 471days for RG1 and 433 days for RG2. The prolonged ensiling period was due to delays in financing of the project. Juice extraction was performed with an in-house (Luke)

mechanical compressor. Silage samples were thawed, packed into mesh bags in 150 g batches, pressed for 2 min and the press-juice was weighed. Three compressions were conducted for each replicate and the pressed components from them were combined before analyses. The samples were analysed using routine methods of Luke as described by Seppälä et al. (2016). Statistical analyses were performed using SAS GLM procedure. Both experiments were analysed separately due to numerous significant interactions between treatments and experiments. Effects of increasing level of enzyme application were evaluated using orthogonal contrasts so that linear, quadratic and cubic effects could be detected, and P-value of the linear effect is referred to as P_L in the following text.

Results and Discussion

The material used in the current experiment was representative for grass regrowth harvested in Northern Europe (see e.g. Huhtanen et al., 2006; Salo et al. 2014) and also the fermentation quality can be considered typical despite the exceptionally long ensiling period. The greatest difference between the herbages was in DM, which was almost 20% higher in RG1 than in RG2 while otherwise the composition was rather similar (Table 1). The higher dry matter (DM) concentration of RG1 herbage was reflected in higher DM concentration of RG1 silages compared to RG2 silages (Table 2). Otherwise the differences between the two experiments in silage chemical composition were rather small. Enzyme application decreased NDF concentrations by 19.9 % in RG1 and 12.1 % in RG2 (P_L<0.001) with increasing level of enzyme application. Enzyme application also affected silage fermentation quality in both experiments (Table 2) as shown e.g. by increases in lactic and acetic acid concentrations (P_L<0.001).

Table 1 Composition of parent herbages

	First regrowth (RG1)	Second regrowth (RG2)
Date of harvest in 2014	4 August	11 September
Dry matter (DM), g/kg	296	241
Addition of formic acid, g/kg FM	0.047	0.016
Buffering capacity, g lactic acid/100 g	5.7	5.9
In DM, g/kg		
Ash	93	105
Crude protein	131	121
Water soluble carbohydrates	103	132
Neutral detergent fibre (NDF)	523	533
Acid detergent fibre (ADF)	260	273
Acid detergent lignin (ADL)	34	20
Hemicellulose (NDF – ADF)	263	260
Cellulose (ADF – ADL)	226	253
Indigestible NDF	77	64
In vitro OMD[1)]	0.729	0.742

[1)]In vitro organic matter digestibility measured by a pepsin cellulose method.

The two batches of grass differed to some extent from each other as fermentation was more intensive in RG2 than in RG1 due to a lower DM concentration of RG2. Further, the formic acid content of RG1 was 0.047 g/kg fresh matter (FM; 5 l/ton) while for RG2 it was only 0.016 g/kg FM (1.6 l/ton), which is clearly below the recommended dose (5 l/ton) and probably contributed to the more extensive fermentation. However, the linear trends in the fermentation profile with increasing enzyme application were similar: pH decreased and concentrations of lactic and acetic acids increased while proportion of ammonia-N in total N

Table 2 Chemical composition and fermentation quality of grass ensiled with increasing level of fibrolytic enzymes

	Enzyme level				SEM[1)]	Statistical significance[2)]		
	Control	Low	Medium	High		L	Q	C
First regrowth (RG1)								
Dry matter (DM), g/kg	284	278	274	272	0.2	0.006	0.277	0.885
pH	4.44	4.34	4.25	4.13	0.024	<0.001	0.624	0.757
In dry matter, g/kg								
Ash	109	106	109	110	2.3	0.626	0.395	0.46
Crude protein	146	148	151	154	0.7	0.001	0.656	0.777
Neutral detergent fibre	516	508	470	413	4.2	<0.001	0.004	0.633
Water sol. carbohydrates	24	27	31	34	1.1	0.003	0.690	0.735
Ethanol	30	28	42	45	2.9	0.009	0.514	0.107
Lactic acid	44	53	60	80	3.3	<0.001	0.178	0.355
Acetic acid	19	21	21	27	0.5	<0.001	0.028	0.031
Propionic acid	0.4	0.5	0.4	0	0.02	<0.001	<0.001	0.024
Butyric acid	0.3	0.5	0.6	0.5	0.08	0.326	0.160	0.980
In total N, g/kg								
Soluble N	364	371	354	337	5.6	0.018	0.094	0.416
Ammonium N	55	54	49	43	2.1	0.013	0.330	0.795
IVOMD[3)]	0.786	0.783	0.778	0.774	0.049	0.146	0.908	0.929
Second regrowth (RG2)								
DM, g/kg	233	232	228	234	0.2	0.835	0.075	0.082
pH	4.09	4.06	4.03	3.96	0.021	0.002	0.404	0.756
In dry matter, g/kg								
Ash	116	115	118	117	0.5	0.038	0.182	0.015
Crude protein	130	131	136	133	1.5	0.049	0.127	0.153
Neutral detergent fibre	509	493	465	447	3.6	<0.001	0.859	0.186
Water sol. carbohydrates	22	19	25	25	4.1	0.486	0.744	0.454
Ethanol	9	10	16	16	1.3	0.003	0.669	0.067
Lactic acid	103	102	117	124	3.5	<0.001	0.278	0.223
Acetic acid	21	23	23	27	1.2	<0.001	0.547	0.356
Propionic acid	0.8	0.6	0.5	0.6	0.11	0.184	0.430	0.905
Butyric acid	4.3	2.4	0.8	1.4	0.56	0.004	0.054	0.452
Isobutyric acid	0.1	0.1	0.1	0.1	0.04	0.762	0.413	0.736
Valeric acid	0.2	0.2	0.2	0.2	0.04	0.945	0.543	0.333
Isovaleric acid	0.3	0.2	0.1	0.2	0.03	0.195	0.100	0.681
Capronic acid	1	0.8	0.4	0.6	0.15	0.034	0.381	0.282
In total N, g/kg								
Soluble N	567	560	552	560	6.4	0.378	0.255	0.577
Ammonium N	91	76	66	68	3.2	<0.001	0.022	0.613
IVOMD	0.802	0.805	0.813	0.808	0.0024	0.046	0.195	0.131

[1)]Standard error of the mean; [2)]L = linear, Q =quadratic and C = cubic effects of level of enzyme application; [3)]In vitro organic matter digestibility measured by a pepsin cellulose method.

decreased. Fibrolytic enzymes release carbohydrates providing additional substrate for lactic acid bacteria (McDonald et al. 1991). It is noteworthy that in the current experiment the effects of enzyme application were clear although both silages were also treated with a formic acid based additive (low dose for RG2 but recommended level for RG1) which is known to effectively restrict silage fermentation.

Press-juice extraction was clearly affected by enzyme application (Table 3). Yield increased linearly (P_L<0.01) with increasing enzyme application in both experiments for all constituents studied [FM, DM, ash, crude protein (CP) and water soluble carbohydrates (WSC)] except for ash in RG2. Effects were more pronounced in RG1 than in RG2 (e.g. 41.7 vs. 7.4 %

Table 3 Extraction results of grass ensiled with increasing level of fibrolytic enzymes

	Enzyme level				SEM	Statistical significance		
	Control	Low	Medium	High		L	Q	C
First regrowth (RG1)								
Press-juice proportion								
Fresh matter	0.163	0.187	0.228	0.285	0.0102	<0.001	0.187	0.987
Dry matter (DM)	0.067	0.080	0.105	0.148	0.0059	<0.001	0.069	0.822
Ash	0.160	0.193	0.231	0.285	0.129	0.002	0.445	0.856
Crude protein (CP)	0.088	0.101	0.121	0.151	0.007	0.003	0.318	0.938
Water sol. carbohydrates	0.137	0.156	0.203	0.253	0.015	0.004	0.363	0.713
DM of the press-juice	110	111	118	127	2.2	<0.001	0.088	0.780
DM of the solid residue	307	314	316	320	2.6	0.008	0.736	0.622
In press-juice, g/kg DM								
Ash	228	224	214	199	3.2	<0.001	0.097	0.943
CP	215	205	200	190	2.7	<0.001	0.895	0.409
Water sol. carbohydrates	31	20	36	30	7.6	0.729	0.761	0.196
Second regrowth (RG2)								
Press-juice proportion								
Fresh matter	0.310	0.321	0.352	0.335	0.006	0.004	0.048	0.038
DM	0.146	0.153	0.181	0.183	0.004	<0.001	0.477	0.034
Ash	0.287	0.298	0.329	0.311	0.007	0.276	0.698	0.133
CP	0.242	0.238	0.266	0.260	0.004	0.009	0.067	0.047
Water sol. carbohydrates	0.191	0.172	0.265	0.219	0.034	0.003	0.774	0.009
DM of the press-juice	117	119	126	141	1.3	<0.001	0.009	0.512
DM of the solid residue	335	332	334	349	2.6	0.020	0.026	0.503
In press-juice, g/kg DM								
Ash	259	253	238	212	2.8	<0.001	0.022	0.824
CP	193	187	174	157	2.8	<0.001	0.102	0.806
Water sol. carbohydrates	49	52	60	59	1.7	0.009	0.380	0.132

[1)]Standard error of the mean; [2)]L = linear, Q =quadratic and C = cubic effects of level of enzyme application.

increase for FM and 41.7 vs. 6.9 % increase for CP), while the absolute level of extraction was lower in RG1 compared to RG2 (0.216 vs. 0.330 for FM and 0.115 vs. 0.252 for CP). Dry matter concentrations of both press-juice and the solid residue increased with increasing level of enzyme application ($P_L<0.05$), while the concentrations of ash and CP in press-juice decreased ($P_L<0.001$) with increasing level of enzyme application. Important qualities of the press-juice as a feed raw material include DM concentration, the amount and quality of protein and concentrations of other nutritionally important compounds such as WSC, lactic acid, minerals and vitamins. Efficient removal of protein and other soluble nutrients may also be important from the point of view of the purity of the press-cake depending on the further use of it in the biorefinery process.

Use of fibrolytic enzymes increased the DM concentration of the press juice, which can be seen as a positive factor. A high water content of the press-juice causes logistic challenges and drying of the press-juice consumes energy and provides additional risks to reduce nutritional quality of the product. Using the press-juice directly at the site of production, i.e. on-farm, either as part of total mixed ration for cattle or part of liquid feed for pigs, would minimize the costs of transportation. Components measured in the current experiment (ash, CP or WSC) did not contribute to the higher DM concentration, but as lactic acid and volatile fatty acid concentrations increased with increasing enzyme application, they are likely contributing to a higher DM of the press-juice. Higher concentration of fermentation acids in the press juice could be considered a positive factor if used as a liquid pig feed, since organic

acids are commonly used as feed additives to stabilize the feed and improve intestinal conditions.

The method of press-juice extraction used in the current experiment was rather inefficient as the proportion of FM extracted was on average 0.273. The efficacy can be much higher if e.g. screw type extraction is used. It is possible that there is an interaction in efficacy of extraction between silage quality and extraction method as unpublished experiences from our lab indicate that with a more efficient extraction method, differences in silage pretreatments may disappear.

To our knowledge, effects of fibrolytic enzymes on press-juice production has not previously been studied although earlier research has indicated higher spontaneous effluent production from enzyme-treated silages (Jaakkola et al., 1991). In our case, ensiling the grass material with fibrolytic enzymes improved silage fermentation quality, restricted CP degradation during the storage period and increased press-juice yields thus proving to be an efficient pretreatment for a green biorefinery. Other important factors affecting the process include DM concentration, plant species and maturity, fertilization, additive treatments, length and temperature of storage, chop length and other options in ensiling. All these factors would need to be optimized to fully utilize the potential of grass silage as a feedstock for a green biorefinery.

Conclusions

Silage fermentation quality was improved by the use of fibrolytic enzymes. Press-juice yield increased, which is beneficial in a biorefinery concept for retrieving valuable nutrients from a grass matrix. In general, the effect of increasing level of enzyme application was linear and only very few quadratic and cubic effects were detected. Optimal ensiling methodology can be seen as a pretreatment for a biorefinery process.

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A note on the response in feed intake and milk yield on increased forage organic matter digestibility

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Introduction

Forage is essential in Danish milk production, and make up the major part of ration dry matter (DM). Therefore, forage quality (digestibility) is essential to achieve a high and efficient milk production. However, forage production is also a major cost. Beside land use, harvest costs are significant and increase with increased number of yearly cuts. Increasing the number of cuts is the common way to increase quality (= digestibility of organic matter), as regrowth length is shortened, forage is harvested at a younger, less fibrous and less lignified stage.

To optimize the harvest strategy, knowledge on expected responses by dairy cows on increased forage digestibility is required. The aim of the present paper is to assess the response by dairy cows in DM intake (DMI) and milk yield of energy corrected milk (ECM) to increased forage digestibility, based on Danish production experiments with forages varying in digestibility.

Materials and Methods

Danish production experiments performed at Aarhus University, Foulum in 2004-2016 were used. Criteria for inclusion were ad libitum feeding with Total Mixed Ration (TMR), at least three levels of forage digestibility per experiment or sub-experiment and concentrate proportion and composition held constant within experiment. The individual experiments are described in Table 1.

Data used were treatment means across parities within experiment. Forage organic matter (OM) digestibility (OMD) was either assessed in sheep digestibility trials (sheep fed at maintenance) or assessed using an in vitro rumen fluid method (Tilley & Terry, 1963) and recalculated to in vivo OMD using equations as given in the NorFor system (Åkerlind et al., 2011). Feed DM was measured at 60^{o}C. Energy corrected milk (ECM, 3.14 MJ/kg) was estimated as proposed by Sjaunja et al. (1991) based on milk yield in kg and concentration of fat, protein and lactose. Models with either DMI or ECM yield as response variables were fitted using random regressions in SAS (Proc Mixed). The models included forage OMD as regression variable, and random intercept and linear effects were allowed with an unstructured covariance structure. Models, also including a fixed non-linear term (the natural logarithm (Ln) of OMD (Ln(OMD))), to allow for non-linear responses, were tested as well. For the non-linear term, both quadratic and Ln terms were assessed and based on AIC, Ln was chosen. For non-linear DMI, Proc Hpmixed was used to obtain convergence. Subject was experiment or sub-experiment, as two of the experiments were divided in sub-experiments. This was done to account for higher DMI and ECM yield on clover compared to grass (Johansen et al., 2016) and to have constant forage/concentrate ratios within subject (Alstrup et al., 2016). Degrees of freedom in Proc Mixed were estimated using the Satterthwaite method.

Table 1 Experiments used for estimating response.

Experiment	Design	Treatments	Observations per treatment	Forage proportion (% DM)	Forage	Days from calving at exp. start, average ±SD	ECM (kg/d) (min-max)	DMI (kg/d) (min-max)	Forage OMD (%) (min-max)	Parity (% 1. Parity)
Hymøller et al. 2005	Group	8	8	60	Maize silage (4 treatments suppl. with 1/3 grass-clover silage)	73±24	29.3-35.8	20.1-23.8	67.9-79.0	50
Weisbjerg, 2009a	Latin sq.	4	16	60	Ryegrass silage	80±38	25.2-31.1	19.1-21.7	67.9-79.0	25
Weisbjerg, 2009b	Latin sq.	4	16	60	Ryegrass silage	74±15	28.3-32.5	19.7-22.4	68.3-79.0	25
Alstrup et al. 2016a	Latin sq.	4	12	80	2/3 grass-clover sil., 1/3 maize sil.	104±28	28.3-33.8	19.0-21.5	74.0-79.0	33.3
Alstrup et al. 2016b	Latin sq.	4	12	50	2/3 grass-clover sil., 1/3 maize sil.	104±28	31.7-33.5	21.2-22.2	74.0-79.0	33.3
Johansen et al. 2016a	Incomp. Latin sq.	4	20	70	Grass silage	83±55	29-9-33.7	18.8-20.3	73.9-83.4	33.3
Johansen et al. 2016b	Incomp. Latin sq.	4	16	70	50-100 % clover, 0-50% grass	83±55	33.3-35.8	20.8-21.7	75.4-82.2	33.3

Results and Discussion

Estimates for regressions are in Table 2. Both linear and non-linear effects were found for ECM (P=0.05 and P=0.04, respectively), but not for DMI (P=0.3 and P=0.2, respectively). However, using the model with only linear term, the linear term was highly significant for both DMI and ECM. Although the non-linear term (and also the other terms) was not significant for DMI, the non-linear model was better than the linear, when testing the difference in -2 Res Log Likelihood using a χ^2 test (P<0.005). Therefore, the non-linear responses for both DMI and ECM are in Figure 1a.

Table 2 Estimates for the fixed parameters in regressions of dry matter intake (DMI) or energy corrected milk (ECM) yield on forage organic matter digestibility (OMD) and natural logarithm (Ln) of OMD.

Response variable	Intercept	P	OMD	P	Ln(OMD)	P	RMSE
DMI (kg/d)	-402	0.2	1.46	0.3	123	0.2	0.66
ECM (kg/d)	-1104	0.03	-4.06	0.05	334	0.04	1.05

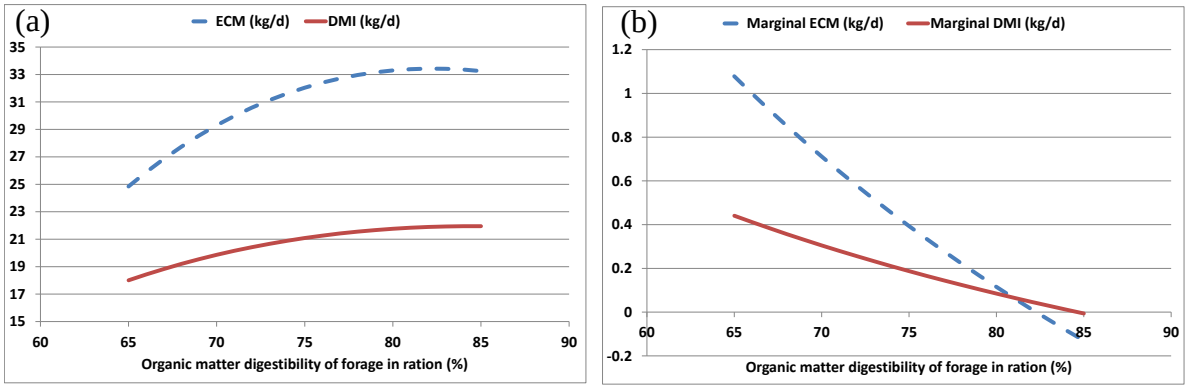

Figure 1 (a) Estimated response in dry matter intake (DMI) and yield of energy corrected milk (ECM) with increased organic matter digestibility (OMD) of forage in ration. Equations given in Table 2. (b) Estimated marginal response in DMI and yield of ECM with increased OMD of forage in ration.

For optimization, the questions is: what is the gain and what it the cost, when digestibility is increased by one %-unit. Marginal costs are not examined in this paper, but marginal responses in DMI and ECM yield are in Figure 1b for the range in forage OMD of 65 to 85%. The forage OMD, at the points of inflection of the response curves (where marginal curves are zero), was 82.3% for ECM and 84.6% for DMI. However, it is also obvious from Figure 1, that near the inflection point, the marginal response in ECM is very small. Therefore, the economically optimal forage OMD is probably considerable lower than at the inflection point, as ECM response must pay for both increased feed intake and a more expensive forage.

Conclusions

In forage rich Danish rations based on grass silage, grass-clover silage, and/or maize silage, feed intake respond positively up to 85% forage OMD, and ECM yield respond positively up to 82% forage OM digestibility. The economic optimal forage digestibility will depend on milk price and costs of increasing forage digestibility.

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Strengthening generation of quality feed-related data and their dissemination: proficiency testing programmes of FAO and its collaborating partners, and Feedipedia

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Introduction

Livestock are vital for food security of millions of people today and will remain important in coming decades. Increasing demand for livestock products is imposing a huge demand on feed resources. Efficient use of available feed resources is key to efficient animal production and food and environment security. Generation of sound quantitative data on livestock and livestock traits and particularly on feed resources is imperative for sustainable development of the livestock sector. Feed-use efficiency and decrease in release of environmental pollutants from livestock production systems rests on preparation and feeding of balanced rations. For this, availability of reliable data on chemical composition and nutritional value of feed resources is a must. Also for reducing wastages, ensuring food safety through enhancing feed safety and promoting international trade, data on presence of microbial contaminants including mycotoxins, heavy metals, antibiotic and pesticide residues must also be strengthened (Makkar and Ankers, 2014). In addition to the generation of sound data from the laboratory, it is equally important to properly manage feed related data in a data base or as Global and/or National Feed Resource Information system, so that data and information on feed resources could be used by extension and development workers, feed industry and researchers for developing feeding strategies, diet formulation and development of livestock development programmes for meeting production targets. Feedipedia is a feed resource information system that aids in dissemination of reliable data to stakeholders of the livestock sector.

Proficiency testing

For generation of sound data on chemical composition and nutritional value of feed ingredients or feeds and on hazards present, if any in them; laboratories should conduct analyses using correct methods in the right manner with good laboratory practices. A proficiency test is an inter-laboratory test that allows evaluation of performance of laboratories and is based on analysis of similar homogeneous samples. It is critical to ensuring quality of analyses performed in a laboratory. A proficiency test is an element of external quality assurance (EQA). EQA promotes both quality improvement and standardization of test procedures. Both EQA and internal quality control (IQC) are essential elements to good laboratory practices. Use of proficiency testing as a tool to assure quality of test results is highlighted in the ISO 17025:2005 standard, Section 5.9. A proficiency testing programme accompanied by regular use of certified reference material and/or internal quality control material as a secondary reference material represent key components of a laboratory quality system. It is in the interest of laboratories to assess their performance, especially using proficiency tests, because it allows them to evaluate their performance *vis-a-vis* their peers

and is a valued step toward certification and accreditation. It also provides assurance to customers that the results they get are the right ones.

Proficiency test result analysis and interpretation

Evaluation of participating laboratories using z score calculation is calculated based on the following equation:

$z = (x - \mu) / \sigma$

where, z is the score to evaluate the individual laboratory performance, x is the mean value of two individual laboratory reported numbers, μ is the assigned value for the corresponding PT item, and σ is calculated based on the following equation:

$\sigma = \{Cx2^{[1 - \log(C)/2]}\}/100$

where, C is the concentration of analyte.

The above equation can be used on the basis of a consensus mean and standard deviation of all laboratories (used in proficiency testing for feed composition) or an assigned value according ISO 17043, B.3 (used in proficiency testing for aflatoxin).

A suggested z score evaluation is:

$|z| \leq 2.0$ satisfactory

$2.0 < |z| < 3.0$ questionable

$|z| \geq 3.0$ not satisfactory

Proficiency testing for feed composition

Since 2014, the Food and Agriculture Organization (FAO) of the United Nations, jointly with the International Analytical Group, Section Feeding Stuffs (IAG), the Austrian Agency for Health and Food Safety (AGES), have invited laboratories to participate in the 'annual' proficiency testing programme for feed analysis laboratories. Proficiency tests, with two feed samples each year, for various constituents (proximate, macro- and microminerals, feed additives, and amino acids) were conducted in 2014 and 2015. In that period, a total of 40 and 50 European and 73 and 63 developing country feed analysis laboratories, respectively, participated in the study. This proficiency test allowed to compare performance of the two groups of countries, European and developing countries, and to use the results of the study to develop strategies and means to enhance quality of data emerging from feed analysis laboratories (Makkar et al., 2016). Higher standard deviation and several-fold higher coefficients of variation were obtained for the developing country laboratories.

The coefficients of variation for chemical composition parameters, macrominerals, microminerals and amino acids were higher by up to 9-, 14-, 10- and 14-fold, respectively, for the developing country laboratories compared with the European laboratories in 2014, while corresponding values for 2015 were 4.6-, 4.4-, 9- and 14-fold higher for developing county laboratories. Also, higher numbers of outliers were observed for developing countries (2014: 7.6−8.7% vs 2.9−3.0%; 2015: 7.7−9.5% vs 4.2−7.0%). These results suggest that there is a greater need for feed analysis laboratories in developing countries to improve quality of data generated (Makkar *et al*., 2014). Higher variability of data generated in developing countries could have severe negative impact on the livestock sector.

Proficiency testing for aflatoxin in feed

Since 2016, three rounds of proficiency testing for aflatoxins (total and aflatoxin B1) in feed samples have been organized by FAO jointly with Texas A&M AgriLife Research, College Station TX, USA. Two rounds have been completed and a third one is in progress. Details on the process of conducting the proficiency testing are available in Herrman and Makkar (2016). In the first round, a total of 84 laboratories participated, and data from 96% of the laboratories were acceptable. Two lab results were eliminated using the Grubbs test for outliers and one result was eliminated using the Cochran test. Participant mean results for total aflatoxin were slightly higher (4 µg/kg than the assigned value and composite relative standard deviation was 35% in this round.

In the second round, laboratories from 5 continents: 55 from Africa, 21 Asia, 22 Europe, 9 North America and 5 South America participated. A total of 175 results were submitted. For total aflatoxin the assigned mean was 34.5 µg/kg while the participants' average was 32.8 µg/kg. The Grubbs test of laboratory means showed no outliers, while the Cochran test of variance of results removed one outlier. For aflatoxin B1, the assigned mean was 31.8 µg/kg while the reported mean was 27.6 µg/kg and the assigned standard deviation of 8.5 µg/kg and the reported standard deviation was 12.3 µg/kg. For aflatoxin B1, there were no outliers.

Five of the z scores for total aflatoxin were greater than 3; while for aflatoxin B1, 7 of the z scores were >3. A score of zero implies a perfect result, approximately 95% of z-scores fall between -2 and +2, and a score outside the range from -3 to 3 should be investigated and accompanied by a corrective action. While only 3 results from the first round had z scores > 3. It may be noted that the number of laboratory results from the first round were approximately half compared with the second round. The composite relative standard deviation was 37% in the second round, slightly higher than that in the first round.

A comparison between continents and testing platforms for total aflatoxin and B1 aflatoxin was performed. African laboratories reported total aflatoxin using test kit platforms with an average relative standard deviation of 33.6%, a composite mean of 33.9 µg/kg, and a composite bias of -0.08, the lowest among the continent groupings. Seventeen of 20 laboratories had z scores < 2 and none of the Z scores were > 3. The Europe laboratories performed equally well for aflatoxin B1 using liquid chromatography with an average relative standard deviation of 34.8%, a mean of 31.3 µg/kg and a bias of 0.23 µg/kg. Unlike for the proficiency testing for feed constituents, the performance of African laboratories was as good as that of the European laboratories. This could be attributed to the strong capacity building activities through the Aflatoxin Proficiency Testing and Control in Africa (APTECA) programme conducted by Texas A&M AgriLife Research, and the Laboratory Quality Systems online course offered by Texas A&M in collaboration with FAO for the last many years.

Implications

Unsound data could adversely impact trade, increase feed wastage, render precision feeding ineffective and make feed industries incapable to resource good quality ingredients or prepare good compound feeds. Feeding of unbalanced rations leads to decrease in profit to farmers, production below the genetic potential of animals, reproductive problems, metabolic diseases, shorter productive life, poor animal health and welfare, and excessive amounts of pollutants

released to the environment. Spin offs of improved data quality are enhanced research and education capabilities of students.

How to improve quality of data

Investment in improving skills of laboratory staff and laboratory infrastructure coupled with implementing good laboratory practices are expected to improve quality and reliability of data. Also, there is a need to further strengthen already ongoing training programs for laboratory staff and to establish formal training programs, if they do not exist. Editors considering manuscripts for publication should also seek information from authors on quality control set up in their research laboratories. Similarly donors, besides supporting efforts that enhance quality control systems in laboratories, should also demand putting in place a proper control mechanism for data generated by laboratories in the framework of their sponsored projects. Use of internal standards will enable laboratory personnel to evaluate quality of data. Also, creation of a network of laboratories within a country and running of annual in-country proficiency tests would contribute to furthering proficiency of laboratories at a relatively low cost. Governments should consider increasing investment for improving laboratory infrastructure and laboratory proficiency. Setting up of a body overseeing quality of data being generated by laboratories and laboratory operations, and by supporting laboratories through investments and capacity development should also be considered (Makkar *et al*., 2016).

Feedipedia

Feedipedia (www.feedipedia.org) is a global feed resource information system developed and maintained jointly by 'Institut national de la recherche agronomique' (INRA), 'Centre de coopération internationale en recherche agronomique pour le développement' (CIRAD), 'Association française de zootechnie' (AFZ) and Food and Agriculture Organization of the United Nations (FAO). It plays an important role in dissemination of feed related data and information to researchers, extension workers, farmers, feed industry and livestock development agents alike.

History

Launched in 2012, Feedipedia is heir to a long series of projects spearheaded by FAO and national research institutions since late 1960s when computers and computerized databases started making inroads in agriculture. In 1973, establishment of INFIC (International Network of Feed Information Centers) resulted in coordination of international efforts aiming at harmonizing, sharing and disseminating feed-related data and information, not only in industrialized countries (where feed tables had been produced for more than a century), but in developing and emerging countries as well (Harris *et al.*, 1974). The publication of feed composition tables for Latin America (McDowell *et al.*, 1974) and the Middle-East (Kearl *et al.*, 1979) and FAO's *Tropical feeds* compendium (Göhl, 1975) are examples of such early work. While INFIC ceased its activities in the 1980s, its legacy lived-on through various national and international projects, notably FAO's AFRIS (Animal Feed Resources Information System), a website created in the mid-1990s that drew largely on *Tropical feeds*, and the French Feed Database. The latter was created in 1989 at the initiative of a consortium of public and private stakeholders, and managed since by AFZ. In 2009, INRA, CIRAD, AFZ and FAO joined forces to create Feedipedia, bringing together experience and databases of

these organizations with the goal to produce a comprehensive resource on animal feeds that would be freely available to the public, leveraging the near-ubiquitousness of internet access.

Content

Feedipedia is an on-line encyclopaedia which provides state-of-the-art scientific information on animal feeds, ranging from common products such as major cereals, oil meals and forages to less conventional or to very local products. The website provides datasheets about a feed or a family of feeds. Datasheets include descriptions of feeds (with images and graphics) and information on occurrence, environmental impact, chemical composition, nutritional value and use in ruminants, pigs, poultry, rabbits, horses, fish and other farmed species. At the time of writing, more than 350 datasheets have been completed. There are about 1400 tables of composition and nutritional values.

Public

Since its creation, Feedipedia has become a reference for all stakeholders looking for neutral, fact-based information on animal feeds worldwide. As such, it is increasingly cited in scientific and technical literature. While it is not meant to replace national or commercial feed tables, which are tailored to specific needs, Feedipedia provides baseline data that users can use as a reference point and original data on less conventional feeds. Feedipedia users are very diverse: they include compound feed manufacturers, feed producers, nutritionists, farmers, extension workers, producer organizations, researchers, students, etc. Its audience has been steadily growing. Since 2012, the site has received 2.6 million visits and served 6 million pages. There are currently more than 3000 visits/day. Feedipedia pages appear on top of search engines results. Its audience is truly international: visitors from Asia, Africa, Europe and the Americas account for 34%, 23%, 23% and 15% respectively. As Feedipedia is written in English, its audience is primarily found in countries where this language is spoken. However, automated translations in other languages are provided by Google.

Datasheet creation

Feedipedia is created by a team of researchers and engineers from INRA, CIRAD and AFZ, with occasional participation of external experts. Feedipedia is strictly evidence-based and texts are elaborated by these researchers from comprehensive reviews of international literature. Indeed, two recent and widely cited peer-reviewed articles (Makkar et al., 2014 and Makkar et al., 2016) were derived from Feedipedia datasheets. More than 15,000 articles, books, reports or dissertations have been used so far for writing the datasheets.

Table creation

Table values are established from a large database containing more than 2.5 million raw data of chemical composition and *in vivo* measurements. Part of these data come from participating organizations, including AFZ, CIRAD and INRA, while other data are collected in the international literature during creation of the datasheets. The values result from a complex, multi-step process. The first step, before adding new data to the database, is to identify and assign correct names for feeds and for chemical and *in vivo* parameters, as a result of a global lack of harmonization in feed and parameter nomenclatures. For instance, terms such as “rice bran” or “tannins” used in a data source can be too imprecise to properly characterize data and, therefore, it is necessary to identify the type of rice bran and the type of analytical method that was used to determine tannin content.

This step is followed by validation of the selected data. Unlike the proficiency testing process described in the first part of this article, validation of feed data remains largely a matter of human expertise and can only be partly automated. The reason for this is that feeds are highly variable materials. Deciding whether a value is correct or not is not a decision that can be based purely on statistics, but one that depends on amount and quality of knowledge that already exists on a particular feed. For instance, decisions tend to be more forgiving for new or little-known feeds than for feeds that are already well documented in the database. However, as the database grows over time and more data become available, it is possible to revise previous decisions and discard or reassign older data.

Data validation starts with identification of trivial errors (such as typos). Once these have been identified and corrected, data go through a first series of feed-independent filters. For instance, the sum of amino acids should be lower or not significantly higher than the crude protein content. The next series of filters are feed-dependent and detect data outside the recorded range for the feed. Human expertise is necessary here to decide whether the new data can be accepted or rejected. At any step of the validation process, potential outliers are examined one by one. They can be found to be valid and kept, or result in reassignment of the sample to another feed, or to the creation of a new feed. Truly erroneous data can be corrected (mistaken unit for instance) or simply eliminated. As some feeds are produced in a more artisanal fashion, *e.g.* by-products of oil extraction from organic farming, they have a more variable composition than that of oil meals from non-organic (conventional) farming. Validation of such products tend to be more forgiving than for corresponding conventional meals. However, organic oil meals should not have too low contents of residual oil because solvent extraction is forbidden in organic oil processing. Even in absence of reference data, an allegedly organic oil meal with an oil content lower than 5% should be viewed with suspicion and possibly reassigned as “conventional”. In the past, fraudulent samples of organic meals have been identified in the database by following this approach.

Once accepted in the database, the data are ready to be used for calculating table values, typically by averaging them. However, this is not a straightforward process. Because analytical parameters are often obtained on different data sets (with different numbers of observations), using only raw means for table values may result in inconsistent profiles. For instance, products with a high fat variability may have average gross energy values widely inconsistent with their average fat content. If the latter is much higher or much lower than the average fat content of the samples used for gross energy calculation, since fat energy is a major component of gross energy and there are typically much fewer available gross energy values than fat values. In order to address this issue, several hundreds of equations that predict one chemical parameter from one or several other parameters have been established from the database or collected from the literature. These equations are used to estimate more accurate table values, resulting in more consistent profiles. A predicted value for gross energy, calculated from the average protein, fat, carbohydrates and mineral contents, will result in a more accurate gross energy value and, as a consequence, more accurate digestible/metabolizable/net energy values that use of gross energy as an input in their own calculations. Likewise, table values for *in vivo* parameters are typically obtained through equations when available. Calculation of table values usually result in a second round of data checking. Averaged values with large standard deviations and/or large ranges are identified and examined. In some cases, scatter graphs are used to visually highlight outliers. This

process is repeated until the data set is stabilized and all outliers have been eliminated, corrected or reassigned to other feeds.

Future

The success of Feedipedia on the internet, and particularly in developing and emerging countries where mobile internet has become a commonplace even in rural areas, shows how important is this sort of knowledge to all stakeholders in the livestock sector. Feedipedia is an ongoing process and more than 300 datasheets still have to be completed and the datasheets written so far will have to be updated in the next few years. Translation of the datasheets into languages other than English will widen dissemination range, resulting in further increase in impact of Feedipedia. Combining Feedipedia with least cost rationing tools would also help livestock farmers to reduce their feed costs as well as impact of livestock on environment.

Conclusion

Proficiency tests and Feedipedia are both FAO initiatives that are meant to improve quality and availability of feed-related data and information, which is the foundation for sustainable development of the livestock sector. While proficiency tests are building laboratories capacity to generate sound data, Feedipedia provides a wealth of already validated information on feeds. Robust data on feed ingredient composition and nutritional value and sound research built on such quality data will further strengthen quality of information in Feedipedia and other national and international databases.

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Current status in Nordic feed analysis, scrutinized through proficiency test results

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Introduction

Quality and suitability of an animal feedstuff is usually assessed through analysis in a laboratory. Usefulness of laboratory results rely heavily upon their trueness i.e. that repeated results from an analytical procedure have god precision and are close to the true value of the analyte in the test samples. Precision can be addressed and improved by actions within the individual laboratories. To evaluate and improve trueness external comparison, i.e. with other laboratories, is needed. Most laboratories analyse certified reference materials and participate in proficiency tests to obtain such comparison. Choice of reference materials with different sample matrices, in our case different types of feeds, is however quite limited. Proficiency tests schemes, offer on the other hand several samples each year and covering many different sample matrices. There is, however, another side to proficiency tests as their results can potentially reveal the current status, good or bad, of analytical test performance in an area or sector. This is of particular importance for empirical methods where the analytical procedure actually defines the outcome.

Proficiency test activities are funded by participation fees and their main function is to aid the participating laboratories in the search for unknown sources of error. Proficiency test results must be treated in a confidential manner to secure test integrity and reduce the risk of data manipulation. Using proficiency test results to provide an overview of analytical quality to parts outside the laboratory sector, may lead to a conflict of interests, unless anonymity of individual proficiency test participants is secured. In the International Union of Pure and Applied Chemistry (IUPAC) harmonized protocol for the proficiency testing of analytical chemistry laboratories (Thompson et al., 2006) this point is addressed under a section on confidentiality. Here it says: "In setting out the confidentiality conditions, organizers should consider the general benefit of open availability of general performance data for the analytical community, and are encouraged to provide for open publication of such information subject to due protection of individual participants".

Effects of differences in analytical procedures used for the determination of test analytes that are on offer in the North European Proficiency Test scheme (NEPT) is now under special investigation. The purpose of this work is to get more uniform results in future rounds of the scheme. This can be done in two ways. Firstly, to work with the participants on harmonizing methods in use and secondly, to use a more strict definition of certain analytes and thus excluding results from participants that are using procedures producing significantly different results from the one prescribed for the proficiency test.

Evaluation of the long term proficiency of test results often involves the estimation of relative errors for example by presenting standard deviation values as percentages of corresponding measurement results for different samples and concentrations. The usefulness of such values depends on the nature of error sources involved in each case. Errors observed in signals produced by an analytical detector or instrument may thus be characterized as either homoscedastic or heteroscedastic. Homoscedastic errors are constant over entire concentration range whereas heteroscedastic errors vary with concentration, most often being

linearly proportional to the detector response and thus to the determined concentration. Relative standard deviation values will often yield valid statistical comparisons for heteroscedastic detectors but it may be better to compare actual standard deviation values (not dividing by the measured concentration) when evaluating results characterized by homoscedastic errors. It however complicates such studies that the overall error of a determination is not merely a detector error, but has contributions from other sources like decomposition, extraction, weighing, diluting, mixing etc. The relative error is however probably the most revealing uncertainty information for the end user of individual analytical results.

Results and Discussion

The numerical data presented here originates from four previous rounds of the NEPT scheme, i.e. Round 9 - 12, with results produced from analysis of ten different samples. The basis for statistical evaluation of a test event (one analyte, one sample) is rather uneven as some average values and standard deviation values, respectively, are compiled from twenty or so determinations whereas other such events involve only one or two measurement results. No statistical evaluation will be presented here for events involving less than seven measurement results that remain after execution of the outlier procedure.

Table 1 Relative standard deviation values, obtained from the analysis of ten samples from four consecutive rounds of the NEPT scheme for the main energy-related analytes. The two bottom rows give a measure of the span of errors for each analyte, estimated as ratios between the highest and the lowest energy term, for the error (standard deviation) and the relative error (standard deviation as % of the average measurement result), respectively.

Sample	Dry matter	Kjeldahl N	Dumas N	Ash	Fat hydrolyzis	Fat direct extraction	Fiber	ADF	NDF	Starch
Chicken feed	0.21	1.4	0.5	2.3	3.1	4.1	9.7	13.0	10.6	3.2
Wheat bran	0.22	1.2	0.7	1.1	4.2	8.7	4.6	5.1	2.5	5.9
Mixed silage	1.05	3.0	2.5	1.8	20.9		5.2		6.4	
Cows feed	0.16	1.6	0.9	2.3	6.8	10.0	11.8			4.3
Grass silage	0.93	1.7	2.3	2.0	4.9		2.6			
Piglets feed	0.27	1.5	1.1	1.8	8.4		8.8		8.6	6.0
Sugar beet pulp	0.21	3.5	2.7	1.8	56.0		6.1		7.8	109
Maize silage	1.27	4.0	4.4	3.8	33.9		5.5		4.2	8.1
Calf feed	0.41	1.4	1.1	1.2	6.4	7.7	9.4		11.5	6.4
Grass silage	1.65	2.2	3.1	1.9	6.3	12.2	5.6		2.4	90
STD max/min	6.1	2.1	4.3	3.6	3.3		4.6		2.9	9.7
%STD max/min	6.0	3.4	9.1	3.6	8.1		4.5		4.7	34

Table 1 gives error estimates for the main energy-related components. Except for the two nitrogen methods, these are gravimetric techniques which one expects to be mostly homoscedastic in nature. A homoscedastic technique is expected to yield a wider span (expressed by the max/min ratio) for the relative error as compared with the corresponding absolute error terms and this is the case for most of the analytes in question. The dry matter content is largely equal for the samples and thus we do not expect a significant difference between the two error ratios. The ratios are also almost equal for ash and fiber content, which might well be explained by undetected outliers. No ratios were computed for fat by direct extraction and for ADF, due to the small number of results. Detector errors for the two nitrogen techniques (titration and thermal conductance) are not expected to be dominating as decomposition and separation processes are also involved and these may well be homoscedastic in nature. It is interesting to note that for all samples with results from both fat measurement techniques, the relative error is greater for direct distillation, indicating that it is the separation of the fat rather than the distillation (very similar for both techniques) which yields a dominating contribution to the error. It may also be pointed out that in seven out of ten cases the Kjeldahl method yields somewhat greater errors than Dumas. The starch determination yields very high relative errors for two of the samples, which both are low in starch. The grass silage contains very little starch and, hence, a high relative error is to be expected. But there must be another error source related to the starch determination of the sugar beet pulp, possibly a high content of sucrose.

Table 2 Relative standard deviation values, obtained from the analysis of ten samples from four consecutive rounds of the NEPT scheme for macro minerals and trace elements. The two bottom rows give a measure of the span of errors for each analyte, estimated as ratios between the highest and the lowest energy term, for the error (standard deviation) and the relative error (standard deviation as % of the average measurement result), respectively.

Sample	P	Ca	Mg	K	Na	Cl	Fe	Mn	Cu	Zn
Chicken feed	10.0	7.3	6.7	9.7	6.5	4.2	6.8	4.9	9.4	6.1
Wheat bran	8.5	15.0	6.1	6.1			7.1	5.4	18.3	7.6
Mixed silage	10.7	5.2	6.7	5.9	9.0	4.0	7.7	6.6	15.1	6.0
Cows feed	4.9	4.6	5.9	5.9	5.7	2.9	8.8	5.2	7.8	10.7
Grass silage	5.0	4.0	5.6	4.6	7.2	3.4	7.3	5.3	16.2	11.0
Piglets feed	4.4	4.7	5.8	9.8	8.3	1.4	8.8	6.3	9.9	10.8
Sugar beet pulp	7.7	4.3	5.1	10.7	9.1		8.4	5.2	21.4	11
Maize silage	4.1	7.3	5.3	14.0	24.9		6.6	3.5	13.7	7.8
Calf feed	3.7	5.2	4.5	8.5	6.4	1.5	6.8	9.5	5.5	8.7
Grass silage	5.8	5.0	7.7	6.7	7.6	3.2	5.7	8.6	9.9	10
STD max/min	15.3	6.6	5.5	3.3	15.8	4.9	14.9	18.3	27.0	10.0
%STD max/min	2.9	3.7	1.7	3.0	4.3	2.9	1.5	2.7	3.9	1.8

Table 2 gives corresponding data for minerals and trace elements. Except for chloride, these were determined spectroscopically and are thus expected to show errors proportional to concentration (heteroscedastic) and the ratios conform to this. For chloride determinations more than one technique was used.

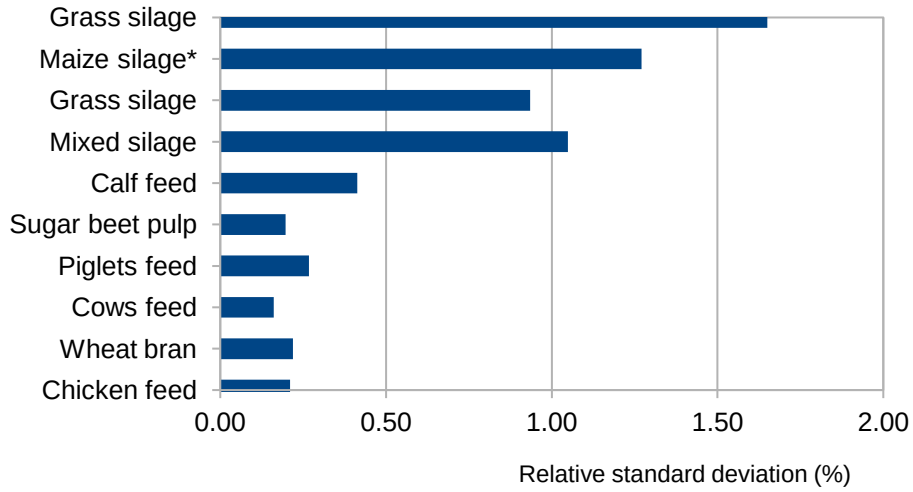

Figure 1 Relative standard deviation (%) for the dry matter determination

The dry matter determination gives a cause for special concern. As may be seen from Figure 1, all silages yield significantly greater z-scores for this analyte than remaining samples. The reason for this seems to be partly due to differences in methodology. All participants report drying at a temperature just exceeding 100°C (102°C - 105°C) for non-silage samples and, all but one, have used the same for the silages. The one participant has dried at least some of the silages at 60°C (in accordance with NorFor recommendations). In addition we have one participant which has (orally) reported that the drying oven used for the task has not been properly calibrated or even adjusted. This participant has thus obtained a number of high z-scores for the dry matter content indicating that the temperature of the oven might have been below 100°C on several occasions. A couple of other laboratories have obtained z-scores for this analyte indicating bias; one a positive one and the other a smaller but negative one. This bias is reportedly not caused by differences in methods used, but may possibly be caused by imperfect adjustment of the drying temperature.

One criterion for evaluating z-scores from several samples/rounds is to evaluate the proportion of scores that are greater than (or less than) zero. In the absence of bias one would expect to obtain 50% of the results with positive z-scores and the remaining with negative scores. For real data, values between 40% and 60% would not cause concern.

Table 3 Z-scores for dry matter, reported by a selection of the participating laboratories

Lab code:	9	14	25	35	40	41
z > 0 (%)	90	100	80	60	10	75
Mean (z)	6.9	1.8	3.0	0.2	-0.8	0.0
Chicken feed	**15.3**	**3.41**	-0.15	-0.86	-0.75	-0.03
Wheat bran	**15.7**	2.25	0.68	-0.10	-0.66	0.02
Mixed silage	1.77	0.66	0.48	-1.65	-1.21	0.14
Cows feed	**25.9**	2.93	-0.58	0.20	-0.91	0.12
Grass silage	1.57	0.90	0.37	-1.93	-0.54	0.10
Piglets feed	0.62	2.99	**11.7**	1.10	-1.34	0.19
Sugar beet pulp	0.08	2.80	**12.7**	0.28	-1.45	-0.44
Maize silage	-0.48	0.65	2.43	2.20	-1.73	0.17
Calf feed	**6.59**	1.11	1.79	0.14	0.69	
Grass silage	1.83	0.47	0.85	2.53	-0.15	

The first three laboratories in Table 3 (codes 9, 14 and 25 respectively), all have a large proportion of positive z-scores for dry matter. The first is the one reporting problems with temperature, resulting in four outlying z-scores. The lab coded 14 has positive z-scores for all samples but only one outlying score. However, seven consecutive z-scores with the same sign and for the same analyte, constitute an outlier. The lab coded 25 has obtained 80% positive scores, two of these severe outliers. Both last mentioned participants are likely to have some issues with controlling their respective drying temperatures. Lab no. 35 is the one reporting lower drying temperature for silages, but only two of the four obtained z-scores for silages are in accordance with this. Participant coded 40 has obtained 9 negative z-scores out of ten. The eight consecutive negative scores collectively constitute an outlier, but none of the individual scores even give cause for a warning (scores numerically exceeding two but less than three. The last column shows a participant obtaining 75% positive scores for dry matter, but all very close to zero and are no cause for alarm. The long-term monitoring of consecutive scores is mandatory by the IUPAC guidelines and obviously useful as well.

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Ringtest shows that in vitro organic matter digestibility in silages varies 3-4%-units between laboratories doing NorFor analysis

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Introduction

Analysis of organic matter digestibility (OMD) is a key input parameter in assessing roughage quality, energy value and intake potential in the NorFor system (Volden, 2011). The reference method for OMD is in vivo measurement of digestibility in sheep fed at maintenance level. Roughage OMD can also be determined by different in vitro analyses if a relationships between in vitro and in vivo OMD have been established within a specific roughage type or across roughage types. VOS (Lindgren, 1983) and IVOS (Møller et al., 1989) are in vitro methods based on rumen fluid used in Sweden and Denmark, respectively. IVOS is a rumen fluid method based on Tilley & Terry (1963) with the only major modification being that OM, instead of dry matter digestibility, is determined. However, enzyme based in vitro methods, not based on rumen fluid, also exist, e.g. the pepsin-cellulase method (De Boever, 1986; Nousiainen et al., 2003), which is used in Belgium and Finland. Today, the majority of roughage samples used in the NorFor system are analyzed in laboratories where NIR-calibrations are based on the IVOS procedure.

In order for laboratories to secure quality in their in vitro analyses of OMD, it is necessary with reference/standard sample material in the form of silages with known in vivo OMD. Such silages can be helpful for validation of in vitro analyses of OMD in existing laboratories but also by new emerging laboratories. Furthermore, silages with known in vivo OMD are also suitable material for performing ringtests in order to monitor inter-laboratory variability for laboratories uploading analyses to NorFor.

The purpose of this project was to: i) collect and store reference silage material, ii) measure in vivo OMD for reference silages, iii) make reference material available to laboratories, iv) organize a ringtest on in vitro OMD and v) compare in vitro and in vivo OMD results

Materials and Methods

Three silages were collected, mixed separately and vacuum packed on a commercial farm. The silages were two grass and one maize silage, all harvested in 2015. The grass silages consisted of a second cut stored in a bunker silo and a fifth cut ensiled in plastic bales. Clover contents in the grass silages were <10% of DM according to NIR analyses performed at 'Kvægbrugets ForsøgsLaboratorium' (SEGES, Agro Food Park 14A, 8200 Aarhus N, Denmark). Each silage was mixed in a vertical auger feed mixer for approximately an hour to obtain a homogenous starting material for before subsampling. The silage was then unloaded inside a shed on a cleaned concrete floor in a string. From the string, silage material were put in plastic bags and vacuum packed for immediate shipment to two universities for storage in freezer containers and later use for in vivo OMD determinations in sheep. Randomly selected bags were subdivided into 400 g portions of silage by coning and quartering. Samples were stored at -20°C and shipped to laboratories for preparation and analysis, i.e. the drying and grinding process at individual laboratories is a part of the test.

In vitro digestibility

Frozen fresh material of the three silages was sent to four laboratories for chemical and for in vitro analysis of OMD according to the protocol of Tilley and Terry (1963) with minor adjustments according to Møller et al. (1989). The laboratories were Eurofins Agro, Holland, Eurofins Agro Testing Denmark A/S, Agrolab, Germany and Aarhus University, Foulum, Denmark.

The NorFor system use in vivo OMD as input and the following relationships are used to calculate in vivo OMD from an in vitro value (IVOS):

Grass and clover grass silage: OMD = 4.10 + 0.959*IVOS (Møller et al.,1989)

Maize silage: OMD = 6.73 + 0.950*IVOS (Søegaard et al., 2001)

In vivo digestibility

OMD was measured in vivo in sheep at Götala Beef and Lamb Research Centre, Swedish Agricultural University, Skara (SLU) and at the Norwegian University of Life Sciences in Ås, Norway (NMBU). At each research facility, trials were conducted according to in house standard procedures (Prestløkken, E., pers. com., Nadeau, E., pers. com.) but a short description is provided below.

The in vivo trials were conducted with nine sheep during 3 weeks in a continuous trial, where each sheep was fed the same silage during the whole 3-week period. Sheep were grouped to achieve similar body weights among groups and the three silages were randomly allocated. The trials used 11 (NMBU) and 14 (SLU) days as adaptation periods, where the sheep were housed and fed individually at ad libitum intake of their respective silage. The silages were completely defrosted before being fed to the sheep. After the adaptation period, all sheep were put in individual cages and fed silage at 80% of ad libitum intake (calculated from the average of the previous 5 days of ad libitum intake) without any protein supplement. Water was available at all times. The cages had a meshed steel floor to allow the faeces to fall through and to be collected in a plastic container underneath the cage. The last four (SLU) and ten (NMBU) days out of the 21 days were used to collect faeces.

Results and Discussion

Results for in vivo determinations of OMD in sheep are in Table 1. In vivo measurements showed relatively low individual variation among sheep with most standard deviations (SD) around 1 and all SD<2% units. There was good agreement between universities as differences were less than 0.5%-units for all silages. Thus, no effect of university was detected (P=0.59) which underlines the robustness of in vivo OMD as reference method.

Table 1 Organic matter digestibility's (%) of two grass silages and one maize silage and individual variation determined in sheep at two universities (Uni_1 and Uni_2)

	Uni_1	SD	Uni_2	SD	Difference	P-value
Grass_1	77.2	1.8	76.8	1.1	0.4	0.59
Grass_2	79.3	0.9	79.3	1.2	0	0.59
Maize	70.1	1.8	69.6	1.0	0.5	0.59

Table 2 shows content of DM and ash in the different silages at the two universities. Surprisingly, there were differences in DM content of >2%-units for two of the silages and

University_1 measured lower DM content than University_2 for all three silages. There was also a consistency in analysed ash content as University_1 measured higher ash content than University_2 for all three silages.

Table 2 Dry matter (DM) and ash content of two grass silages and one maize silage analysed at two universities

	DM (%)			Ash (% of DM)		
	Grass_1	Grass_2	Maize	Grass_1	Grass_2	Maize
University_1	25.2	37.1	24.6	14.3	9.5	4.0
University_2	26.4	40.8	26.7	12.8	9.0	3.4
Average	25.8	39.0	25.6	13.5	9.2	3.7
Difference	1.2	3.7	2.1	1.5	0.5	0.6

Table 3 shows in vitro values for the four laboratories. Normally, seven laboratories are needed to calculate z-scores, however, it was only possible to find four laboratories that offer IVOS-analyses based on Tilley and Terry (1963). None of the analysed values were considered as outliers and the results did not reveal any z-scores near or above 2 which is typically considered a critical level. However, there was a difference of approximately 3%-units between lowest and highest laboratory for the two grass silages and 4%-units for the maize silage. Across the three silages, Lab_3 reported the highest values (76.4%) and Lab_3 and Lab_4 had systematically 2-3%-units higher values than Lab_1 and lab_2.

Table 3 In vitro organic matter digestibility (IVOS, %) measured in four laboratories

	Grass_1		Grass_2		Maize		All
	IVOS	Z-score	IVOS	Z-score	IVOS	Z-score	Average
Lab_1	73.2	-0.6	77.9	-0.7	69.2	-0.8	73.4
Lab_2	72.4	-1.1	77.6	-0.9	69.4	-0.7	73.1
Lab_3	75.5	0.9	80.3	1.3	73.3	1.3	76.4
Lab_4	75.3	0.8	79.2	0.3	71.4	0.3	75.3
Average	74.1		78.7		70.8		
Median	74.2		78.5		70.4		
SD	1.5		1.2		1.9		

The z-score was calculated as: (Lab – average)/SD. None of the values were considered as outliers and therefore all four values were included in the calculations. A z-score below 2 is typically considered acceptable.

Comparisons of in vitro and in vivo values are shown in Table 4, where in vivo OMD has been used to estimate in vitro values by rearranging the equations from Møller et al. (1989) for grass silage and Søegaard et al. (2001) for maize silage (see earlier). Thus, the in vivo values in Table 4 are the reference values for these silages and can be used by laboratories to quality assure their IVOS or other in vitro method. All laboratories showed a higher digestibility for Grass_2 compared to Grass_1 and the lowest digestibility for the maize silage, i.e. all laboratories ranked silages correctly. Lab_3 and Lab_4 values for the grass silages were close to the in vivo derived values but compared poorly for the maize silage with 5-7%-units higher values. Lab_1 and Lab_2 reported lower IVOS values for grass silages and higher for the maize silage compared to in vivo derived values.

Table 4 In vitro organic matter digestibility (IVOS, %) measured in four laboratories compared to IVOS values estimated from in vivo organic matter digestibility

	Grass_1		Grass_2		Maize	
	IVOS	Diff.	IVOS	Diff.	IVOS	Diff.
Lab_1	73.2	-2.8	77.9	-0.5	69.2	2.8
Lab_2	72.4	-3.6	77.6	-0.8	69.4	3.0
Lab_3	75.5	-0.5	80.3	1.9	73.3	6.9
Lab_4	75.3	-0.8	79.2	0.8	71.4	5.0
In vivo[1]	76.0		78.4		66.4	

[1] IVOS estimates from in vivo averages reported by the two universities (Table 1). These values are reference values and can be used by laboratories to quality assure their IVOS or other in vitro method.

Conclusions

There was good agreement between NMBU and SLU for the in vivo OMD on grass and maize silage. On the basis of these three silages and four IVOS-laboratories, the following is concluded: The test did not reveal any z-scores near or above 2, indicating that all four laboratories performed acceptable IVOS analyses. Two laboratories had systematically 2-3%-units higher IVOS-values for all three silages than the two other laboratories. There was a difference of approximately 3%-units between lowest and highest laboratory for the two grass silages and 4%-units for the maize silage but all laboratories ranked silages consistently. All laboratories measured higher (3-7%-units) in vitro values than corresponding in vivo values for maize silage. For the grass silages there was less consistency between in vitro and corresponding in vivo values.

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Finnish feed evaluation system and Feed Tables

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Introduction

The driver of the development of Animal Science as we know it now has been the need to compare the production potential of different feeds and to develop evaluation methods for that purpose. The history of feed evaluation, particularly from a Nordic perspective, was reviewed by Weisbjerg et al. (2010) at the 1st Nordic Feed Science Conference. The aim of this article is to describe the current situation of feed evaluation and the update practises of Feed Tables in Finland with some examples of the system with emphasis on dairy cows.

Feed value legislation in Finland

EU (2009a) has stated that if the energy value and/or protein value of a feed are indicated, such indication shall be in accordance with the EC method, if available or with the respective official national method in the Member State where the feed is placed on the market, if available. EU has not harmonised feed values except for poultry (EU, 2009b) and it is considered a task for the industry itself. According to the legislation by the Finnish Ministry of Agriculture and Forestry (MMM, 2012), Luke (previously MTT Agrifood Research Finland) is responsible for publishing the basis of feed value calculations and constants such as digestibility coefficients and efficient protein degradability (EPD) values for feeds. Presenting energy and protein values is voluntary for the industry at EU level, but if they are presented in Finland, they need to be calculated as described by Luke.

A permanent expert group consisting of representatives from Luke, University of Helsinki and Ministry of Agriculture and Forestry follows and guides development of feed evaluation work in Finland. Research professor Marketta Rinne is the chairman and Dr Kaisa Kuoppala the secretary of that group. The group has expertise from different livestock species and feed evaluation work is considered as a statutory service in Luke since 2016.

Web service delivers up-to-date information

In Finland, Feed Tables were initially published as appendices in agricultural handbooks. The first independent version of Feed Tables was published in 1982 (Salo et al., 1982). An overview of the series of Feed Tables published and major updates are presented in Table 1.

Currently, the major user interface is the web service, which is freely available to the public in Finnish (www.luke.fi/rehutaulukot) and in English (www.luke.fi/feedtables). An edited publication is published intermittently in Finnish; the last version being from 2015 (Luke, 2015). The animal species covered include ruminants (dairy cows, growing cattle and suckler cows, sheep and goats), pigs (piglets, fattening pigs, breeding pigs and sows), poultry (chicken and turkeys), fur animals (fox and mink) and horses. The service presents equations for feed value calculations, tables of typical feed materials with their chemical composition and feed values, as well as the nutrient requirements of the different animal species and groups. Compositional data of feed materials is shared among animal species, but selection of feeds shown for each species is tailored (e.g. no forages shown for poultry). Information on amino acids, minerals and vitamins is also provided.

For ruminants and horses, energy values are presented as metabolizable energy (ME) according to MAFF (1975). For forages, one kg digestible organic matter is assumed to provide 16 MJ ME to the animal. For concentrate feeds, digestibility coefficients for crude protein, crude fibre, crude fat and nitrogen free extracts are provided in the Feed Tables as well as contents of ME of the different digestible components. The protein values for ruminants are based on the Nordic AAT/PBV (amino acids absorbed from the small intestine / protein balance in the rumen) system with national modifications (Luke, 2015; Luke, 2017). This is a so called "metabolizable protein" system which takes into account amount of microbial protein synthesized in the rumen and undegraded feed protein, which together provide amino acids available for absorption in the small intestine of the ruminant.

For horses, protein values are presented as digestible crude protein. Digestibility coefficients from ruminants are used for horses both for energy and protein, which results in biased absolute values, but this is balanced by adjustments of the requirements.

In 2014, Finland adopted the French INRA-AFZ system in feed evaluation for pigs. A Finnish version of EvaPig® program is available for use. Feed energy is presented as net energy for growing and adult pigs and protein values as standardized ileal digestible amino acids. For poultry, a common European system (WPSA 1986) is applied with energy values presented as metabolizable energy and protein values as crude protein. Digestible phosphorus values of feed materials are also given for pigs and poultry.

Table 1. Major developments in the history of Finnish feed evaluation since 1982

Publication / year of update	**Major features and updates**
Salo et al., 1982	Compilation of feed tables and feeding recommendation for ruminants, pigs, poultry and fur animals into an edited publication.
Tuori et al., 1995	Ruminants changed from fattening feed units to metabolizable energy in energy evaluation and from digestible crude protein to AAT/PBV in protein evaluation after a comparison with several international systems using production data. Dutch system for pig feed evaluation was adopted. Nutrient requirements of horses were included.
1999	Ministry of Agriculture and Forestry gave a mandate to MTT (currently Luke) to publish the calculation methods ad coefficient for feed values.
2001	First Internet based Feed Table user interface was published.
MTT, 2004	Major modernization of Feed Tables, update of energy and protein requirements of dairy cows, reduction of phosphorus requirements of dairy cows.
MTT, 2006	Update of energy and protein requirements of pigs, update of mineral tables.
2011	Simplification of AAT/PBV calculations, feed unit replaced by presenting energy values as MJ, updates of energy and protein requirements and energy intake correction equation for dairy cows. Major modernization of Feed Tables. Increment of the energy recommendations of growing bulls, abandonment of AAT requirement for growing cattle, adjustment of P requirements of pigs in response to phytase inclusion in the diet.
2014	INRA-AFZ feed evaluation system adopted for pigs
2015	Feed tables for fur animals renewed.
2017	Feeding recommendations for suckler cows launched
2018 (plan)	Update of macro mineral and trace mineral information.

Maintenance of the Feed Tables

The evolution of the compositional data of feeds aims at presenting values that represent, as accurately as possible, feeds used in Finland. As experimental and practical data accumulate, values are updated. When changes are made, e.g. in the processes of important by-product producers, information is transferred to Feed Tables in cooperation with industry. New feeds

are added when a need is recognized (e.g. data is accumulating from minor crops and insect based feeds currently in the ScenoProt project). These updates are based on expert assessments and number of samples behind each mean varies greatly.

For the most important feed materials, e.g. grains and forages such as grass silage, several different options are presented (Figure 1). The values have been calculated based on empirical equations created from Finnish sample sets. An important benefit of national Feed Tables is that environmental conditions under which the domestic feeds are produced can be featured. The selection of different feeds is rather limited in Finland and values are affected by climatic conditions. For example, grass silage is produced mainly from timothy and meadow fescue, and digestibility values are higher at northern latitudes (Deinum et al., 1981). On the other hand, typical European forages such as ryegrass or maize silage are practically not used in Finland currently.

Rehutaulukot

Feed Table for ruminants - energy and protein values

If a value is missing, it is unavailable.

Feed code	Feed	DM	ME	AAT	PBV	EPD	D-value
		g/kg	MJ/kg DM	g/kg DM	g/kg DM		g/kg DM
07001	Grass silage, early 1st cut	250	11,5	88	39	0,85	720
07002	Grass silage, average/early 1st cut	250	11,0	84	35	0,85	690
07003	Grass silage, average/late 1st cut	250	10,6	80	26	0,85	660
07004	Grass silage, late 1st cut	250	10,1	75	18	0,85	630
07005	Grass silage, very late 1st cut	250	9,6	71	14	0,85	600
07006	Grass silage, high digestibility 2nd cut	250	10,9	84	45	0,85	680
07007	Grass silage, average digestibility 2nd	250	10,4	80	36	0,85	650
07008	Grass silage, low digestibility 2nd cut	250	9,9	76	32	0,85	620
07009	Grass silage, 3rd cut	250	11,2	87	46	0,85	700
07010	Italian ryegrass silage	340	10,6	82	40	0,85	661
07021	Red clover silage, early cut	250	11,2	101	85	0,80	700
07022	Red clover silage, average cut	250	10,4	93	71	0,80	650
07023	Red clover silage, late cut	250	9,6	85	62	0,80	600
07024	Red clover (0.25) silage, early 1st cut	250	10,7	89	35	0,80	670
07025	Red clover (0.25) silage, normal 1st cut	250	10,2	84	27	0,80	640
07026	Red clover (0.25) silage, late 1st cut	250	9,6	78	21	0,80	600
07027	Red clover (0.50) silage, early 1st cut	250	10,9	93	54	0,80	680

Figure 1 Screenshot from the Finnish Feed Table web service showing different quality grass and red clover silages (Luke 2017).

Case: correct estimation of the AAT value for different protein supplements

Soya bean based feeds are globally the most important protein feeds and commonly used in livestock feeding also in the Nordic countries. Experimental data has shown that protein supply and production responses of dairy cows fed soya bean feeds are lower than expected based on calculated protein values (e.g. Shingfield et al., 2003; Broderick et al., 2015, Rinne et al., 2015). Indeed, a meta-analysis by Huhtanen et al. (2011a) revealed that milk protein production responses were greater with rapeseed compared with soya bean based feeds (136 vs. 98 g milk protein per kg increase in CP intake).

In the Finnish Feed Tables, EPD values of soya bean meal and rapeseed meal have been adjusted to 0.75 and 0.63, respectively, which results in similar AAT values (169 g/kg DM) for both feeds. EPD values are not directly derived from in situ incubations, but they are adjusted based on physiological and production experiments. This approach helps in creating rations that are biologically, environmentally and economically sound. As a consequence,

very little soya bean meal is used in Finland for dairy cows and protein supplements are mainly based on rapeseed meal. A correct protein value for soya bean meal is of great importance because often protein value of other protein sources are compared with that of soya bean meal.

Further, if the EPD of a feed is modified by processing, e.g. heat treatment, treatment efficacy must be verified by production experiments before a corrected EPD value is included in the Feed Tables.

CowCompass is based on the Finnish feed evaluation system

A ration formulation program CowCompass (in Finnish 'KarjaKompassi', in Swedish 'KoKompassen') was launched by ProAgria in 2011 to be used for dairy herds. The program was developed in cooperation with ProAgria, Mtech, Luke, University of Helsinki, Valio Ltd., SLU and TTS Työtehoseura.

CowCompass is based on Finnish energy and protein evaluation systems presented by Luke (2015, 2017) and the feed data base used by the program is synchronized with Luke. Further, data from farm feeds is provided by feed laboratories and feed companies update their own products in the data base. The Finnish feed evaluation system does not require complicated analyses or calculations and is factorial. Interactions between feeds are mimicked using an empirical correction equation for energy intake, in which level of DM intake, energy value of feeds used and diet CP concentration are taken into account (Huhtanen et al., 2009).

Rations can be optimized by e.g. least cost, maximal milk production or minimal environmental load, but the most common choice is "milk income over feed cost". This requires accurate estimates of feed intake (Huhtanen et al., 2007, 2008, 2011b) and milk production responses (Huhtanen & Nousiainen, 2012), which are based on meta-analyses generated using large data sets on diets similar to those used in Finland. These are incorporated into the Lypsikki® model (Nousiainen et al., 2011, Huhtanen et al., 2011c), which is the core of ration optimizing in CowCompass. The theoretical principles of the approach have been reviewed by Huhtanen & Nousiainen (2014).

Figure 2 shows trends in dairy cow feeding and ECM production in Finland in 2010's according to statistics from ProAgria and official milk recording. Trends of both feed intake and ECM production are pointing upwards, with values for 2016 of 7341 kg DM consumed and 9735 kg ECM produced per cow per year. Trends in consumption of different types of feeds have not been very clear. In 2016, the proportion of forages (silage, grazed grass, hay and straw) was 0.55 of total DM consumption.

Conclusions

Correct feed values are important components of rational livestock production, ensuring nutritionally balanced, environmentally effective and economically sound rations. Feeding decisions also affect quality of animal products from human nutrition points of view. Feed Tables are used in modelling activities and scenarios used in different disciplines of agricultural and environmental sciences and are subsequently bases for political decisions regarding e.g. environmental protection. They are also used as default values in several statistics such as national greenhouse gas and ammonia emission calculations, which are reported to international bodies to monitor fulfilment of agreements, indicating the multiple uses of this data.

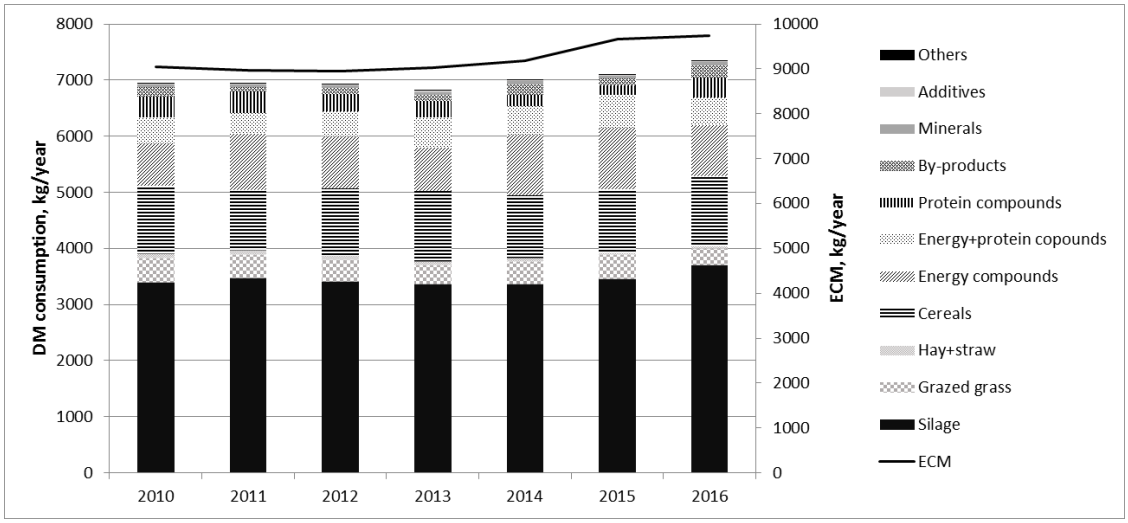

Figure 2 Development of feed consumption and energy corrected milk (ECM) production of dairy cows in Finland according to statistics from ProAgria during 2010-2016.

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The NorFor feed database

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Introduction

The Nordic feed evaluation system (NorFor) is operated by NorFor A.m.b.a (public limited corporation), owned by The Icelandic Agricultural Advisory Center, SEGES in Denmark, TINE SA in Norway and Växa Sverige in Sweden. NorFor A.m.b.a. develops and supports NorFor and is formed by experts in animal nutrition and information technology from the Nordic countries.

The feedstuff table (FST) of NorFor provides composition and nutritive value of 4300 feed materials, roughages and commercial compound feeds and it is available on-line. In addition, feed analyses from farmers are stored in the NorFor feed database. Results from feed analyses and feeds from FST are automatically transferred to the ´Herd feedstuff table´. This table is the base for users when formulating rations to dairy cows and growing cattle.

The aim of the FST is to systematically provide dairy and beef farmers with accurate and updated information about compound feeds and feed materials. In order to speed up the process, IT tools and interfaces have been developed and offered to laboratories and feed companies.

Laboratories send analytical results to NorFor's Feed Analysis System via a web service link. Feed companies upload their products and update them via a web service or manually. Updates of roughage values and some feed materials (concentrate feeds) into the NorFor FST are based mostly on feed analyses originating from farms.

4300 feedstuffs and 300000 feed analyses

Part of the NorFor FST is public and available at www.norfor.info/feed-table/. More than 500 feed materials (grains, oil seeds, legume seeds, tubers, roots, fruits, minerals, by-products, etc.) and roughages are shown here. Approximately 3800 commercial compound feeds are also included in the FST, but are not shown publicly. These are only displayed in the ration optimizer tools divided into country of origin and are shown by the Norwegian software ´TINE Optifor´, the Swedish ´IndividRAM´, the Danish software ´DMS´ or the international ´NorFor feed ration optimizer´ (NFRO).

There are other users of NorFor FST than the shown above. Swedish Sheep Breeders' Association uses a copy of Swedish feedstuffs in their feed ration formulation software for sheep, ´ElitLamm´, which is mainly used in Sweden, but to a small extent also in Denmark and Finland. Also, the Swedish Board of Agriculture uses a copy of Swedish feedstuffs with focus on reducing nitrogen and phosphorus losses from farmland within the project Focus on nutrients (´Greppa Näringen´). Likewise, in Denmark the FST serves as input for calculating environmental load in terms of nitrogen/ammonia excretion from cattle. And, it is further used by individual dairy and beef producers to document reduced environmental load, allowing them to have more cows per hectare.

In addition, approximately 35000 feed analyses from laboratories in Denmark, Germany, Iceland, the Netherlands, Norway and Sweden are uploaded to the NorFor feed database every year (Figure 1). These are mostly analyses from of roughages from dairy and beef farms.

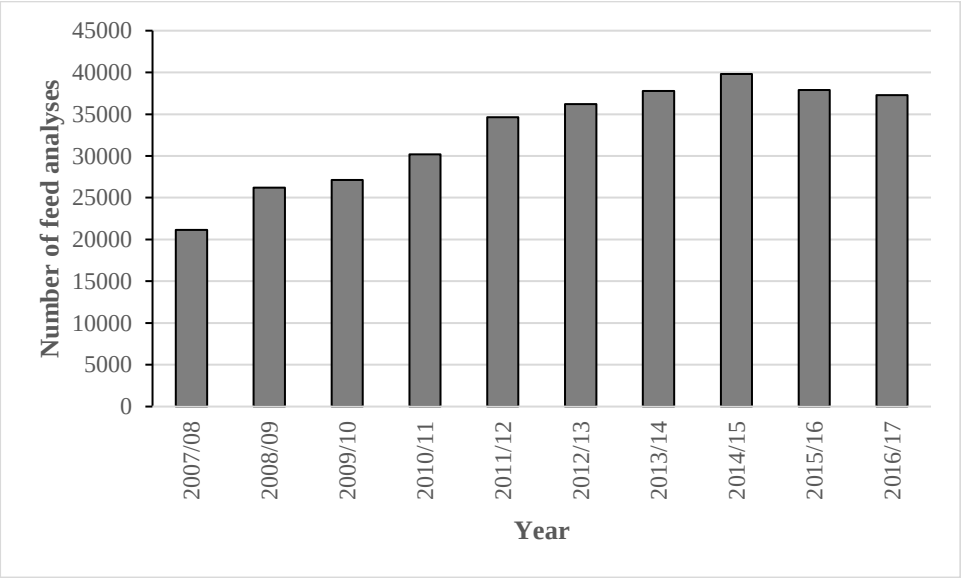

Figure 1 Number of feed analyses uploaded yearly by laboratories to the NorFor feed database. Each analytical result was automatically transferred to the herd feedstuff tables, eliminating the need of typing them manually while also avoiding typing errors.

Data on forage analyses, commercial products and table values of feed materials together represent an important base of feeds for accurate ration formulation by advisors or farmers using any of national software tool.

Updating feedstuffs on-line

All updates of feedstuffs are done on-line and are immediately available for use in the ration optimizer tools. Feed analyses, purchased by farmers, provide the largest number of samples for updating values of roughages and some of the feed materials in the public NorFor FST. Nordic researchers, feed companies and NorFor A.m.b.a. also contribute to this base. All analysis results are carefully reviewed before updating table values for a specific feedstuff.

Each feed company has a login user name and access to their own feed group in the NorFor FST. Here, feed companies can upload and continuously update their products. To facilitate and speed up the updating process, many feed companies are using the web service. On average, 30% of the commercial feedstuffs are updated every year. Laboratories also use a web service function for delivering results to NorFor's feed analysis system (Figure 1). This automatic transfer of analytical results from the laboratories to a specific herd in the software saves advisor time and eliminate risks of typing errors. It is, thereby, timesaving for farmers and dairy advisor and reduces costs. Also, data in the herd feedstuff table provides opportunities for farmers to monitor quality of their own silages between years.

Likewise, that compound feeds can be uploaded by feed companies into FST, saves time and ensures correct input when they are transferred into the herd feedstuff table for further use.

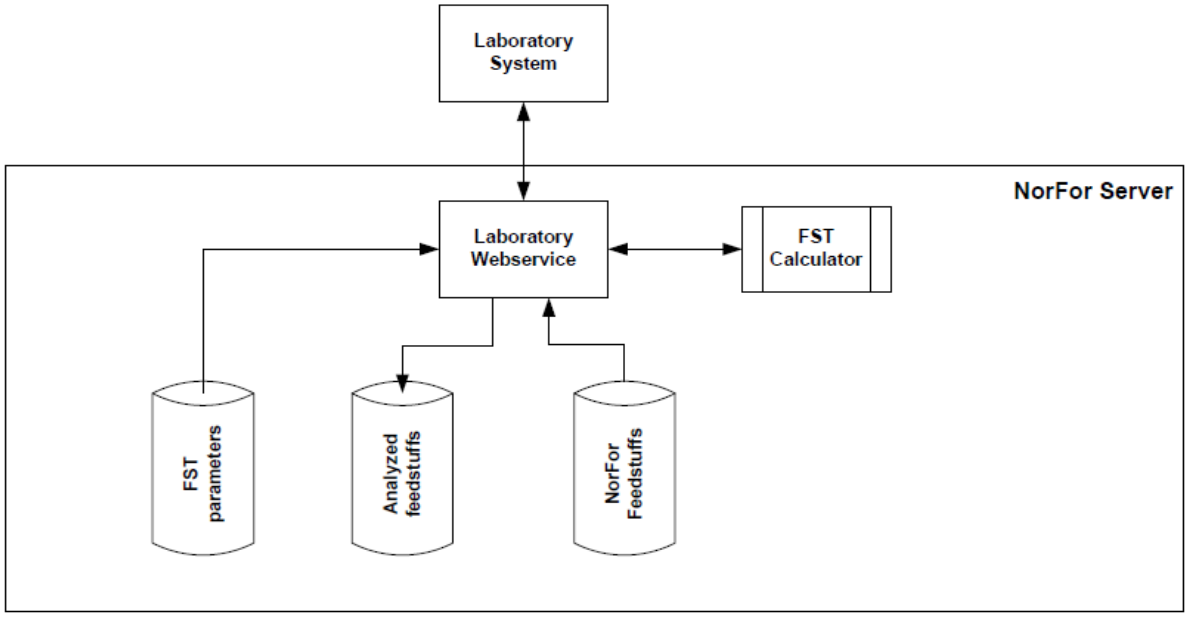

Figure 1 Overview of NorFor's feed analysis system (FAS). A laboratory sends analytical results from its own laboratory system to FAS via a web service link. In FAS, the results are merged with table values of the corresponding feedstuff in the NorFor feedstuff table (FST) and standard feed values are calculated. Calculated feed values are sent back to the laboratory and are also stored, together with the analytical results, in the NorFor feed database.

Feed characteristics

The NorFor model is a semi-mechanistic feed evaluation system, where several characteristics of the feedstuffs are needed beyond conventional analytical information. Selection of analytical methods have been assessed in collaboration with researchers, who have evaluated methods for characterizing feedstuffs for use in the NorFor model. Recommendations are to use EU methods (EC No. 152/2009) for the conventional analyses of crude ash, crude protein (CP) and crude fat. Analyses for dry matter (DM), NDF and starch are more specific and are described in the NorFor book (Volden, 2011). The drying temperature for DM determination of roughages is set to 60˚C and the DM of silages should be corrected for volatiles (Volden, 2011). For concentrates, the conventional drying temperature of 103˚C is recommended (EC No. 152/2009). Neutral detergent fiber (NDF) should be determined as aNDFom, described by Mertens (ISO 16472:2006 IDT), where samples are treated with sodium sulphite and amylase with ash excluded. The method for starch is based on an enzymatic method (Bach Knudsen *et al.*, 1997) and measured by an YSI apparatus (Yellow Springs Instrument, Ohio, USA). A large part, 10 to 20 % of DM, is normally not recovered by the analyses, and is called the rest fraction. It consists mainly of pectin, water soluble carbohydrates, and organic acids (Udén, 2013). In roughages, the organic matter digestibility is of particular importance, and there are several *in vitro* methods established such as Tilley and Terry (1963), IVOS (Søegaard *et al.*, 2001), EFOS (Weisbjerg and Hvelplund, 1993) and VOS (Lindgren, 1983), to mention a few.

Also, more thorough characteristics of CP, NDF and starch are needed. Crude protein is divided into soluble (s), potential degradable (pd), indigestible (i) fractions and the degradation rate (kd) of the pd fraction. Starch and aNDFom are each divided into pd and i fractions, and kd of the pd fraction. The NorFor book (Volden, 2011) describes rumen *in situ*

analysis for determining iNDF and kd of pdCP and pdNDF as well the mobile nylon bag technique for determining iCP. Since 2013, starch degradation characteristics are determined from *in vivo* trials (Moharrery, et al., 2014) and abandoning *in situ* determinations of starch (Volden, 2011).

For feeds shown in the public FST, values for 18 amino acids and 10 fatty acids are presented, preparing for future ration optimizations, based on individual amino acids and iodine values. It is also possible to upload 15 different minerals and three vitamins in the public FST.

In order to compare feedstuffs, feed values of net energy, metabolizable protein and protein balance in the rumen (NEL, AAT and PBV, respectively) are calculated for each feedstuff. Feed values, mostly used, are based on standardized conditions for a cow of 600 kg body weight, consuming 20 kg DM with 50% concentrates, yielding fixed digesta passage rates of NDF etc. These values are labelled NEL20, AAT20, PBV20, etc. (Volden, 2011).

When new analytical methods are introduced, it is important to have close and open dialogues with laboratories. Organisations representing farmers, buying feed analyses from laboratories, are encouraged to demand laboratories that participate in proficiency testing and conduct internal quality control. This will ensure quality of analyses and stimulate laboratory improvements.

Structure of the feedstuff table

NorFor A.m.b.a. has developed a comprehensive feedstuff table (FST). From the NorFor server, FST is available to each software tool and, when working on a herd-specific feed plan, feedstuffs are exported from the FST into the herd feedstuff table for further use.

For external users, FST is available publicly at www.norfor.info/feed-table. The FST is the most visited page of the homepage, with 300 to 400 viewers per month. Viewers are primarily from the Nordic countries, but also from Germany, US, UK, Russia, France and the Netherlands (countries with more than 150 viewers). Half of these are new and half of them are the so-called returning viewers.

An overview of the hierarchal directory system is shown in Figure 2, in which the highest level is region, dividing the feedstuffs into country specific and a common NorFor category. The feed directory is further divided into 15 feed groups with each feed groups divided into feed types. For example, ´Grains´ is divided into three feed types: ´Grains´, ´Dry grain by-products´ and ´Wet grain by-products´. The ´Forages and roughage´ feed group is divided into seven feed types: ´Pasture grass and clover grass´, ´Grass and clover–grass´, ´Whole crop´, ´Grass and clover-grass silage´, ´Whole crop silage´, ´Hay and straw´, and ´Grass pellets´.

Figure 2 also shows an example of possible parameter settings of ´NDF´ characteristics. These settings are used to generate reports on variables such as NDF, starch, protein, amino acids, fatty acids, fermentation products, minerals, vitamins or total characteristics (Volden, 2011).

In the non-public part of FST there are additional 75 feed groups, each one representing a feed company.

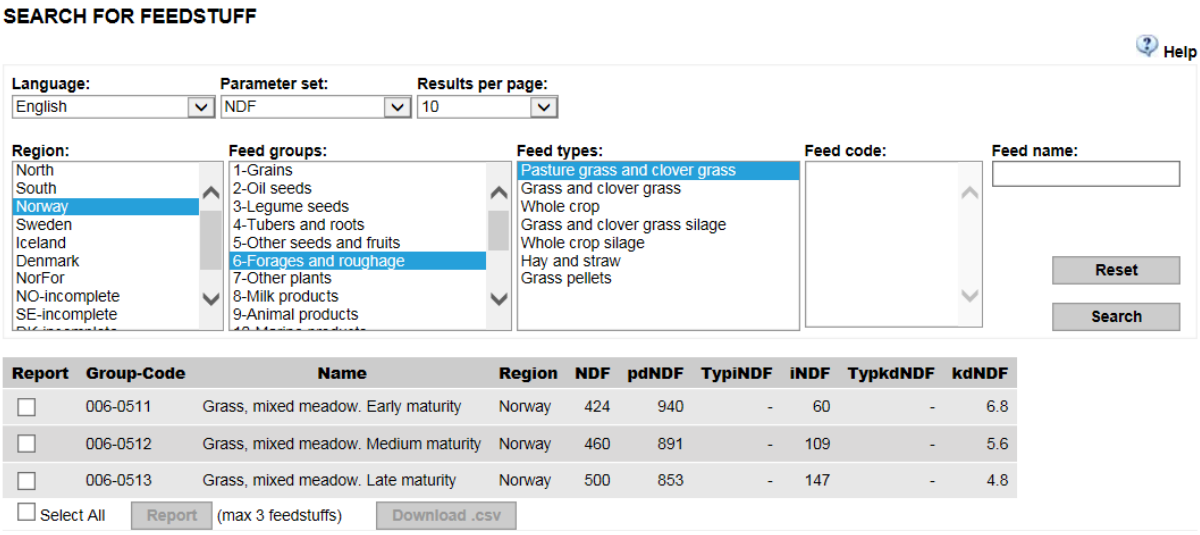

Figure 2 Overview of the public NorFor feedstuff table and example of NDF characteristics of feedstuffs selected from region "Norway", feed group "Forages and roughage", and feed type "Pasture grass and clover grass".

The structure of FST facilitates its administration and new parameters are, for instance, easily included. The latest additions to this table are molybdenum, standard feed values on NDF digestibility, histidine, lysine and methionine. Today, you can select between English, Danish, Icelandic, Norwegian and Swedish languages for all feed names and parameter names, but FST can be expanded with more languages in the future.

Another area for use of the FST could be the estimation carbon foot prints of individual farms. There is a plan to add CO_2 equivalents (CO2e) for each feedstuff, making it easier to estimate climate impact from both feed cultivation and enteric emissions.

Conclusions

The NorFor feed database is central to herd economy and feeding of dairy and beef cattle. Nutrient values, energy contents and prices for each feedstuff are used for feed planning and follow-ups in individual herds. Automatic transfer of feed analyses from a laboratory to an individual herd feedstuff table, and the feed companies' responsibility for values of their own products in FST, are timesaving for advisors and farmers using the software and also eliminates the risk of typing errors. The flexibility of the FST IT structure makes it easy to administer and enables further development and expansion.

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The importance of pectin and organic acids in plants

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Introduction

In NorFor, feeds are divided into ash, neutral detergent fibre, crude protein, starch, lipids, fermentation products and a rest fraction (RestCHO), which is calculated by difference. Water soluble carbohydrates (WSC) are included in RestCHO but is also a separate entity for estimating rumen load and metabolizable energy content. Other components, assumed to be found in RestCHO are pectin and β-glucanes in some grains (Volden, 2011). The NorFor Swedish feed tables (accessed 2011-12-21) revealed that forages had an average RestCHO fraction of 239 and 142 g/kg DM with WSC excluded (Table 1). Distillers grains, lupins and sugar beets contained very high levels of WSC-corrected RestCHO. It is well known that sugar beets contain high levels of pectin (Odensten, 2001) and that lupine α-galactoside content can be on the order of 200 g/kg DM (Zalewski et al., 2001). However, the WSC-corrected RestCHO values cannot be fully explained by the presence of these carbohydrates and for distillers grains, no candidate is available to explain the high value.

The non-WSC portion of RestCHO is assigned a fermentation rate of 0.1 /h, which is considerably slower than for WSC (1.50 /h). The proportion of carbohydrates and non-carbohydrates in RestCHO impact on NorFor predictions. The NorFor Scientific Advisory Group is aware of this problem and its potential effect on ration formulations.

Recently, an attempt was made to investigate the composition of the non-WSC portion of the RestCHO fraction in forages (Udén, 2017). Apart from pectin, one obvious component of this portion in green plants should be plant organic acids. Also soluble phenolic substances may be present as well as soluble fibre in the form of α-galactosides (rhamnose family oligosaccharides: lupins, peas, soybean and faba beans) and various gums, such as galactomannans in numerous other leguminous seeds. However, this paper examines only pectin and organic acids with respect to their analysis, occurrence and properties.

Pectins

Pectins are part of the plant cell wall and are often esterified with calcium (Jarvis, 1984). They will normally be extracted by chelating agents such as EDTA, oxalic acid, etc., even though recalcitrant pectin exists in lucerne stems (Hatfield, 1995). Pectin is composed mainly of galacturonic acid but also contains some rhamnose as well as minor amounts of other sugars, depending on origin (Chesson and Monro, 1982; Leitao et al., 1995). The proportion of galacturonic acid in pectin differs among plants and can be as low as 40% in potato tubers and as high as 90% in peach pectin (Voragen et al., 1995). No simple unequivocal assay exists for the analysis and researchers, therefore, often report the galacturonic acid content or use the proportion of galacturonic acid in citrus pectin of 83% to estimate total pectin content (Bucher, 1984). We have used a method recommended by Bucher (1984), which is based on the colorimetric reaction between galacturonic acid and meta-hydroxydiphenyl (MHDP). It was superior to an enzymatic method based on pectin lyase and the change in absorbance at 235 nm upon hydrolysis of polygalacturonic acid (Odensten, 2001). Unfortunately, the

MHDP method is published only in the form of a dissertation. A description of the method is available upon request from this author.

Table 1 The size of the unaccounted rest fraction with (+) or without (-) water soluble carbohydrates (WSC) included, based on NorFor Swedish tables accessed 2011-12-21 (g/kg DM)

Feed	Form	N	+WSC	-WSC
	Forages			
Grasses		29	243	136
Clovers		8	268	224
Grass-clover		40	230	130
Wheat	Whole crop	1	282	162
Mean			239	142
	Seeds and roots			
Grains	Whole	15	40	16
	By-prod.	12	69	27
	Distillers grains	5	217	197
	Brewers grains	3	42	22
Rape seeds	Whole	1	49	-11
	Expeller	4	170	89
	Meal	1	191	91
Soya	Whole	2	164	114
	Expeller	1	210	87
	Meal	2	221	100
	Hulls	1	126	107
Oil palm	Expeller	1	45	15
	Meal	1	51	19
Lupins	Whole	3	265	218
Peas	Whole	1	103	62
Field beans	Whole	2	61	57
Sugar beets	Pulp	2	427	387
	Molassed pulp	3	494	322

Pectin is not included in the neutral detergent fibre (NDF) as it is mostly solubilized by the EDTA. It is partially precipitated by acid detergents which can explain unusually high acid detergent fibre (ADF) values, relative to NDF, in certain low-hemicellulose, high-pectin samples. It is therefore advisable to analyse for ADF in such samples sequentially after a neutral detergent extraction (Van Soest, 2015).

Some literature data on pectin levels in feeds are shown in Table 2. The values from Udén (2017) are from analyses of 20 ley and whole crops and, as can be seen, levels for the clovers were considerably higher than for those reported by Chesson and Munro (1995). Pectin levels reported by Udén (2017) corresponded to approximately 60% of the WSC-corrected RestCHO fraction.

Ruminal degradation rates of pectin are scarce in the literature. Hatfield and Weimer (1995) found *in vitro* uronic acid degradation rates of 0.4 and 0.5 /h for lucerne and commercial

citrus pectin, respectively. Odensten (2001) found variations in *in vitro* degradation rates of pectins of different origins. The values varied from 0.23 to 0.41/h for samples of legumes, beet pulp and citrus pulp.

Table 2 Pectin or galacturonan contents in some feeds (g/kg dry matter)

Item	Pectin	Galacturonans	Reference
Citrus pulp	240		Odensten (2001)
Molassed beet pulp	150		
Soybean hulls	150		
Lucerne meal	80		
Lucerne		113	Åman (1993)
Lucerne		80	Chesson and Munro (1982)
Red clover		57-81	
Red clover	180		Udén (2017)
White clover	150		
Grass meal		23-25	Bach Knudsen (1997)
Grasses		25-34	Åman (1993)
Timothy	50		Udén (2017)
Whole crop barley	40		
Whole crop maize	50		
Whole crop wheat	40		

Pectin is defined as a dietary fibre source, according to AOAC (DeVries and Rader, 2005), which also includes non-structural components such as fructans and resistant starch. Ruminant nutritionists have found it hard to accept pectin as a source of fibre and have tended to classify it more as a rapidly fermenting carbohydrate with similar ruminal properties to starch. However, Strobel and Russel (1986) found no *in vitro* lactic acid production, contrary to the fermentation of starch. This has made pectin an interesting candidate for replacing starch in the diet. One Swedish feed company has even balanced dairy cow rations with respect to pectin and in the US, citrus pectin has been used to replace dietary starch (Arthington et al., 2002). Hall et al. (1997) presented an analytical scheme to estimate soluble fibre, with particular application to the vast quantities of citrus pulp available in Florida, US. She assayed it as: ethanol-water insoluble organic matter (EIROM) – NDFom – crude protein (CP) in EIROM + CP in NDFom – starch in EIROM. The method has practical virtues, but fructans in temperate grasses (and many tubers) will be included and errors from multiple analyses will reduce precision.

Organic acids

Organic acids (OA) in plants have mainly attracted interest by plant physiologists. It constitutes a heterogonous group of tricarboxylic acid (TCA) cycle intermediates as well as oxalic, cyclohexanecarboxylic acids (quinic and shikimic acids), malonic acid, etc. (Fig 1). The major plant OA are di- or tricarboxylic acids which are matched by metal cations (Playne and McDonald, 1966; Dijkshoorn, 1973). Dijkshoorn (1973) has suggested that ash alkalinity can be a rough indicator of OA levels in plants. Storage occurs in cell vacuoles and OA serve as energy reserves but can also be metabolized to carbohydrates, secondary compounds or amino acids and also play a role in maintaining redox potential (Igamberdiev and Eprintsev, 2016).

Malonic acid is important for N fixation in legumes and as a precursor of fatty acid synthesis (Li and Copeland, 2000; Igamberdiev and Eprintsev, 2016). Quinic acid is used for synthesis

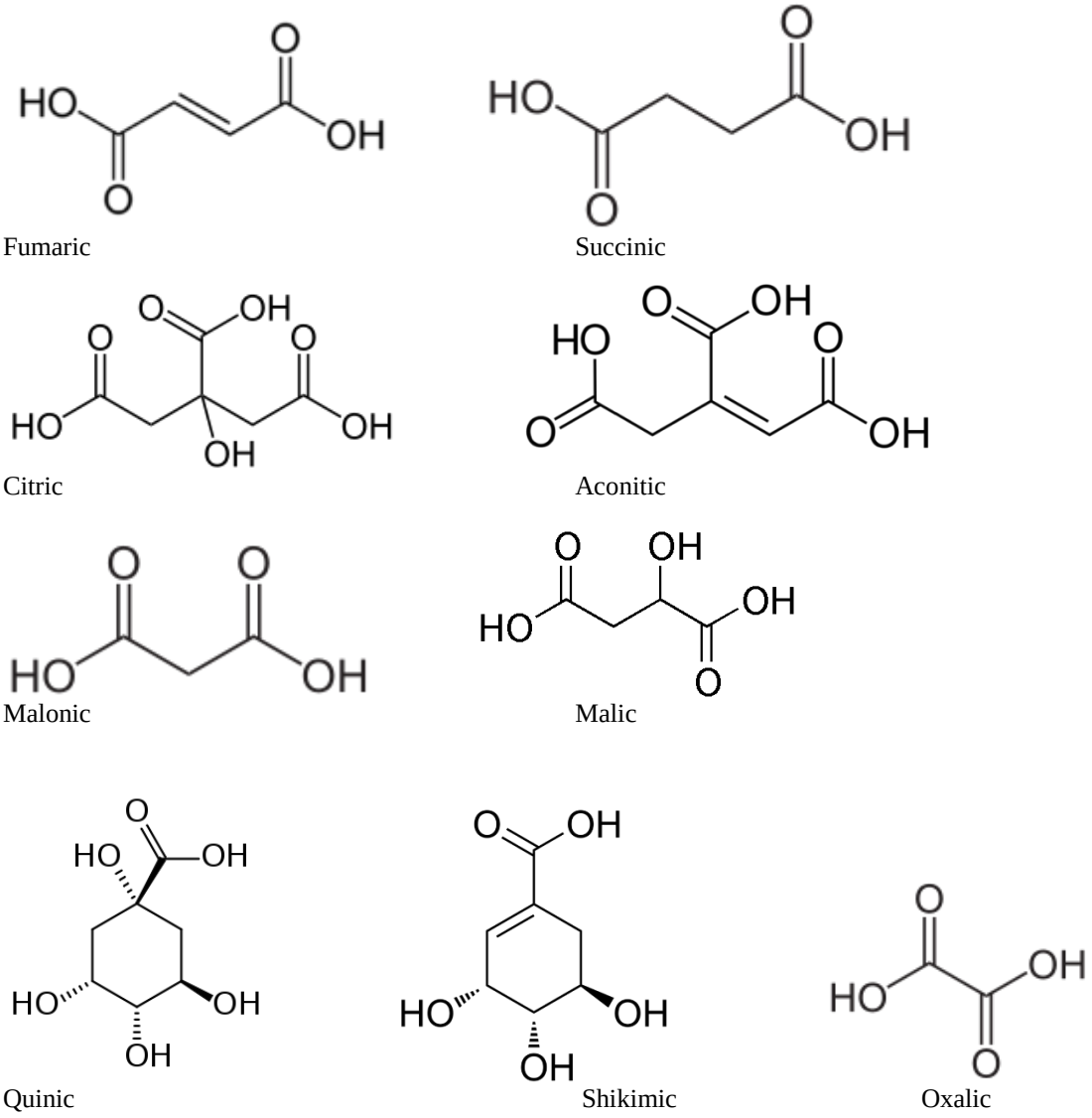

Figure 1 Structural formulas of some important plant organic acids.

of shikimic acid (Marsh et al., 2009), which in its turn is a precursor of aromatic amino acids. Both quinic and shikimic acid are involved in synthesis of a range of secondary compounds (Thoge et al., 2013). Oxalic acid accumulates in many plants as calcium oxalates and play a role in calcium regulation, protection from grazing, metal detoxification, etc. (Nakata, 2003). Cis- and trans-isomers of aconitic acid, with the latter dominating, play a role in disease resistance and protection against insects and Al toxicity (Rémus-Borel et al., 2009).

The scant information on OA levels in forage plants is generally from older studies while non-forage plants have been studied from metabolism points of view. Dijkshoorn (1973) reviewed forages and found total levels of OA of 13–72 in temperate grasses and 30–100 g/kg DM in legumes. Metabolic intermediates of the TCA cycle dominated but also shikimic, quinic and malonic acids were present. In a study of Udén (unpubl.), quinic, malonic, malic

and citric acid dominated in the 20 grass, clover, whole crop wheat and barley crops, but not in maize. In maize, aconitic and oxalic acid were instead present. In the same study, total OA levels were 65 (51–98) and 44 (14-70) g/kg DM for leys and whole crops, respectively. These levels corresponded to approximately 40% of the non-WSC proportion of the NorFor RestCHO fraction. Information on oxalate levels in forage plants are more prevalent due adverse effects when consumed by animals. A review was published by Libert and Franceschi (1987). Levels as high as 100-160 g/kg DM have been found in beet (Beta vulgaris) tops and in spinach (Spinacia oleracea); 10-40 g/kg DM in some C-4 grasses but <10 g/kg DM in legumes. In a study of Mengistu (2001) with cactus (Opuntia ficus indica), analysis of cladodes showed 109 g oxalic acid/kg DM (unpubl.).

The nutritional value of OA to ruminants is not well understood. The majority should be metabolised in the rumen (Van Soest, 1994) even though they may not all serve as energy sources for the host animal and even require detoxification in the liver. Russell and Van Soest (1984) demonstrated rapid *in vitro* fermentation of citric, malic and aconitic acid and that malonic, quinic and shikimic acids disappeared relatively slowly *in vitro* with nearly half of malonic acid still present after 30 h. Martin (1982) found that quinic and shikimic acids were partially metabolized in the rumen of sheep and excreted as benzoic acid in urine. However, little is known about malonic acid metabolism *in vivo*. Oxalic acid can be metabolized in the rumen and up to 40 g/d can be tolerated by adapted sheep, according to Allison et al. (1977). Albeit, a high dietary proportion of cactus, high in oxalic acid, lowered urinary pH markedly and also gave signs of diarrhoea in sheep, probably as a result of insufficient metabolism in the rumen (Mengistu, 2001). This could potentially cause urinary calculi after prolonged consumption of cactus rich diets. Ingestion of trans-aconitic acid has been of interest to ruminant nutritionists. It is metabolized to tricarbalyllic acid and that tricarbalyllic acid is absorbed into the blood (Russell and Van Soest, 1984), which could cause inhibition of acetate oxidation in the liver (Russell and Forsberg, 1986). Also grass tetany has been implicated from ingestion of trans-aconitic acid (Koseki and Takahashi, 1980) as a result of chelation of Mg in the blood by tricarbalyllic acid. However, the quantitative importance of this is uncertain (Cook et al., 1994).

Estimation of metabolisable energy (ME) values of OAs are not simple. It is likely that citric and malic acids are completely converted to volatile fatty acids (VFA) but that quinic and malonic acid have considerably lower conversion factors in the rumen, on the order of 20%. Using data from Russell and Van Soest (1984) and assuming a complete conversion of VFA energy to ME, an OA mixture of an average ley crop from Udén (unpubl.) may contribute on the order of 0.4 MJ ME/kg crop DM.

Silage metabolism

Forage ensilability is partially dictated by substrate availability and buffering capacity. Pectin and OA concentrations may be of some importance in these respects. Ensiling in mini-silos caused an average 36% reduction of pectin in the 20 forages examined by Udén (2017). This was contrary to Ben-Ghedalia and Yosef (1989) and Ben-Ghedalia et al. (1993) who found no pectin fermentation during ensiling. Reasons for these contradictory results are not known. Organic acids are fermented in the silo. Playne and McDonald (1966) found approximately 90% loss of malic and citric acids in unwilted ryegrass and red clover while Udén (unpubl.) only found approximately 45% loss of these acids and an average of 35% loss of total OA (Udén, 2017). However, this latter value included a 7.5-fold increase of succinic acid in the

silages from succinic acid production by the microorganisms (McDonald et al., 1991). Similar to WSC, wilting also causes a loss of OA (Playne and McDonald, 1966), which may be due to volatilization and plant respiration.

Organic acids could have a buffering effect during silage fermentation but not pectins as galacturonic acid has a pKa value of 3.5 (Kohn and Kovac, 1978). The common plant OA have one pKa value above 5.5 making them relatively potent buffers during ensiling. But, as at least citric and malic acid also serve as substrates, fermentation of these acids would lower buffering capacity in the region of pH 4-6 (Playne and McDonald, 1966). Unfortunately, the resulting negative cation-anion balance would require additional fermentation acids such as lactic and acetic acid, which would restore buffering capacity (Playne and McDonald, 1966).

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A comparison of first, second and third cut timothy silages in the diets of finishing beef bulls

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Introduction

Grass silage is the basic component of diets for growing cattle in Northern Europe. A three-cut strategy, which provides better utilisation of the entire growing season than a two-cut strategy (Hyrkäs et al., 2015), is becoming more common also in northern Finland. Hyrkäs et al. (2015) reported that over the whole growing season, three cuts provided higher average digestibility than two cuts. Thus, the feeding value of the autumn cut grass silage should be high. However, in some recent studies third cut silage has resulted in lower milk production than expected based on feed analysis (Sairanen & Juutinen, 2013; Sairanen et al., 2016). Sairanen et al. (2016) concluded that the high energy content of the autumn cut silage was not realised as milk production and a low total feed dry matter intake (DMI) was the main reason for the relatively low milk yield with third cut silage. Furthermore, Sairanen et al. (2016) speculated that undesirable weather conditions during autumn could affect the field flora negatively resulting in a poor silage quality and a decreased silage intake. These types of quality parameters do not necessarily affect chemical analysis and cause variation in experimental results.

For growing cattle, the importance of grass silage digestibility has been demonstrated by Steen et al. (1998) and Huuskonen et al. (2013). Huuskonen et al. (2013) observed that the effects of forage quality characteristic on DMI are quite similar in growing cattle and dairy cows. However, there is a lack of published information on performance of finishing bulls when autumn cut grass silage harvested under northern climatic conditions is used in feeding. Therefore, the objective of the present experiment was to study the effects of the third cut grass silage compared with the first and second cut silages on intake, performance and carcass characteristics of finishing bulls. Based on earlier milk production experiments (Sairanen & Juutinen, 2013; Sairanen et al., 2016) it was hypothesised that DMI and gain of the autumn cut silage fed bulls would be lower than expected based on feed analysis.

Materials and Methods

A feeding experiment was conducted using 45 Simmental bulls with an initial live weight (LW) of 475 (±36.8) kg. At the start of the feeding experiment, the animals were on average 328 (±13.9) days old. During the experiment, the bulls were housed in an uninsulated barn in pens (10.0 × 5.0 m; 5 bulls in each pen), providing 10.0 m^2/bull. A GrowSafe feed intake system (model 4000E; GrowSafe Systems Ltd., Airdrie, AB, Canada) was used to record individual daily feed intakes so that each pen contained two GrowSafe feeder nodes.

Experimental grass silages were produced at the experimental farm of Natural Resources Institute Finland (Luke) in Ruukki (64°44'N, 25°15'E) and harvested in three cuts from a first year timothy (*Phleum pratense* cv. Tuure) stand at early heading on 25 June, 11 August and 3 October 2015. All silages were cut by a mower conditioner (Elho 280 Hydro Balance), harvested with an integrated round baler wrapper (McHale Fusion 3) 24 hours after cutting and treated with a formic acid-based additive (AIV ÄSSÄ; Eastman Chemical Company,

Oulu, Finland; 589 g formic acid, 199 g propionic acid, 43 g ammonium formate and 25 g potassium sorbate per kg) applied at a rate of 5.8 kg/t of fresh forage.

At the beginning of the feeding experiment, the bulls were randomly allotted to pens which were then randomly allotted to three feeding treatments (three pens and 15 bulls per treatment). The three dietary treatments included either first (GS1), second (GS2) or third cut (GS3) grass silage (550 g/kg DM), rolled barley (435 g/kg DM) and a mineral-vitamin mixture (15 g/kg DM). The bulls were fed a total mixed ration *ad libitum* allowing approximately 5% refusals. Total mixed rations were fed using a mixer wagon once a day.

During the feeding experiment, silage sub-samples were taken twice a week, pooled over periods of approximately four weeks and stored at –20 ºC prior to analyses. Thawed samples were analysed for DM, ash, crude protein (CP), neutral detergent fibre (NDFom) exclusive of residual ash, silage fermentation quality [pH, water-soluble carbohydrates (WSC), lactic and formic acids, volatile fatty acids (VFA), soluble and ammonia N content of total N], and digestible organic matter (DOM) in DM (D-value) as described by Pesonen et al. (2013). Barley sub-samples were collected weekly, pooled over periods of eight weeks and analysed for DM, ash, CP and NDFom. The metabolisable energy (ME) concentration of the silages was calculated as 0.016 × D-value. The ME concentration of barley was calculated based on concentrations of digestible crude fibre, CP, crude fat and nitrogen-free extract described by Luke (2017). Amino acids absorbed from small intestine (AAT) and protein balance in the rumen (PBV) were calculated according to Luke (2017). The relative intake potential of silage DM (SDMI index) was calculated as described by Huhtanen et al. (2007).

The bulls were weighed on two consecutive days at the beginning of the experiment and thereafter approximately once every 28 days. Before slaughter, the bulls were weighed on two consecutive days. The target for average carcass weight was 400–410 kg, which is currently the average carcass weight for slaughtered Simmental bulls in Finland. The bulls were selected for slaughter based on LW, and slaughtered in the Atria Ltd. commercial slaughterhouse in three batches. All feeding treatments were represented in all batches. After slaughter, the carcasses were weighed hot. The cold carcass weight was estimated as 0.98 of the hot carcass weight. The carcasses were classified for conformation and fat using the EUROP classification.

The data were subjected to analysis of variance using the SAS GLM procedure. The statistical model used was $y_{ijkl} = \mu + \delta_j + \alpha_i + \theta_{ijl} + \beta_{xijk} + e_{ijkl}$, where μ is the intercept and e_{ijkl} is the residual error term associated with kt^h animal. α_i, is the effect of i^{th} diet (GS1, GS2, GS3), while δ_j is the effect of the slaughtering batch (j=1, 2, 3) and θ_{ijl} is the effect of pen. The effect of pen was used as an error term when differences between treatments were compared because treatments were allocated to animals penned together. Initial LW was used as a covariate (β_{xijk}) in the model for intake, gain and feed conversion parameters. When the dressing proportion, carcass conformation and carcass fat score were tested, carcass weight was used as a covariate. Tukey's t-test was applied for multiple comparison among the treatment means considering $P<0.05$ as significant.

Results

Due to the weather conditions during harvesting, DM concentrations of the second cut and third cut silages were 47 and 41% higher compared to the first cut silage, respectively (Table 1). According to the feed analyses, the third cut silage had 5–7% higher ME concentration

and 22–26% higher CP concentration compared to the first and second cut silages. Further, the third cut silage had 16 and 10% higher SDMI index compared to the first and second cut silages, respectively.

The fermentation characteristics of all three silages were good, as indicated by the low concentrations of ammonia N in total N and total fermentation acids (Table 1). All silages were restrictively fermented with a high residual WSC concentration and low lactic acid concentration. However, the second and third cut silages had clearly higher WSC concentrations compared to the first cut silage. Due to differences in composition of the experimental silages, the GS3 ration contained slightly more ME and CP and less NDFom compared to GS1 and GS2 rations. In all rations, the PBV value fulfilled the Finnish recommendation for growing cattle (PBV of the diet above –10 g/kg DM for animals above 200 kg LW).

Table 1 Chemical composition and feeding values of the ingredients and total mixed rations (calculated) used in the feeding experiment

	Feeds				Total mixed rations		
	GS first cut	GS second cut	GS third cut	Barley grain	GS1	GS2	GS3
Number of feed samples	5	5	5	3			
Dry matter (DM), g/kg feed	222	326	314	872	334	453	441
Organic matter (OM), g/kg DM	945	932	917	971	958	949	940
Crude protein, g/kg DM	152	147	186	115	135	132	154
Neutral detergent fibre, g/kg DM	592	533	446	211	420	388	340
Metabolisable energy, MJ/kg DM	11.2	11.0	11.8	12.9	12.0	11.8	12.3
AAT, g/kg DM	85	82	92	95	90	88	93
PBV, g/kg DM	26	24	49	-27	2	1	15
Digestible OM in DM, g/kg DM	701	685	740	821	755	746	776
Silage DM intake index	99	105	115				
Fermentation quality of the experimental silages							
pH	3.90	4.26	4.56				
Volatile fatty acids, g/kg DM	15	8	8				
Lactic + formic acid, g/kg DM	49	37	32				
WSC, g/kg DM	65	115	148				
Ammonium N, g/kg N	66	56	53				
Soluble N, g/kg N	543	485	427				

GS = grass silage; GS1 = first cut grass silage (550 g/kg DM), rolled barley (435 g/kg DM), mineral-vitamin mixture (15 g/kg DM); GS2 = second cut grass silage (550 g/kg DM), rolled barley (435 g/kg DM), mineral-vitamin mixture (15 g/kg DM); GS3 = third cut grass silage (550 g/kg DM), rolled barley (435 g/kg DM), mineral-vitamin mixture (15 g/kg DM); AAT = Amino acids absorbed from small intestine; PBV = Protein balance in the rumen; WSC = Water soluble carbohydrates

The feeding experiment lasted 128 days and slaughter age of the bulls was 456 days on average (Table 2). Daily DMI was approximately 11% higher when GS1 and GS3 were used instead of GS2. There were no differences in feed intake between GS1 and GS3 treatments. Energy intake of the GS1 and GS3 bulls was 12 and 15% higher, respectively, compared to the GS2 bulls. There were no differences in ME intake between GS1 and GS3. Due to

differences in CP composition of the experimental silages, GS3 bulls received clearly more CP compared to the GS1 and GS2 bulls.

The average live weight gain (LWG) and carcass gain of the bulls was 2021 and 1257 g/d, respectively. Both LWG and carcass gain of the GS1 and GS3 bulls was approximately 11% higher compared to the GS2 bulls. There were no differences in growth parameters between GS1 and GS3 treatments. Dietary treatments had no significant effects on DM or energy conversion rates (Table 2). However, CP conversion was better in GS1 and GS2 bulls compared to the GS3 bulls. The carcass weight, dressing proportion, carcass conformation score and carcass fat score of the bulls were, on average, 406 kg, 556 g/kg, 10.2 and 2.3, respectively, and there were no significant differences among the feeding treatments. Nevertheless, the carcass conformation of the GS3 bulls tended to be 9% higher (P<0.10) compared to the GS1 bulls.

Table 2 Intake, growth, feed conversion and carcass traits of the bulls fed different rations

	Diets				
	GS1	GS2	GS3	SEM	*P*-value
Number of bulls	15	15	15		
Duration of the experiment, d	124[a]	131[b]	128[ab]	3.3	0.04
Initial live weight, kg	482	470	472	9.7	0.64
Final live weight. kg	731	721	738	5.9	0.10
Slaughter age, d	449[a]	463[b]	457[ab]	5.0	0.05
Intake					
Dry matter (DM), kg/d	11.47[a]	10.38[b]	11.57[a]	0.299	0.006
Metabolisable energy, MJ/d	138[a]	123[b]	142[a]	3.6	0.01
Crude protein, g/d	1586[a]	1363[b]	1794[c]	41.8	<0.001
Neutral detergent fibre, g/d	4715[a]	3999[b]	3936[b]	119.8	<0.001
Live weight gain, g/d	2097[a]	1883[b]	2082[a]	52.5	0.005
Carcass gain, g/d	1299[a]	1169[b]	1304[a]	36.2	0.01
Feed conversion					
kg DM/kg carcass gain	8.83	8.88	8.87	0.334	0.96
MJ/kg carcass gain	106.2	105.2	108.9	3.98	0.71
g crude protein/kg carcass gain	1221[a]	1166[a]	1376[b]	44.5	0.006
Carcass characteristics					
Carcass weight, kg	406	400	413	4.7	0.15
Dressing proportion, g/kg	554	559	556	3.4	0.40
Conformation score, EUROP	9.7	10.4	10.6	0.26	0.054
Fat score, EUROP	2.4	2.2	2.3	0.12	0.51

GS1 = first cut grass silage (550 g/kg DM), rolled barley (435 g/kg DM), mineral-vitamin mixture (15 g/kg DM); GS2 = second cut grass silage (550 g/kg DM), rolled barley (435 g/kg DM), mineral-vitamin mixture (15 g/kg DM); GS3 = third cut grass silage (550 g/kg DM), rolled barley (435 g/kg DM), mineral-vitamin mixture (15 g/kg DM); SEM = standard error of the mean; Between treatment comparisons (Tukey, P<0.05): estimated means with the different letters were significantly different (P<0.05)

Discussion

Due to the rainy weather conditions during the first cut, DM concentration of the first cut silage was clearly lower compared to the second and third cut silages. This difference also

affected the SDMI index as the meta-analysis by Huhtanen et al. (2007) implied that SDMI is independently affected by silage DM concentration.

In the present experiment, the third cut silage had higher estimated digestibility and SDMI index compared to the first and second cut silages. Based on this, intake and production responses of GS3 should have been higher compared to GS1 and GS2. This was also realised when comparing GS2 and GS3. Nevertheless, there was no difference in DM or energy intake between GS1 and GS3 so SDMI index was not able to predict the differences in DMI in this case. However, in previous large scale meta-analyses, SDMI index has generally predicted silage DMI in both dairy cows (Huhtanen et al., 2007) and growing cattle (Huuskonen et al., 2013) well.

Contrary to the earlier milk production experiments (Sairanen & Juutinen, 2013; Sairanen et al., 2016), DMI was not decreased in the present study when the third cut silage was used instead of the first and second cut silages. The observed daily DMI was clearly higher when the GS1 and GS3 were used instead of the GS2. This may partly have been an effect of digestibility, which was the lowest in the second cut silage. Feed digestibility is one of the most important factors affecting silage intake (Huhtanen et al., 2007). One possible reason for the decreased DMI in GS2 could have been an impaired microbiological quality of the herbage. Earlier, Kuoppala (2010) discussed that microbiological quality of regrowth grass may differ from that of primary growth because regrowth contains more dead plant material. In Finland, weather is typically warmer later in the summer when the second cut is generally harvested, compared to the first cut in early summer or third cut in autumn. Therefore, the second cut may have contained more dead plant material than first and third cuts. However, the occurrence of leaf spot infections was not evaluated in the present study.

Higher daily DMI of the GS1 and GS3 bulls compared to the GS2 bulls was reflected also as larger daily ME and nutrient intakes. Observed difference in ME intake is probably a crucial explanation for the improved LWG and carcass gain of the GS1 and GS3 bulls compared to the GS2 bulls. Based on the meta-analysis of feeding experiments, Huuskonen & Huhtanen (2015) found that energy intake was clearly the most important variable affecting LWG of growing cattle, whereas they showed only marginal effects of protein supply on growth.

Protein conversion rate was better in GS1 and GS2 bulls compared to the GS3 bulls because also in GS1 and GS2 rations the PBV value fulfilled the protein recommendation for growing cattle. Therefore, the bulls could not utilise the additional protein obtained through feeding the third cut silage. A recent meta-analysis (Huuskonen et al., 2014) indicates that the currently recommended PBV could even be reduced without adverse effects on performance.

Previous reports have shown no effects of silage digestibility on dressing proportion, carcass conformation and fat scores (e.g. Cummins et al., 2007; Manninen et al., 2011). However, increasing energy intake has often increased carcass conformation and carcass fatness of finishing cattle (Pesonen et al., 2013; Huuskonen & Huhtanen, 2015) but these effects could not be demonstrated in the present experiment.

Conclusions

Daily DM and energy intakes, as well as growth rates of the bulls, increased when either first or third cut timothy silages were used in total mixed ration instead of a second cut silage. This was probably due to digestibility, which was lowest in the second cut silage. There were no differences in intake or growth between the first and the third cut silage based rations,

although based on feed analysis, the third cut silage had a higher digestibility and DM intake index compared to the first cut silage. Thus, as hypothesised, intake and growth of the autumn cut silage fed bulls was lower than expected based on feed analysis.

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Effects of silage additives on intake, gain and carcass traits of growing and finishing dairy bulls fed grass silage and barley grain based rations

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Introduction

Voluntary feed intake of cattle has a great impact on animal performance and energy intake is the most important variable affecting growth performance of growing cattle (Huuskonen and Huhtanen, 2015). Improving fermentation quality of silage has been shown to increase feed intake and performance. In a meta-analysis of data from silage fermentation studies in dairy cows, Huhtanen et al. (2003) observed that both the extent and type of in-silo fermentation influenced milk production variables. However, in favourable harvesting conditions wilting grass to a dry matter (DM) content of 300 g/kg can support achievement of relatively good fermentation quality and feeding value also without additives (Heikkilä et al., 2010). Nevertheless, in spite of low butyric acid and ammonia N content of untreated bale silage having relatively high DM content (380 g DM/kg), use of inoculants or formic acid based additive improved milk production and sensory quality of milk (Heikkilä et al., 1997) which demonstrates that fermentation parameters of high DM silage insufficiently describe the value of silage in animal production.

Relative to dairy cows, there are only few reports available where high DM silages treated with different additives have been fed to growing and finishing bulls. Therefore, the objective of the present experiment was to study effects of two silage additives compared with a control silage without any additive on intake, animal performance and carcass characteristics of growing and finishing dairy bulls. It was hypothesized that the use of additives would increase the feed intake and gain and improve carcass traits of bulls. We also hypothesized that there would be no interactions between additive treatments and breed on growth performance and carcass characteristics.

Materials and Methods

A feeding experiment was conducted using 45 Nordic Red (NR) and 45 Holstein (HO) bulls with an initial live weight (LW) of 290 (±24.5) kg. During the experiment, bulls were housed in an uninsulated barn in pens (10.0 × 5.0 m; 5 bulls in each pen), providing 10.0 m^2/bull. A GrowSafe feed intake system (model 4000E; GrowSafe Systems Ltd., Airdrie, AB, Canada) was used to record individual daily feed intakes so that each pen contained two GrowSafe feeder nodes.

Experimental silages were produced at the experimental farm of Luke in Ruukki (64°44'N, 25°15'E) and harvested from timothy (*Phleum pratense*) stands (on 18 June and 6 August 2014, primary growth and regrowth, respectively). The stands were cut by a mower conditioner (Elho 280 Hydro Balance, Pännäinen, Finland) and harvested with an integrated round baler wrapper (McHale Fusion 3, Ballinrobe, Ireland) approximately 24 hours after cutting.

Three additive treatments were used: (i) control without additives (CON), (ii) a commercial additive containing sodium benzoate (200 g/kg), potassium sorbate (100 g/kg) and sodium nitrite (50 g/kg) (Safesil/Ab Hanson & Möhring, Sweden) applied at a rate of 3.4 kg/t of fresh forage (SALT), and (iii) a commercial additive containing formic acid (589 g/kg), propionic acid (199 g/kg), ammonium formate (43 g/kg) and potassium sorbate (25 g/kg) (AIV ÄSSÄ/ Eastman Chemical Company, Finland) applied at a rate of 5.8 kg/t of fresh forage (ACID).

At the beginning of the feeding experiment, both NR and HO bulls were randomly allotted to pens (animals from the same breed were housed together) which were then randomly allotted to three feeding treatments (CON, SALT, ACID; three NR pens and three HO pens per treatment; 30 bulls per treatment). The diets included experimental silages (600 g/kg DM), rolled barley grain (385 g/kg DM) and a mineral-vitamin mixture (15 g/kg DM). The primary growth of timothy was fed during the early part of the feeding experiment (135 days) and regrowth during the late part of the experiment (124 days). Thus, the whole feeding experiment lasted 259 days. The bulls were fed a total mixed ration *ad libitum* (proportionate refusals of 5%). Two HO bulls (one SALT and one ACID bull) were excluded from the study due to pneumonia and one HO bull (SALT) due to several occurrences of bloat.

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. Thawed samples were analysed for DM, ash, crude protein (CP), neutral detergent fibre (NDFom) exclusive of residual ash, silage fermentation quality [pH, water-soluble carbohydrates (WSC), lactic and formic acids, ethanol, volatile fatty acids and ammonia N content of total N], and digestible organic matter (DOM) in DM (D-value) as described by Seppälä et al. (2016). Barley sub-samples were collected weekly, pooled over periods of eight weeks and analysed for DM, ash, CP and NDFom. Metabolisable energy (ME) concentration of the silages was calculated as 0.016 × D-value. The ME concentration of barley was calculated based on concentrations of digestible crude fibre, CP, crude fat and nitrogen-free extract described by Luke (2017). Amino acids absorbed from small intestine (AAT) and protein balance in the rumen (PBV) values were calculated according to Luke (2017). The relative intake potential of silage DM (SDMI index) was calculated as described by Huhtanen et al. (2007).

The bulls were weighed on two consecutive days at the beginning of the experiment, in the middle of the experiment when silages were changed and before slaughter. All bulls were slaughtered on the same day, and the target for average carcass weight was 330–335 kg which was the average carcass weight for slaughtered dairy bulls in Finland. After slaughter, carcasses were weighed hot. Cold carcass weight was estimated as 0.98 of hot carcass weight. Dressing proportions were calculated from the ratio of cold carcass weight to final LW. Carcasses were classified for conformation and fat using the EUROP classification.

Data was subjected to analysis of variance using the SAS GLM procedure. The model used was $y_{ijkl} = \mu + \alpha_i + \gamma_j + (\alpha \times \gamma)_{ij} + \theta_{ijl} + \beta_{xijk} + e_{ijkl}$, where μ is the intercept and e_{ijkl} is the residual error term associated with l^{th} animal. α_i, γ_j and $(\alpha \times \gamma)_{ij}$ are the effects of i^{th} diet (CON, SALT, ACID) and j^{th} breed (NR, HO) and their interaction, respectively, while θ_{ijl} is the effect of pen. The effect of pen was used as an error term when differences between treatments (diet, breed and their interaction) were compared because treatments were allocated to animals penned together. Initial LW was used as a covariate (β_{xijk}) in the model for intake and gain parameters. When dressing proportion, carcass conformation and carcass fat score were tested, carcass weight was used as a covariate. Differences between treatments

were tested using orthogonal contrasts: (i) NR vs. HO, (ii) CON vs. additives (SALT + ACID), (iii) SALT vs. ACID, (iv) interaction between breed and (ii) and (v) interaction between breed and (iii). As the interactions between breed and feeding treatments were not statistically significant, the *P*-values of the interactions are not presented.

Results

Relatively high average DM contents (362 and 389 g/kg for primary growth and regrowth, respectively) were achieved (Table 1). There were only minor differences in chemical composition and feeding values among additive treatments. Fermentation characteristics of all silages were good as indicated by low pH values and low concentrations of ammonia-N in total N. Further, low concentrations of total fermentation acids suggested high intake potential (Huhtanen et al., 2007). All silages were restrictively fermented with high residual WSC concentration and relatively low lactic acid concentrations. There were some differences in fermentation characteristics among treatments. The ACID silage contained slightly less lactic acid and acetic acid and more WSC compared to CON and SALT silages. The ACID additive contained some propionic acid and ammonia, but results presented in Table 1 have not been corrected for amounts added.

Table 1 Chemical composition and feeding values of timothy silages and barley grain

	Silage primary growth			Silage regrowth			Barley
	CON	SALT	ACID	CON	SALT	ACID	
Number of feed samples	5	5	5	4	4	4	5
Dry matter (DM), g/kg feed	356	358	371	390	399	378	882
Organic matter (OM), g/kg DM	933	936	942	925	921	926	971
Crude protein, g/kg DM	162	154	161	177	174	173	122
Neutral detergent fibre, g/kg DM	528	542	535	564	578	564	211
Metabolisable energy, MJ/kg DM	11.2	11.1	11.2	9.9.	9.7	9.9	13.2
AAT, g/kg DM	88	87	88	85	84	85	98
PBV, g/kg DM	31	24	29	51	51	47	-25
Digestible OM in DM, g/kg DM	698	695	703	618	604	617	
Silage DM intake index	110	110	114	95	93	97	
Fermentation quality of experimental silages							
pH	4.24	4.28	4.27	4.59	4.74	4.52	
Lactic acid, g/kg DM	55.6	49.3	37.4	39.8	33.1	27.2	
Formic acid, g/kg DM	0.1	0.1	3.6	0.1	0.1	5.4	
WSC, g/kg DM	79.4	94.8	129.5	53.5	53.7	73.3	
Ethanol, g/kg DM	8.8	7.1	6.8	3.8	2.5	2.6	
Volatile fatty acids, g/kg DM	14.6	12.7	11.1	14.5	15.7	10.9	
Acetic acid, g/kg DM	13.5	11.9	9.1	13.5	14.7	8.3	
Propionic acid, g/kg DM	0.41	0.33	1.44	0.30	0.42	1.92	
Butyric acid, g/kg DM	0.35	0.30	0.29	0.36	0.29	0.34	
Ammonium N, g/kg N	54.8	52.4	47.5	66.2	71.2	56.6	

CON = silage without additives; SALT = silage with sodium benzoate, potassium sorbate and sodium nitrate based additive; ACID = silage with a mixture of mostly formic acid and propionic acid based additive; AAT = Amino acids absorbed from small intestine; PBV = Protein balance in the rumen; WSC = Water soluble carbohydrates.

Breed did not affect intake during early or late part or total experimental period but additive treatments affected intake parameters (Table 2). During the early part of the experiment DM and energy intake of CON bulls was 7% higher ($P<0.01$) compared to SALT and ACID bulls. However, there were no differences between SALT and ACID treatments in feed or nutrient intake. During the late part of the experiment there were no treatment differences in DM or ME intakes (Table 2). During the total experimental period, average DM and ME intakes were 10.1 kg/d and 117 MJ/d, respectively. No significant differences among treatments were observed.

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

	Diet			Breed		SEM	Contrasts (*P*-values)		
	CON	SALT	ACID	NR	HO		1	2	3
Number of bulls	30	28	29	45	42	-	-	-	-
Slaughter age, d	508	511	509	510	508	1.7	0.35	0.43	0.47
Dry matter (DM) intake, kg/d									
Early part (135 d, primary growth of timothy)	9.51	9.03	8.76	9.18	9.06	0.136	0.43	0.002	0.15
Late part (124 d, regrowth of timothy)	11.07	11.46	11.29	11.41	11.15	0.209	0.30	0.26	0.57
Total experimental period (259 d)	10.23	10.18	9.96	10.21	10.03	0.152	0.32	0.39	0.32
Metabolisable energy intake, MJ/d									
Early part	114	108	106	110	109	1.6	0.43	0.002	0.24
Late part	125	128	127	128	125	2.3	0.30	0.35	0.89
Total experimental period	119	117	116	119	116	1.8	0.32	0.27	0.54
Live weight (kg)									
Initial	287	295	290	289	293	2.2	0.63	0.38	0.49
Middle (after 135 days)	503	496	496	500	498	3.9	0.67	0.24	0.75
Final	650	641	636	643	642	5.6	0.90	0.17	0.43
Live weight gain (LWG), g/d									
Early part	1601	1490	1524	1558	1519	26.4	0.23	0.01	0.43
Late part	1185	1167	1130	1157	1166	24.0	0.74	0.27	0.29
Total experimental period	1408	1340	1341	1371	1355	20.5	0.52	0.02	0.94
Carcass gain, g/d	747	736	741	750	732	14.8	0.33	0.64	0.81
Feed conversion rate (total experimental period)									
Kg DM/kg LWG	7.23	7.60	7.43	7.45	7.40	0.186	0.76	0.36	0.43
MJ/kg LWG	84.5	87.3	86.5	86.8	85.6	2.15	0.76	0.44	0.58
Kg DM/kg carcass gain	13.69	13.83	13.44	13.61	13.70	0.408	0.98	0.76	0.35
MJ/kg carcass gain	159.3	159.0	156.5	158.7	158.5	4.73	0.99	0.66	0.46
Carcass characteristics									
Carcass weight, kg	336	336	333	337	334	3.7	0.45	0.84	0.54
Dressing proportion, g/kg	517	524	524	524	519	2.9	0.33	0.16	0.95
Conformation, EUROP	4.77	5.11	5.00	5.13	4.79	0.083	0.004	0.01	0.29
Fat score, EUROP	2.60	2.43	2.38	2.42	2.51	0.102	0.56	0.21	0.79

SEM = standard error of the mean; CON = timothy silage without additives (600 g/kg DM), rolled barley (385 g/kg DM) and mineral-vitamin mixture (15 g/kg DM); SALT = timothy silage with sodium benzoate, potassium sorbate and sodium nitrate based additive (600 g/kg DM), barley (385 g/kg DM) and mineral-vitamin mixture (15 g/kg DM); ACID = timothy silage with a mixture of mostly formic acid and propionic acid based additive (600 g/kg DM), barley (385 g/kg DM) and mineral-vitamin mixture (15 g/kg DM); NR = Nordic Red; HO = Holstein; orthogonal contrasts: 1 = NR vs. HO, 2 = CON vs. additives (SALT + ACID), 3 = SALT vs. ACID.

Breed had no effects on LWG or carcass gain of the bulls (Table 2). During the early part of the experiment, LWG of CON bulls was 6% higher ($P=0.01$) than that of SALT and ACID bulls but there was no difference between SALT and ACID bulls. There were no treatment

differences in LWG during the late part of the experiment. During the total experimental period, LWG of CON bulls was 5% higher ($P<0.05$) compared to bulls fed additive treated silages but there were no treatment differences in carcass gain and energy conversion rates among treatments.

Average carcass weight and dressing proportion was 335 kg and 522 g/kg, respectively, and there were no differences among treatments. Carcass conformation of SALT and ACID bulls was 6% higher compared to CON bulls but there was no difference between SALT and ACID bulls. There were no differences in carcass fat score among additive treatments (Table 2) but conformation score of NR bulls was 7% higher compared to HO bulls ($P<0.01$) but there was no difference in carcass fat score between breeds.

Discussion

In the present experiment, CON silages had good fermentation quality showing relatively low pH, restricted amount of fermentation products and low amounts of ammonia-N. Increasing DM content by wilting has been shown to restrict extent of fermentation (Heikkilä et al., 2010; Seppälä et al., 2016), which reduces possibilities to manipulate fermentation by silage additives. However, Seppälä et al. (2013) showed that formic acid can still restrict fermentation when silage DM is as high as 340–360 g/kg. Similar to the current trial, Heikkilä et al. (2010) concluded that wilting grass to a DM content above 300 g/kg supports achievement of relatively good fermentation quality even without use of silage additives. Also, Seppälä et al. (2016) stated that for high DM silages (504–573 g DM/kg) benefits of additives were not clear. More positive effects on silage quality parameters with the same SALT treatment as in the present study were detected by Knicky and Spörndly (2009). Their observations indicate that a mixture of sodium benzoate, potassium sorbate and sodium nitrate efficiently improved silage quality for crops with both high and low DM content.

Explanation for a decreased feed intake of bulls fed additive treated silages during the early part of the experiment is unclear. Although the primary growth of the ACID treatment had slightly higher SDMI index compared to CON silage, total DMI of the CON bulls was higher compared to ACID bulls. Higher daily DMI of CON bulls during the early part of the experiment compared to SALT and ACID bulls was reflected also in higher daily ME intakes. Observed difference in ME intake is probably a crucial explanation for improved LWG of CON bulls compared to bulls fed with treated silages. In spite of improved LWG of CON bulls, there were no differences in carcass gain among treatments. Previous results comparing untreated and treated silages in diets of growing cattle are somewhat conflicting. Agnew & Carson (2000) reported that additive treatment increased carcass gain when cattle received no concentrate. However, there was no increase in gain with supplement levels above 1.5 kg/d. O'Kiely & Moloney (1994) observed that formic acid and an acid-complex increased LWG in Experiment 1. However, in their Experiment 2, additives had no effects on overall LWG. Generally, the significance of silage fermentation quality is highlighted if silage is fed as a sole feed.

Conclusions

Use of silage additives did not increase feed intake of the bulls. Furthermore, there were no differences in carcass gain or feed conversion among additive treatments. However, use of additives improved carcass conformation. The experiment demonstrated that there was only

little benefit from silage additives in animal performance when timothy silage was successfully ensiled in round bales at DM concentration of 350–400 g/kg.

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In situ dry matter degradation kinetics of tropical forages

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Introduction

Information on the dynamics of organic matter (OM) degradation in the rumen is a major determinant of energy and nutrient supply to ruminants from fiber-rich forages. This type of information is also the basis for ration formulation and for prediction of metabolizable nutrient and energy intakes in many feed evaluation systems such as National Research Council (NRC, 2001), Cornell Net Carbohydrate and Protein System (CNCPS; Fox et al., 2004) and the Nordic Feed Evaluation System (NorFor; Volden, 2011). Current methods of ration formulation in Pakistan (Jabbar *et al.*, 2013) use nutrient availability values reported in foreign based feed evaluations systems (e.g. NRC, 2001). These values may not be correct in circumstances where environment, agronomic, animal and dietary conditions are different from those which form the basis for these feed evaluation systems. As a consequence, animals are often under- or over-fed, resulting in lower feed efficiency and economic losses to the farmers. Therefore, accurate estimates of the coefficients of OM and nutrient degradation of local feeds in the rumen are required under the local conditions. The *in situ* technique is widely used to study the fractional rate of ruminal disappearance of OM and various nutrients (Ørskov and McDonald, 1979) and despite some limitations, it utilizes the actual ruminal environment (Nocek, 1988) and is considered more reliable for measuring rumen degradation by the different feed evaluation systems than the *in vitro* techniques. The objective of this study were to: 1) evaluate chemical composition and dry matter degradability of commonly used tropical forages as affected by forage species and forage family and 2) determine relationship between in situ parameters and effective rumen degradability values.

Materials and Methods

A total of 30 forage samples including 18 cereal and 12 legume fodders were collected from three locations i.e. Rawalpindi, Lahore and Bahawalpur representing northern, central and southern regions of the Punjab province, respectively. Three replicate samples (~ 10 kg each) were taken by harvesting three randomly selected areas (~ 100 m apart) in each location. The harvested herbage was chopped and laid down under shade to reduce moisture content within recommended range for drying e.g. for three to seven days. The dried samples were ground to pass a 2-mm screen using a hammer mill (POLYMIX® PX-MFC, Kinematica AG, Germany) and incubated in the rumen of each of eight rumen cannulated (Bar Diamond, Parma, ID, USA) lactating buffaloes and cattle (four from each species). The incubations started in June 2016 and continued until October, 2016. *In situ* dry matter (DM) degradation parameters for all feed samples were determined according to NorFor standards (Åkerlind *et al.*, 2011; Volden, 2011). In brief, about 1 g air dried and milled feed samples, were incubated for 0, 4, 8, 16, 24 or 48 h in sewed and glued Polyester (Dacron) bags 11 × 8.5 cm^2 (10 × 7.5 effective size), pore size 33 µm (PES material 140/35 with 25% open bag area, Beyaztaş PES 140/35

(Beyaztaş Fabrika Malzemeleri San. Ve. Tic. AŞ, Istanbul, Turkey). At end of each incubation interval, bags were washed with tap water and stored at -18ºC. After all bags had been removed, they were thawed and washed in a washing machine twice for 12 min each with tap water (25ºC). Residues were dried at 100ºC for 24 h to determine DM loss.

The cannulated animals (daily milk yield of cows: 3.34±0.271 kg/day and buffaloes: 5.63±0.207 kg/day) were offered a standard diet at maintenance level as per NorFor standards for cannulated animals. The diet consisted (g/kg DM) of fresh green forage of sorghum (844), lucern hay (88), cotton seed cake (30) and a commercial concentrate mixture (37) with a roughage to concentrate ratio 80:20 on a DM basis. The chemical composition was (g/kg DM); DM (302), crude protein (CP; 58), ether extract (EE; 18), neutral detergent fibre (aNDF, 580) non-fibre carbohydrates (NFC; 230) and ash (113). All animals were tied up, individually fed and given access to fresh clean water as per requirements. The cannulated animals were kept at Livestock Farm of University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur and cared for according to the instructions of the Animal Care and Management Committee. Diet allocation was 30 g DM/kg body weight. However, actual intake was 20 g DM/kg body weight. .

Dry matter of fresh forages was measured after drying at 60ºC for 48 h. The DM of dry feeds (both ration and in situ incubation) was reported after drying at 105ºC for 16 h (Association of Official Analytical Chemists (AOAC), 1984; method 7.003), Ash was analyzed at 525ºC for 6 h (AOAC, 1984; method 923.03), CP (AOAC 1984; method 7.015) and EE (AOAC 1984; method 7.062). The aNDF concentration (ash included) was determined using the method of Van Soest *et al.* (1991) as modified by Mertens *et al.* (2002) with the addition of sodium sulphite and heat-stable alpha-amylase CP (CAS No. 9000-90-2, Junsei Chemicals, Japan).

In situ degradation data were divided into a washable fraction (a; the 0 h) and non-washable fraction. The non washable fraction was further divided into potentially degradable (b) and indigestible fraction, represented as the disappearance and residue at final incubation interval, respectively. The *in situ* degradation data were fitted to a first-order kinetic model:

$Yt = a + b \times (1\text{-}exp\ (-kd \times t))$

The model was fitted using Table Curve 2D (ver. 5.0®, SPSS Inc. NY). Yt denotes the degraded fraction at a given time t and kd denotes the fractional degradation rate of fraction b. Effective ruminal DM degradability (DMD1) was calculated as $DMD1 = a + b \times kd/(kd + kp)$, assuming the fractional rate of passage (kp) to be 0.05/h for forages (a 20-h rumen retention time), as used in several protein evaluation systems (e.g. Madsen *et al.*, 1995; Hvelplund and Weisbjerg, 2000). Dry matter degradability (DMD2) was calculated from the *in situ* data according to a 2-compartment model as suggested by Allen and Mertens (1988),

$DMD2 = a + [(b \times kd)/(kd+kp) \times (1+kp/kd+kr)]$,

in which kd = fractional rate of DM degradation (1/h), kp = fractional rate of passage [a value of $1/(0.6 \times 20) = 0.083$/h was used], and kr = fractional rate of release from the non-escapable fraction to the escapable fraction [a value of $1/(0.4 \times 20) = 0.125$/h was used]. This implied a total rumen residence time of 20 h for forages distributed between the 2 compartments in a ratio of 40:60.

The statistical analyses were performed using the GLM procedure of Minitab® 16.1.1.0. The data on chemical composition of rations and incubation samples were analyzed by descriptive

statistics while the data on *in situ* parameters were analyzed using the following model considering each buffalo and cattle as an experimental replicate:

$$Yijkl = \mu + F(T)i + Tj + Lk + Eijk$$

where Yijkl is the dependent variable, μ is the overall mean, F(T)i is the effect of ith forage species, Tj is the effect of jth family of forage (cereal vs. legume) nested under forage species, Lk is the effect of kth location and Eijk is the residual error. Results were presented as least square means with standard error of the means and were considered statistically significant when the P-value was <0.05.

Results and Discussion

In situ DM degradation parameters of the forages are presented in Table 1. Forage species, family and location and location × family influenced (P<0.001) all fractions. The a-fraction ranged from 0.26 (maize) to 0.34 (wheat) for cereals (average 0.29) and from 0.28 (jantar) to 0.46 (lucern) for legumes (average 0.38). The b-fraction ranged from 0.50 (wheat & millet) to 0.59 (oats) for cereals (average 0.53) and from 0.31 (mustard) to 0.44 (jantar) for legumes (average 0.36). The kd averaged 0.056 for cereals and 0.11/h for legumes. The DMD and DMD1 ranged from 0.53 and 0.79 (millet) and 0.61 and 0.93 (oats) for cereals (average 0.56 and 0.85, respectively) and from 0.56 and 0.70 (mustard) and 0.68 and 0.82 (lucern) for legumes (average 0.61 and 0.77, respectively). Forages ranked of the same for DMD and DMD2: oats > wheat > barley > maize > sorghum > millet (cereals) and lucern > berseem > jantar > mustard (legumes). Our results agree with those of Sarwar et al. (1996) who used cannulated Nili-Ravi Buffalo calves and observed higher values of kd and rumen DMD for legumes than cereals.

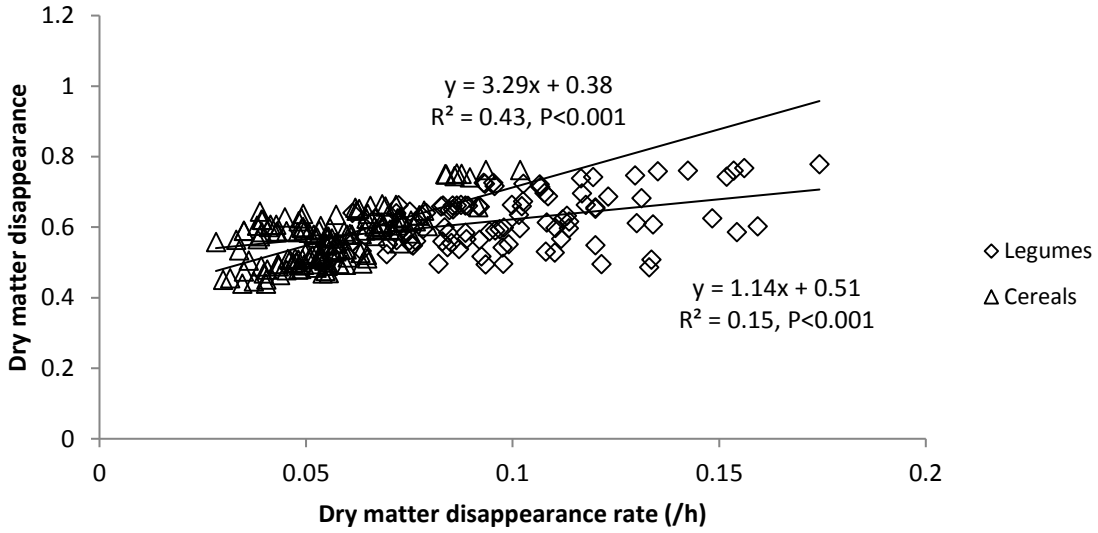

Figure 1 Relationship between dry matter degradability and rate of degradation (/h).

Habib et al. (2013) applied the *in situ* technique in buffalo steers to evaluate oilseeds, cereal grains, and animal-origin by products to study interactions among effective rumen degradability of DM at various time intervals and applied regression equations to determine relationships between DMD determined using various kp and *in situ* parameters. They found differences in rumen degradation kinetics and DMD within by-products of oilseeds, cereal grains, and animal origin feed sources.

Figure 1 and 2 show relationships between DMD or DMD2 and kd, respectively. It is apparent from the Figure 1 that a moderate (R^2 = 0.43) but significant (P<0.001), and a poor (R^2 = 0.14) and significant (P <0.001) relationship exists for cereals and legumes, respectively. Figure 2 shows no relationship between DMD2 and kd for cereals and legume forages.

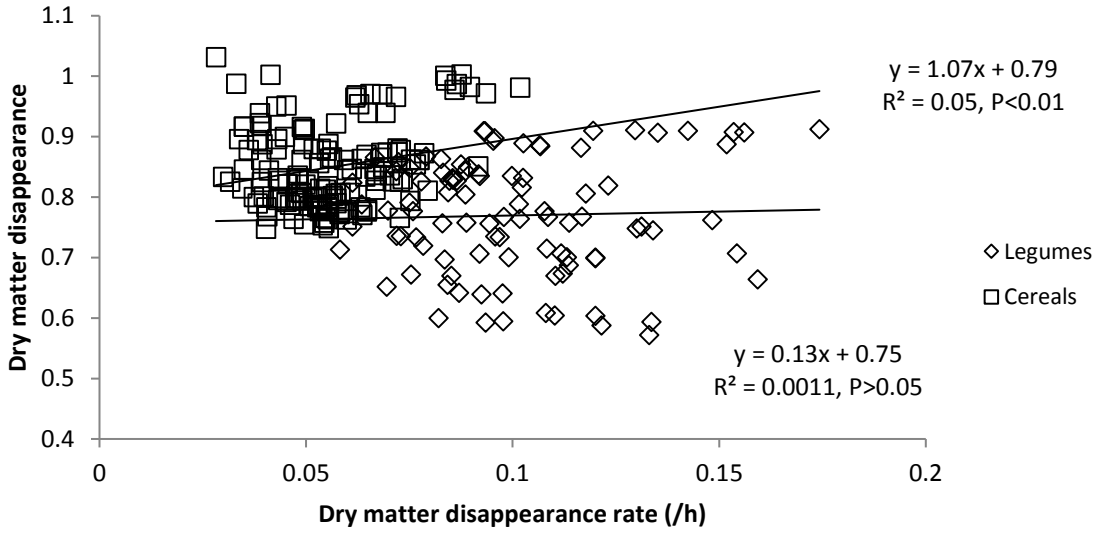

Figure 2 Relationship between dry matter degradability (DMD2) and rate of degradation (/h).

Conclusions

The rumen *in situ* is a powerful technique to describe rumen degradation characteristics of forages. A great variation in degradation characteristics was observed among forage species, between cereal and legumes and also among geographical locations. Using rate of degradation to determine dry matter degradability presents a good agreement for cereals, however, this assumption may not work for legumes with a large variation in degradation characteristics.

Table 1 Effect of forage species, type and location on in situ dry matter degradation kinetics and effective degradability of cereal and leguminous forages collected from different locations in Punjab province

In situ parameters[1]		a	b	Kd	DMD[2]	DMD[3]
Cereals						
Barley		0.30	0.54	0.05	0.57	0.86
Oat		0.31	0.59	0.06	0.61	0.93
Wheat		0.34	0.50	0.06	0.61	0.87
Maize		0.26	0.54	0.06	0.55	0.84
Millet		0.27	0.50	0.05	0.53	0.79
Sorghum		0.26	0.52	0.06	0.54	0.81
Legumes						
Barseem		0.42	0.36	0.12	0.67	0.81
Lucern		0.46	0.34	0.12	0.68	0.82
Mustard		0.36	0.31	0.09	0.56	0.70
Jantar		0.28	0.44	0.10	0.56	0.75
SEM		0.008	0.014	0.007	0.008	0.011
P-value	Forage species	<0.001	<0.001	0.042	<0.001	<0.001
	Type	<0.001	<0.001	<0.001	<0.001	<0.001
	Location	<0.001	<0.001	0.001	<0.001	<0.001
	Location × Type	0.001	<0.001	0.010	<0.001	<0.001

[1]*In situ* parameters described according to the model by Ørskov and McDonald (1979), where a = washable fraction representing the portion of dry matter (DM) that had disappeared at time 0, and b = potentially degradable DM fraction. The estimate of kd from the *in situ* method represents the fractional rate of disappearance of fraction b; [2] Effective DM degradability (DMD) was calculated from the *in situ* data assuming the fractional rate of passage (kp) to be 0.05/h for forages, as used in several protein evaluation systems (e.g. Madsen *et al*., 1995; Hvelplund and Weisbjerg, 2000); [3] Effective DMD was calculated from the *in situ* data according to a 2-compartment model as suggested by Allen and Mertens (1988).

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Can manipulation of growth patterns of dairy bulls bring benefits to beef production?

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Introduction

Economic profitability is one of the principal challenges in beef production. Improving feed efficiency by restricting feed intake, either during the growing period (Rossi *et al*., 2001, Trial 1) or growing and finishing period (Murphy and Loerch 1994, Trial 2) is one method to improve beef production. If growth rate is manipulated with feed restriction and subsequent realimentation, animals may exhibit compensatory growth (Owens *et al*., 1993). This can improve growth but also feed efficiency (Rossi *et al*., 2001, Trial 1). However, utilization of compensatory growth has had variable effects on animal performance (Sainz *et al*., 1995; Manni *et al*., 2013; Keogh *et al*., 2015). The objective of this experiment was to determine the effects of feed allocation regime on performance of growing dairy bulls by achieving even, increased or decreased growth patterns.

Materials and Methods

The feeding experiment comprised in total 32 Finnish Ayrshire bulls with an initial mean live weight (LW) of 123 (s.d. ±8.9) kg and age of 114 (s.d. ±6.9) days. At the beginning of the experiment, the bulls were divided according to LW into eight blocks of four animals each. Within blocks, bulls were randomly allotted to one of the four treatments. During the feeding experiment, the animals were housed in a tie stall barn. All bulls completed the entire study.

Feeding was based on grass silage and rolled barley grain including also 100 g of a mineral-vitamin mixture. Animals had free access to water. The silage was prepared from a timothy and meadow fescue sward, cut at heading stage of timothy using a mower without conditioner, slightly wilted, harvested using a precision-chop forage harvester, treated with a formic acid based additive applied at a rate of 5 l/tonne of fresh grass and ensiled in bunker silos. All feeds were analysed as described by Manni et al. (2016) except for N, which was measured by the Kjehldal method. Metabolisable energy (ME) concentration of the silage was calculated as 0.016 × digestible organic matter in dry matter (DM) (D-value) and for barley, it was estimated from its chemical composition (MAFF, 1984). Crude fibre and crude fat concentrations and digestibility coefficients of barley grain were taken from the Finnish Feed Tables (Luke, 2017). Metabolisable protein (MP) and protein balance in rumen (PBV) were calculated according to the Finnish feed protein evaluation system (Luke, 2017).

The whole experimental period was divided into two parts - early and late. The feeding treatments consisted of four feed allocation regimes:

1. A: *Ad libitum* feeding. *Ad libitum* (daily proportionate refusals of 10%) grass silage allowance during the whole experimental period. The amount of rolled barley grain supplementation was 93 g DM/kg$^{0.60}$ LW per animal per day during the whole experimental period.

2. R: Restricted feeding. Restricted grass silage and barley grain allowance during the whole experimental period, equivalent to 80% of group A intake at corresponding LW.

3. I: Increasing allowance. Feeding as group R up to 430 kg LW. After that (from 430 kg LW to slaughter), feeding as for group A.

4. D: Decreasing allowance. Feeding similarly as A up to 430 kg LW. After that (from 430 kg LW to slaughter), as for group R.

Target slaughter carcass weight was 300 kg and after slaughter, carcasses were weighed hot and cold carcass weight was estimated as 0.98 of hot carcass weight. Dressing proportion was calculated from the ratio of cold carcass weight to final LW. Carcasses were classified for conformation and fatness using the EUROP quality classification (EC 2006). Live weight gain (LWG) was calculated as the difference between the means of initial and final LW divided by the number of days. Estimated rate of carcass gain was calculated as the difference between the final cold carcass weight and carcass weight at the beginning of the experiment divided by the number of growing days. Carcass weight at start of the experiment was assumed to be 0.50 × initial LW.

Results are shown as least squares means. Normality of analysed variables was checked using graphical methods: box-plot and scatter plot of residuals and fitted values. Data was subjected to analysis of variance using the SAS GLM procedure (version 9.4, SAS Institute Inc., Cary, NC). Differences between treatments were tested using Tukey's test. Significant differences were assumed at $P<0.05$ and $P<0.10$ was regarded as a tendency.

Results

The grass silage used was of average nutritional quality (161 g crude protein (CP), 575 g neutral detergent fibre, 81 g MP and 10.4 MJ ME per kg DM). Fermentation characteristics were good (pH 3.77, 47 g lactic and formic acid and 14 g volatile fatty acids per kg DM and 42 g ammonia N and 422 g soluble N per kg N). The barley had a typical chemical composition and feed values (13.0 MJ ME, 130 g CP and 92 g MP per kg DM).

Restricted DM intake (DMI), either during the whole growing period (R) or in the early (I) or late (D) part of it, decreased average total daily DMI by 27, 14 and 10% ($P < 0.05$), respectively, compared to treatment A (Table 1). As a consequence of decreased DMI, ME, CP and MP intakes and PBV also decreased ($P < 0.05$). During the late period, Treatment I and A received the same diet but DMI of I was 12% higher ($P < 0.05$) than that of A as a consequence of restricted feeding of I during the early period.

Different feeding strategies affected growth rates and growth patterns (Table 2). Average LWG during the whole growing period decreased 27, 14 and 11% ($P < 0.05$) in R, I and D compared to A. There were no significant differences in LWG between I and D. During the early part of the growing period when DMI was restricted in R and I, average LWG of these treatments was 31% ($P < 0.05$) lower compared to A and D. During the late part of the growing period, LWG in A bulls was 18 and 45% ($P < 0.05$) higher compared to the bulls in R and D, respectively. Bulls on Treatment I exhibited compensatory growth and LWG was 40% ($P < 0.05$) higher compared to A. When D was compared to R, LWG was 18% ($P < 0.05$) lower in D.

There were no differences among treatments in DM or energy conversion rates over the whole growing period (Table 2). During the early part of the growing period, DM and energy conversion rates of A and D bulls were higher ($P < 0.05$) compared to the R and I bulls. During the late part of the growing period DM and energy conversion rates of I bulls were

higher ($P < 0.05$) compared to the bulls on Treatment A. Bulls on Treatment D had lower ($P < 0.05$) DM and energy conversion rates than bulls on R.

Restricted DMI during the whole or part of the growing period increased feeding days compared to A ($P < 0.05$). There were no differences in carcass weight, dressing proportion and carcass conformation score among the treatments. However, bulls on I tended to have 6% higher carcass weights compared to the other treatments ($P < 0.10$). In addition, bulls on A tended to have slightly better conformed carcasses compared to R and I bulls ($P < 0.10$). Carcass fat score of bulls on A was 29% higher ($P < 0.05$) compared to R bulls.

Table 1 Feed, energy and nutrient intake of growing dairy bulls on different feed allocation regimes

Treatments	A	R	I	D	SEM	*P*-value
Number of observations	8	8	8	8	-	-
Duration of the experiment, days	377[a]	494[b]	464[c]	409[d]	7.6	<0.001
Dry matter intake						
Early part						
Silage, kg/d	3.86[a]	2.80[b]	2.85[b]	3.80[a]	0.076	<0.001
Barley grain, kg/d	2.68[a]	2.16[b]	2.18[b]	2.62[a]	0.022	<0.001
Total, kg/d	6.63[a]	5.05[b]	5.12[b]	6.51[a]	0.090	<0.001
Total, g/kg$^{0.60}$ live weight (LW)	226[a]	172[b]	174[b]	224[a]	2.6	<0.001
Late part						
Silage, kg/d	5.43[a]	3.59[b]	6.64[c]	4.18[d]	0.133	<0.001
Barley grain, kg/d	3.94[a]	3.15[b]	3.91[a]	3.16[b]	0.030	<0.001
Total, kg/d	9.46[a]	6.83[b]	10.64[c]	7.43[d]	0.144	<0.001
Total, g/kg$^{0.60}$ LW	225[a]	165[b]	251[c]	179[d]	3.1	<0.001
Total experimental period						
Silage, kg/d	4.41[a]	3.04[b]	3.83[c]	3.95[c]	0.066	<0.001
Barley grain, kg/d	3.13[a]	2.46[b]	2.63[c]	2.85[d]	0.022	<0.001
Total, kg/d	7.63[a]	5.59[b]	6.55[c]	6.89[d]	0.079	<0.001
Total, g/kg$^{0.60}$ LW	227[a]	169[b]	191[c]	208[d]	2.4	<0.001
Nutrient intake						
Metabolisable energy, MJ/d	87.3[a]	63.6[b]	74.0[c]	78.6[d]	0.86	<0.001
Crude protein, g/d	2889[a]	1981[b]	2380[c]	2616[d]	38.8	<0.001
Metabolisable protein, g/d	1546[a]	1063[b]	1265[c]	1386[d]	20.1	<0.001
Protein balance in the rumen, g/d	608[a]	400[b]	497[c]	546[d]	10.1	<0.001

A = *Ad libitum* grass silage allowance during the whole experimental period. The amount of the barley grain supplementation was 93 g (kg$^{0.60}$ LW)$^{-1}$ per animal per day during the whole experimental period.
R = Restricted silage and barley allowance (0.8 × treatment A intake) during the whole experimental period.
I = Feeding similar as group R up to 430 kg LW. After that feeding similar as group A.
D = Feeding similar as group A up to 430 kg LW. After that feeding similar as group R.
SEM = Standard error of the mean.
[a, b, c, d] Means in the same row with different superscript letters are significantly different ($p<0.05$).

Table 2 Growth performance, feed conversion and carcass characteristics of growing dairy bulls on different feed allocation regimes

Treatments	A	R	I	D	SEM	*P*-value
Number of observations	8	8	8	8	-	-
Age, days						
At the beginning of the experiment	113	114	114	115	1.0	0.845
At the end of the early part	357^{a}	463^{b}	463^{b}	359^{a}	1.0	<0.001
At the end of the late part	490^{a}	607^{b}	577^{c}	524^{d}	7.6	<0.001
Live weight, kg						
At the beginning of the experiment	124	123	122	122	1.2	0.838
At the end of the experiment	578^{ab}	559^{b}	604^{a}	559^{b}	7.5	0.001
Live weight gain (LWG), g/d						
Early part	1280^{a}	880^{b}	898^{b}	1295^{a}	25.5	<0.001
Late part	1066^{a}	900^{b}	1491^{c}	737^{d}	44.7	<0.001
Total experimental period	1209^{a}	884^{b}	1041^{c}	1073^{c}	17.8	<0.001
Carcass gain, g/d	628^{a}	480^{b}	544^{c}	575^{c}	10.7	<0.001
Feed conversion rate						
Early period						
Kg dry matter/kg LWG	5.18^{a}	5.74^{b}	5.70^{b}	5.03^{a}	0.108	<0.001
MJ metabolisable energy/kg LWG	59.4^{a}	65.8^{b}	65.3^{b}	57.5^{a}	1.22	<0.001
g crude protein/kg LWG	2152	2136	2047	2083	58.0	0.526
Late period						
Kg dry matter/kg LWG	8.87^{ab}	7.59^{bc}	7.14^{c}	10.08^{a}	0.403	<0.001
MJ metabolisable energy/kg LWG	101.3^{ab}	85.3^{bc}	79.1^{c}	114.7^{a}	4.54	<0.001
g crude protein/kg LWG	2715^{b}	2463^{b}	2483^{b}	3343^{a}	152.9	0.009
Total experimental period						
Kg dry matter/kg LWG	6.31	6.32	6.29	6.42	0.131	0.908
MJ metabolisable energy/kg LWG	72.2	71.9	71.1	73.3	1.45	0.772
g crude protein/kg LWG	2390	2241	2286	2438	50.0	0.058
Carcass characteristics						
Carcass weight, kg	298	298	313	296	4.8	0.052
Dressing proportion, g/kg	515	534	519	529	6.4	0.098
Conformation score, EUROP	4.5	4.0	3.9	4.4	0.19	0.081
Fat score, EUROP	2.8^{a}	2.0^{b}	2.4^{ab}	2.3^{ab}	0.14	0.012

A = *Ad libitum* grass silage allowance during the whole experimental period. The amount of the barley grain supplementation was 93 g (kg$^{0.60}$ LW)$^{-1}$ per animal per day during the whole experimental period.
R = Restricted silage and barley allowance (0.8 × treatment A intake) during the whole experimental period.
I = Feeding similar as group R up to 430 kg LW. After that feeding similar as group A.
D = Feeding similar as group A up to 430 kg LW. After that feeding similar as group R.
SEM = Standard error of the mean.
$^{a, b, c, d}$ Means in the same row with different superscript letters are significantly different (p<0.05).

Discussion

Voluntary DMI greatly affects performance of growing cattle, which was also observed in the present experiment. When DMI was restricted, ME intake decreased and explained the decreased LWG of R, I and D bulls compared to A bulls. According to Huuskonen and Huhtanen (2015), energy intake is the most important variable affecting LWG of growing cattle.

Typically, if growth rate is not manipulated by restricting intake, growth accelerates until puberty and then becomes slower (McDonald *et al*., 1988). This trend was confirmed in the present experiment. If growth is divided into periods by restricting feed intake, the

manipulated growth rate of animals depends on energy and nutrient intake (Cummins *et al*., 2007) and growth pattern changes, as in the present experiment.

Increasing DMI after period of restricted feeding resulted in compensatory growth in the present experiment. During compensatory growth, LWG normally increases when feed or nutrient intake increases (Hornick *et al*., 2000). Compensatory growth in Treatment I was not sufficient to reach slaughter weight at the same time as for A. It is normal that restricted animals require more time to reach target slaughter weight, despite compensatory growth, when compared to non-restricted animals (Hornick *et al*., 2000).

Our results indicate that high DM and energy intakes during the growing period when animals have high growth capacity does not necessarily reduce feed efficiency. Decreased feed efficiency when DMI was restricted only during the late part of the growing period (D) may result from an increased weight of visceral organs before the restricted period. As a consequence, more energy was used for maintenance than for growth, which resulted in decreased LWG and impaired feed efficiency (D vs. R) during the restriction period. Feed conversion is usually more effective in young animals and declines as cattle approach maturity and growth rate declines. Our results are consistent with numerous findings in the literature (Keane, 2010; Manni *et al*., 2013; Manni *et al*., 2016).

Bulls on Treatment I had higher DMI, LWG and feed efficiency compared to A during the late part of the growing period. Reasons for this may have been a reduction in maintenance energy requirements, increase in net efficiency of tissue growth, increased feed intake and also gut fill (Carstens, 1995). Consistent with the present experiment, improved feed efficiency has found to be related to compensatory growth in earlier experiments (Sainz *et al*., 1995; Keogh *et al*., 2015).

Consistent with the meta-analysis of Huuskonen and Huhtanen (2015), increased ME intake improved carcass conformation. It is generally accepted that increased energy intake of growing cattle increases carcass fatness (Nogalski *et al*., 2014; Huuskonen and Huhtanen, 2015), which is in line with the current results when comparing to Treatment A with R.

Increased deposition of protein relative to fat (Ryan, 1993) and decreased carcass fatness (Carstens, 1995; Keogh *et al*., 2015) are expected compensatory growth phenomena. In the present experiment, compensatory growth had no effect on carcass characteristics. The conflicting results may originate partly from differences in severity and duration of the growth restriction and also from genetic and age differences of the animals during restriction and realimentation (Hornick *et al*., 2000).

Conclusions

Lack of improved feed efficiency by restricting feed intake indicates that *ad libitum* silage intake supplemented with concentrate is a relevant method to produce beef effectively. It improves growth and decreases the number of growing days of dairy bulls compared to restricted feeding strategies. However, if there is a temporary lack in amount and/or quality of feeds offered, it necessarily does not have major harmful consequences on beef production.

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Near infrared reflectance spectroscopy as a tool for estimating quality parameter of legume feeds

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Introduction

In recent years, demands for locally grown protein feeds have increased in husbandry and poultry farming of Latvia. Peas, faba beans, chickpeas and lupines are the four key cool-season grain legume species that are widely used throughout Europe (Murphy-Bokern et al., 2014), including Latvia. Beans and peas are valuable protein sources for dairy cows, swine and poultry. These legume grains are interesting ingredients in dairy cow diets, because of their rapid degradation in the rumen and readily available energy (Osmane et al., 2016). It has been shown that near-infrared spectroscopy (NIRS) has a great potential to estimate several grain quality attributes and has proven to be a fast, reliable, accurate and economical analytical technique (Singh *et al*., 2006). Most research performed with this technique on seeds has until now been focused on quality parameters (protein, starch, fiber, amino acids, β-glucans etc.) of cereals (barley, wheat, rye, and oats). There are also some examples where chemical composition of legume seeds have been investigated (Aulrich and Bohm, 2012; Asekova *et al*., 2016).
The aim of this study was to develop NIRS calibrations for dry matter and crude protein contents of field beans (*Vicia faba* L.).

Materials and Methods

In total, 138 field bean samples were collected from different landraces, varieties, breeding lines and advanced cultivars during 2014 and 2015.

Preparation of samples for NIRS scanning

An amount of 30 to 50 g of samples (85% dry matter or higher) was ground to pass a 1-mm sieve (Kinematica, PX-MFC 90D, Luzern, Switzerland). Field bean samples with large seeds were first ground on a hammer mill (Hawos Pegasus 400, Getreidemuhlen Reisinger, Austria) to < 4-6 mm. The coarse ground samples were then ground using a Laboratory Mill 3100 (Perten, Hägersten, Sweden) to ≤1 mm. All samples were kept in hygroscopic environment until analysis.

Chemical analysis

Dry seed samples were evaluated for protein contents according to international standard LVS EN ISO 5983-2 "Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content - Part 2: Block digestion and steam distillation method (ISO 5983-2:2009)". Dry matter of samples were measured according to ISO 6496:1999 "Animal feeding stuffs - Determination of moisture and other volatile matter content ". Chemical analyses were carried out at the Research Laboratory of Biotechnology of Latvia University of Agriculture.

NIRS scanning

Each sample was scanned in triplicate on a near infra-red spectrometer (Rapid Analyzer XDS, FOSS, Hillerød, Denmark) with a spectral range from 400 to 2498 nm. Spectral data were exported to WinISI 4 software (FOSS, Hillerød, Denmark). The spectra were first examined visually to eliminate abnormal scans before development of calibration equations.

Development of calibration model

The calibration process was initiated by identifying those spectra that were statistically different from the rest. This was followed by converting the spectra to principal component scores.

Calibration equations for protein and dry matter content were developed by modified partial least square regression (MPLS) on about two-third of the samples. The calibration equations were then validated against the remaining sample sets. Global regression equations with full spectrum was used for calibration model development. Spectra were pre-treated using moving average smoothing (1,4,4,1), scatter correction, standard normal variate (SNV) conversion and detrending.

Statistical evaluation of acquired calibration equations

The calibrations were evaluated using standard error of calibration **(SEC),** coefficient of determination **(RSQ),** standard error of cross validation **(SECV)** and variance or 1 minus the variance ratio **(1-VR).** The statistics used for comparison of predicted and reference values were standard error of prediction **(SEP), bias** and **slope.**

Spectral data corresponding to calibration set were analyzed by principal component analysis (PCA). Anomalous spectra were detected by applying Mahalanobis distance (H-statistics). Samples with H-values greater than 3 may be considered as not belonging to the population from which the equations were developed and in this case, the equations should not be used to make any prediction (Martin et al., 2014).

Results and Discussion

The optimum calibration model for DM and CP of beans were acquired. The DM range was 87-91% (mean, 89), and CP content 26-36% (mean, 31) in the reference samples (Table 1).

Table 1 Statistical parameters of the calibration equations constructed for field beans

Const.	N	Mean (%)	SD	Est min	Est max	SEC	RSQ	SECV	1-VR	SEP	Bias	Slope
DM	299	88.87	0.757	86.60	91.14	0.049	0.996	0.062	0.993	0.074	-0.002	1.010
CP	393	31.32	1.685	26.27	36.38	0.523	0.904	0.601	0.875	0.712	-0.041	0.919

N=number of samples; SD =standard deviation; SEC=standard error of calibration; RSQ=coefficient of determination; SECV=standard error of cross validation; 1-VR=variance; SEP=standard error of prediction.

The standard deviation of the difference between the reference and NIR values estimated from the calibration models (SEC) was small (Table 1), indicating that predictions of constituents were accurate. It is referred in literature that models with an RSQ between 0.66-0.81can be used for screening, but between 0.83-0.96 for most applications, including research and quality assurance (Williams, 2001). The RSQ of 0.996 for DM and 0.904 for CP

are high and means that 99.6% of variance in DM contents and 90.4% of variance in CP contents of calibration dataset was explained by our equations.

SECV and SEC values were similar (0.062 and 0.049, respectively for DM; 0.601 and 0.523, respectively for CP) indicating high accuracy of the prediction models. 1-VR is near to 1 (0.993 for DM; 0.875 for CP) which means 99.3% of variance in DM contents, and 87.5% of variance in CP contents of calibration dataset were explained by the calibration equations during the cross validation process (LVS EN ISO 12099; Williams, 2001; NIR White paper).

A SEP value of 0.712 for CP is high and indicates a high prediction error, but a SEP of 0.074 for DM is low indicating a low prediction error.

The difference between reference prediction means by the NIRS model (Bias) for both DM and CP were very small (-0.002 and -0.041, respectively), and were not greater than the confidence level specified (0.600).

Relationship between the measured and predicted CP values is in Figure 1. CP concentration of faba beans could be predicted with high accuracy (R^2=0.90). The relationship between the reference values and the NIRS predicted values only had a minor bias, with a slope close to 1 (Table 1, Figure 1).

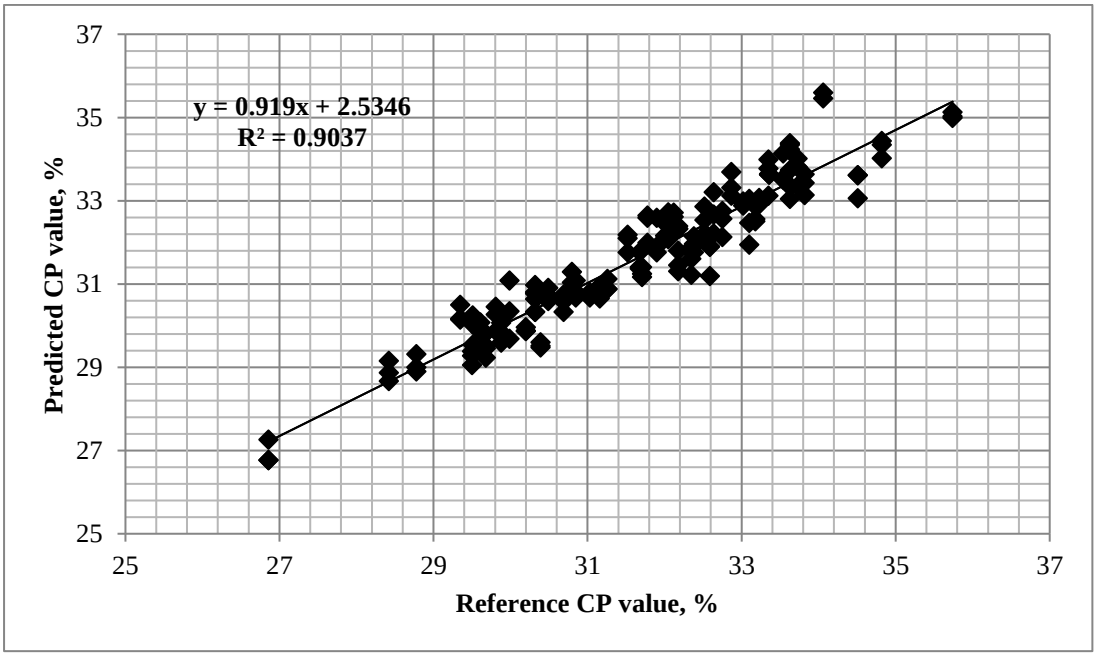

Figure 1 Predicted CP vs reference CP of bean samples.

The model needs to be further tested against new samples with known reference values to improve accuracy of the calibration equation of CP.

Conclusions

The results obtained indicate that the NIRS can be successfully used for dry matter and protein determination of faba beans. It is desirable to test the model with new samples with known reference values.

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In vitro evaluation of agro-industrial by-products replacing barley in diets to dairy cows

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Introduction

A rapidly growing world population will demand a secure and increased global food supply in the future. Ruminant animals can utilize fibrous plant material not edible to humans efficiently and convert it into highly nutritious food for human consumption. A large number of by-products from the agricultural industry can thereby be suitable feed ingredients in diets to dairy cows. However, the use of agro-industrial by-products in feed rations to dairy cows have to be complementary to basal feed ingredients/efficient in terms of nutrient utilization and not lower production. Several *in vitro* techniques have been developed to enable rapid and cost-effective evaluation of feed resources as alternative to experiments with live animals. Recently, there has been great progress in the development of the automated gas *in vitro* technique, which enables comparison of treatment effects on diet digestibility, ruminal fermentation, digestion rate (k_d; Huhtanen et al., 2008) and CH_4 production (Ramin and Huhtanen, 2012). The aim of this study was to evaluate the effects of replacement of barley by some common agro-industrial by-products in diets based on grass silage on true organic matter digestibility (TOMD), volatile fatty acids (VFA) concentrations, diet k_d and CH_4 production *in vitro*.

Materials and Methods

Experimental diets for the gas *in vitro* incubation were composed from a basal diet of grass silage and rolled barley in the ratio 700:300 g/kg of diet dry matter (DM). Grass silage and barley were replaced by one of rolled barley, palm kernel cake (PKC), molasses, wheat bran or sugar beet pulp (SBP) in levels of 200 and 400 g/kg of diet DM. Replacements were such that the ratio of forage:concentrate was kept constant in all diets.

Two lactating Swedish Red cows fed a diet of 600 g/kg grass silage and 400 g/kg concentrate on DM basis *ad libitum* were used for the *in situ* incubation and for collection of rumen fluid for the *in vitro* incubations. Rumen fluid was collected from the same cows for all three *in vitro* incubations. The collected rumen fluid from each cow was strained separately through a double layer of cheesecloth into steel thermoses pre-heated to 39°C that had previously been flushed with CO_2. In the laboratory, rumen fluid was filtered through four layers of cheesecloth, mixed with a buffer-mineral solution (Menke and Steingass, 1988) and held in a water bath at 39°C under CO_2 saturation. The volume ratio of rumen fluid to buffer was 1:2. The experimental diets were subjected to *in vitro* incubations in which gas production was automatically recorded and corrected to normal atmospheric pressure (101.3 kPa; Cone et al., 1996). Dietary ingredients had previously been dried at 60ºC for 48 h and thereafter ground to pass a 1-mm screen using a Retsch SM 2000 cutting mill (Retsch GmbH, Haan, Germany). Diet samples of 1000 mg were weighed directly in 250 ml serum bottles (Schott, Mainz, Germany) and were incubated in 60 ml of buffered rumen fluid for 48 h. Incubations were conducted at 39°C and the bottles were continually agitated. All samples were incubated in

duplicate in three consecutive runs. All runs included triplicate bottles with blanks. Samples were randomly allocated to the different *in vitro* incubations flasks but never incubated in the same flasks in different runs. Mean blank gas production within run was subtracted from the sample gas production. Digestion rate was calculated from the cumulative gas production curve of each replicated experimental diet and predicted digestibility from a dynamic mechanistic rumen model as described by Huhtanen et al. (2008).

Gas samples were drawn from each bottle by a gas tight syringe (Hamilton, Bonaduz, Switzerland) at 2, 4, 8, 24, 32 and 48 h of incubation. Predicted *in vivo* CH_4 production was calculated as described by Ramin and Huhtanen (2012). Liquid samples of 0.6 ml for NH_3-N analysis were taken at 24 h of incubation and preserved with 0.024 ml of H_2SO_4. Another sample of 0.6 ml of buffered rumen fluid was collected at 48 h of incubation from the bottles and immediately stored at -20°C until processed for VFA determination. Samples for VFA analysis from the duplicate bottles in each run were pooled before analysis. The individual and total VFA productions were calculated by subtracting mean blank VFA concentration from the sample concentration. The TOMD was determined for all samples in all runs by analysing the neutral detergent fibre (NDF) concentrations in the residues after the 48 h incubations. Mean blank true *in vitro* digestibility within run was subtracted from the sample *in vitro* TOMD.

Residual moisture of all feed samples was determined by oven drying for 16 h at 105°C. Ash concentration was determined by ignition of the dried sample at 500°C for 4 h. Indigestible NDF (iNDF) concentration was determined by a 12-d *in situ* ruminal incubation according to the procedure of Krizsan et al. (2015). Samples were analyzed for NDF using a heat stable α-amylase (Mertens et al., 2002) in an ANKOM^{200} Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA). Values of NDF and iNDF were expressed on an ash-free basis. Concentrations of N were determined by Kjeldahl digestion of 1.0 g sample in 12 *M* sulfuric acid using Foss Tecator Kjeltabs Cu (Höganäs, Sweden) in a Block Digestion 28 system (SEAL Analytical Ltd., Mequon, WI, USA) with determination of total N by continuous flow analysis using an Auto Analyzer 3 (SEAL Analytical Ltd., Mequon, WI, USA). Individual VFA concentrations in rumen fluid samples were determined using a Waters Alliance 2795 UPLC system as described by Puhakka et al. (2016), and NH_3 according to the method provided by the SEAL Analytical (Method no. G-102-93 multitest MT7) using the AutoAnalyzer 3.

Data was analysed using the GLM procedure (SAS Inc. 2002-2003, Release 9.2; SAS Inst., Inc., Cary, NC, USA) by a model correcting for effect of run and experimental diet. Polynomial contrasts were included for evaluation of linear and quadratic responses to level of barley and by-product ingredient in the experimental diet, and diets with barley vs. by-products.

Results and Discussion

Chemical composition of experimental feed ingredients is presented in Table 1. Silage, barley and by-product ingredients displayed chemical composition within expected ranges (NRC, 2001; Alimon, 2004). *In situ* iNDF values indicated a potential digestibility of the NDF fraction of 705, 782 and 920 g/kg for PCK, wheat bran and SBP compared with 824 g/kg for barley. Crude protein concentrations were higher in PCK and wheat bran compared to barley, while both molasses and SBP displayed lower concentrations. Further, non-fibre

carbohydrates concentrations were much higher for molasses and SBP compared to PKC and wheat bran.

Table 1 Chemical composition of experimental dietary ingredients (g/kg DM)

			By-product feeds			
	Silage	Barley	PKC	Molasses	Wheat bran	SBP
Dry matter	259	779	922	718	896	917
Organic matter	919	972	948	877	936	924
Crude protein	143	129	179	101	139	79
Neutral detergent fibre (NDF)	552	239	606	NA	487	339
Non-fibre carbohydrates[a]	200	582	88	773	267	495
Indigestible NDF	NA	42	179	NA	106	27

PKC = palm kernel cake; SBP = sugar beet pulp; NA = not analysed; [a]Calculated using tabulated values of ether extracts for all feeds and for NDF for molasses from NRC (2001) and Alimon (2004).

Measurements derived from the gas *in vitro* incubation of the basal diet (grass silage and barley) and with replacement of grass silage and barley by barley and by-product feed ingredients at two levels of inclusion are in Table 2. Ammonia-N measured in buffered rumen fluid at 24 h after start of the incubation decreased (P=0.02) with increased barley and by-product inclusion. Changes in NH_3-N in buffered rumen fluid can be difficult to explain and can, in addition to diet degradation, be a result of degradation of feed particle from the rumen fluid medium, or at later time points, be due to microbial lysis and degradation. There was a quadratic increase (P=0.04) in total VFA production with the replacement of basal diet with barley and by-product feed ingredients indicating that diets were more fermentable at the 200 g/kg inclusion level. There was a linear decrease (P<0.01) in propionate with increased dietary inclusion level. As a result of the lower propionate, there was an increase in molar proportion of butyrate (P<0.01) with increased dietary inclusion level. Further, molar proportions of branched-chain VFAs increased quadratically and caproic acid increased linearly (P<0.01) with increased barley and by-product dietary inclusion level (P≤0.04). Predicted CH_4 production increased quadratically (P<0.01) with greater dietary supplementation. The PKC and wheat bran diets were lower in TOMD when compared to barley diets (P≤0.02). Otherwise, the lower potential digestibility of NDF in PKC and wheat bran, and the higher NFC in molasses and SBP than when compared to barley, were reflected in the fermentation profile and predicted CH_4 *in vivo*. The higher concentration of acetate (P<0.01) and generally lower concentration of butyrate (except PKC; P <0.01) for the by-product supplemented diets reflect a shift in fermentation when exchanging starch in barley to either more fibre or sugar containing dietary ingredients. Ertl et al. (2015) reported lower butyrate production from feeds containing hemicelluloses and pectins compared to those containing starch. Ruminal branched-chain VFA (i.e. isobutyric, isovaleric) and valeric and caproic acid primarily originate from dietary protein according to Tedeschi et al., (2000), and was generally decreased in by-product containing diets compared to diets only supplemented with barley, especially for molasses and SBP (P<0.01).

Conclusions

Replacing barley with molasses and SBP in grass silage-based diets did not decrease diet TOMD in buffered rumen fluid *in vitro*. However, both molasses and SBP inclusion changed

rumen fermentation profile towards more acetate and less butyrate, which might affect the production by dairy cows and beef cattle.

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Table 2 Measurements derived from the automated gas *in vitro* system of basal diet (grass silage and barley) replaced in two levels of diet dry matter (DM) with barley (B), palm kernel cake (PKC), molasses (M), wheat bran (WB) and sugar beet pulp (SBP)

	Basal	200 g/kg diet DM					400 g/kg diet DM						P-value[a]					
Item		B	PKC	M	WB	SBP	B	PKC	M	WB	SBP	SEM	C1	C2	C3	C4	Lin.	Quadr.
TOMD, g/kg	862	867	829	869	829	888	878	823	911	837	867	15.0	<0.01	0.25	0.02	0.73	0.83	0.57
NH_3-N_8, mg/l	247	407	329	210	377	219	313	385	153	426	215	53.0	0.96	<0.01	0.44	0.01	0.49	0.35
NH_3-N_{24}, mg/l	555	455	542	657	583	433	364	274	527	303	525	70.0	0.98	0.02	0.64	0.33	0.02	0.26
Total VFA, mmoles	1.95	2.24	2.12	2.20	2.12	2.58	2.22	1.82	2.25	1.90	2.24	0.150	0.10	0.99	0.16	0.24	0.78	0.04
Molar proportions, mmole/mole																		
Acetate	601	587	598	595	595	615	581	598	600	601	623	4.5	<0.01	<0.01	<0.01	<0.01	0.91	0.38
Propionate	242	236	220	238	232	228	230	201	244	227	224	3.4	<0.01	0.03	0.38	0.05	<0.01	0.24
Butyrate	117	132	136	128	127	116	144	151	122	128	116	2.4	0.03	<0.01	<0.01	<0.01	<0.01	0.06
Isobutyric acid	9.2	10.8	9.9	8.6	11.0	9.9	10.7	9.5	7.3	10.7	8.6	0.45	0.03	<0.01	0.81	<0.01	0.69	0.03
Isovaleric acid	7.4	9.0	8.1	6.7	9.2	7.7	8.5	7.6	5.1	8.7	6.6	0.50	0.10	<0.01	0.66	<0.01	0.44	0.04
Valeric acid	19.3	21.0	20.4	19.2	20.8	19.0	20.9	20.5	17.6	20.3	18.0	0.49	0.31	<0.01	0.41	<001	0.83	0.07
Caproic acid	4.0	5.1	7.5	4.6	4.6	4.6	5.6	12.0	4.4	4.6	4.7	0.32	<0.01	0.02	0.03	0.04	<0.01	0.60
k_d, 1/h	0.117	0.104	0.101	0.117	0.097	0.118	0.119	0.091	0.139	0.102	0.145	0.0100	0.12	0.12	0.24	0.06	0.54	0.14
CH_4[b], ml/g DM	35.3	39	36.1	40.5	38.1	40.0	40.9	36.7	43.5	36.3	41.8	0.53	<0.01	<0.01	<0.01	0.09	<0.01	<0.01

SEM = standard error of mean; TOMD = true organic matter digestibility, NH_3-N_8 = ammonia N in sampled rumen fluid 8 h after start of incubation; NH_3-N_{24} = ammonia N in sampled rumen fluid 24 h after start of incubation; Total VFA = volatile fatty acids (sum of all individual acids); k_d = diet digestion rate. [a]C1 = B vs. PKC; C2 = B vs. M; C3 = B vs. WB; C4 = B vs. SBP; Lin.= linear effect of supplementary inclusion level; Quadr. = quadratic effect of supplementary inclusion level; [b]Predicted CH_4 *in vivo*.

Precision feeding strategies in dairy cows: TMR and auto feeder use

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Introduction

A total mixed ration (TMR) is a common feeding strategy in dairy cattle farms. In TMR, all forages, concentrates and additives are combined to obtain a specified diet offered "ad libitum" to the animals (Formigoni, 1990; Linn, 2013). This feeding technique offers many advantages to farmers. However in this situation, all cows receive the same ration with the risk of over- / under-feeding those cows in specific stages of lactation (Formigoni and Mordenti, 1995). In order to overcome this, computerized concentrate feeders can be used. This system allows automatically identification of cows and the software makes it possible to set a certain amount of concentrate to be distributed at specific intervals. The use of some concentrates as a supplement over the TMR could enhance the use of forages for lower producing animals, and should improve efficiency of use of the most expensive part of the rations like protein sources and additives for higher producing animals. The aim of this study was to test the possibility to apply a "precision feeding system" in a dairy herd fed with TMR and adding some concentrates according to individual milk production.

Materials and Methods

Forty dairy cows, milked 2 times a day, were involved in the study (DIM 122.0±107 d; lactation number 1.8±1.1; milk production 38.0±8.4 L/d; BW 615.0±73.0 kg, divided in two homogeneous groups TMR+C and TMR) in a cross-over design (two experimental periods consisting of two weeks of adaptation and five weeks of data collection). The TMR+C group received a basal TMR (Table 1) and a variable amount of concentrate offered in an auto feeder as a pellet (Table 2) using the following scheme:

- $\leq$ 30 kg of milk/day: 2.0 kg of concentrate
- 30.1-35.0 kg of milk/day: 3.5 kg of concentrate
- 35.1-40.0 kg of milk/day: 4.5 kg of concentrate
- 40.1-45.0 kg of milk/day: 5.5 kg of concentrate
- > 45.1 kg of milk/day: 7.0 kg of concentrate

The TMR group received a ration that included the average amount of concentrate (4.5 kg/head/day) supplied to the TMR+C. Every day, average dry matter intake (DMI) was recorded as well as milk yield, milk quality, rumination and resting time by monitoring system designed to collect data (Afikim®, Afilab®, AFIACT and SCR system).

Every week four, different animals per group were moved for five days to a tie stall to record individual dry matter intake using an automatic system (Dinamica Generale, Poggio Rusco, Italy). During this period, the supplement of concentrate in the TMR+C diet was given according to milk production every 6 h in four equal portions. Rations were formulated using the Dinamilk® software (Fabermatica, Cremona, Italy), based on the CNCPS model (Cornell Net Carbohydrate and Protein System, Ithaca, NY).

Table 1 TMR composition (kg/h/d).

	TMR+C	TMR
Grass hay	11.5	11.5
Feed mix*	5.5	7.5
Corn flakes	5.0	5.0
Molasses (cane and beet, 50:50)	1.0	1.0
Auto-feeder pellet	4.5	...

*Feed mix composition: Sorghum meal 29%, wheat bran 29%, soybean meal (44%CP) 22%, soy full fat flaked 15,5%, calcium carbonate 2%, sodium bentonite 1%, NaCl 1%, magnesium oxide 0.25%, microminerals 0.24%, vitamins ADE 0.01%.

Table 2 Composition of concentrate used as supplement in auto-feeder and added to TMR

Ingredients	% of dry matter
Sorghum meal fine	25.00
Barley meal fine	16.00
Soybean meal (44% crude protein)	13.00
Soybean full fat flaked	13.00
Soy hulls	13.00
Beet pulp	12.79
Molasses (cane and beet, 50%:50%)	2.50
Maltose	2.50
Salt (NaCl)	2.00
Microminerals	0.20
Vitamins ADE	0.01

TMR was prepared and quantities adjusted to obtain 5-8% orts. Feed bunks were cleaned daily and refusals weighed to calculate average daily dry matter intake for each experimental group. TMR composition was representative of rations used in farms were milk is used for Parmigiano Reggiano cheese production. In this production, silages are forbidden to avoid spoilage by spores. In order to limit cow sorting of TMR, forages were finely chopped as recommended by Fustini et al. (2016). For the TMR+C group, number and duration of meals in the auto-feeder (Afilab®, Afikim® - Israel) were recorded. Rumination time (SCR Engineers Ltd., Netanya, Israel), production parameters (milk quantity, fat and protein percentage, lactose, somatic cells and electrical conductivity), activity, resting time and body weight data were recorded daily (Afilab®, Afikim® - Israel).

Chemical and physical analyses of feedstuffs

Feed ingredients were sampled and analysed at the beginning of the experiment and before each new lot of feed. The TMRs were sampled twice a week. Samples were analysed for dry matter (DM), amylase treated ash-free neutral detergent fiber (aNDFom), acid detergent fiber (ADF), acid detergent lignin (ADL), undigested neutral detergent fiber after 240 h ($uNDF_{240h}$), starch, sugars, crude protein, lipids and ash.

TMR particle size distribution was evaluated using the Pennsylvania State Particle Separator and the Ro-Tap (W.S. Tyler, Mentor, Ohio, USA). The latter was used to measure the percentage of particles retained by a 1.18 mm sieve (physical effective factor, pef) and physical effective NDF was calculated as: peNDF = pef x NDF (Mertens, 1997).

Fecal samples were collected when the cows were housed in the tie stall at 12-h intervals for the last two days (4 samples). Feed and fecal samples were analysed for in vitro aNDFom digestibility at 24 h and 240 h according to the procedure described by Palmonari et al. (2016). The in vitro digestibility of NDF (IVNDFD) at 240 h was used to determine the $uNDF_{240h}$ as marker for total tract pdNDF (potentially digestible NDF) digestibility (TTpdNDFD) as follow:

*TTpdNDFD, % pdNDF = 100 – [(dietary $uNDF_{240h}$ / fecal $uNDF_{240h}$) * (fecal pdNDF concentration / dietary pdNDF)]*

where both *pdNDF* and *$uNDF_{240h}$* are expressed in % of DM.

Data were analysed with a repeated measures mixed model, using statistical software JMP-12 (SAS Institute Inc., Cary NC). Treatment, period and lactation number were used as fixed effect and animal as random effect. $P < 0.05$ values were considered significant.

Results and Discussion

Tables 3 and 4 show the results of the chemical analysis of the concentrates and TMR ration. Data obtained were very close to those expected theoretically.

Table 3 Chemical composition of feedstuffs used in the experiment

	Hay	Corn flake	Feed mix	Supplemented concentrate
Dry matter, % of fresh matter	89.51	88.60	90.05	89.95
Crude protein, % of DM	8.99	6.92	22.31	18.63
Fat, % of DM	1.68	3.55	3.76	3.45
aNDFom[1], % of DM	53.06	15.80	17.57	21.14
$uNDF_{240}$[2], % of DM	20.55	…..	…..	…..
Starch, % of DM	2.06	66.81	26.67	25.73
Ash, % of DM	9.91	1.12	7.60	6.28

[1]aNDFom = amylase- and sodium sulfite-treated NDF, corrected for ash residue[2] uNDF240 = unavailable NDF estimated via 240h in vitro fermentation.

TMR particle size distribution (Table 5) was very low as expected and typical for dry rations like the one fed in this study. Fustini et al. (2010) showed that the peNDF content of a diet for

adequate levels of rumination time and rumen pH, can be lower compared to what was suggested by Mertens (1997).

During the experiment, animal behaviour was not influenced (data not reported) by the different feeding strategies and similar time of resting and activity were recorded even though the primiparous cows used the auto-feeder more frequently compared to the multiparous (9.31 vs. 6.26 times/day; $P< 0.0001$).

Table 4 Chemical composition of TMR used in the two treatments

	TMR	TRM+C
Dry matter, % of fresh matter	87.76 ± 1.36	87.94 ± 1.13
Crude protein, % of DM	15.52 ± 0.81	15.70 ± 1.04
Fat, % of DM	2.34 ± 0.22	2.26 ± 0.30
aNDFom[1], % of DM	30.20 ± 1.77	30.30 ± 2.69
$IVNDFD_{24h}$[2]	65.08 ± 3.93	64.40 ± 3.31
$uNDF_{240h}$[3]	10.51 ± 1.12	10.62 ± 1.02
pdNDF, % of DM	19.61 ± 1.08	19.64 ± 2.17
Starch, % of DM	22.11 ± 1.46	21.98 ± 2.10
Sugar, % of DM	7.24 ± 0.40	7.11 ± 0.40
Ash, % of DM	9.59 ± 0.28	9.48 ± 0.41

[1]aNDFom = amylase- and sodium sulfite-treated NDF, corrected for ash residue; [2] IVNDFD = in vitro NDF digestibility; [3] uNDF240 = unavailable NDF estimated via 240h in vitro fermentation.

The TMR+C strategy in the free stall allowed a higher intake (2.4 kg/d) and a shorter rumination time (Table 6); during the tie stall periods these results were only partially confirmed with a tendency of higher intake observed using the concentrate supplement. The results can be justified to the less fill effect of the TMR+C ration due to the lower intake of forages.

Table 7 shows milk production and composition of the cows in the free stall. Milk yield was higher in the TMR+C treatment ($P<0.01$), while fat percentage was lower ($P<0.01$). This result underlines the potential benefit of a precision feeding system that can promote higher production levels. Under the tie-stall conditions milk production (data not reported) was not influenced by the different feeding strategies. This was probably due to the short period of this phase and to the stress derived from the change in housing conditions.

Supplementing the TMR with concentrates depressed TTpdNDFD (73.3 vs 67.8 in TMR vs TMR+C respectively; $P <0.001$). Fiber digestibility is influenced by many factors and particularly by level of forage intake and the use of more concentrate can reduce total fiber intake and reduce TTpdNDFD. The results highlight the importance of a more appropriate supplement formulation to enable an adequate ruminal fiber retention and, hence, a high fiber digestibility.

Table 5 Particle size distribution of the TMR in the two treatments.

*PSPS**	TMR	TMR+C	SEM	P-value
19 mm, % of DM	1.32	1.52	0.2613	0.4454
8 mm, % of DM	11.46	13.06	1.3445	0.2451
4 mm, % of DM	25.96	19.51	0.7674	<.0001
Bottom, % of DM	61.25	65.62	1.5894	0.0101
Ro-Tap				
>1.18 mm % DM	51.56	49.16	1.4127	0.1000
<1.18 mm % DM	48.44	50.84	1.4127	0.1000
peNDF % DM	15.66	15.75	0.5961	0.8720

Table 6 Dry matter intake (DMI) and rumination time recorded during the experiment.

Groups	TMR	TMR+C	SEM	*P-value*
Free stall				
DMI, kg/d	23.07	25.43	0.25	<0.0001
TMR, kg/d	23.07	21.72		
Pellet, kg/d	…	3.71		
Rumination time, min/d	543.9	532.2	0.01	<0.0001
Tie stall				
DMI, kg/d	25.46	26.05	0.44	n.s.
TMR, kg/d	25.46	21.86		
Pellet, kg/d	…	4.19		
Rumination time, min/d.	443.7	445.7	9.48	n.s.

Table 7 Milk yield and composition

	TMR	TMR+C	SEM	P-value
Milk, kg/d	38.99	39.76	0.10	<0.01
Protein, %	3.00	3.01	0.01	=0.74
Fat, %	3.60	3.56	0.01	<0.01
ECM, kg/d	41.42	42.16	0.15	<0.01

Conclusions

Data obtained in this trial showed that efficiency of a TMR feeding system could be improved by individual concentrate supplementation. Thanks to the use of an auto feeder, we achieved a higher DMI and milk production. Precision feeding strategies need to be carefully evaluated by nutritionist and farmers in order to fully make use of home produced forages,

which could be used at higher levels for low-productive cows, while the most expensive feeds can supplement only diets to the more productive animals. Furthermore, it will be necessary to investigate effects on, particularly, fiber digestibility which can be affected when low forage proportions are used in the rations.

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The effect of additive treatments of grass silage and total mixed ration (TMR) on the aerobic stability of TMR

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Introduction

Good aerobic stability is necessary to maintain nutritive value and hygienic quality of silage in a silo during feed-out period and of total mixed ration (TMR) in a mixer and a feed bunk. The use of additives altering silage fermentation profile and the use of TMR stabilizers are means to affect warming of silage-based TMR (Kung 2010). Total mixed rations containing barley silage (Taylor et al. 2002) or alfalfa silage (Kung *et al.*, 2003) treated with *L. buchneri*, or high-dry matter (DM) grass silage treated with an inoculant containing heterofermentative strain of *L. brevis* (Seppälä *et al*., 2016) showed better aerobic stability than the TMRs containing untreated silage. Results indicated that additive treatment accumulating acetic acid in the silage may improve aerobic stability of TMR.

Seppälä *et al.* (2013) showed that propionic and formic acid-based preservatives can improve the aerobic stability of grass silage-based TMR when the hygienic quality of the ingredients of TMR are good. However, when the number of yeasts and aerobic bacteria was high in the TMR ingredients (silage or brewer's grain), the benefit of using preservative decreased. Thus, there is a need to develop stabilizers effective also in challenging situations when TMR is prone to warm up due to low microbial quality of ingredients. The aim of the trial was to assess efficacy of acid based stabilizers in TMRs based on grass silages ensiled without or with silage additives and brewer's grains as one of the ingredients.

Materials and Methods

The study was arranged using a 3 x 3 factorial design of treatments with three silage treatments and three TMR treatments. Second cut timothy-meadow fescues grass was ensiled after 29 h wilting either untreated (Control) or after treatment with formic acid based additive (AIV 2 Plus 5 l/t, containing formic acid 760 g/kg and ammonium formate 55 g/kg, Oulu, Finland, Taminco Finland Oy, subsidiary of Eastman Chemical Company) (FA) or lactic acid bacteria based additive containing *Lactobacillus plantarum* and *Pediococcus acidilactici* 6 x 10^5 cfu/g and xylanase (Inoculant). Eight replicate laboratory silos (800 g grass/1.5 l silo) were prepared per treatment.

After an ensiling period of 11 months, eight replicate silage samples were pooled over silage additive treatment and thereafter mixed with concentrates to prepare TMRs. Concentrate consisted of brewer's grain and a mixture of barley, oats, rapeseed meal, faba mean meal and minerals. The composition of TMR was in DM basis grass silage 500 g/kg, grain mixture 400 g/kg and brewer's grains 100 g/kg.

Four replicate TMRs were subjected to an aerobic stability test lasting for 12 days either untreated or after treatment with a stabilizer containing formic acid 590 g/kg, ammonium formate 40 g/kg, propionic acid 200 g/kg and potassium sorbate 25 g/kg (StabA, AIV Ässä, Oulu, Finland, Taminco Finland Oy, subsidiary of Eastman Chemical Company) or formic

acid 473 g/kg, sodium formate 150 g/kg and propionic acid 200 g/kg (StabB). The additives were applied to each replicate sample at the rate of 3 l/t fresh weight of TMR. Samples of TMRs (560 g) were placed in styrofoam boxes (volume 1.0 dm^3). On top of each box was a hole (Ø 2 cm) for air to penetrate. Temperature changes were monitored using data loggers (Rosh Ha'ayin, MicroLite, Fourtec - Fourier Systems Ltd., Israel) placed inside the TMR. Temperatures were recorded every 15 minutes. Aerobic stability was defined as the time for sample temperature to reach 2°C above ambient temperature (20.5ºC) after opening the silos.

Pre-ensiling sample of untreated chopped grass was collected for analyses of DM, ash, neutral detergent fibre (aNDFom), crude protein (CP), water soluble carbohydrates (WSC), *in vitro* digestibility, soluble nitrogen and buffering capacity. Silages from two replicate silos of same treatment were pooled and samples were collected for determination of DM, pH, ash, CP, WSC, lactic acid, volatile fatty acids, ethanol, ammonia-N and microbial composition (aerobic bacteria, total count of yeasts and moulds). The mixture of TMR's dry components and brewer's grains were sampled and analysed for DM, ash, NDF, CP and microbial composition (aerobic bacteria, total count of yeasts and moulds). Chemical analyses were performed as described by König *et al.* (2017). Microbial analyses were conducted in a commercial laboratory by plate cultivation methods (Bionautit, Helsinki, Finland).

Data for silage fermentation characteristics were analysed by ANOVA using the Mixed procedure of SAS (SAS 9.3, Institute Inc., Cary, NC, USA). Differences between the silage additive treatments in silage composition, fermentation quality and microbial quality (log-transformed data) were further analysed by pairwise comparisons using Tukey's test. Residuals from analysis of TMR aerobic stability data were not normally distributed and thus ln-transformation was used to improve normality. Sums of squares of treatment effects were further separated into single degree of freedom comparisons using following orthogonal contrasts: Silage additives vs Control (1), silage additives FA vs Inoculant (2), TMR stabilizers vs untreated TMR (3) and TMR stabilizers StabA vs StabB (4), and interactions: 1 x 3, 2 x 3, 1 x 4 and 2 x 4.

Results and Discussion

After wilting, the DM content of grass was 279 g/kg (Table 1). The content of water soluble carbohydrates of grass was 27.3 g/kg in fresh weight basis indicating that the ensiled grass was moderately difficult to ensile according EFSA (2006). The rather high buffering capacity (742 mEq/kg DM) suggested some challenges in ensiling.

Clear differences were achieved in the fermentation pattern of silages (Table 1). Fermentation was restricted in FA silage compared to both Control silage and Inoculant silage as evidenced by a higher content of residual WSC ($P<0.001$) and a lower contents of lactic acid ($P<0.001$), acetic acid ($P=0.07$ vs Control, $P<0.001$ vs Inoculant) and ammonia-N ($P<0.01$) in FA silage. Inoculant treatment resulted in higher pH and higher content of acetic acid, and lower contents of residual WSC and lactic acid compared to Control ($P<0.01$ for all). The proportion of lactic acid in the fermentation acids differed between silages being 0.81 in Control, 0.74 in FA and 0.61 in Inoculant. Acetic acid was the only volatile fatty acid detected in the silages and the proportion of ammonia-N was always below 90 g/kg N.

Table 1. Chemical composition of concentrates, grass and silages, and fermentation quality of silages, g/kg dry matter (DM) unless otherwise stated

				Silage additive treatment				Statistical significance (Tukey)		
	Grain mixture	Brewer's grains	Grass	Control	FA	Inoculant	SEM	FA vs Control	Inoculant vs Control	FA vs Inoculant
Dry matter, g/kg	878	307	279	289	284	288	0.2	0.267	0.864	0.508
pH				4.24	4.31	4.38	0.023	0.162	0.005	0.114
In dry matter, g/kg										
Ash	60.0	37.4	98.4	107	108	111	0.1	0.764	0.051	0.017
Neutral detergent fibre	238	536	539							
Crude protein	150	251	152	144	151	146	1.2	0.009	0.486	0.058
Water soluble carbohydrates			97.9	21.6	50.6	6.3	2.09	<0.001	0.002	<0.001
Lactic acid (LA)				111.2	56.8	92.0	2.46	<0.001	0.001	<0.001
LA/total fermentation acids				0.81	0.74	0.61	0.012	0.001	<0.001	0.001
Acetic acid				26.6	20.0	58.2	1.82	0.069	<0.001	<0.001
Fermentation acids total				137.8	76.8	150.3	2.56	<0.001	0.018	<0.001
Ethanol				2.73	6.98	3.58	0.465	<0.001	0.436	0.002
Ammonia-N, g/kg N				86.6	71.1	86.9	1.89	0.001	0.996	0.001
Aerobic bacteria, log cfu/g	6.10	6.35		5.28	5.37	7.66	0.177	0.928	<0.001	<0.001
Moulds and yeasts, log cfu/g	4.84	4.85		0.60	0.81	0.60	0.163	0.469	1.000	0.469
Soluble N, g/kg N			499							
BC, mEq/kg DM			742							
DOMD, g/kg DM			693							
Silage intake index[1]				98	106	96	0.3	<0.001	<0.001	0.010

Control = untreated; FA = formic acid based additive; Inoculant = lactic acid bacteria and enzymes; BC = buffering capacity; DOMD = digestible organic matter in DM

[1] Silage intake index calculated according Huhtanen *et al*. (2007).

According DLG-quality criteria (Kaiser *et al.*, 2006), Inoculant silage was graded as bad while FA and Control were regarded as very good. From the view of animal production, it is notable that FA silage could improve silage DM intake of dairy cow by 780 g or by 940 g compared to Control and Inoculant respectively as calculated based on the amount of fermentation acids (Huhtanen *et al.*, 2007).

There were no differences in total contents of yeasts and moulds between silages while the content of aerobic bacteria was higher in Inoculant as compared both to FA and Control. Grain mixture and brewer's grains contained relatively high amounts of all the micro-organisms analysed which obviously increased the susceptibility of TMRs to heating. Yeasts and moulds are known to cause silage aerobic deterioration and increase temperature (Pahlow *et al*., 2003).

Results show that aerobic stability of TMRs containing additive treated silage was, on average, better than those containing untreated silage (P<0.001) (Table 2). However, interactions between TMR stabilizer treatment and silage additive treatment were observed. On average, TMR stabilizers improved aerobic stability compared to untreated TMR (P<0.001). The positive average effect of the two stabilizers on aerobic stability of TMR compared to untreated TMR was similar with TMRs containing Control silage or additive treated silages (interaction not significant, P>0.05). Further, the positive effect of the stabilizers on aerobic stability was similar to TMRs including FA silage or Inoculant silage (interaction not significant, P>0.05).

Table 2. Aerobic stability time of the TMRs treated with different stabilizers and containing silages treated with different silage additives

Silage additive	TMR stabilizer	Time, hours
Control	Untreated	18.9
	StabA	59.0
	StabB	72.5
FA	Untreated	26.1
	StabA	109.9
	StabB	65.1
Inoculant	Untreated	25.1
	StabA	106.1
	StabB	97.3
	SEM	1.13
Statistical significances of the contrasts		P-value
	Silage additives vs Control (1)	<0.001
	FA vs Inoculant (2)	0.265
	TMR stabilizers vs untreated (3)	<0.001
	StabA vs StabB (4)	0.303
	Interaction 1x3	0.572
	Interaction 2x3	0.331
	Interaction 1x4	0.006
	Interaction 2x4	0.041

Control = untreated; FA = formic acid based additive; Inoculant = lactic acid bacteria and enzymes; StabA and StabB = acid based stabilizers.

However, when StabA and StabB were compared, aerobic stability of TMR was slightly better with StabB than StabA when Control silage was used in TMR (difference 14 h) while the effect of StabA was on average better with additive treated silages (difference 27 h) (interaction P=0.006). Further contrast showed that FA silage and Inoculant silage based TMRs responded differently to StabA and StabB (interaction P=0.041). With FA silage stability time was 45 h longer with StabA than StabB while the difference between stabilizers in favour of StabA was only 9 h with Inoculant silage. These results suggest that FA silage based TMR benefited from less buffered and potassium sorbate containing StabA while Inoculant based TMR was almost equally stable with StabB because of a higher acetic acid content in Inoculant silage than in FA silage.

Conclusions

Ensiling time in this trial was 11 months potentially strengthening differences between silage additive types. Formic acid restricted fermentation, while Inoculant had the opposite effect.

Silage fermentation pattern was reflected in the aerobic stability of TMR. A higher content of lactic acid in Control silage compared to FA and Inoculant silages explains at least partly the poorer aerobic stability of TMR containing untreated silage compared to TMR containing FA or Inoculant silage.

The positive effect of stabilizers on TMR aerobic stability was observed irrespective of the fermentation quality of silages used in the TMRs. Interactions revealed that the efficacy of the stabilizers to maintain aerobic stability of TMR depends on silage additive treatment and thus on silage fermentation quality. Due to high acetic acid content of Inoculant silage, aerobic stability of TMR was less sensitive to composition of stabilizers.

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Improving the usability of carrot by-products as animal feeds by ensiling

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Introduction

Food by-products have been an important source of feed for livestock throughout history and interests in even more efficient use of by-products and side streams is encouraged by economic and environmental incentives. Vegetable and fruit residues are a rather challenging type of by-products as they are easily perishable and typically moist, sometimes extremely wet. Their production may be seasonal, and in many cases they are produced by small or medium size companies, resulting in rather small batches of the side streams, which makes efficient utilization of them challenging. FAO (Wadhwa and Bakshi, 2013) has estimated that nearly 50% of all fruits and vegetables in the European Union go to waste, with losses occurring during agricultural production, processing, distribution and by consumers.

Vegetable residues may be composted and used as soil amendments with only a small added value. One option to add value to these products is to preserve them by ensiling for use as livestock feeds. Indeed, Oroz & Davies (2015) stated that "it would be immoral" if not considerable efforts were taken to maximize the use of these nutrients by optimal storage and feeding. To be able to recycle these residues back into the food chain requires high hygienic quality of the products and good stability to allow efficient logistics.

Carrots are widely used as food and remarkable amounts of carrot by-products and discarded carrots are generated. They are rich in sugar making them very palatable and traditionally, they have been used as a supplemental feed for horses. They are also readily consumed by ruminants and fit well e.g. in TMR for growing cattle. The aim of the current study was to evaluate ensilability of a carrot by-product from a steam-peeling process either with or without silage additives (lactic acid bacteria strains or formic acid).

Materials and methods

We used carrot by-product from steam-peeling, which had been washed and heated so that the hygienic quality of the raw material was high. The by-product was ensiled immediately after receiving it from the company. Five additive treatments were used including a control without additive, two commercial LAB inoculants [heterofermentative (LAB1) and homofermentative (LAB2)], an in-house isolated LAB mix (LAB3) and a commercial formic acid based product (Acid). Details of the additives are presented in Table 1.

The carrot mass was ensiled in 3 replicates in 1.5-l glass jars which were allowed to ferment at +20 °C for 28 days. After opening the jars, solid and liquid fractions were separated by draining before analyses, which were conducted as presented by Seppälä et al. (2016). The N content of the original material was used to express ammonia-N proportions in total N after fermentation. Due to lack of material, water soluble carbohydrates (WSC) of the liquid fraction were analysed from samples combined across replicates.

Small 100-g glass bottles were used to evaluate dynamics of fermentation. Three replicates for each additive treatment were prepared for 0, 2, 6, 14 and 28 day fermentation periods to evaluate microbiological quality and aerobic stability at different time points. Carrot samples were analysed for enterobacteria, yeasts and moulds using standard methodologies. Aerobic stability of the carrot samples at days 0, 2, 6, 14 and 28 was measured by placing a 2 cm layer

of the carrot mass in a plastic container which was covered by a perforated plastic film and maintained at +20°C. Aerobic stability was evaluated once daily by visually observing growth of yeasts and moulds on the surface of the carrot mass. Additional three replicates were used to manually measure gas production from the bottles using a syringe to produce cumulative gas production curves over the 28-day ensiling period.

Table 1 Additives used in the carrot ensiling experiment

Abbr.	Name	Source	Composition	Amount used
LAB1	Bonsilage alfa	Schaumann Eurotrading	1k2071 *Lactobacillus plantarum* (DSM 21762), 1k2076 , *Lactobacillus paracasei* (DSM 16245), 1k2075 *Lactobacillus buchneri* (DSM 12856), 1k2082, *Lactococcus lactis* (NCIMB 30160) In product at least $1.25 \cdot 10^{11}$ bacteria/ g	2.5×10^5 CFU/g
LAB2	Josilac Classic	Josera GmbH & Co.	*Lactobacillus plantarum* LSI (NCIMB 30083 /1k20736), *Lactobacillus plantarum* L256 (NCIMB 30084 /1k20737), *Pediococcus acidilactici* P11 (DSM 23689 /1k1011), *Pediococcus acidilactici* P6 (DSM 23688 /1k1010) Enzyme 43 000 HET/g fresh matter: Xylanase from *Trichoderma longibrachiatum* MUCL 39203 (EC 3.2.1.8) (1k)	6 g/t 6×10^5 CFU/g
LAB3		Luke	A mixture of strains isolated from vegetables by Luke	ca. 1×10^6 CFU/g
Acid	AIV® 2 Plus	Eastman Chemical Company	76% formic acid, 5.5% ammonium formate, 18.5% water	5 l/t

Results and Discussion

The carrot by-product contained less DM and ash than the reference value in the Feed Tables (Luke, 2017), which can be explained by the processing of the material, but crude protein and crude fibre concentrations were quite similar (Table 2). Carotenes were analysed from the bottles at 0, 2, 6 and 14 d of ensiling resulting in 1.06, 0.85, 0.59 and 0.38 mg/g fresh matter and showing a clear decreasing trend with extending ensiling period.

The liquid was separated from solids by drainage. The method we used was not very efficient, but Acid treatment resulted in higher liquid separation than LAB. Dry matter concentration of the solid fraction was on average 84.3 and that of the liquid fraction 60.5 g/kg, i.e. a rather small difference. We only measured WSC and some fermentation end-products from the two fractions and they represented 0.108 of the solid fraction and 0.328 of the liquid fraction in the control and LAB treated samples, respectively. The Acid treated samples differed clearly from the other treatments, mainly due to high residual WSC concentrations. For the solid fraction, analyses recovered 0.413 and for liquids, certainly due to analytical errors, the recovery was 1.048.

Carrots are highly digestible and half of the DM was in the form of WSC. Such a material is readily fermentable, and indeed, the fermentation of the untreated material was very strong

and resulted in very low pH values and high concentrations of fermentation acids (Table 3). Virtually all WSC were fermented during the process. There were no differences between the three different LAB treatments in this experiment, and also very few differences between them and the control treatment, except the lower lactic, acetic and butyric acid concentrations in the liquid fraction of them compared to the control, and the higher pH (Table 3). Also DM concentration of both solid and liquid fractions were higher in LAB treated than in control samples.

Treating the material with Acid resulted in a totally different type of fermentation which was much more restricted and dominated by ethanol formation with virtually no lactic acid, but limited amount of acetic acid being produced. A sizeable amount of WSC was left after 28 days of fermentation (Table 3). The higher ammonia concentration of the Acid treated silages originates partly from the additive.

The cumulative production of fermentation gases illustrates clearly differences in type of fermentation among treatments (Figure 1). All LAB treatments increased rate and final volume of gas produced, compared to the control, while formation of gas from Acid treated material was very slow and did not reach a plateau during the 28-day observation period (Figure 1).

Table 2 Composition of the carrot by-product used in the current experiment compared with Feed Table (Luke 2017) values for carrots

	Carrot by-product	Carrot (Luke 2017)
Dry matter (DM), g/kg	87	120
pH	6.10	
In DM, g/kg		
Ash	48	80
Crude protein	93	100
Crude fat	6	15
Water soluble carbohydrates	501	
Crude fibre	127	100
Neural detergent fibre	177	
Feed values for ruminants		
IVOMD[1)], g/g	0.877	0.873
Metabolizable energy, MJ/kg DM	13.4	12.6
AAT, g/kg DM	94	92
PBV, g/kg DM	-50	-39

[1)]Pepsin-cellulase method calculated with the general equation of Huhtanen et al. (2006).

The fresh carrot by-product spoiled already after 2 days, but aerobic stability was greatly improved by Acid (Figure 2). Ensiling slightly increased stability of control and LAB materials but decreased that of Acid. Stability was 7 days after a 28-day fermentation period for all treatments except LAB3. The efficacy of formic acid based additives in improving aerobic stability of grass silages is well established (see e.g. Seppälä et al., 2016). In the current experiment, the advantages of using Acid disappeared by day 28 of fermentation, which may be related to accumulation of acetic acid in the other silages, which is known to improve aerobic stability.

Table 3 Gas production (GP) during fermentation, and composition and fermentation quality of the solid and liquid fractions of ensiled carrot by-products after 28 days of fermentation

	Control	LAB1	LAB2	LAB3	Acid	SEM	C1	C2	C3
GP, ml/g DM	13.7	23.9	20.0	19.0	21.4	1.09	<0.001	<0.001	0.765
Liquid proportion	0.027	0.015	0.020	0.015	0.039	0.0048	0.089	0.123	0.003
Solid fraction									
DM, g/kg	81.1	85.4	83.1	84.7	87.3	0.95	0.013	<0.001	0.024
pH	3.37	3.42	3.41	3.45	3.68	0.014	0.005	<0.001	<0.001
Amm. N, g/kg N	55	58	56	56	85	2.0	0.614	<0.001	<0.001
In DM, g/kg									
WSC	13.9	15.4	14.5	19.1	304	9.27	0.822	<0.001	<0.001
Ethanol	36.6	38.3	38.3	40.1	95.7	4.22	0.648	<0.001	<0.001
Lactic acid	172	158	175	152	1.1	80.9	0.215	<0.001	<0.001
Acetic acid	40.0	40.6	38.7	32.6	7.7	1.18	0.076	<0.001	<0.001
Propionic acid	1.7	1.9	1.7	1.8	4.1	0.10	0.600	<0.001	<0.001
Butyric acid	0.9	0.8	0.8	0.9	0.9	0.02	0.420	0.105	0.013
Liquid fraction									
DM, g/kg	59.1	68.2	64.0	70.8	40.5	0.00	<0.001	<0.001	<0.001
pH	3.21	3.20	3.19	3.19	3.38	0.013	0.437	<0.001	<0.001
Amm. N, g/kg N	79	79	73	63	161	5.2	0.252	<0.001	<0.001
In DM, g/kg									
WSC	4.7	5.4	4.5	7.8	823				
Ethanol	35.1	20.9	26.9	18.8	198	16.8	0.519	<0.001	<0.001
Lactic acid	282	244	241	197	3.0	9.19	<0.001	<0.001	<0.001
Acetic acid	63.0	55.2	52.6	49.5	16.3	3.61	0.029	<0.001	<0.001
Propionic acid	0.2	0.1	0.1	0.1	6.4	0.30	0.753	<0.001	<0.001
Butyric acid	0.8	0.5	0.6	0.6	1.0	0.08	0.024	0.254	0.002

For treatment explanations, see Table 1; SEM = Standard error of the mean; C1 = Control versus all LAB treatments; treatments: C2 = Control versus Acid, C3 = All LAB treatments versus Acid.

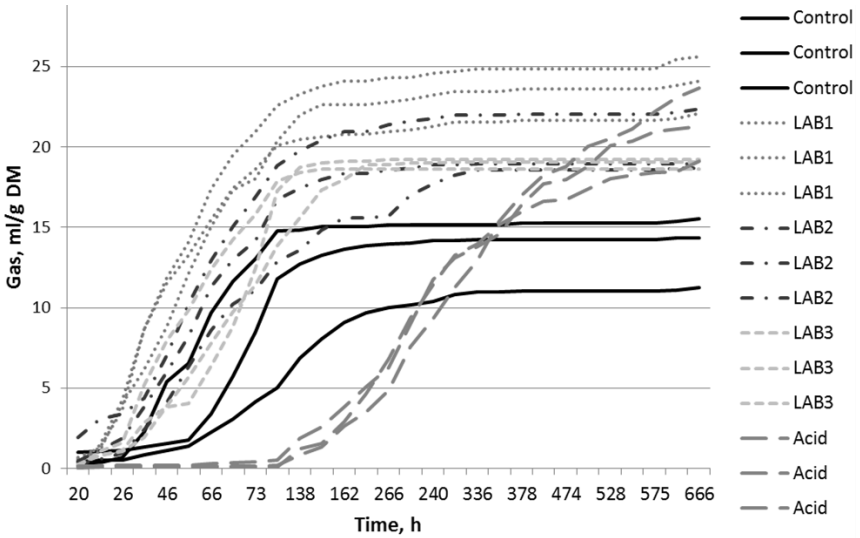

Figure 1 Cumulative gas production curves from carrot by-product ensiled using different additives (for explanations, see Table 1).

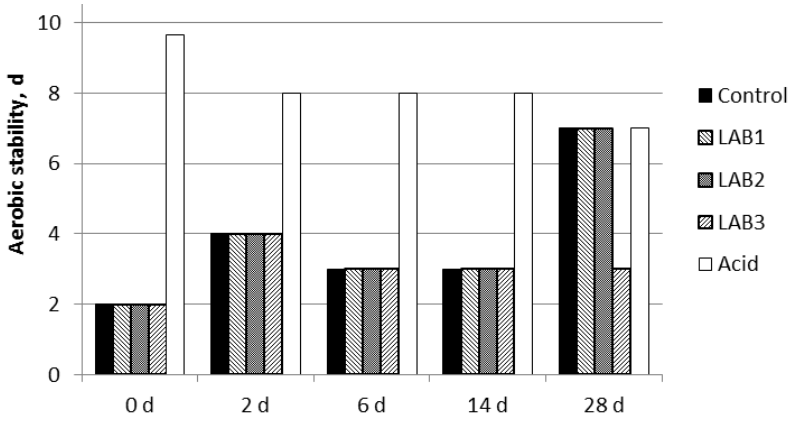

Figure 2 Aerobic stability of carrot by-product after different ensiling periods and additive treatments (for explanations, see Table 1)

Hygienic quality of samples was evaluated by determining enterobacteria, moulds and yeasts from the fresh product and after 2, 6, 14 and 28 days of ensiling. Microbial counts clearly decreased with time for all treatments. Counts of moulds and enterobacteria from fresh product were 6.4 x 10^3 cfu/g and 1.0 x 10^3 cfu/g, respectively. After 14 days, counts of all samples were below detection limit. Likewise, lower counts of yeasts were detected after ensiling. Fresh products had yeast population of 6.6 x 10^5 cfu/g while after 28 days, counts ranged between <10 cfu/g (LAB2) and 1.7 x 10^5 (Acid).

A novel idea that we wanted to test was to prepare a liquid feed that could be used for pigs. Although the liquid fraction has certain interesting qualities as a pig feed, it has a very low DM content and it may be difficult to ensure stable and sufficient supply to large pig units. In our experimental setting, the yield of liquid fraction remained very low, but that could be increased by technical solutions.

A general challenge with the carrot by-product is its dilute nutrient content. Dewatering would be a logical first step in further processing of the by-product, and ensiling the dry residue would probably be more successful than that of the original moist product. Companies therefore need to consider if it is profitable to invest in such processing.

A practical solution could be to use fresh by-product for cattle, and to use acid based silage additives to improve the stability for reduced delivery intervals. Carrot mass (fresh or ensiled) could also be a good source of digestible fibre for finishing pigs and gestating sows in extensive pig rearing systems. Liquid effluent from carrot mass could create a major practical problem. Co-ensiling carrots with some absorbents such as straw, hay, cereals or other dry by-products could alleviate this problem as was demonstrated in Spain in utilizing moist tomato by-products for feeding of ruminants (SOLID, 2017).

By-products, such as carrot derived feed materials, may also have positive effects in feeding of livestock as it is readily consumed and is rich in e.g. carotene. In case of fermented products, LAB and acids may stabilize the liquid feed prepared for pigs and have positive effects in the intestinal health of pigs, but for ruminants extended fermentation is known to restrict voluntary feed intake (Huhtanen et al., 2007).

If a vegetable company markets by-products as feeds, EU legislation requires them to register to ensure safety of the feed, which is the responsibility of its producer.

Conclusions

The carrot by-product could be preserved by ensiling, but the fermentation was very intensive if not restricted by Acid. Only minor effects could be detected from using different LAB compared to the Control. Stability of the fresh carrot by-product was remarkably extended by applying Acid. Many concerns in utilizing vegetable by-products as feeds for livestock remain, and solutions need to be found fitting each individual case both from producer and end-user point of views.

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Relation between seal integrity and hygienic quality in silage bales and differences between baling techniques

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Introduction

Bale silage is widely used in many countries on small and medium sized cattle farms and for horses. About 8 million bales are produced annually in Sweden, corresponding to approximately 45% of the total silage production of 4.5 million ton DM (Pettersson, 2006; SCB, 2016). Sufficient protection against air intrusion during storage is essential in all ensiling, and since introduction of the round bale technique, seal integrity of bales has continuously been improved. When the technique to make silage in bales was introduced, bales were inserted in plastic bags but this was soon replaced by the stretch film technique. During the last two decades, the polyethylene industry has continuously developed new stretch film qualities which have been tested on round and square bales of grass at the Department of Animal Nutrition and Management, SLU. Degree of air-tightness of bales, seal integrity have been measured in these studies by the time it takes for an induced under-pressure to disappear in the bales. Silage quality has thereafter been analyzed in terms of standard chemical analysis of fermentation parameters such as pH, ammonia nitrogen and volatile fatty acids and hygienic standard in terms of cell counts of yeast and mould.

The main purpose of the present work was to evaluate the relation between seal integrity, measured as pressure equalization time, and fermentation parameters and hygienic quality. Furthermore, data was used in a meta-analysis to study how silage quality was affected by number of stretch film layers and by using round- or square bales.

Materials and Methods

Data consisted of 29 experiments where seal integrity and silage quality parameters were analysed. Square bales had been used in 7 of the 29 experiments, while round bales were used in the remaining experiments. Crops were pure grass or grass/clover leys where clover proportion varied from 5 to 40% and had been harvested in first or second harvests in the southern half of Sweden (55º to 59ºN). Bales were made with 4, 6 or 8 layers of 750 mm wide stretch film with a thickness from 17-25 µm. The plastic was in most cases applied on netted bales but in some experiments, mantel film was used (Spörndly & Nylund, 2016). White stretch films were most common but, occasionally, some light green films were used and in two experiments, black films were used. Combined machines for pressing and wrapping were used in all experiments. All treatments were made with 6 replicate bales and in many experiments, several stretch films with different chemical compositions were tested for company product development purposes. Data comprised analyses of 1193 bales.

Bales were stored for at least 100 days after which they were subjected to seal integrity measurements where an under-pressure of -200 Pa was applied via a non-return valve. Level of air tightness was determined by time in seconds for under-pressure to be reduced to -150 Pa by air penetrating the seal. In the process of gas evacuation, carbon dioxide content (% of volume) in the bales was measured with a portable gas analyser GA 2000 (Geotechnical Instruments, Warwickshire, UK). After removing the stretch film, visible spots of yeast and

mould on bale surfaces were measured and areas of visible yeast or mould was expressed as percent of total bale surface area. Six cores (35 mm wide, 700 mm deep) per bale were then drilled and pooled into one sample per bale for chemical analyses. Dry matter (DM) and water soluble carbohydrates (WSC) were analysed on the pooled samples and pH, ammonia nitrogen (NH_3-N), ethanol, 2,3-butanediole, lactic, acetic, butyric and succinic acid were analysed on the liquid phase. All analyses were performed with wet chemistry methods as described by Åkerlind et al. (2011).

Correlation calculations between seal integrity (s) and surface yeast and moulds, CO_2 content and chemical fermentation parameters were performed using PROC CORR statement (SAS, 2014). Statement PROC GLM was used to investigate effects of bale type and number of stretch film layers. In all statistical calculations, bale was considered an experimental unit and effects were considered as statistically significant when $P < 0.05$.

Results and Discussion

Seal integrity was negatively correlated with yeast and mould on bale surface and positively correlated to carbon dioxide concentration and to lactic acid, ammonia nitrogen and succinic acid content in silage (Table 1). Correlations were low but still significantly different from zero. This implies that the method for measuring seal integrity works. A better seal integrity gives a better barrier between the atmosphere rich in carbon dioxide inside the bale and the outside air, resulting in less visible mould growing on the bale surface. The magnitude of the correlation was as mentioned lower than expected. It could be due to the variable dry matter content of bales in the different experiments, varying from 22 to 80%. Dry matter content is the main factor determining fermentation processes and this was not accounted for by simple correlation coefficients.

Table 1 Pearson correlation coefficients (r) of seal integrity and dry matter content with visible yeast and mould on bales, carbon dioxide content and a number of silage fermentation characteristics

Seal integrity

	Yeast	Mould	CO_2	DM	pH	Amm-N	Lactic acid	Acetic acid	Butyric acid	Succinic acid	Ethanol	2.3-butandiole
r	-0.07	-0.13	0.32	0.02	-0.03	0.08	0.10	0.02	0.02	0.30	-0.05	0.08
Sign.	*	***	***	Ns	Ns	*	**	Ns	Ns	***	Ns	Ns
N	1182	1180	490	1183	1183	698	754	692	330	243	646	543

Dry matter content

	Yeast	Mould	CO_2	Seal integrity	pH	Amm-N	Lactic acid	Acetic acid	Butyric acid	Succinic acid	Ethanol	2.3-butandiole
r	0.03	0.07	-0.27	0.02	0.77	-0.82	-0.55	-0.53	-0.45	-0.44	-0.17	-0.46
Sign.	Ns	*	***	Ns	***	***	***	***	***	***	***	***
N	1192	1190	490	1183	1193	703	761	699	335	243	651	545

N=number of observations. Ns=p>0.05. * = p<0.05. **=p<0.01. ***p<0.001.

Correlations between DM content and silage characteristics are shown separately in Table 1. The dominating influence of dry matter content was probably the main reason for the low correlation between seal integrity and effects on silage.

Analysis of effect of bale type and number of stretch film layers are in Table 2. Compared to square bales, round bales were better sealed with more than three times longer equalization times. Number of stretch film layers also had a clear effect on seal integrity. The major improvement took place when the number of layers increased from 4 to 6 layers, whereas further increase in air-tightness from 6 to 8 layers was considerably less. Increasing layers and a better seal integrity was also reflected in a higher carbon dioxide content in the bales and less mould growing on the surface.

Table 2 Effect of bale type and number of bale stretch film layers on seal integrity, carbon dioxide content and areas of yeast and mould on bale surface. N= number of observations

	Effect of bale type				*Effect of stretch film layers*				
	N	Square	Round	p<	N	4	6	8	p<
Seal integrity, sec	1183	149^{a}	547^{b}	0.001	1183	112^{a}	410^{b}	564^{c}	0.001
CO_2, %	490	56.8^{a}	43.2^{b}	0.001	490	40.1^{a}	57.6^{b}	69.9^{c}	0.001
Yeast , % of bale surface	1192	0.05^{a}	0.09^{b}	0.048	1192	0.11	0.07	0.04	0.211
Mould, % of bale surface	1190	1.88	1.98	0.604	1183	1.11^{a}	0.44^{b}	0.17^{b}	0.001

Superscripts (a, b, c) on the same row within effect indicate significant difference at p<0.05.

The better seal integrity of round bales compared to square bales was not reflected in higher carbon dioxide content or less yeast and mould growth. For carbon dioxide, this can possibly be explained by the fact that carbon dioxide measurements were introduced relatively late in the series of experiments and were, therefore, only measured in one of the seven experiments with square bales. This was, however, not the case for yeast and mould measurements but for mould, there was no difference and for yeast, the effect was barely significant (P=0.048).

Conclusions

The method for measuring seal integrity of silage bales by pressure equalization time was positively correlated to measurements reflecting other signs of seal integrity, such as bale carbon dioxide concentration. It was also shown that seal integrity measured in this way was associated with growth of yeast, and particularly, growth of mould. The meta-analysis of 29 experiments clearly showed that more layers of stretch film resulted in bales with a better protection from air intrusion and growth of mould. Improvements were greater when increasing from 4 to 6 layers than when increasing from 6 to 8 layers.

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