Proceedings of the 7th Nordic Feed Science Conference, Uppsala, Sweden

Nordic Feed Science Conference

June 14 - 15, 2016

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Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

Rapport 293
Report

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Department of Animal Nutrition and Management
Swedish University of Agricultural Sciences (SLU)
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Organising Committee:
Peter Udén
Rolf Spörndly
Bengt-Ove Rustas
Torsten Eriksson

Edited by:
P. Udén
T. Eriksson
B-O. Rustas
R. Danielsson

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Foreword

This year we hold the 7th annual Nordic Feed Science Conference in Uppsala. The aim of the conference is to create an arena for Nordic feed scientists to meet and discuss ruminant feeds and feeding. For this and future conferences, we have decided not to include horse feeding as this area is, nowadays, well covered in other conferences. We have been able to acquire some funding this year to include Analytical Quality of Feeds as a separate session and to invite Dr Arngrimur Thorlacius from Iceland as a keynote speaker. A session on Sub-Acute Ruminal Acidosis is also a very important event in the program this year. To cover this topic, we are extremely pleased that Prof. M. Oba from the University of Alberta, Canada, Prof. T. Mottram, Royal Agricultural University, Cirencester, UK and Dr. A.M. Denscher, University of Copenhagen have accepted invitations to speak at our conference. We wish to welcome these eminent scientists and thank them for coming all the way to join us in this year's conference.

A total of 25 papers have been received, covering topics, apart from those previously mentioned, related to feed conservation, processing of forages, animal models, 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/en/departments/animal-nutrition-management/news/nordic-feed-science-conference-2016/) where you also find a list of all titles.

Uppsala 2016-06-09

Peter Udén

The Nordic Feed Science Conference was initiated by Dr. Peter Udén and has been held annually at the Swedish University of Agricultural Sciences in Uppsala since 2010. It is now established as a valuable forum with an informal atmosphere, where PhD students have many opportunities to present their ongoing work. At the same time, a high scientific level is guaranteed by the leading international scientists that act as keynote speakers. This has been made possible only by Dr Udén's unique experience as a scientist and scientific editor and by his extensive international network in the animal science community. When Dr. Udén now retires from regular lecturing duties at our University, we hope that he will continue with his invaluable work for the Nordic Feed Science Conference.

Uppsala 2016-06-09

The Organising Committee for the 7th Nordic Feed Science Conference

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Improving feed analytical quality through proficiency testing

A.Thorlacius
Agricultural University of Iceland, Department of Environmental Science, Hvanneyri, 311 Borgarnes, Iceland.
Correspondance: arngrimur@lbhi.is

Introduction

A proficiency test involves the quantitative determination of one or more analytes in one or more homogenized test samples by several or many laboratories. Proficiency test samples are, prior to sample dispatch, put through a homogeneity test procedure to ensure that any significant difference between reported results for a particular sample and analyte, respectively, are not due to differences between the sample portions that are distributed to participants. Most proficiency tests are organized as regular schemes with sample dispatch at least once each year. Such tests are an integral and important part of the external quality assurance at any routine laboratory and their main function is to trace systematic errors which are not easily detected through internal quality control procedures. The presence of such systematic errors can rather be considered a rule than a rarely occurring event. This is a well-documented fact established through a countless number of inter-laboratory comparisons.

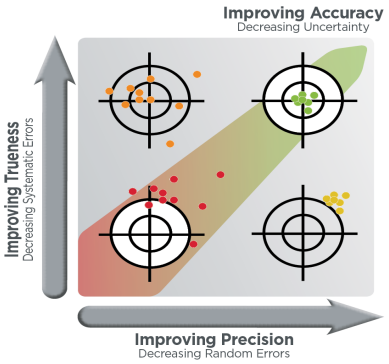

Figure 1 The relationship between the terms precision, trueness and accuracy, illustrated with analogy to marksmanship.

The words precision, accuracy and trueness all are, in context with measurement results, well defined concepts (ISO Guide 5725-1, 1994), with meaning different from their traditional use. Here precision refers to repeatability i.e. the smaller the random error, the more precise is the measurement. Reducing the systematic error on the other hand leads to improved trueness and reducing both types of errors gives better accuracy which is the overall goal. Fig. 1 may be helpful in clarifying these concepts. Improving trueness of measurements requires the reduction of systematic errors and one of the most important tools for this is the participation in proficiency tests. Collaborative tests, involving several laboratories, are used to test analytical methods before accepting them as standard or reference methods according to internationally accepted protocols. A mandatory feat here is to evaluate the within-laboratory as well as the between-laboratory error. Both error terms are estimated as standard deviation values and as a rule, the between-lab variation is much greater than the within-lab contribution to the error, even though all laboratories participating in such method-performance studies are regarded as highly proficient for performing the type of analysis being investigated. Such reference methods do in general have a more stringent and detailed

description of requirements and tasks involved in the determination than other less tested procedures. Moreover, laboratories offered or asked to participate in such studies are expected to both master and practice elaborate routines of internal quality assurance and they are required to do multiple determinations of each sample: Yet, their respective average results vary and to a significantly greater extent than repeated values produced by any single laboratory. This outlines an intrinsic problem of routine analysis and underlines the importance of proficiency testing.

In 1993, the International Union of Pure and Applied Chemistry (IUPAC) published "The International Harmonized Protocol for the proficiency testing of (chemical) analytical laboratories" and this has since formed a backbone of such activities worldwide. In 2006, IUPAC published a revised version (The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories). The harmonized protocol contains guidelines and recommendations rather than strict rules, but in some cases there is only one recommendation which then in practice may become a rule. One such case is that the only type of score recommended by the protocol is the z-score, underlining the need for a universally accepted and understood method of evaluating proficiency test results and undermining the various other types of estimates that have been introduced by interests other than those of quality assurance.

The z-score measures individual results as deviations from an accepted true value and is expressed in units of standard deviations. The score is sign-sensitive i.e., a negative z-score means that the result in question is lower than the accepted true value. The letter z refers to the z-distribution and the interpretation of z-scores assumes that the scores are normally distributed with a mean value of zero. Consequently one should expect about 95% of z-scores to fall within the range ±2. Scores numerically greater than three should then be extremely unlikely and the underlying analysis termed a failure. Values numerically between 2 and 3 are termed questionable.

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. Results from such tests have, however, often been used for external purposes either by the laboratories themselves or by stakeholders such as buyers of laboratory services, accreditation bodies or control authorities. Such external involvement may, however, undermine the main goal of the tests. When proficiency test results are being scrutinized by parties outside the laboratory, various non-scientific forces may come into action resulting from marketing issues, leadership pressure or even derailed professional pride, etc. Any of these may give incentive for data manipulations or even falsifications. Known issues of this sort are the reporting of averages from multiple analysis when single determinations are assumed and collusion between laboratories, i.e., two or more laboratories share results and repeat analysis, before reporting, when agreement between their respective results is poor. All data manipulation may be considered sabotage of the scheme since all involved laboratories, as a result of this, are not participating on equal grounds and also because most proficiency test schemes use participant consensus to decide upon accepted "true" values. For accredited laboratories, the participation in proficiency tests is mandatory and even this may affect the motivation of their analysts. Instead of being driven only by desire to locate hidden problems in their work routines or instrumentation, their participation in the proficiency test is partly or even mainly to fulfil an external requirement in order to maintain accreditation.

Proficiency test results must be treated in a confidential manner to secure test integrity and reduce the risk of data manipulation. The revised IUPAC Harmonized Protocol is quite clear on the requirement of confidentiality by both the scheme organizer and individual participants, respectively: "*The scheme provider shall not disclose the identity of a participant to any third party, including other participants, without the express permission of the participant concerned. Participants shall be identified in reports by code only. Participants may communicate their own results, including the regular scheme reports, privately to a laboratory accreditation or other assessment body when required for the purpose of assessment, or to clients for the purpose of demonstrating analytical capability. Participants may publish information on their own performance, but shall not publish comparative information on other participants, including score ranking."*

On the other hand the same Protocol also states, that: "*.. the 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 participant information*."

This author has been involved in organizing proficiency tests for feed laboratories for almost twenty years; first on behalf of the NJF (Nordic Association of Agricultural Scientists) and later through a free-standing scheme called NEPT (North European Proficiency Testing). A part of this work has been development of basic traits of the testing scheme itself, resulting in some unique features including the outlier testing procedure and the d-score parameter. These are discussed below.

Results and Discussion

The North European Proficiency Test scheme for the chemical analysis of feed stuffs has now completed its tenth round, involving twenty-five different samples of compound feeds, raw materials and silages, respectively. For several years, there has been an informal contact between NEPT and NorFor, regarding such matters as choice of analytes and sample matrices. Now, the two parts have decided upon a more formal contact where we will together attempt, by using proficiency test results as well as by other means, to improve the quality of analytical results used for the feed evaluation. It is therefore in its place here to give a brief overview of the status of affairs, as may be judged from these first ten rounds of proficiency testing. We have presently 31 different analytes available for reporting, except for the first two rounds, where the number was 28. Table 1 shows average values for the relative standard deviation, for the major energy components. It may be noted that the values are not to be used for statistical inference, since they are obtained as arithmetic means, without regard to the different population sizes among the raw values.

Table 1 Results for average values of the relative standard deviation, obtained from ten consecutive rounds of the NEPT scheme for the main energy-related analytes. (Kj.) and (D.) stand for respectively, Kjeldahl and Dumas and (h.l.) and (d.e.) for respectively acid hydrolysis and direct extraction

Item	Water	N (Kj.)	N (D.)	Ash	Fat (hl)	Fat (de.)	Fiber	ADF	NDF	Starch
Silages (av. % std.)	134	2.1	2.4	1.4	12.8	9.0	4.9	7.2	5.2	63.7
Qther feeds (av. % std.)	3.1-11.3	2.1	1	1.7	7.0	11.5	12.8	17.9	14.2	18.8
Conc. range (%)	3.1-11.3	1.3-10.0	1.4 - 10.2	1.4 - 19.7	2.2-26.5	2.2-25.5	0.3-26.4	0.7-29.6	1.8-51.1	0.5-66.8

Scrutiny of the underlying data set, led to a separation into two parts i.e. silages on one hand and compound feeds and raw materials on the other. The relative standard deviation for water content is surprisingly large for the silages. On the other hand, for fibre components, we get much smaller relative deviation for the silages as compared with the compound feeds and raw materials, respectively. For starch, this is the other way around. This is as expected since the silages are generally low in starch and high in fibre. Somewhat surprising though, is the poor reproducibility of starch at high levels i.e. in the raw materials and compound feeds. Here it would seem that some degree of harmonization might be in order, as the list of methods used is all but uniform.

Table 2 Results for average values of the relative standard deviation, obtained from ten consecutive rounds of the NEPT scheme, for macro minerals and some trace elements

Item	P	Ca	Mg	K	Na	S	Cl	Fe	Mn	Cu	Zn	Se
Silages												
(av. % std.)	7.1	8.1	6.6	7.6	14.6	11.1	4.1	11.0	8.8	19.6	15.7	72.2
Qther feeds												
(av. % std.)	5.8	12.5	5.9	7.7	27.6	20.2	9.3	13.0	9.4	15.3	9.9	51.8
	……			…%.....				……		..mg/kg.		
Conc.	0.2-	0.0-	0.1-	0.4-	0.0-	0.1-	0.0-	5-	4-	0-	0-186	0.0-
range	1.8	4.0	0.6	2.9	3.5	09	6.3	1814	174	155		2.4

Table 2 gives corresponding data for minerals and trace elements. Except for sulphur, results are for the most part as to be expected. Most relative standard deviation values lie between five and ten percent and where averages exceed this range, it is because of contributions from low concentration levels approaching the detection limit for the analyte and thus yielding high relative standard deviation estimates. This can be clearly seen for sodium, chloride and even calcium, though these are in most samples in well measurable concentrations, and not surprisingly for the trace analytes copper, zinc and selenium. The rather high values for iron may possibly be traced to laboratory contamination. The surprisingly high deviation observed for sulphur in many samples has a different explanation. When the number of reported results for an analyte is less than seven, we don´t employ the outlier procedure and no z-scores are calculated. This has been the case for the sulphur analysis for almost half of the samples. Moreover, for small populations, a single outlier has a rather dramatic effect, exaggerating estimates of the standard deviation. The remaining analytes are five amino acids (Cys, Met, Thr, Lys, Trp), soluble crude protein (sCP), digestible organic matter, indigestible NDF and ammonia. All these have in common that the number of reported results is low, hence, outlier testing is not applicable and compilation of relative errors are not meaningful.

The Harmonized Protocol puts a rather small emphasis on outlier procedures. This is in line with general practice of statistics where we often have to choose between the risk of falsely retaining data that contains only noise or outliers and falsely discarding relevant data. In hypothesis testing, this is often referred to as an error of Type I and Type II, respectively. When experiments are carried out to test some hypothesis, it may seem tempting to discard a point or two if the remaining data then "comply" better with your hypothesis. One may then accept and publish a theory based on an error of Type II, followed by most likely a short era

of glory. Generally, problems with Type I error are easier to handle. As a consequence, all texts on elementary statistics stress the importance of using outlier rejection cautiously or preferably not at all. In proficiency tests, the problem is quite the opposite. If one falsely rejects a result as an outlier, the consequence is a smaller standard deviation value and correspondingly an elevation of al z-scores calculated from this value. The laboratory most likely to obtain an outlying score for this reason will then have to go through its routines looking for error sources. This laboratory would have had the highest z-score of all even without the rejection and may well be in need of such scrutiny anyway. Retaining a true outlier, on the other hand, gives a too lenient scoring and may well render the whole exercise useless for the analyte in question.

Table 3 Results for chloride from sample no. 3 in NEPT, round no. 5 and for soluble crude protein (SCP) from sample no. 3 in NEPT round no. 9. Z-scores are not calculated when the number of results is < 7.

Chloride		sCP
(%)	Z-score	(g/kg)
1.12	0.57	84.00
0.66	-1.91	82.00
1.11	0.48	44.00
0.67	-1.85	83.00
1.04	0.12	84.00
1.11	0.48	
1.12	0.53	
1.13	0.59	
1.10	0.45	
1.12	0.53	

An example of this is given by the chloride results in Table 3. The number of results is ten, which evokes the outlier procedure, but due to masking, the two low results are retained and we accept a set of replicates with an almost two-times difference between the highest and lowest values. The remaining eight results are very closely grouped. In contrast to visual inspection, corresponding z-values for all ten measurements indicate that this is a perfectly acceptable set of replicates. For soluble crude protein we get four almost identical results and one severely deviating. As we don´t have any scores, the odd result raises no alarm. In a monograph by Iglewicz and Hoaglin (1993), entirely devoted to outlier testing, a procedure termed Generalized Extreme Studentized Deviate Test (Generalized ESD) is recommended. When comparing this test with z-scores based outlier test used in the NEPT scheme, and using a large number of test occasions from previous NEPT rounds, very similar results were obtained, but the Generalized ESD is more time consuming and it requires decision making by the data analyst. Further work on improving outlier detection for the NEPT scheme is, however, underway.

The d-score is a parameter, unique to the NEPT scheme, which is used to evaluate precision in proficiency testing. It requires that participating laboratories report duplicate results. The majority of results received are in fact reported as duplicates. The d-score is similar to the z-score and is calculated by the expression:

$$d_i = |A_i - B_i| / s\_d = |d_i| / s\_d$$

where, the index 'i' refers to laboratory number i, 'A' and 'B' are single measurements forming a duplicate and 's_d' is the within-lab standard deviation, calculated by one-way ANOVA. The sign of the difference has no meaning, hence the absolute value. The d-scores are interpreted analogous to z-scores. Thus, a d-value greater than 3 is unsatisfactory and a value between 2 and 3 is suspect or questionable. The d-score gives additional information to that conveyed by the z-score and may be particularly useful when an unfavourable z-score has been obtained. If you obtain a high d-score it tells you that your determination of the analyte in question is less precise than those made at other laboratories. If you also have obtained a bad z-score for the same determination the reason for this may in fact be some instability rather than a systematic error. In general, high d-scores indicate that one has room for improvements in one's internal quality assurance. As pointed out in the Harmonized Protocol (Thompson et al., 2006) validation of an analytical method can be based on proficiency test result if certain criteria are met. This may be a very cost-effective alternative to formal collaborative tests. Added information in the form of d-scores makes this utilization even more feasible.

Conclusions

Participation in proficiency tests is an absolute necessity for any routine laboratory striving to produce accurate results. The present work, as well as numerous other studies, has demonstrated that proficiency test results can be utilized to reveal inconsistencies even amongst repute laboratories. It has further revealed that the choice of outlier procedure used to pre-treat test data, can greatly influence the outcome and usefulness of the tests. Proficiency test data may also be utilized to validate methods used for producing the analytical data, thus giving the methods added value.

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Are the nutrients and energy values for commercial compound feeds in the NorFor feedstuff table correct? – a NIR screening conducted using different laboratories

M. Åkerlind[1] and N. I. Nielsen[2]
[1] *Växa Sverige, Box 7024, 750 07 Uppsala, Sweden.*
[2] *SEGES, Dairy and Beef Research Centre. Agro Food Park 15, 8200 Aarhus N, Denmark.*
Correspondence: maria.akerlind@vxa.se

Introduction

The NorFor model has been used in ration formulation for cattle using different software in the Nordic countries for nearly 10 years. The increased use of NorFor during these years has led to an increased focus on the energy value of compound feeds, as the energy content in relation to price is a key parameter (kr/MJ) when dairy and beef farmers purchase feeds. In NorFor, energy values are calculated under a set of standard conditions which implies a cow weighing 600 kg and consuming 20 kg DM/d (Åkerlind and Volden, 2011). This standard energy value is listed as a NEL20 value in the NorFor FeedStuff Table (FST; www.norfor.info). The NorFor FST is unique in the sense that it contains nutrient and energy values for approximately 2000 commercial compound feeds. Feed companies themselves upload and update their own compound feeds and NorFor has established guidelines on how to do this (www.norfor.info/feed-company).

Due to the increased focus on energy values for compound feeds in the NorFor system, some concrete cases of discrepancies between NEL20 listed in the NorFor FST and on the delivery note/sales materials have been reported. Farmers and advisors have occasionally asked: "why does my compound feed have one NEL20 value in the sales materials/product sheet and another value in the NorFor feedstuff table (FST)?". Also feed companies have been puzzled by the NEL20 or AAT20 values reported by other feed companies in the NorFor FST. Furthermore, some advisors have noticed that compound feeds from specific feed companies often are chosen in least cost ration optimizations at the expense of compound feeds from other companies.

In Denmark, the energy content of commercial compound feeds has been analyzed for more than 20 years in order to ensure that the farmer gets the quality he/she has paid for. This analysis is done by use of an enzymatic method (EFOS) that measures organic matter digestibility which can be further used in the calculation of Feed Units (Weisbjerg and Hvelplund, 1993). The latest analyses of compound feeds in Denmark by NIR at Kvægbrugets ForsøgsLaboratorium (KFLab) contained 90 compound feeds with declared feed units (FU) and 291 compound feeds with declared fat and protein contents (Kristensen and Thøgersen, 2015). Thus, the EFOS method helps to ensure that the declared energy content is correct in commercial compound feeds and therefore such a method helps the farmer and the advisor to choose the most profitable compound feed based on a transparent energy content and price. Unfortunately, such a method is not available at the moment for measuring NEL20 in the NorFor system.

The objectives of this investigation were:

- to compare nutrients and energy values in the NorFor FST with analyzed values for commercial compound feeds
- to identify if the magnitude of the differences between analyzed and FST values were related to specific feed companies
- to identify possible differences in NIR analyzes between laboratories

- to identify possible pathways to determine the NEL20 content in compound feeds with unknown composition of ingredients

Materials and Methods

Sampling
In 2015, the collection of compound feeds from dairy farms in Denmark (DK; n=15), Norway (NO; n=10) and Sweden (SE; n=15) and from feed factories in Iceland (IS; n=6) was initiated and a total of 46 samples were collected by farmers and/or advisors. The intention was, within each country, to select commonly used compound feeds that would represent different feed companies and a large variation in energy content and nutrients in terms of crude fat (Cfat), crude protein (CP), NDF and starch. A total of 20 feed companies were represented: 7 from DK, 3 from IS, 4 from NO and 6 from SE with 1 (n=4), 2 (n=8), 3 (n=6) or 4 samples (n=2) per company.

A*nalyses*
All samples were analyzed by NIR at KFLab in Denmark and the SE and NO samples were also analyzed by NIR at Blgg AgroXpertus (Blgg) in the Netherlands.

Comparison of NorFor feedstuff table values with analyzed values
The information on nutrients and energy values for the specific compound feeds were extracted from the NorFor FST on the same day as the sample was collected on farm. The NorFor FST and the NorFor model are available via national software tools where ration formulation is done. The software tools where nutrients and energy values were extracted were: DMS in DK, IndividRAM in SE and Optifór in IS and NO. In order to have a reference for differences between NorFor FST values and analyzed values, EU tolerances for cattle feeds were chosen (Table 1).

Table 1 EU tolerances (EU 939/2010) for cattle feeds that were applied when comparing analyte values from the NorFor feed stuff table with analyzed values

Declared content	Lower tolerance (too low content)	Upper tolerance (too high content)
Crude protein		
>24%	3% units	3% units
8-24%	12.5%	12.5%
<8%	1% unit	1% unit
Crude fat		
>24%	3% units	6% units
8-24%	12.5%	25%
<8%	1% unit	2% units
Ash		
>24%	No lower tolerance	3% units
8-24%		12.5%
<8%		1% unit
Crude fiber and NDF[1]		
>20%	3.5% units	3.5% units
10-20%	17.5%	17.5%
<10%	1.7% units	1.7% units
Starch		
>20%	3.5% units	7% units
10-20%	17.5%	35%
<10%	1.7% units	3.4% units

[1] NDF is not part of the EU-regulation (EU 939/2010) but was here assumed to have same tolerance as crude fiber.

Results and Discussion

Composition of the compound feeds, estimated by NIR, is presented in Table 2. The range was wide for crude protein, crude fat, NDF, starch and estimated energy (NEL20).

Table 2 Nutrients and energy content of 46 compound feeds from 20 feed companies in Denmark (n=15), Iceland (n=6), Norway (n=10) and Sweden (n=15). Values represent analyzed values from KFLab

	Mean	Min.	Max.	s.d.
Crude protein, g/kg	219	138	406	67
Soluble CP, g/kg CP	231	152	366	43
Crude fat, g/kg	60	27	142	21
NDF, g/kg	204	87	297	47
Starch, g/kg	212	5	397	110
NEL20, MJ/kg	7.54	6.6	8.7	0.48

Figures 1a-d show comparisons between nutrients from the NorFor FST and analyzed values for 46 commercial compound feeds. In order to visualize how small/big the differences are between analyzed values and FST values, the EU-tolerances (EU 939/2010) for declared nutrients in cattle feeds were applied. Generally, values of commercial compound feeds in NorFor FST are updated once or twice a year by the feed companies. However, the composition of ingredients can change many times during the year and therefore, small deviations between values in FST and values in the compound can be expected.

The investigation of crude protein contents (Figure 1a) showed that 10 samples were outside the minimum and maximum EU tolerances. It could be argued that the EU permitted tolerances might be too narrow and too strict for FST values that are updated once or twice a year, but this was chosen to have some sort of reference point.

The investigation of crude fat (Figure 1b) showed that 5 samples had less analyzed fat compared with the value in NorFor FST. There was one extreme outlier (Figure 1b). Swedish compound feeds stand out in the analysis of crude fat from KFLab, i.e. KFLab reported lower crude fat contents than what the feed companies have listed in NorFor FST (Figure 1b). However in general, KFLab measured 9% lower crude fat than Blgg on the Swedish samples (data not shown). In Sweden, it is common to use fat in the form of calcium soaps in compound feeds and therefore a hydrolysis is needed to break the calcium ester links to get correct analytical results. However, both laboratories present NIR results based on chemical analysis of fat that include hydrolysis, so this does not seem to be the explanation. One can speculate that either chemical analysis of crude fat between laboratories are done differently (although the method should be the same) and/or the fat sources in the compound feeds used for the NIR calibration at the two laboratories differ substantially.

The investigation of NDF (Figure 1c) showed that 9 samples were outside the minimum and maximum levels corresponding to the EU limits for crude fiber.

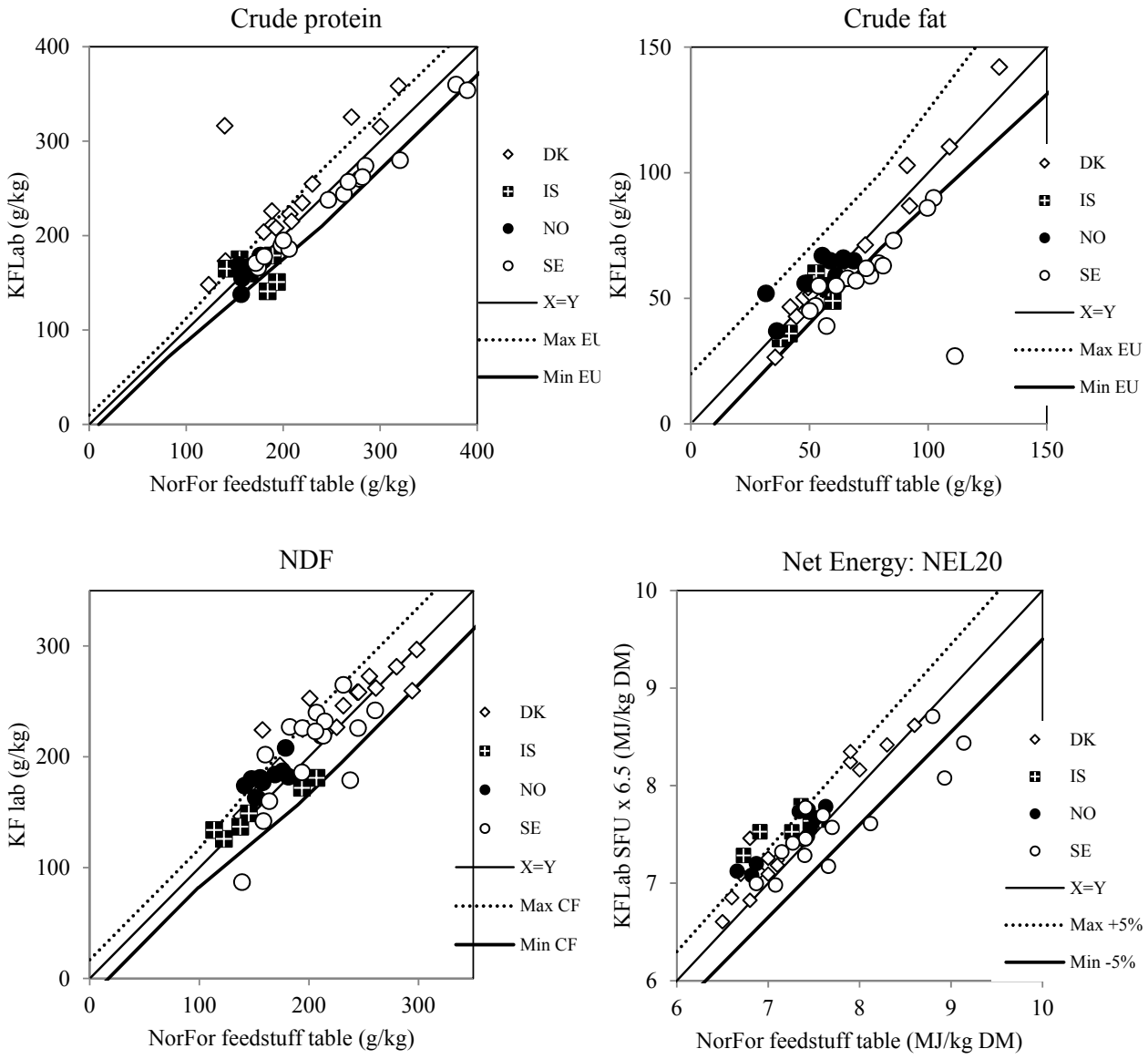

Figure 1 a-d Comparisons between NIR analyzed nutrients from Kvægbrugets ForsøgsLaboratorium (KFLab) and corresponding nutrients from the NorFor feed stuff table for 46 compound feeds from 4 countries (DK=Denmark; IS=Iceland; NO=Norway; SE=Sweden). The max and min EU line indicate upper and lower tolerances, respectively, for declared contents in cattle feeds. SFU=Scandinavian Feed Unit

The investigation of net energy (NEL20) (Figure 1d) showed that 9 samples were outside the minimum and maximum levels. Results from one of the samples could be explained by an extreme outlier for crude fat. It is noteworthy that three of the samples, which were below the minimum line, i.e. higher NEL20 values in NorFor FST than analyzed, came from one feed company. This deviation could not be explained by analyses of nutrients in this screening. There is no analytical method for NEL20 at the moment and therefore, NEL20 was estimated from the NIR results of EFOS and crude fiber via calculation of the Scandinavian Feed Unit (SFU), according to Weisbjerg and Hvelplund (1993). Based on raw materials from the

NorFor FST and data on compound feeds from Weisbjerg and Hvelplund (1993), a relationship between SFU and NEL20 was established (Figure 2).

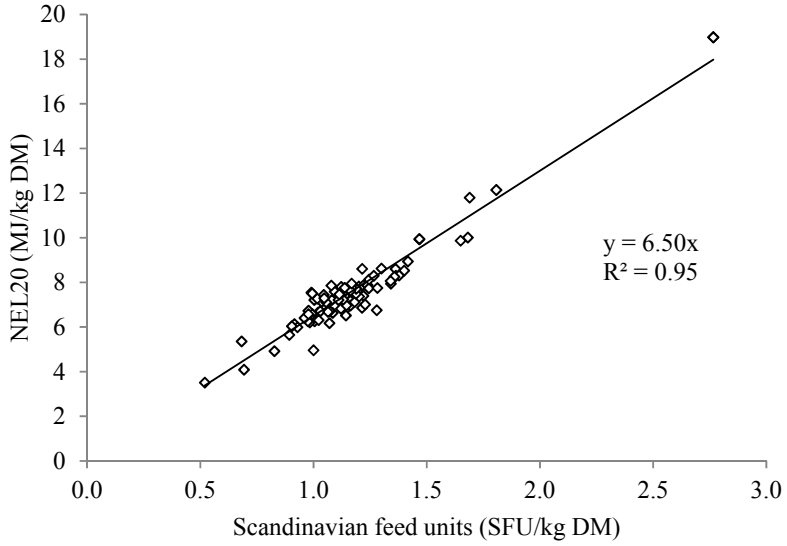

Figure 2 The relationship between Scandinavian feed units (a Danish energy value for cattle feeds) and NEL20 based on 78 raw materials from the NorFor feedstuff table and 23 compound feeds from Weisbjerg and Hvelplund (1993).

An evaluation of the NIR analyses from the two laboratories showed in general good agreement, except for soluble CP (Table 3).

Table 3 Evaluation of the correspondence between NIR analyses performed by Blgg and KFLab on 21 compound feeds from Norway and Sweden

	MPE (%)	RMSPE	Intercept	Slope	R^2
DM, g/kg	1	8	332	0.62	0.41
Ash, g/kg	14	9	26	0.54	0.46
Crude protein, g/kg	6	13	-1.0	1.03	0.97
Soluble CP, g/kg CP	26	60	182	0.13	0.07
Crude fat, g/kg	11	7	8.5	0.82	0.82
NDF, g/kg	13	24	38	0.81	0.81
Starch, g/kg	9	18	10	0.98	0.98
Crude fiber, g/kg	12	10	-2.3	0.93	0.93
OMD, % of OM	2.8	2.3	31	0.63	0.68
NEL20[1], MJ/kg DM	3.6	0.26	0.32	0.98	0.86

[1]NEL20 was not analyzed but calculated from a relationship between NEL20 and SFU (see Figure 2).

Conclusions

- Some caution should be exercised as results are based on a small sample size (46 out of ~2000) and also on analysis by NIR
- Generally, there was a reasonable agreement between declared nutrients in FST and analyzed values:
  - 10 samples (22%) were outside EU-tolerances for protein
  - 5 samples (11%) contained less fat than the EU-tolerance
- Indications that one feed company (out of 20) declared higher energy values in FST than found by estimations

- Good agreements were found between NIR results from Blgg and KFLab, except for soluble CP.
- This investigation has led to a specification of guidelines for updating compound feeds to the NorFor FST
- There is a need for a laboratory method to determine NEL20. A possible way forward is to use an *in vitro* OMD analysis.

Perspective
NorFor will pursue efforts to find a cheap and fast method with good accuracy and precision that can quantify the energy content in compound feeds in terms of NEL20.

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A note on sample preparation in the analysis of nitrate and nitrite in forage

R. Spörndly, T. Grönqvist, M. Knicky & B. Ericson
Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Feed Science Division, Box 7024, 750 07 Uppsala.
Correspondence: Rolf.Spörndly@slu.se

Introduction

Nitrate content in forage is of interest as it pose a risk of poisoning for ruminants at levels above 1000 mg NO_3-N per kg DM (Strickland et al, 1995; Undersander et al, 1999). Analysing nitrate in forages by colorimetric methods have been questioned during decades due to interference of coloured substances in the forage (Wiseman & Jacobson, 1962; Wegner, 1972; Anderson & Case, 1999). Nevertheless, the colorimetric method using flow injection analysis (FIA) with a Cu-Cd reduction column has become a standard method in analysing nitrite and nitrate after reduction to nitrite (MacKown & Weik, 2004). The problem of diverging results in nitrate analysis has often appeared as a result of different nitrate extraction techniques, as opposed to differences due to detection methods after extraction (Anderson & Case, 1999). The sample preparation and analytical method in use at the departments of Soil and Environment, Crop Production Ecology and Animal Nutrition and Management at the Swedish University of Agricultural Science has been water extraction of dried samples followed by the nitrite and nitrate analysis according to the ISO method 13395;1996. As nitrate, and in particular nitrite, are unstable compounds, doubts have been raised concerning the influence of the sample preparation step. The present study was made with the aim to compare result of nitrite and nitrate analyses of dried or un-dried samples and extraction with boiling water or room tempered water.

Materials and Methods

Samples of fresh mixed grass-red clover forage were treated as in Figure 1.

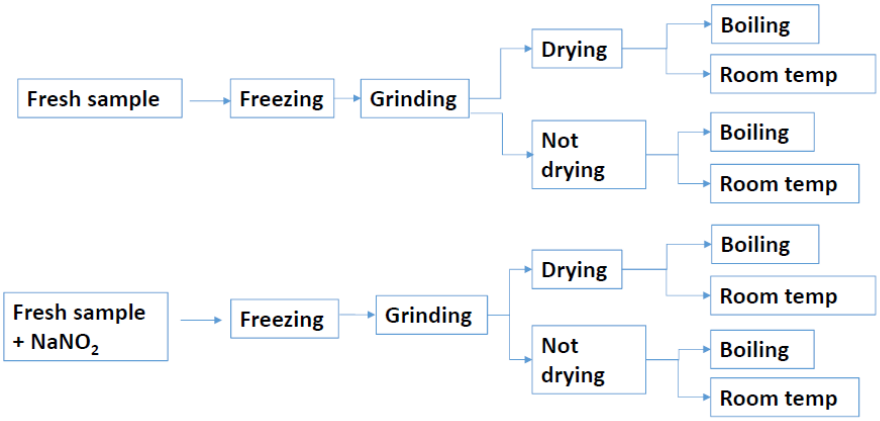

Figure 1. Set up of experiment to evaluate the effect of drying and extraction on the level of nitrite and nitrate content in a grass- clover forage crop.

Two kg of the fresh forage crop was wilted to 50% DM and divided in two 1 kg portions. To one portion, 3 ml Safesil® (equivalent to 30.435 mg NO_2-N/kg) was added in the form of an aerosol to cover the entire forage surface and mixed inside an inflated plastic bag. Both portions were frozen to -20ºC and ground frozen in a meat grinder and thoroughly blended.

These two portions, one blank and one nitrite spiked, were each of them treated as follows. One half was dried at 60ºC for 16 h and two 1-g sub-samples were mixed with 100 ml distilled water each. The other half was not dried and 2 samples, equivalent in size on DM basis to the dried samples, were mixed with 100 ml distilled water each. Extraction was performed as follows: one of each parallel water suspension was boiled for 10 minutes and one was shaken for 1 h at room temperature after which they were diluted with distilled water to 250 ml and filtered through filter paper (Whatman 602H½).

After extraction, all samples were analysed without delay according to the procedure prescribed in the ISO method 13395:1996 as follows: liquid extracts were analysed for nitrite-N by a computerized flow injection analysis (FIA) system (FIAstar™ 5000 Analyzer with the PC software 'soFIA' from FOSS, www.foss.dk). The colorimetric detection was based on nitrite with sulfonilamide forming an azo dye product with N-(1-naphthyl)ethylenediamine (Griess reagent). Absorbance was determined at 540 nm. Nitrate was then reduced to nitrite in a copper-cadmium column and nitrite-N was analysed again. Nitrate was then calculated by difference. Absorbance for all samples was also determined without reagents and deducted from the gross determinations of nitrite. Finally, the nitrate- and nitrite-N were calculated to as per mg/kg DM.

Results were processed by the GLM procedure by SAS using nitrite-treatment, preparation method and extraction method as class variables (SAS ver. 9.4. SAS Institute Inc., Cary. NC. USA).

Results and Discussion

The fresh crop selected was very low in nitrate and nitrite. The values were all very close to the detection limit and are presented in Table 1. Nitrite treatment increased both nitrate and nitrite levels ($p<0.0028$). No difference could be seen between extraction methods ($p>0.8156$). However, preparation method made a difference ($p<0.0035$), as drying the samples resulted in considerably lower nitrate and nitrite levels compared to no drying. Sample drying seemed to reduce nitrate content with by on average 36% and the nitrite content by 90%.

Table 1 Nitrate and nitrite in fresh crop samples with or without added NO_2, with or without drying and extraction in boiling or room temperate water (average ± SEM of two replicates)

		NO_3-N, mg/kg DM		NO_2-N, mg /kg DM	
Sample	Extraction	Dried	Un-dried	Dried	Un-dried
Fresh crop	Boiled	61 ± 1.0	61 ± 1.0	7 ± 0.5	10 ± 0.5
Fresh crop	Room temp	60 ± 1.0	59 ± 0.5	3 ± 0.0	4 ± 0.0
Fresh crop + NO_2	Boiled	72 ± 2.0	181 ± 2.0	11 ± 0.0	80 ± 1.5
Fresh crop + NO_2	Room temp	68 ± 0.0	188 ± 2.0	5 ± 0.0	85 ± 2.5

The low basal nitrate and nitrite content of the crop was not, or only slightly, affected by the drying process. For samples where $NaNO_2$ was added, the drying process reduced the nitrate and nitrite content ($p<0.05$).

After evaluation of the effect on fresh crop similar analyses continued with silages. As the extraction method did not affect the result of nitrate and nitrite analyses, it was decided only to evaluate the effect of sample preparation (dried or un-dried) on recovery of nitrate and nitrite in silages. The result from 4 silage samples from the Czech Republic and 7 silages from the USA are presented in Table 2.

Table 2. Nitrate and nitrite in silage samples prepared with or without drying. All samples were extracted in boiling water

	NO_3-N, mg/kg DM		NO_2-N, mg/kg DM	
	Dried	Un-dried	Dried	Un-dried
Silage Czech republic 1	1155	1134	0.7	0.5
Silage Czech republic 2	892	908	0.7	0.5
Silage Czech republic 3	849	827	0.7	0.6
Silage Czech republic 4	123	95	0.7	0.6
Silage USA 1	182	191	0.7	1.6
Silage USA 2	19	25	3.9	5.6
Silage USA 3	237	270	5.1	9.6
Silage USA 4	253	246	5.6	10.1
Silage USA 5	11	14	4.2	5.5
Silage USA 6	21	32	5.9	8.9
Silage USA 7	23	26	7.1	8.8

No clear difference between dried and non-dried samples in nitrate and nitrite results can be seen in the Czech Republic samples. These samples were maize silages. In the samples from USA, which were alfalfa silages, a tendency for 7% lower nitrate values of the dried compared to un-dried samples were seen. Nitrite was affected to a greater extent by drying showing a 34% reduction.

In this experiment, nitrate and nitrite analysis according to ISO 13395:1996 was performed immediately upon extraction. In a preliminary investigation, we observed that freezing to -18ºC, followed by thawing, decreased both nitrate and nitrite content in the sample. This observation, together with the main results in this paper suggest that further detailed studies are needed in order to establish complete recommendation of sample preparation for nitrate and nitrate analysis.

Conclusions

No differences in the nitrate and nitrite levels of fresh forage were observed if the extraction step was made by boiling the sample in water for 10 minutes or shaking in water at room temperature for 1 hour. Effects of drying the samples prior to extraction were however noticed. When $NaNO_2$ was added to the fresh crop, drying at 60ºC for 16 h resulted in nitrate loss of 36% and nitrite loss of 90%. In preparation of silage samples, the drying process resulted in a 13% loss of nitrate and 63% loss of nitrite in some maize and alfalfa silages. It is

recommended not to use drying during sample preparation for nitrate and nitrite analyses. Instead extractions of fresh or frozen samples should be made.

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Different experimental designs in testing of silage additives

M. Knicky[1], R. Spörndly[1], F. Eide[2] & B. Gertzell[2]
[1-2]Department of Animal Nutrition & Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences, S-753 23 Uppsala, Sweden
[2]AB Hanson & Möhring, P.O. Box 222, S-301 06 Halmstad, Sweden

Introduction

Quality of silage fermentation and consequent aerobic stability of silages is still a common problem of many types of silage. Experimental testing of silage additives is commonly conducted under routine ensiling condition with properly consolidated forages and airtight silos. Consequently, it is not surprising that results of these trials do often not display a potential of a product in the agricultural practice. Punctures and other damages of silo cover as well as uneven forage consolidation in a silo are common. These defects make ensiling conditions more difficult and challenge a silage additive to fulfil its purpose. It has been observed that silos which were not tight under the fermentation process are more prone to be aerobically unstable (Jonsson & Pahlow, 1984). Based on this observation, a German system for evaluation effects of silage additives (DLG, 2009) applies a design where silage additives are tested under difficult ensiling condition by two times of air ingress into a mini-silo for 8-12 hours combined with a very low packing density. This condition, however, does not properly reflect silo un-tightness. It is more common that a silo is exposed to a weak but constant air ingress. This condition is more closely reflected by a design with a 2-hours weekly air ingress used by Pauly and Hjelm (2015) in testing efficiency of silage additives on conservation of crimped maize.

Therefore, the objective of this study was to compare the impact of ensiling challenged by weekly aeration in a silage additive test to improve forage conservation.

Materials and Methods

A mixture of timothy (15%, head visible), perennial ryegrass (30%, vegetative stage), meadow fescue (16%, head visible), and red clover (vegetative stage, 39%) was harvested with a scythe on 10th of June 2015. The crop was directly chopped in a stationary cutter to approx. 2 cm particle length. After chopping, the forages were mixed with a suspension of *Clostridium tyrobutyricum* spores at a rate of 1 x 10^5 per g fresh matter (FM) and partitioned into two fractions. One fraction was left untreated and served as a control. Remaining fraction was treated with the additive Safesil (20% sodium benzoate, 10% potassium sorbate, and 5% sodium nitrite) at a rate of 3 L/t (FM). The silage additive was applied by hand with a spray bottle on the forage spread out on a sheet of plastic film and mixed thoroughly. Sub-samples (5 kg FM) from each fraction were then ensiled in 6 mini-silos each (1.7 L volume with a fermentation lock in the lid) and ensiled under two ensiling conditions. Half the mini-silos were tightly sealed with a fermentation lock during the entire storage time (DLG design for testing efficiency of silage additives WR1, DLG, 2009). Silos in the challenged ensiling condition were packed at the same density as the tight silos, but had lids fitted with rubber stoppers (≈ 6 mm), when removed, allowed air ingress into the silos. The rubber stoppers were removed for two hours every week during the storage period.

Each treatment consisted of 3 replicates and the silos were stored for 98 days at a room temperature of 21˚C. At the end of storage, silages samples were extracted and analysed for dry matter, volatile fatty acids, lactic acid, ethanol, pH, water soluble carbohydrates, lactic acid bacteria, clostridia spores, yeasts and tested for aerobic stability.

Results and Discussion

The chemical and microbiological composition of the forage, prior to ensiling, is presented in Table 1. Chemical composition of fresh forage represented a common composition found in first cut grass crops in Sweden except for the low nitrate content. Calculated fermentation coefficient of 38 characterizes the forage as slightly above the limit for a difficult crop to ensile (Weissbach et al., 1974).

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

Analyses	Unit	Clover-grass
DM	%	19.9
Ash	%	9.5
CP	%	11.6
WSC	%	15.7
NDF	%	44.8
Nitrate-N	mg/kg DM	2.1
ME	MJ/kg DM	11.1
Ammonia-N	% total N	1.2
Buffering capacity	g LA/100 g DM	7.1
LAB-homofermentative	log cfu/g FM	6.2
LAB-heterofermentative	log cfu/g FM	3.9
Clostridia spores	log cfu/g FM	3.8
pH of fresh forage		5.8
Fermentation coefficient		38

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

Results from chemical and microbiological analyses of the silages are presented in Tables 2 and 3. As expected, low DM content of the crop caused extensive fermentation, signified by low pH, high levels of fermentation products and depletion of WSC.

Results also show major differences between control and additive treatments. Additive treated silages were found to have a lower silage pH and a higher concentration of lactic acid. Butyric acid concentrations were below the detection limit in all additive treated silages and significantly less in comparison with controls. As butyric acid is considered to be a major product of clostridia, it is not surprising that control silages were found to have significantly higher counts of clostridia spores than additive treated ones.

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

Treatment	DM	pH	NH_3-N*	Lactic acid	Acetic acid	Butyric acid	2.3-butanediol	Ethanol	WSC
	%		% of TN	% of DM					
Control	18.1	4.5	10.9	9.3	2.6	1.7	2.9	2.0	0.7
Safesil	19.4	4.1	4.9	11.6	1.4	0.0	0.1	0.4	6.4
Control-Air	17.9	4.6	11.1	5.4	6.5	1.0	3.0	1.9	0.8
Safesil-Air	19.3	4.1	5.9	11.2	2.4	0.0	0.2	0.5	5.6
$LSD_{0.05}$		**0.06**	**0.38**	**0.88**	**0.69**	**0.19**	**0.44**	**0.27**	**0.25**
P-additive		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
P-air		0.01	0.001	0.001	0.001	0.001	0.4	0.6	0.003
P-add+air		0.02	0.01	0.001	0.001	0.001	0.8	0.1	0.001

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

Table 3 Aerobic stability and microbiological composition of silages after 98 days of storage at an ambient temperature of 20.2˚C (n=3)

Treatment	Time (h) until temp. rise of 3˚C	Max-temp (˚C)	Max. temp-increase (˚C)	pH after stability	Yeasts	Clostr. spores	LAB Homoferm.	Heteroferm.	Weight loss
					log cfu/g				% DM
Control	216	20.5	0.0	4.5	<1.7	4.6	<4.7	7.4	14.7
Safesil	216	20.7	0.2	4.1	<1.7	1.7	<4.7	6.2	2.4
Control-Air	84	37.1	16.5	7.5	3.6	4.3	<4.7	7.9	15.1
Safesil-Air	216	20.7	0.1	4.1	<1.7	1.8	<4.7	4.7	4.2
$LSD_{0.05}$	**26.2**			**0.62**	**0.29**	**0.21**	**-**	**0.29**	**0.45**
P-additive	0.001			0.001	0.001	0.001	n.s.	0.001	0.001
P-air	0.001			0.001	0.001	0.2	n.s.	0.001	0.001
P-add+air	0.001			0.001	0.001	0.02	n.s.	0.001	0.001

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

The results confirm results from previous studies (Knicky & Spörndly, 2009, 2011) of the ability of the present additive composition to eliminate clostridia. A similar development, as for butyric acid, was observed for ammonia-N formation where control silages displayed higher ammonia-N levels than additive treated silages. Reduced formation of undesirable ensiling products such as butyric and acetic acid, ethanol and 2,3-butanediol were probably the reason for significantly lower silage losses in the additive treatments compared to controls (Fig. 1).

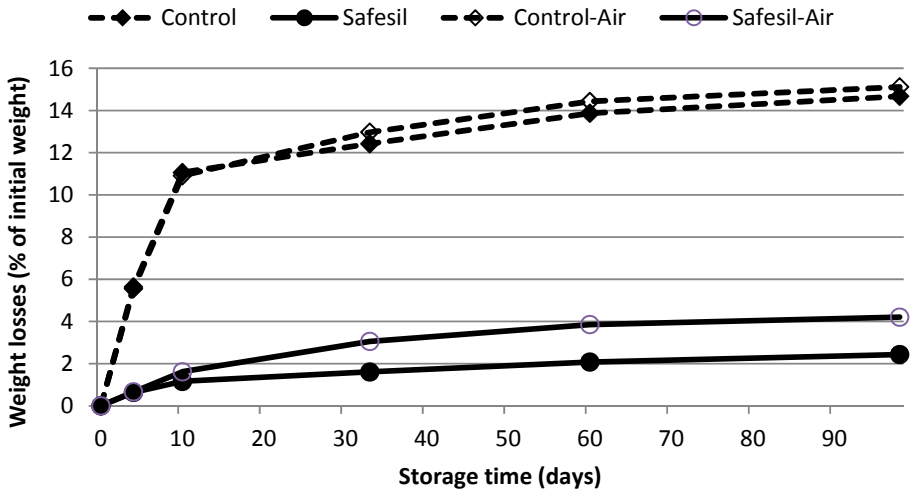

Figure 1 Weight losses of silages stored for 98 days (n=3).

The air ingress during ensiling mainly influenced silage quality parameters of the control treatments. Control aerated silos were found to have a higher pH, a lower concentration of lactic acid and butyric acid, but a higher concentration of acetic acid than control silages without aeration. Increased formation of acetic acid can be attributed to different LAB fermentation pathways. One likely pathway can be associated with *L. plantarum* that possesses the ability to oxidize lactate to acetate (McDonald et al., 1991). Although elevated acetic acid formation, control aerated silages were the only silages containing yeasts and were found to be less aerobically stable than control silages without aeration and other treatments as well. Air ingress affected fermentation parameters of the additive treated silages to a lesser extent. Aeration increased formation of acetic acid and ammonia-N and significantly decreased number of heterofermentative LAB in comparison with additive treated silages without aeration. These changes in fermentation patterns of aerated silages were reflected in increased silage losses in comparison with silages without aeration (Fig. 1).

Conclusions

Two hours weekly air ingress sufficiently challenged the ensiling condition by promoting the growth of undesirable yeasts. The application of the silage additive improved silage fermentation, reduced silage losses and maintained silages aerobically stable under both ensiling conditions.

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Bacterial composition of grass, red clover and maize, a pilot study

K. Mogodiniyai Kasmaei
Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Feed Science Division, Box 7024, 750 07 Uppsala, Sweden.
Correspondence: Kamyar.Mogodiniyai.Kasmaei@slu.se

Introduction

Fermentation quality and aerobic stability of silage are affected by sources of forage and field flora. For instance, maize silages usually have a poor aerobic stability compared to legume silages. Field flora is defined as microflora entering a silo with the forage, comprising forage epiphytic microflora and any soil microflora.

There is little information on bacterial composition of different field floras. In this pilot study, bacterial composition of single-source samples of grass, red clover and maize, representing three unique sources of field flora, was compared using *16S rRNA* gene as a molecular marker.

Materials and Methods

Sampling

One sample of timothy (*Phleum pratense*)-meadow fescue (*Festuca pratensis*) at early maturity, one sample of red clover (*Trifolium pratense*) at late flowering and one sample of whole maize crop (*Zea mays*) at early dent were harvested by hand in 2013. The perennial forage samples were from fields nearby Uppsala and the maize sample was collected from southern Sweden. Samples were chopped to a theoretical length of 3-5 cm and frozen immediately at -20°C.

DNA extraction

Three kg of frozen samples was divided in two and from each replicate 100 g was taken. The 100-g samples were freeze-dried and ground in a kitchen blender. An amount of 2 g of ground samples was used for DNA extraction as described by Schenk et al. (2010). It included, in short, incubation of samples in CTAB buffer and purification and precipitation of extracted DNA by chloroform and 2-propanol, respectively. After washing with ethanol, the DNA was re-suspended in MilliQ water.

16S rRNA gene amplification

The technique described by Hugerth et al. (2014) was used. In brief, the V4 region of the gene was amplified using 515´F (Hugerth et al., 2014) and 805R (Herlemann et al., 2011) primers equipped with linker sequences. The protocol of polymerase chain reaction (PCR) was: an initial denaturation at 95°C for 5 min; 35 cycles of denaturation (95°C for 30 sec), annealing (60°C for 30 sec) and elongation (72°C for 1 min); and a final elongation step at 72°C for 5 min. After purification of PCR products, barcodes and Illumina adaptors were attached to the linker sequences by the means of a second amplification. For primer description, please see Hugerth et al. (2014). The PCR protocol was: an initial denaturation at 95°C for 5 min; 9 cycles of denaturation (95°C for 30 sec), annealing (62°C for 30 sec) and elongation (72°C for 1 min); and a final elongation step at 72°C for 5 min. After purification, PCR products were pooled and sequenced by the Illumina MiSeq sequencing technique.

Bioinformatic analysis

The method of Müller et al. (2016) was used. In short, after quality check by Cutadapt (Martin, 2011), paired-end reads were analysed using Quantitative Insights into Microbial Ecology (QIIME, 1.7.0/1.8.0) pipeline (Caporaso et al., 2010). The workflow comprised joining the paired-end reads, operational taxonomic unit (OTU) picking, exclusion of chimeric sequences and taxonomy assignment.

Results and Discussion

A total number of 847 OTUs was detected, with 5740 sequence reads per sample. Bacterial compositions of the three field floral sources at the family level are shown in Figure 1. The maize sample had a higher abundance of species from the *Enterobacteriaceae* family than the grass or red clover sample. On the other hand, the abundance of species from the *Sphingomonadaceae* family was higher on the grass or red clover sample. Species from the *Pseudomonadaceae* family were more abundance on the maize and red clover samples than on the grass sample. An interesting observation was that the *Streptococcaceae* and *Leuconostocaceae* families, belonging to lactic acid bacteria (LAB), were more abundant on the maize sample than on the grass or red clover sample. The abundance of species from the *Lactobacillaceae* family, a family with highest interest for ensiling, was <1% on all the three field floral sources.

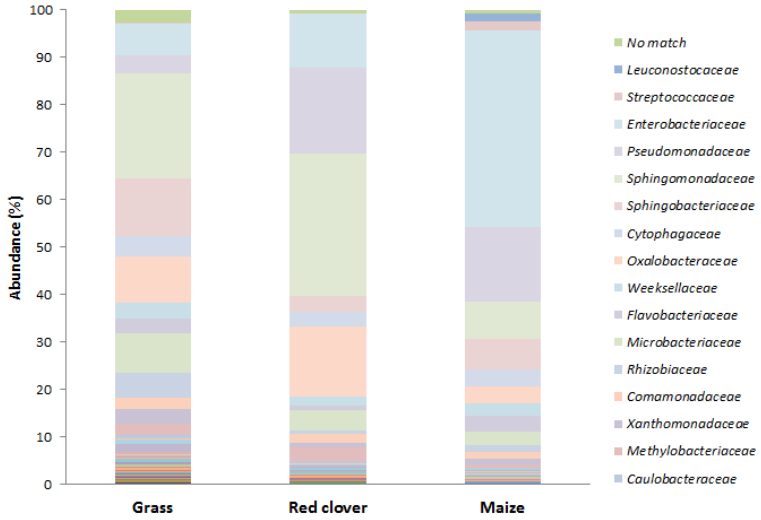

Figure 1 Relative abundance of bacterial families on single-source samples of grass, red clover and maize. Values represent the relative abundance at the family level and are mean of the two replicates. Only 16 families are shown in the legend.

The higher abundance of LAB on the maize sample is in accordance with previous observations obtained from culture dependent methods (Andrieu and Gouet, 1991; Lin et al., 1992). This suggests that maize forage probably provides better conditions for the growth of LAB. Such associations might also exist between abundance of other bacterial groups (e.g. fungi, enterobacteria) and forage type, noteworthy of investigation.

Conclusions

Bacterial composition of the unique grass, red clover and maize samples differed. The maize sample had a higher abundance of LAB than the grass or red clover sample. Amplification of

the *16S rRNA* gene proved useful to study bacterial composition of forages. The results presented here are preliminary as they were obtained from single forage sources and extrapolation should hence be done with caution.

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Test scheme for the evaluation of ensiling agents which have potential to improve aerobic deterioration

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

Introduction

Different ensiling agents have different modes of action and, hence, might come useful if applied to prevent problems they were developed for. Contrary to many on-farm situations, silage agents are often tested under rather favourable environmental conditions. However, one of the advantages of lab-scale experiments is that environmental conditions in a silo can be altered in such a way that more or less favourable conditions can be created in the silos. If compared to a farm-sized bunker silo, this would mean that lab silos can mimic different spots in a bunker, e.g. a spot close to the surface or corner of the silo (air challenged silage) or a spot in the lower centre (airtight storage). If silage agents are tested under more challenging conditions, preferably in repeated trials, their potential with respect to silage quality and aerobic stability might stand out much clearer.

The German DLG commission for silage additives has developed and applied different test protocols for the certification of silage additives since the 1970's (DLG, 2013). Test guidelines exist for additives which claim to improve silage fermentation or aerobic stability, reduce effluent production, improve feeding value (including animal performance) and increase methane production in biogas digesters. The DLG test protocol for improved aerobic stability includes two air stress treatments for 24 hours at 28 and 42 days post ensilage and silo opening and sampling after 49 days. This air stress treatment is usually sufficient to render control silages aerobically unstable, unless fermentation has resulted in elevated levels of butyrate or acetate, since these acids have the ability to improve aerobic stability.

The objectives of this study was to examine three additives with claims to improve aerobic stability and propionic acid on crimped grain by applying two aerobic stress levels and a negative control in the form of airtight storage. A similar test protocol for forages is also presented in these proceedings by Knicky et al. (2016).

Materials and Methods

Maize grain was combine harvested Dec. 2015 in southern Sweden at 33% moisture content (MC) and was sent (cold storage at 6-8°C) about 650 km north to Uppsala, where the grain was moisturized to 37.4% MC while stored at 4-6°C under a period of 13 days before it was crimped (crimper: Murska 350 S2, Aimo Kortteen Konepaja Oy, Ylivieska, Finland) and ensiled in 1.7-L glass jars with water-filled siphons (gas vents) mounted on the silo lids. Three samples of the grain were analysed for MC, crude protein (CP), pH and viable counts of lactic acid bacteria (LAB), yeasts and moulds. MC was determined by drying at 103°C for 6 h after subsequent cooling in a desiccator and viable counts of LAB were determined on Rogosa agar (Merck 1.05413, pH 5.5, pour plates, anaerobic cultivation at 30°C) and yeast and moulds on DG18 agar (Merck 1.00465, pH 5.6, surface spread, aerobic cultivation at 25°C).

The test protocol was identical to the trial presented at the 6th NFSC conference (Pauly & Hjelm, 2015). The following silage additives were used:

- ProSid MI700 (MI; Perstorp AB) containing 66% propionic acid, 26% propionic acid glycerol esters, 2% formic acid and 1% glycerol,
- ProMyr NT611 (NT; Perstorp AB, Sweden) containing 25% propionic acid and 23% Na-formate
- Competitor's additive (CS) contained 42% formic acid, 30% ammonium formate, 10% propionic acid and 2.2% benzoic acid and
- propionic acid (PA), >99% purity.

All additives were applied at a rate of 4.0 mL/kg fresh matter (FM) equivalent to 4.0 L/metric ton FM. Each additive treatment was exposed to 3 air stress levels:

1. no air leakage (no),
2. one stopper in the jar bottom and one in the lid (6.0 mm hole Ø) removed for 2 hours once a week (1/w) and
3. three stoppers in the jar bottom and one in the lid removed 3 times per week for 2 hours (3/w). The air stress treatments started 14 days after ensilage.

Additives were applied to pre-weighed portions of grain as described by Pauly & Hjelm (2015). They were sprayed over the grain, which was spread out inside large transparent bags, followed by shaking of the closed, inflated bag for approx. 20 s. After approx. 30 minutes in the closed bag the treated grain was filled into autoclaved glass jars (triplicates). A relatively low fill density was chosen to facilitate the movement of air inside the silos during air stress treatments (density: 565 kg FM/m^3 or 350 kg DM/m^3).

Silos were stored for 62 days at 20±1°C. During storage, all silos were frequently weighed and assessed for presence of moulds with the help of a mould score system (0 = no visible moulds, 5 = more than 50% of silo surface visibly mouldy; Pauly & Hjelm, 2015). Weight losses during storage were calculated in % of the initial grain DM content after silo filling.

Silos were opened and sampled for DM, pH, ammonia-N, organic acids (formic, acetic, propionic, lactic, butyric) and alcohols (ethanol, 1,2-propanediol, 2,3-butanediol). Organic acids and alcohols were determined by HPLC according to Ericson & André (2010), ammonia-N using flow-injection analysis (ISO method 11732:2005) and DM and MC by drying as with the fresh grain. In addition, all samples were exposed to an aerobic stability test that was planned for a period of 14 days but was extended to 42 days (ambient temperature 18.3±0.1°C). Grain samples (600 g FM) were aseptically transferred from silos to ethanol-treated PVC-pipes (1.3 L), which were covered with a piece of autoclaved geotextile at the bottom. PVC-pipes were inserted into a Styrofoam block for insulation. The block was covered (top + bottom) with 10 mm thick Styrofoam boards. Air could pass through the PVC-pipes via 2 holes (10 mm Ø), one at the bottom and one at the top of each pipe. Grain temperatures were recorded every 2nd hour via electronic sensors (thermo-couples, type T) inserted into the centre of each pipe. An increase of temperature indicated the onset of aerobic microbial activity. Maximum grain temperature during the test and time (days) until grain had reached +3°C above ambient temperature were used as indicators of aerobic stability.

The data was analysed statistically using the software package SAS 9.3 (SAS, 2014). Since most of the data were not normally distributed, the collected data could not be analysed by

analysis of variance as planned. Instead, the data was first ranked (PROC RANK) and then, the ranked data was analysed with PROC GLM. Results were expected to be similar to the Wilcoxon Kruskal-Wallis test (acc. to U. Olsson, pers. comm.), a test often applied with data that is not normally distributed.

Results and Discussion

Analyses made on the freshly crimped grain (Table 1) showed a moisture content of 37% and high viable counts of LAB and yeasts.

Table 1 Composition of crimped maize (means of 3 samples) before ensilage.

MC (% FM)	pH	WSC (% DM)	CP (% DM)	True protein[1] (% CP)	LAB (cfu/g FM)	Yeasts (cfu/g FM)	Moulds (cfu/g FM)
37.4	5.89	1.5	8.3	80	9.4×10^6	7.8×10^6	1.4×10^5

[1]True protein = CP – NPN (non-protein nitrogen)

When silages were sampled 62 days later, all silages were mould-free except air stressed CS- and the intensively aerated NT-grain (Table 2). It was unexpected that the air stressed controls (C) were mould-free, but it is possible that the air stress led to increased yeast activity, as indicated by elevated ethanol contents (0.2 g/100 g FM) in the airtight C-silos, while all other treatments were ethanol-free. Yeast growth may have consumed the oxygen in the silos and inhibited mould growth. Inhibition of moulds by yeasts has been observed in yeast-inoculated grain by Druvefors & Schnürer (2005). It is likely that lactate-assimilating yeasts were responsible for the reduced lactate concentrations in air stressed treatments, since these yeasts are able to metabolize lactate in the presence of oxygen. Lactate contents appear to be reduced and pH values increased in relation to the intensity of air stress during storage.

During storage, pH values decreased from 5.9 to 3.7-4.1. The quantity of lactic acid required to accomplish this pH reduction was relatively small (see Table 2), probably owing to the low buffering capacity of maize grain (1.5 g lactic acid/100 g DM to reach pH 4.0). This would indicate a rapid acidification of the grain and might explain the low weight losses.

Losses were generally low and increased in all additive treatments in relation to the intensity of air stress. The effect of the different air stress treatments on chemical composition was generally small except for lactate and pH (Table 2), but aerobic stability was strongly decreased by the air stress treatments (Table 3). Differences in aerobic stability between no air leakage and the lower air stress level (1/w) appeared considerably larger than between the low and high air stress level.

Lactic acid fermentation was restricted when the additive contained a high proportion of formic acid like CS (Table 2). This led to low DM losses in airtight silos but to mould growth during storage after these silos were exposed to air. The NT-additive contained comparatively less formic and more propionic acid, which improved the NT-grain stability towards mould growth and aerobic deterioration, since propionic acid possesses anti-fungal activity and formic acid not. Formic acid might even have been responsible for the reduction of yeasts during the first 2 weeks when all silos were airtight and no air stress was applied. The lack of yeasts might have given moulds a chance to grow when air stress treatments commenced.

The best aerobic stability response was demonstrated by MI and PA, which were comparable in effect. Even the intensively air stressed grain treated with these additives (4 L/ton FM) was mould-free after 62 days of storage and didn't heat up during the following 42 days of aerobic exposure. Only in intensively air stressed MI-silos, single mould spots became visible at the end of the aerobic stability test (42 d).

The most compelling reason to apply an additive at crimping is to minimize problems with heating and moulding during feed-out. Two options that would improve aerobic stability would be a) to minimize air leakage into silos and b) to apply an additive with a high antifungal activity such as ProSid MI700 or propionic acid.

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

Addi-tive [a]	Air stress	MC	pH	Am-N	Lactic	Acet.	Form.	Prop.	Sum	Loss	Mould scores
		(% FM)		(% N)	(g/100 g FM)					(% DM)	
C	no	38.5	3.76	4.7	0.48	0.07	<0.01	<0.01	0.62	0.73	0
C	1/w	38.4	3.80	4.3	0.46	0.05	<0.01	<0.01	0.70	1.94	0
C	3/w	38.6	3.88	3.9	0.39	0.03	<0.01	<0.01	0.62	2.73	0
NT	no	37.5	3.79	4.5	0.48	0.07	<0.01	0.01	0.59	0.37	0
NT	1/w	37.7	4.01	4.2	0.32	0.07	<0.01	0.01	0.45	0.53	0
NT	3/w	37.8	4.04	4.2	0.31	0.05	<0.01	0.01	0.42	0.92	0.7
MI	no	37.5	3.69	3.9	0.45	0.06	<0.01	0.03	0.55	0.26	0
MI	1/w	37.6	3.89	3.9	0.32	0.07	<0.01	0.03	0.43	0.32	0
MI	3/w	37.0	3.92	3.9	0.30	0.07	<0.01	0.03	0.41	0.47	0
CS	no	37.9	3.88	6.7	0.26	0.04	0.06	<0.01	0.36	0.11	0
CS	1/w	38.0	4.09	6.7	0.15	0.03	0.06	<0.01	0.25	0.16	0.7
CS	3/w	38.0	4.13	6.6	0.13	0.03	0.06	<0.01	0.22	0.48	2.0
PA	no	38.1	3.71	4.0	0.43	0.05	<0.01	0.04	0.53	0.19	0
PA	1/w	37.5	3.87	3.8	0.30	0.06	<0.01	0.04	0.41	0.24	0
PA	3/w	38.0	3.89	4.3	0.32	0.06	<0.01	0.05	0.44	0.43	0
Probabilities of effects:											
Additive:		*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Air stress:		*0.77*	*<0.001*	*0.21*	*<0.001*	*<0.001*	*0.54*	*<0.001*	*<0.001*	*<0.001*	*0.005*
Additive x Air:		*0.40*	*<0.001*	*0.004*	*<0.001*	*<0.001*	*0.12*	*<0.001*	*<0.001*	*<0.001*	*0.007*

[a]C=Control ; NT=ProMyr NT611; MI=ProSid MI700; CS=competitor's additive; PA=propionic acid.

Table 3 Results of the 42-d aerobic stability test of the crimped grain after silo opening. Mould scores were assessed at silo opening (before) and after the stability test (after). Values represent means of 3 samples

Additive[a]	Air stress	Max. grain temp. (°C)	Time until +3°C (d)
C	no	35	29.4
C	1/w	38	3.9
C	3/w	41	1.0
NT	no	19	>42
NT	1/w	38	13.8
NT	3/w	34	8.7
MI	no	18	>42
MI	1/w	18	>42
MI	3/w	20	41.8
CS	no	19	>42
CS	1/w	35	19.1
CS	3/w	37	8.8
PA	no	18	>42
PA	1/w	18	>42
PA	3/w	18	>42
Probabilities of effects :			
Additive:		*<0.001*	*<0.001*
Air stress:		*<0.001*	*<0.001*
Additive x Air:		*0.04*	*<0.001*

[a]C=Control; NT=ProMyr NT611; MI=ProSid MI700; CS= competitor's additive; PA=propionic acid.

Conclusions

- Air leakage during storage had a strong positive effect on heating and moulding after silo opening. When the level of air stress was low (2 h/week), changes in chemical composition were small compared to airtight silos, but aerobic stability was severely compromised.
- Grain treated with ProSid MI700 or with propionic acid at 4 L/ton did not mould during storage even when exposed to an intensive air stress treatment (3 x 2 h/ week). After silo opening and full exposure to air, the MI- and PA-grain did not heat or mould during the following days of aerobic exposure.
- The lower air stress level, *i.e.* 2 h once a week, appears to be a suitable treatment to test the ability of additives to extend the aerobic stability of fermented grains without compromising fermentation quality. Long aerobic stability has no value if it is caused by poor fermentation quality.

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Ensiling of whole crop field beans (*Vicia faba*) as affected by silage additives

C. Kalzendorf[1], A. Milimonka[2] & W. Richardt[3]
[1]Landwirtschaftskammer Niedersachsen, FB Grünland und Futterbau, Mars-la-Tour-Straße 1 – 13, 26121 Oldenburg Germany, [2]ADDCON Europe GmbH, Parsevalstr. 6, 06749 Bitterfeld Germany, [3]LKS Lichtenwalde, August-Bebel-Str. 6, 09577 Niederwiesa
Correspondence: Andreas.Milimonka@addcon.com

Introduction

Production of home grown protein is in discussion since some years and is focused now by the EU agriculture policy. Home grown protein may help to slow down the shift in global land use. In general, legumes have positive effects in crop rotation like phyto-sanitary aspects, save mineral nitrogen and reduce the global warming potential by biological nitrogen fixation and may help to improve farm economy (Phelan et al., 2015). Besides greening aspects in the North West of Germany, field bean may also help to control *Alopecurus myosuroides* in the crop rotation.

Grain legumes can provide ruminants as well as monogastrics with farm grown protein. Grain legumes are characterized by a relatively high protein content, whereas the energy content is variable and depending on ripening stage and variety. Especially for monogastrics, the nutritive value is limited by oligosaccharides and secondary plant ingredients like tannins or alkaloids (Doblado et al., 2003, Gefrom, 2013a). Silage fermentation may help to reduce these traits, but phytin bound phosphorus obviously can't be broken by silage fermentation (Gefrom, 2013b) and don't get phosphorus more available for monogastrics.

The ripening of grain legumes under humid climate conditions is uneven and result in considerable field losses or the harvested grains may require expensive drying. These problems could be overcome by ensiling. However, the grain legumes are characterized by high protein contents and have a high buffer capacity (BC). Grains of field beans and whole crop soja beans have a BC (g lactic acid/kg DM) of 40 and 80, respectively (Anonymus, 2014, Gefrom, 2013a). Besides a high buffer capacity, the content of water soluble carbohydrates (WSC) is low. Consequently, the ensilability of whole crop grain legumes seems to be difficult. On the other hand, fermentation is also affected by the DM content and a low BC and a sufficient high DM may result in a good fermentation coefficient. The low content of rapidly fermenting sugars can be enhanced by adding molasses. With tropical shrub legumes, fermentation was improved considerably by using molasses and lactic acid bacteria (LAB) (Heinritz et al., 2012). Lactic acid was raised and the proportion of NH3-N of total N as well as pH were significantly reduced. But butyric acid was still high (9.5 g/kg DM) in inoculated silage. That was obviously affected by low nitrate contents (<2 g/kg DM). In case of grain legumes, the addition of carbohydrates in the form of sorghum or molasses combined with inoculants could control the butyric acid fermentation (Gonzales et al., 2012).

At dairy farms it is more easy to harvest the whole crop because of the technique available at the farms. But data about the not easy ensilable whole crop grain legume are not so much available. So, we analysed the effect of different silage additives in whole crop field beans.

Materials and Methods

Field beans (*Vicia faba L.*) were grown on loamy soils in the North West of Bremen. The plant material (Tab. 1) was harvested at the 14th of August 2014. Most of the beans reached

yellow ripeness and in the foreland late dough ripeness. Immediately after harvest the plant material was brought to the field laboratory of the Agricultural Chamber in Lower Saxony, Oldenburg. The material was chopped and ensiled in 2.5-L glass jars in a randomized block design with 3 replications.

The jars were stored for 103 days at constant temperature of 25°C. The treatments were: a1) C = control (sprayed with 100 ml tap water), a2) LABho = homofermentative LAB with 1x105 cfu/g forage applied with 100 ml/g forage, a3) HMT = chemical additive (hexamethylene tetramin and sodium nitrite, 3 l/t), a4) LABho/he = combination of homo- and heterofermentative LAB with 2.5 105 cfu/g forage, applied with 100 ml/g forage, a5) BeSo = chemical additive (benzoate, sorbate, acetate, 2 l/t).

Table 1 Plant parameters of the field beans (*Vicia faba*) before ensiling

Item	*Vicia faba*, yellow ripe	*Vicia faba*, late dough ripe
DM (%)	37.0	69.4
Crude protein (% of DM)	22.5	24.0
Crude fiber (% of DM)	17.8	14.1
ELOS (%)	74.7	82.0
WSC (% of DM)	3.5	2.8
Starch (% of DM)	27.7	38.4
Nitrate (% of DM)	0.54	0.29
Buffer capacity (g LA/100 g DM)	4.2	2.8
WSC/BC	0.8	1.0
Fermentation coefficient	44	77

ELOS=enzyme soluble organic matter; WSC=water soluble carbohydrates; BC=buffer capacity; LA=lactic acid

Chemical analysis of the plant material was performed according to the official German standards for feed evaluation (Anonymus, 2011). Silage DM was measured and corrected for loss of volatiles during drying (Weißbach and Kuhla, 1995). The pH-value was analysed by a pH-electrode. Determination of the fermentation acids was done by HPLC for lactic acid and by GC for volatile fatty acids and alcohols. Ammonia was analysed photometrically. The crude protein fractions were analysed according to Licitra et al. (1996) and dividing the crude protein according to the CNCPS (Sniffen et al., 1992) into five different fractions A, B1, B2, B3, C. As true protein the sum of the fractions A, B and C was used. The rumen undedradeable protein (RUP) was calculated for a rumen passage rate of 5% h-1, according to Kirchhof et al. (2006) The DM losses during fermentation were calculated according to Weißbach (2005). Aerobic stability was measured by the temperature method (Honig, 1990).

Data were evaluated by using procedure MIXED of SAS. Differences among means were compared at a significant level of $P <= 0.05$.

Results and Discussion

After 103 days of fermentation, all silages were of a good fermentation quality. The DM losses were reduced by the HMT and LABho/he treatments (Fig. 1b). Fermentation losses during the anaerobe phase typically come from the metabolism of the groups of the enterobacteria and clostridia. Because of the low butyric acid concentration in the silages, acetic acid fermentation probably contributed to these losses most. In case of HMT, the low fermentation losses corresponded to a low acetic acid concentration in the silage. With LABho/he on the other hand, we found the highest amount of acetic acid and low fermentation losses. That may also have come from a heterofermentative metabolism of the lactic acid bacteria (LAB) in the inoculant. In this experiment, we did not analyse 1,2

propanediole to describe a possible *Lactobacillus buchneri* type effect (Weiß and Krause, 2011). However, also a heterofermentative metabolic pathway gives more DM losses compared to a homofermentative lactic acid fermentation. Therefore, it's not clear why high acetic acid concentrations corresponded with low DM losses in case of the LABho/he treatment.

The pH value was lowest in the HMT silage which corresponded with the highest amount of lactic acid and lowest content of acetic acid (Tab. 2). Not in line with the data in literature were the inoculant treatments (Gefrom 2013a; Gonzales et al, 2012) resulting in higher pH levels and lower lactic acid concentrations, compared to the control. These treatments with the highest pH value also had the highest acetic acid concentrations. The control and all tested treatments showed quite low contents of butyric acid (<0.1 % of DM). Heinritz et al. (2012) found quite high amounts of butyric acid in grain legume silages but low amounts of nitrate <2 g/kg DM. The plant material used for ensiling (Tab. 1) was characterized by a sufficient nitrate concentration (>4.4 g/kg DM). The strong positive impact of nitrate on butyric acid free silages and the related fermentation pathway was described by Kaiser et al. (1997).

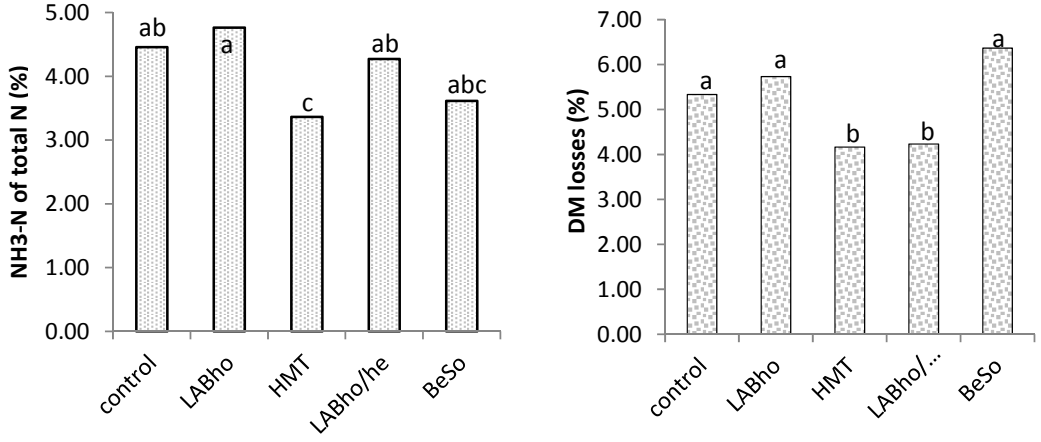

Figure 1 Effect of different additives on proportion of ammonia-N of total N (left) and DM losses (right) of a field bean (*Vicia faba*) whole crop silage. HMT=chemical additive hexamethylene tetramin and sodium nitrite; LABho=homofermentative lactic acid bacteria; LABho/he=homo and heterofermentative lactic acid bacteria; BeSo=chemical additive, benzoate, sorbate, acetate; different letters show significant differences at P<= 0.05

Table 2 Effect of different additives on fermentation acids and pH of a field bean (*Vicia faba*) whole crop silage

	Lactic acid	Acetic acid (% of DM)	Butyric acid	pH value
Control	5.16a	1.57a	0.08a	4.40a
LABho	3.21b	2.67b	0.00b	4.93b
HMT	6.51c	1.49a	0.00b	4.23c
LABho/he	1.71d	3.17c	0.08ab	5.40d
BeSo	2.04e	3.08c	0.00b	5.30e

HMT=chemical additive hexamethylene tetramin and sodium nitrite; LABho=homofermentative lactic acid bacteria; LABho/he=homo and heterofermentative lactic acid bacteria; BeSo=chemical additive, benzoate, sorbate, acetate; different letters show significant differences at P<= 0.05

The ammonia-N in silage was only affected by the HMT additive (Fig. 1a). If we consider that proteolysis only occurs up to a minimal pH of 5 (Seyfarth et al., 1989), the long-lasting

(103 days) low pH may have affected protein break down. However, the ammonia-N concentrations in the other treatments were around 4 and did not really indicate any severe protein break down.

The protein fractions of the silages was lowest for fraction A and highest for B1 in the LABho/he inoculant compared to the other treatments (Fig. 2). That might come from a more rapid interruption of the protein degradation. However, the ammonia-N and pH values were not in line with these findings. The HMT additive reduced pH but had no clear effects on the protein fractions. Significantly higher true protein contents against the control were found in the LABho/he and BeSo treatments (Fig. 2). These type of additives are designed to control heating in silage and not primarily to reduce pH. Heating may cause Maillard products and if there was a heat stress during fermentation, it should have been visible in fraction C. But, fraction C was unaffected by the treatments. Therefore, the positive effect on the true protein fraction of this additive cannot easily be explained.

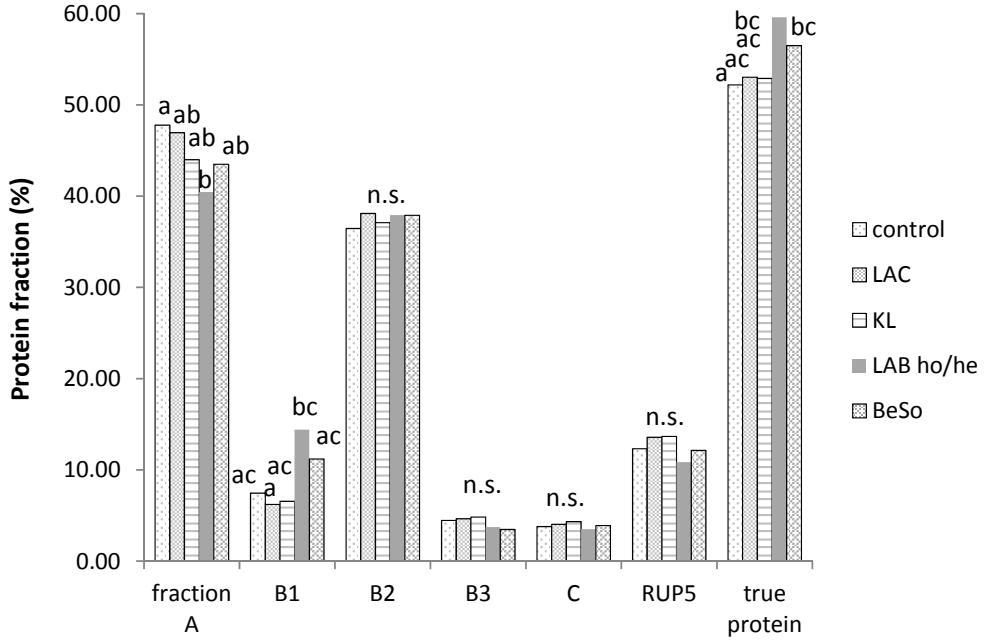

Figure 2 Effect of different additives onto different fraction of true protein of a field bean (*Vicia faba*) whole crop silage; different letters show significant differences at P<= 0.05, n.s.= not significant

In addition to that experiment, we also analysed a field bean crop during ripeness (Tab. 1). The DM content in that silage was 58.8 % compared to the plant material used in the experiment which had a DM content of 33.8 %. This drier material showed a strongly limited proteolysis. Fraction A reached only 35.2 % compared to 44 % in case of the more wet material. The true protein was 64.8 %.

As expected the aerobic stability of the silages was lowest in the HMT treated silage. But also in the LABho/he treatment which reached as well 5 days the aerobic stability was unexpected low. Also in the other silage treatments, temperature was stable only for 6.5 days.

Conclusions

Whole crop field beans can be preserved by ensiling. The DM losses are at an acceptable level and can be reduced by using a suitable additive such as HMT. With HMT as an additive, the highest lactic acid and lowest acetic acid levels were found in the silages as well as the lowest DM losses. The pH value was low and no butyric acid was formed when the HMT treatment was applied. The amount of true protein was highest with the additives designed to control heating but the reason for that effect could not be clarified. An undesirable fermentation can be prohibited by suitable additives, but there is a risk of heating and hygienic deterioration.

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Temperature development and dry matter losses of grass silage in bunker silos

R. Spörndly & R. Nylund
Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management. BOX 7024, 750 07 Uppsala, Sweden.
Correspondence: Rolf.Sporndly@slu.se

Introduction

Ensiling is a preservation method for crops based on anaerobic fermentation where epiphytic lactic acid bacteria convert water soluble carbohydrates to lactic acid which decreases the pH. To store silage and protect it from oxygen, several structures, such as tower silos, bunker silos, tube silos and round bales, are used. Aerobic conditions are detrimental to silage quality and bunker silos are often difficult to keep hermetically sealed (Woolford, 1990). Air intrusion during the storage period, normally 2 to 10 months, results in deterioration of the silage already in the silo (Honig, 1991). After unloading, the silage is fully exposed to oxygen and aerobic deterioration processes start. Stability of silage during the feed-out period is an important factor determining quality and usability of the fodder (Wilkinson & Davies, 2012) and silage handling in warm climates require special attention in order to limit vast losses (Ashbell et al, 2002). The present paper consists of two independent parts: a) a laboratory method designed to mimic unloading of bunker silos is described and the effect of silage DM content on temperature development is reported, b) temperatures in 5 farm-scale bunker silos were logged throughout the ensiling period and total mass entering and leaving the silos were recorded.

Materials and Methods

a) Laboratory method

A grass forage crop was wilted to 30 and 50 % DM and packed into 2-m PVC tubes (diameter 0.16 m) at a density of 150 kg DM/m^3. The tubes were stored horizontally at 20ºC and insulated with 5 cm Styrofoam in order to mimic an environment inside a bunker silo (Figure 1). The tubes were fitted with temperature loggers every 0.15 m and 4 water filled gas locks to let out CO_2 but restrict air to enter. The forage consisted of a regrowth of *Poa pratensis* and *Festuca pratense* from a pasture that had been grazed earlier in the season.

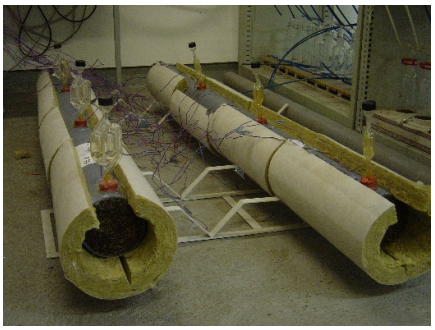

Figure 1. PVC tubes (Ø 0.16 m)

Before filling the tubes, half of each of the two crops (30 % DM and 50 % DM crop) was treated with two lactate assimilating species of the yeast genera *Candida* and *Hanensula (Pichia)* and the other half was left untreated. The four treatments were (30 and 50% DM with and without yeast) were duplicated, resulting in a total of 8 tubes. The tubes were stored 120 days after which they were opened by cutting off one of the ends. Thereafter, 0.15 m of each tube was cut 10 times at 7-day intervals using a saw and the content weighed and sampled.

After sampling, samples were transferred to a storage stability test unit to monitor increase in temperature. Temperature in silages were measured in 1300 ml PVC containers covered at the bottom with a PE fiber net and filled with silage in relation to their DM contents,

according to the equation: filling weight (g fresh matter) = -205.57 x ln(%dry matter) + 1061. The containers were placed in an insulating Styrofoam block and kept at 20ºC during 9 days and thereafter weighed before being discarded. The green crop and all silages were analysed for dry matter (DM) and as well as for presence of yeast and mould cultured aerobically at 25ºC on Malt Extract Agar (MEA, Merck) supplemented with Penicillin G (30 mg/L agar) and Streptomycine sulphate (30 mg/L agar) (Sigma).

b) Full scale silo measurements

Five farm scale silos were investigated. The dimensions were (length x width x height): 30x6x3 (2), 43x8x3 (2) and 42x12x3 (1) m and were located at three farms in central Sweden. The forage crops of grass and clover were weighed with a calibrated vehicle scale at loading and a sample was taken from each wagon load put into the silos. Each silo was fitted with temperature loggers recording and storing t temperature data at 4-h intervals throughout the ensiling and unloading period. The temperature loggers were placed in the centre of the silo and along one side and at two levels from the bottom. At unloading for feeding, total weights were recorded daily and samples were taken for analysis three times per week. At unloading, the temperature loggers were collected and temperature data was downloaded. Samples of crops at harvest and silages at unloading were analysed for DM, ash, crude protein (CP) and neutral detergent fibre (NDF), according to standard methods. Digestible organic matter was analysed with the Swedish 'VOS' method (Lindgren, 1979).

In both studies, the GLM procedure of SAS (SAS, 2014) was used for analysing statistical differences, where appropriate.

Results and Discussion

a) Laboratory method

Loss of DM during the 120-d storage period assumed that it consisted of only CO_2 exiting via the water locks. Accumulated weight loss during unloading was obtained by weighing all ten 0.15 m cuts during the 63-d emptying of the silos. Results are presented in Table 1 which shows that the dryer forage gave higher fermentation losses during the storage period. The addition of yeast did not make any difference. The losses after the unloading period of 63 days further increased total losses of the wetter silage by almost 5 times and of the dryer silage by more than 10 times

Table 1 Total DM loss after 120 days airtight storage and accumulated DM loss after a 63-d unloading time of the silos

	Weight loss at opening (% of crop DM)	Accumulated weight loss during a 63-d unloading (% of crop DM)
Untreated 30 %	6.1[a]	29.2[a]
Yeast 30 %	6.2[a]	29.2[a]
Untreated 50 %	1.6[b]	21.4[b]
Yeast 50 %	1.9[b]	19.3[b]

[a, b] Different superscripts in the same column differ at $p<0.05$

The temperatures recorded in the silo tubes during the storage period when silos were closed were similar to the ambient temperature. When unloading started by cutting off one end of the tube, the temperature in the wetter silages continued to be unaffected. The dryer silages were also unaffected during the first two weeks. Thereafter, the silage that was due to be

unloaded started to show 2 to 4 degrees higher temperatures than the ambient about one week in advance.

Figure 1 illustrates the result of the storage stability test performed with silage unloaded the first day and the third week (day 22) after silo opening. All silages (30% and 50 % DM) tested after unloading on the first day after silo opening were stable throughout 48 hours. When unloading three weeks later, the dryer silages (50% DM), starting at a slightly elevated level as mentioned above, immediately increased in temperature, reaching 32ºC after approximately 44 hours while the wetter silages maintained their stability.

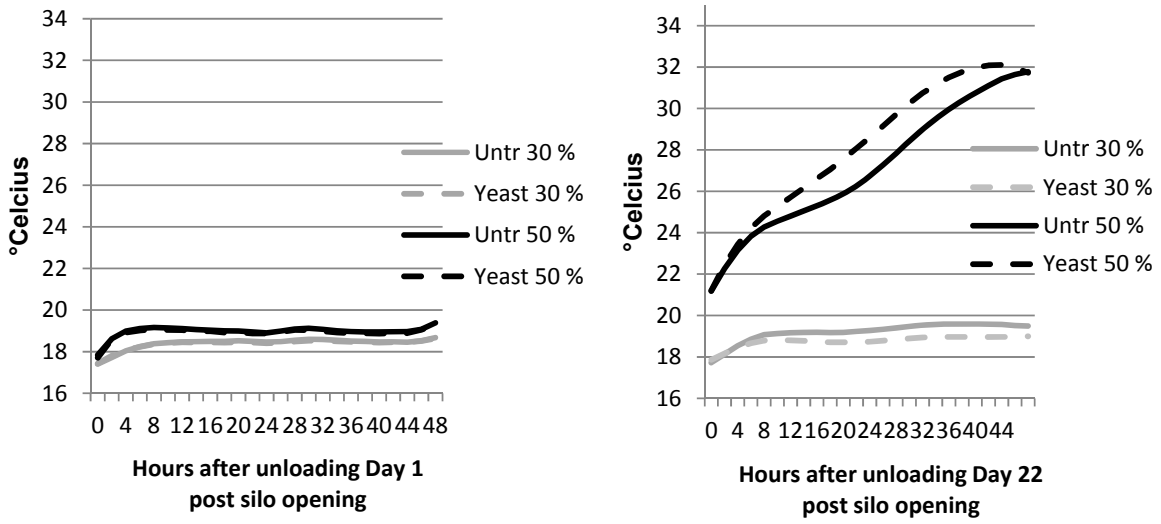

Figure 2 Storage stability after unloading silage Day 1 and Day 22 after opening the silos

The results from the 50% DM silage can be compared with a full size silo packed at a density of 150 kg DM/m^3. At opening, the porosity will allow air from the face to penetrate the silage. This will start up aerobic microbial processes and, when the silage is fully exposed to air at unloading, aerobic deterioration will be rapid. For the wetter silage, this does not seem to happen. The increased yeast counts found in the unloaded silage at week three supports this finding, < log 1.7 cfu/g in the 30% DM silages and log 4.4 cfu/g in the 50% DM silages. The yeast content in the green crop was 4.4 and 4.8 cfu/g in the 30 and 50% DM, respectively. No effect at all was observed for the addition of extra yeast to the green crops, probably because of an abundant occurrence of yeast present on the crop.

b) Full scale silo measurements

Recording temperatures in full scale silos typically show an initial rise in temperature followed by a slow decrease during the remaining storage and unloading periods. Figure 3 illustrates such a pattern in one of the silos. The silo was filled September 12, opened January 23 and was completely emptied May 19. In this silo, the temperature loggers were placed 1 meter above the bottom and the silo measuring 30x6x2.4 m (LxWxH). Note that the silo temperature was approximately 20ºC in January when the outdoor temperature was -15ºC.

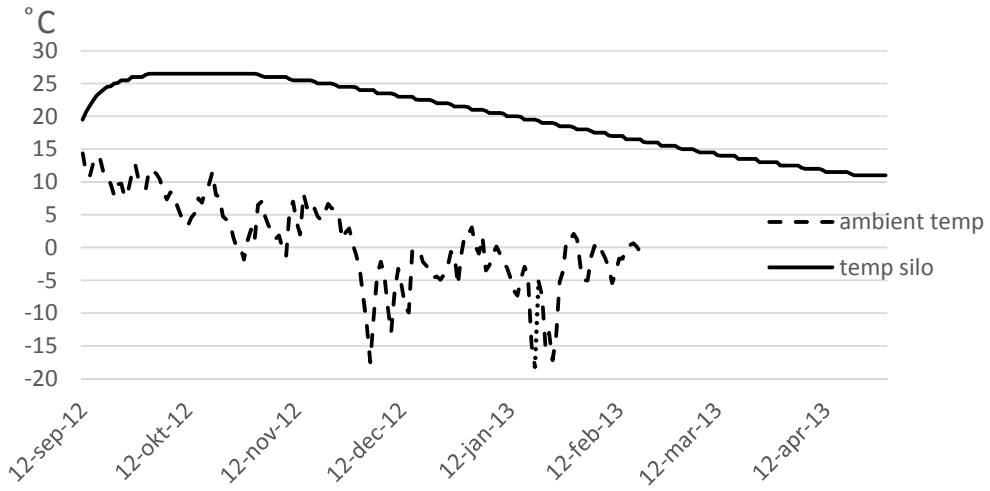

Figure 3 Ambient and the in-silo temperature at 1.0 m from the bottom

Figure 4 illustrates a silo where the loggers were placed close the bottom (0.5 m) and close to the top (1.90 m) in a silo measuring 43x8x2.55 m (LxWxH). This silo was filled July 9, opened and emptied October 16 the same year. The temperature in the upper layer soon exceeded the lower layer which may indicate some aerobic activity due to air leakage from poor sealing.

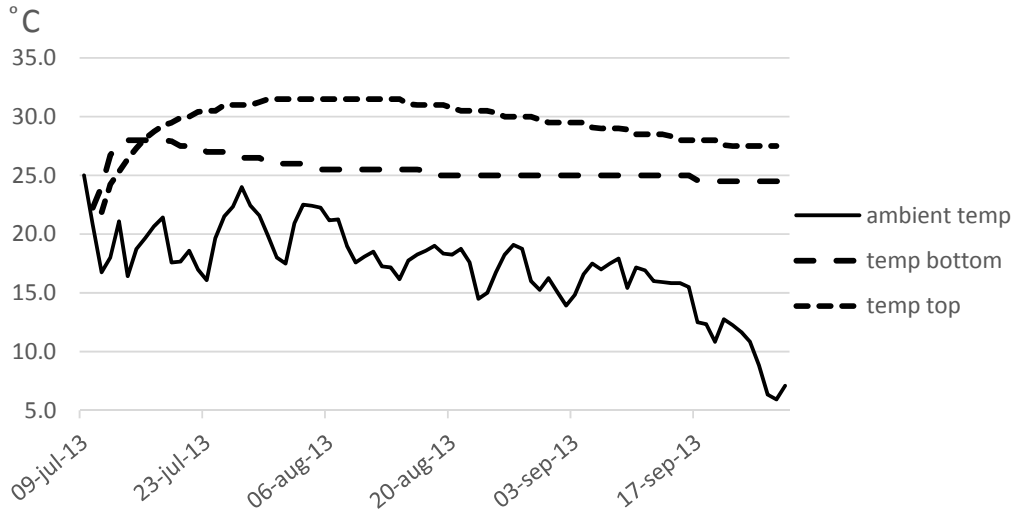

Figure 4 Ambient and in-silo temperature in the bottom (0,5 m) layer and top (1,90 m) layer.

Figure 5 illustrates temperature development in a silo where a second harvest (July 22) filled half the silo. In September, the plastic film was removed and a third harvest was loaded on top of the second harvest, where after the silo was re-sealed. January 8, the silo was opened and stayed open until May 20 for unloading. The temperature in this silo elevated to about

40ºC. The logger close to the surface of the second harvest was heated a second time when the third harvest entered.

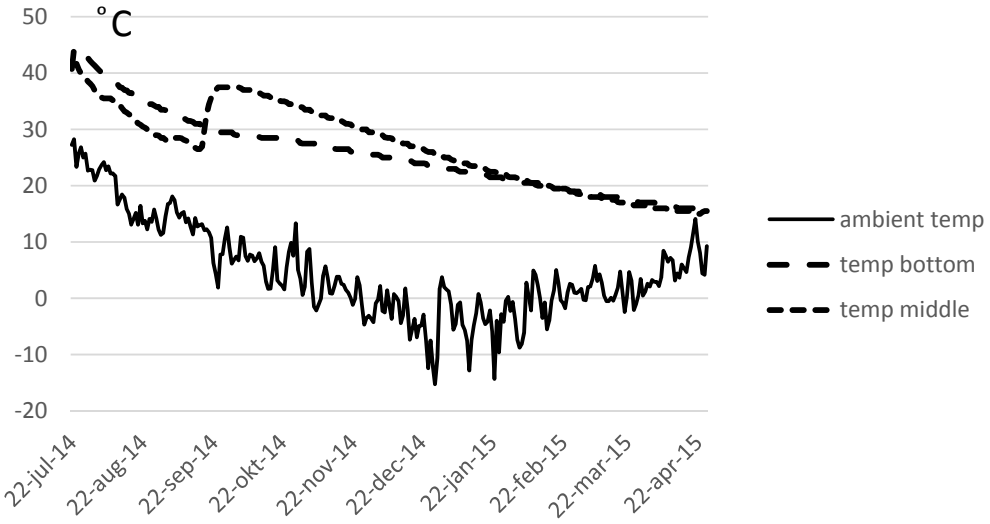

Figure 5 Ambient and in-silo temperature

The calculated silo balances, kg DM unloaded from the silo minus kg DM filled into the silo, are presented in Table 2. Temperature recordings do not seem to have the potential to indicate losses in the silos investigated in this study. In some farms, the discarded quantity of silage was higher due to farmer preferences. Therefore, it could be a better criterion to use DM losses where discarded silage is not included. Dry matter loss, excluding the discarded quantity, is therefore also included in the table. In this case however, the difference was not significant and no relation was found between the in-silo temperature and losses recorded. Daytime temperature sum (DTS), the sum of the daily average silo temperatures, were also tested as an alternative to finding a relationship between temperature and losses. Furthermore, an index of the difference between in-silo DTS and ambient DTS divided by silo surface area was also tested as an indicator of silage losses. However, neither of these alternative variables improved the correlation between the temperature and recorded DM losses.

Table 2 Mean and maximum temperature and DM loss recorded in 5 silos at three farms.

				Mean temperatur (˚C)				Max temp (˚C)		DM loss (%)	
		Date of			Silo			Silo			Excl.
Silo	Farm	Filling	Empty	Ambient	Mean	Lower	Upper	Lower	Upper	Total	discard
1	A	09-sep	08-apr	1	22,6	22,6	-	26,5	-	3,1	1,5
2	B	12-sep	19-maj	-0,1	19,8	18	21,7	25,5	28,5	18,1	15,3
3	A	15-jul	04-dec	16,1	26,7	24,2	28,7	28	30,5	3,4	2,7
5	B	10-jul	16-okt	10,9	23,1	20,8	25,3	30,1	34,9	16,0	12,4
8	C	22-jul	20-maj	4,7	26,2	25,6	26,8	44,5	41,7	6,4	5,6

There was, however, a clear effect of farm on DM losses. Farm A differed from other farms by displaying very low DM losses. At this farm, filling of the silos was done very slowly resulting in a long packing time with a tractor. The practice on this farm was also to not close

the silo immediately when full. Instead, packing continued the day after with the visible effect of pressing the surface another 0.2 meter downwards. The silos were finally covered with 0.15 m of sand on top of the plastic film.

Conclusions

A laboratory method is presented where the pattern of unloading bunker silos can be mimicked and monitored. Porous silages, due to a high DM content or poor compaction, were sensitivity to oxygen intrusion during the unloading period. This resulted in a silage with poor stability, heating up fast after unloading and leading to considerable DM losses. Temperature recording in full-scale silos did not prove to be a valid indicator of DM losses. However, large differences in losses among farms were observed, indicating that thorough and meticulous compaction of the silo may be more important to minimize losses than rapid sealing. Controlled experiments are required to establish if this indication can be verified.

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Karoline model as a useful tool in predicting methane in cattle

M. Kass, M. Ramin & P. Huhtanen
Swedish University of Agricultural Sciences (SLU), Department of Agricultural Research for Northern Sweden, SE-901 83 Umeå, Sweden.
Correspondence: marko.kass@slu.se

Introduction

Methane (CH_4) is a greenhouse gas contributing to global climate change. Ruminant livestock is one of the largest contributors to greenhouse gas emissions at the global level. Methane emission in ruminants is also associated with an energy loss of about 2-12% of gross energy intake depending upon type of diet and intake level (Johnson & Johnson, 1995). As the direct measurement of CH_4 (e.g in respiration chamber) is relatively expensive and labour-intensive, numerous empirical equations and mechanistic models to predict CH_4 emission are reported in the literature. The Nordic dairy cow model Karoline is a dynamic and mechanistic model that describes digestion and metabolism in dairy cows (Danfær *et al*., 2006), and its fundamental underlying principles were first published by Danfær (1990). The model predicts the amounts of nutrients absorbed from the digestive tract and metabolized in various tissues, and milk production. Although the model was constructed to predict nutrient supply and milk production in dairy cows, it can also be used to predict absorption of nutrients in growing cattle. Moreover, the previous study by Ramin & Huhtanen (2015) confirmed that the Karoline model is a useful tool in predicting CH_4 emissions in ruminants. Therefore, the aim of the current study was to update the data set previously used by Ramin & Huhtanen (2015) with data recently published in the literature to predict CH_4 emission in growing and lactating cattle with the Karoline model.

Materials and Methods

Original data and prerequisites of data set from 31 papers published between 1964-2013 with a total of 184 observations is described in more detail by Ramin & Huhtanen (2015). Additional survey of literature was performed in Web of Science with results of a total of 90 treatments published 2013-2015. Among the 24 papers, 16 were studies conducted with dairy cows and 8 studies with growing cattle.

Additional papers included in the previous data set by Ramin & Huhtanen (2015) are given at the end of the reference list. To accept a study to be included in the data set, the following prerequisites were used: a) methane emissions were estimated in a respiration chamber, b) treatments did not include feed additives (e.g., monensin, tannins etc.), c) dietary ingredient composition and forage analytical values for fibre (e.g. NDF, ADF) were reported and d) dry matter intake (DMI) was reported. Chemical composition of diets were collected from the published papers and when compositional data were not reported, tabulated values from the National Research Council (NRC, 2001) and the Cornell Net Carbohydrate and Protein System (CNCPS; Tylutki et al., 2008) were used. Data of rumen fermentation characteristics, body weight (BW) and diet digestibility were added to the dataset, if reported. For certain other parameters, such as protein fractions, indigestible NDF, passage rate of potentially digestible NDF, etc., tabulated values were used or estimated from equations published in the literature. The objective was to compile a data set in order to cover a wide range of dietary composition from experiments that had studied the effects of feeding level, proportion of concentrate supplementation, protein and fat supplementation, carbohydrate composition of

concentrate supplements, forage type, maturity of forage crops at harvest and silage fermentation quality. Studies with diets including more than 75% concentrate on a dry matter basis were excluded from the final data set. Methane production was expressed as grams per day, litres per day, MJ per day or as a proportion of GE or DE. The following factors were used in converting methane: 1 g = 1.40 L = 55.5 kJ. The values are based on a molar mass of 16.04 g and gas volume of 22.4 L/mol.

For the prediction of CH_4 emissions, the Nordic dairy cow model Karoline was used (Danfær *et al*., 2006; Huhtanen et al., 2015). Originally, the Karoline model was built using the graphic Powersim® software, but the current study was performed using the translated version of the Karoline model in MS Excel (Huhtanen, 2015). Moreover, detailed information of the sub-model predicting CH_4 emission in the Karoline model is given by Huhtanen *et al*. 2015.

The relationships between predicted and observed CH_4 emissions were assessed by using the FIXED model analysis. Root mean square prediction error (RMSPE) was calculated as follows: $RMSPE = \sqrt{[\sum (Observed - Predicted)^2 / n]}$

The error was expressed as a proportion of the observed mean to give an estimate of the overall prediction error. Also, residual analysis was conducted for CH_4 emissions by regressing the centred predicted values against the residuals (observed–predicted).

Results and Discussion

Dietary and animal characteristics collected and used in the current study covered the ranges of typical cattle diets (Table 1), and displayed large variation in intake, diet composition and digestibility. For example, there were diets included to the dataset without any concentrate up to 70 percentage of concentrates in diet. Also, the large variation in BW and DMI values were described with indication to the animal type, being the mean values of BW for dairy cows 589±78.6 kg and for growing cattle 398±129.4 kg, and of DMI 17.1±4.21 and 7.2±1.86, respectively.

Table 1 Statistical description of the diets and other parameters in the observed dataset (n=274).

Item	n	Mean	SD	Min	Max
DM intake, kg/d	274	14.2	5.82	3.42	27.2
Proportion of concentrate, %	274	34.6	18.35	0	70.0
Body weight, kg	267	531	131.1	175	740
Rumen fermentation, mol/mol					
Acetate	110	0.64	0.037	0.549	0.770
Propionate	110	0.20	0.027	0.145	0.271
Butyrate	110	0.12	0.022	0.065	0.200
Organic matter digestibility	157	0.73	0.050	0.545	0.836
CH_4 emissions, g/d	274	307	118.7	90.5	540

DM=dry matter; OM=organic matter; CP=crude protein; NDF=neutral detergent fibre; CH_4=methane; SD=standard deviation.

The relationship between observed and predicted emissions are presented in Figure 1. The Karoline model performed well with the current dataset with a high correlation between predicted and observed emissions (R^2=0.92) and RMSPE=34.1 g/d (11.1% of the observed mean).

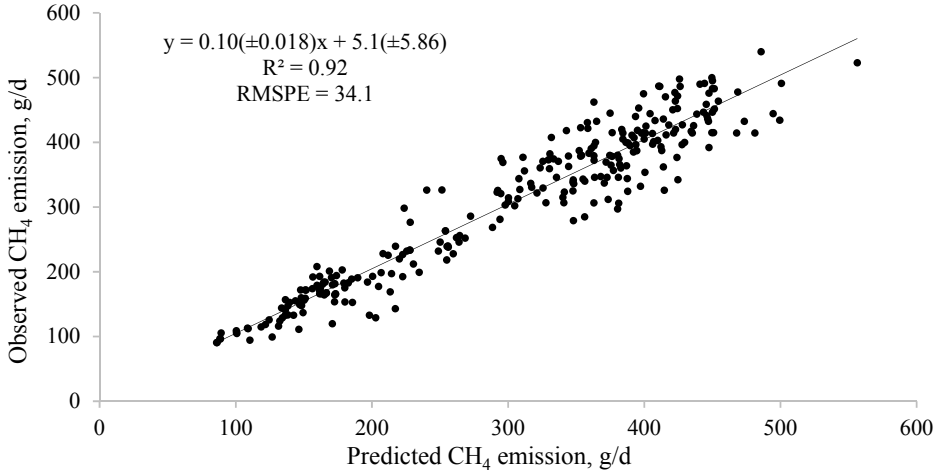

Figure 1. Relationship between predicted and observed CH_4 emissions (n=274).

Using the fixed regression model following linear relationship was constructed:

observed CH_4 emission (g/d) = 0.10 (±0.018) × predicted CH_4 emission (g/d) + 5.1 (±5.86)

In the previous study (Ramin & Huhtanen, 2015) with a smaller dataset using the Karoline model the values were R^2=0.93 and RMSPE=42.8 L/d. The greater error (11.1 % vs. 10.1% of the observed mean) of the present study compared to Ramin & Huhtanen (2015) could be due the fact that a relatively large proportion of papers added to initial dataset did not report or partly reported the chemical composition of feeds. However, compared to other mechanistic models (Benchaar *et al*., 1998; Mills *et al*., 2001) the previous evaluation showed higher R^2 and lower RMSPE values. As mentioned before, mechanistic models such as the Karoline cow model could be a useful tool to understanding the reasons affecting CH_4 emissions as it simulates the emissions using background information of ruminal fermentation patterns.

However, relating the residuals of CH_4 emission to factors influencing CH_4 emission the most, e.g. DMI, OMD and dietary carbohydrates could yield valuable information. This could elucidate how well the model is in predicting the effect of the respective factor on CH_4 emission.

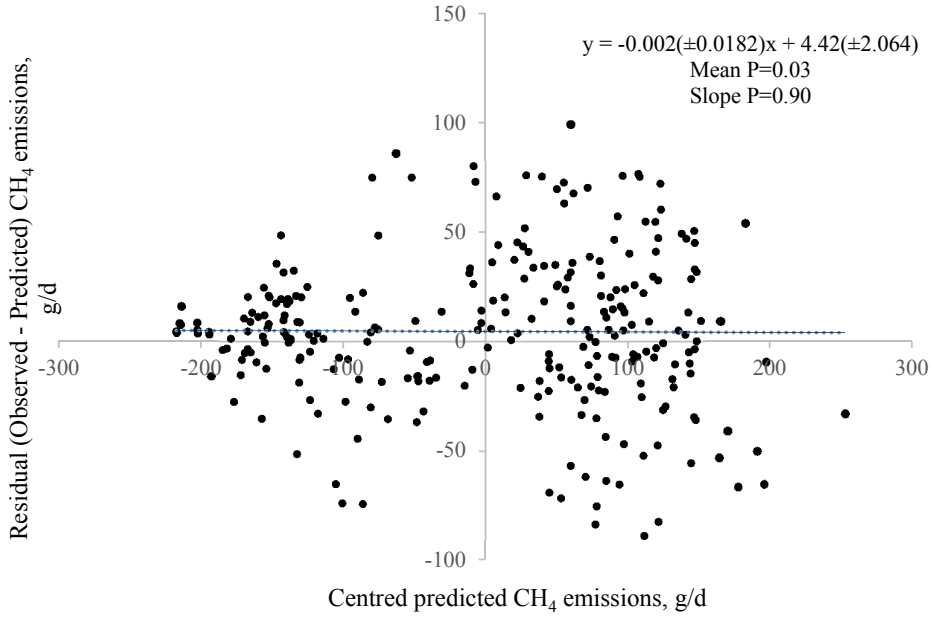

Figure 2. Relationship between centred predicted values and residuals of methane emissions (g/d) n=274.

Conclusions

It is concluded that the Nordic dairy cow model Karoline is a useful tool in predicting CH_4 emissions from dairy and growing cattle. Moreover, the model has the potential to be used for the development of mitigation strategies and teaching purposes.

Acknowledgments

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Importance of accurate and correct quantitative measurements in a new volumetric gas measuring technique for *in vitro* assessment of ruminant feeds

J. Liu[1,2*], S. Strömberg[1], J.R. van Gorp[1] & M. Nistor[1]
[1] *Bioprocess Control AB, Scheelevägen 22, 22363 Lund, Sweden*
[2] *Division of Biotechnology, Faculty of Engineering, Lund University, Getingevägen 60, 22100 Lund, Sweden*
[*] *Correspondence: jl@bioprocesscontrol.com or Jing.Liu@biotek.lu.se, tel. +46 46 163951*

Introduction

In vitro rumen incubation analysis have already been used for years to evaluate the nutritive qualities of feeds, originally employing end-point measurements focusing on feedstuff digestion. The relation between accumulation of fermentation gases and metabolisable energy content of the feed was established in the 1970s. Since then, measurement techniques based on *in vitro* gas production have been further developed for feed evaluation experiments. Much of these early reports rely on a manometric gas measurement principle. Not many publications on liquid displacement systems based on a volumetric gas measuring principle are available due to the limitations of the instrumentation setup.

This work describes a new volumetric gas measuring technique specially developed for monitoring production of ultra low gas volumes, with various applications in batch fermentation tests. The technique has been successfully applied and validated for quantifying biochemical methane potential from various biodegradable organic matters. Its high measurement accuracy and precision, as well as its unique feature on gas volume normalisation can potentially match the analysis demand for *in vitro* digestibility test. An automated measuring system based on this volumetric measuring technique can offer continuous monitoring of gas production from *in vitro* digestibility tests with high throughput and significant reduction of labour and time intensity. A wide range of applications of such an automated system is expected in ruminant feed evaluation, including continuous monitoring of gas production for extracting process kinetic information, determination of feed digestibility and its metabolisable energy content, comparing different technological pre-treatments of feed compounds, optimisation of feed composition and nutrient content for livestock, screening a large range of feeds or additives before testing these *in vivo*, etc. In this study, the effect of various parameters on the measurement technology are discussed, and the method is used for a short term *in vitro* digestion test. Preliminary results from a long term validation tests are discussed as well.

Background

Ruminants contribute substantially to the human food supply, accounting for almost all of the milk and a large part of the meat production world wide. A lot of research has therefore been completed on the digestive system of ruminants, increasing the understanding of its functioning and optimising feed efficiency for increased production. The close association between rumen fermentation and gas production was recognised several decades ago and has since been well studied. The gas production measurement technique has been widely used to determine organic matter digestibility, protein degradation, or to predict metabolisable energy content and feed digestion rate (Cone et all., 1996; Getachew et all., 1998; Getachew et all., 2008; Murray et all., 2014). Other applications include testing the use of dietary supplements in support of, for instance, greenhouse gas mitigation (Martínez-Fernández et all., 2014;

Romero-Perez et all., 2014). There are many protocols available on how to perform *in vitro* digestibility tests. Some of them are adapted for the utilisation of the gas measurement technique, but they differ in the experimental set up and are generally modified and adapted to the specific researcher´s purpose. Because of this, it is often difficult to evaluate results from different studies and values can vary substantially. Thus, there is a need for a test standard and general procedure, but also for a measurement quality standard of the gas measurement technique for *in vitro* digestibility tests.

One issue that is not fully addressed in the current protocols is the equipment and experimental set up that is used for these kinds of tests. Many times these are developed in house and specific for each laboratory. A solution to minimise the differences is the use of a complete lab platform such as the Gas Endeavour (Figure 1). The Gas Endeavour is specially designed for low gas volume and flow analysis and includes everything needed to perform *in vitro* digestibility tests; i.e. temperature controlled continuously stirred test vessels, optional vessels for carbon dioxide removal when methane analysis is performed, and a robust and reliable gas measuring system with a resolution of approximately 2 ml. In a study where three different ways of measuring the biochemical methane potential of cellulose were tested, the Gas Endeavour´s predecessor, AMPTS (Automatic Methane Potential Test System), provided the highest accuracy and repeatability (Esteves et al., 2011). Examples of studies where the AMPTS has been used are: investigation of methane potential from algae farming on available sludge streams from a waste water treatment plant (Rusten & Sahu, 2011); evaluation of different pre-treatments of sugarcane bagasse (Badshah et al., 2012) and evaluation of the effects from different chemical and biological additives on a substrate mixture (Strömberg et al., 2011).

A common problem when comparing results from different sources is the various ways of presenting the quantitative gas measurements (Walker et al., 2009; Wulf et al., 2011). Corrections to standard conditions for temperature and pressure are often poorly described or presented using different standard values which could lead to differences of up to 10% in the corrected volume. Another factor, that is not addressed in many corrections, is the water content of the gas. At 20°C and 1 atmosphere, roughly 2.3% of the gas volume consists of water and thus, should not be considered as gas in the reported values. It is also important to get an accurate value of the instant temperature and pressure throughout the test period in order to adjust gas volumes correctly in real time. Many times a fixed room temperature and pressure is assumed but in fact, these values can vary substantially. In a 30-day long experiment, carried out in a well sealed and shielded lab in Lund, Sweden, the pressure varied between 99-106 kPa, which could lead to a difference of up to almost 7% if the extremes were used. Clearly, there are many factors that need to be addressed in order to present quantitative gas measurements in a correct way. For the Gas Endeavour, all these issues are addressed and fully automatic.

System and Calculations

The Gas Endeavour has been used to perform a number of methane potential tests for biogas production on various types of substrates. As can be seen in Figure 1, the Gas Endeavour consists of two major parts, of which the first part is the temperature controlled water bath with 15 reactors of 500 ml, which can be seen on the left hand side of Figure 1. Each bottle has a mixer with a motor that can be run in either continuous or intermittent mode. The second part, which can be seen on the right hand side of Figure 1, is a gas measurement unit

where gas is collected in a cell through water displacement. When a pre-defined gas volume has been accumulated, the cell opens and releases the gas which is registered in the embedded CPU. Every opening corresponds to roughly 2 ml of gas and for each opening the ambient temperature and pressure are registered for calculations of normalised values (0°C, 1 atmosphere and zero moisture content).

Figure 1 The Gas Endeavour

Ambient pressure

The variation of ambient pressure can significantly influence gas volume and flow measurement. To minimise the influence of ambient pressure difference and variation among different testing sites and labs, the gas volume is usually corrected to standard conditions using the ideal gas law. However, it should be considered that there are two common standard conditions which differ from each other on reference temperature (i.e. 0ºC or 20ºC). This can lead to differences of up to 10 % (Walker et al., 2009).

In order to meet demands for high accuracy and precision, it is not sufficient that pressure is measured by off line spot checks. It should be measured continuously at each measuring point in real time, to be sure that a correct value is registered. The ambient pressure can vary from day to day which will impact on both the dynamic profile and the accumulated volume.

Temperature

As with pressure, temperature at the measuring point will affect the volume of the gas and should be adjusted to standard conditions using the ideal gas law. Equation 1 shows how to adjust a gas volume to standard volume and pressure based on the ideal gas law.

$$V_{STP} = \frac{p_{STP}}{p_{gas}} * \frac{T_{gas}}{T_{STP}} V_{gas} \quad (1)$$

In Equation 1, V_{STP} is the volume adapted to standard temperature and pressure, p_{STP} is the standard pressure, p_{gas} is pressure of the measured gas, T_{gas} is the temperature of the measured gas, T_{STP} is the standard temperature (which is 0ºC for the Gas Endeavour) and V_{gas} is the measured volume.

Water content

Gas produced from anaerobic digestion and *in vitro* digestibility tests is assumed to be saturated with water vapour and, in order to give accurate and correct gas measurements, this water should be removed (Walker *et al.,* 2009). At the ranges where an anaerobic digestion test and *in vitro* digestibility tests normally are performed (i.e. 0.9-1.1 bar and 10-40°C), the vapour pressure of water can satisfactory be approximated using the Antoine equation

(Equation 2). In Equation 2, p_{vap} is the fraction of water in the gas and T_{gas} is the temperature of the gas in °C.

$$p_{vap} = 10^{8.1962-\frac{1730.63}{233.426+T_{gas}}} \quad (2)$$

Relative error

The error introduced by assuming either the temperature or pressure constant or including water vapour in the gas volume measurement, is calculated according to Equation 3.

$$Relative\ error = \frac{V_{mL} - V_{NmL}}{V_{NmL}} * 100\% \quad (3)$$

In Equation 3, V_{mL} is the measured accumulated volume at a certain time in mL, for a scenario where either the pressure or temperature is assumed constant or where the water content is included in the measurement. V_{NmL} is the measured accumulated volume in normalised mL.

Materials and Methods

Biochemical methane potential test

The sample (banana stems) was mixed with an inoculum in 500 ml bottles to reach a liquid volume of 400 ml with an inoculum to substrate ratio of 2:1 (based on volatile solids content). The inoculum was collected from a sewage treatment plant in Sweden (Ellinge sewage plant, Sweden), which receives municipal wastewater and vegetable residues from the food industry, and was stored at room temperature for five days to reduce as much of its organic content as possible. Triplicates of each sample were used and the bottles was incubated, at 37°C with continuously mixing of approximately 80-100 rotations per minute. Triplicates with only 400 ml of inoculum was included to remove background production from fermentable material contained in the inoculum. No additional external nutrients or trace elements were added to the reactors. Before starting the test, the headspace was flushed with nitrogen gas for 1 minute to achieve anaerobic conditions. The produced biogas was led through 80 mL of 3 M sodium hydroxide solution to remove carbon dioxide and hydrogen sulphide to allow measurement of only methane. The test was performed for 35 days during which the gas volume, together with temperature and pressure, was continuously recorded with the Gas Endeavour´s predecessor AMPTS II.

Methods short-term incubation

Samples were incubated with 200 ml rumen fluid and 200 ml of VOS buffer (Lindgren, 1979), containing per litre: 8.50 g $NaHCO_3$, 5.80 g K_2HPO_4, 0.50 g $(NH_4)_2HPO_4$, 1.00 g NaCl, 0.50 g $MgSO_4$•7 H_2O, 0.01 g $FeSO_4$•7 H_2O and 0.10 g $CaCl_2$. Rumen fluid was from a maintenance fed non-lactating cow and collected at about 17:00 h, after last feeding. The rumen fluid was transported to the lab and strained through a kitchen strainer (approx. 1-mm openings).

Wheat starch and urea (both from Kebo, Stockholm) were incubated at three different levels: 3 g starch + 200 mg urea, 6 g starch + 400 mg urea and 9 g starch + 600 mg urea. In addition, a grass sample with in vitro digestible organic matter (IVDOM) concentration of 840 g/kg OM and a blank (only rumen fluid and buffer) were incubated. All incubations were in triplicate.

Feed samples were added first to the incubation bottles and VOS buffer was added after flushing the bottles with CO_2. Rumen fluid was thereafter added to a triplicate of incubation vessels, tubing and stirring motors were connected and gas measurement started for that triplicate. About 5 minutes elapsed from rumen fluid addition until gas production was being logged for a triplicate of vessels.

Methods 96 h incubation

This incubation involved both gas measurement and gravimetric determination of organic matter disappearance in the incubation vessels. It was performed in conjunction to the lab's weekly routine IVDOM determination of forage samples according to the 96 h VOS procedure (Lindgren 1979; Åkerlind et al., 2011). Proportions of rumen fluid, buffer and sample were similar to the VOS procedure with 10 ml rumen fluid, 290 ml VOS buffer and 4 g of air-dry sample.

A set of six calibration samples with IVDOM 686-901 g/kg OM that are included in each IVDOM batch at the lab were incubated in duplicate and so was a barley straw sample with IVDOM 505 g/kg OM.

The rumen fluid was from a maintenance fed non-lactating cow and collected in the morning. Handling of rumen fluid and buffer was similar to the lab's IVDOM procedures with straining of rumen fluid through a 1 mm screen, mixing with buffer and dispensing into incubation bottles without previous CO_2 flushing. Incubation was conducted over 96 hours.

After incubation termination, each bottle was split into three glass filter tubes with porosity P1 (100-160 μm) and rinsed according to the VOS procedure with hot water and acetone. The samples were then dried overnight at 103°C and ashed for 3 h at 500°C according to the standard procedures for VOS to get a measure of remaining organic matter amount and hence organic matter digestibility in vitro.

Results and Discussion

In this section, the results from the biochemical methane potential test are presented. Focus is directed on showing the effect of the different factors influencing the results, and a feed digestibility test performed using standard samples.

Three different aspects that influence the recorded gas volume were studied: assuming a fixed ambient temperature (22ºC) and pressure (1 atm) *vs* continuous measurements of the two parameters as well as considering or not considering the water vapour content of the gas. The recorded variation of pressure and temperature from the biochemical methane potential test can be seen in Figure 2. Pressure varied considerably, whereas temperature remained more stable due to the temperature controlled environment inside the lab.

Figure 3 shows the difference in the measured accumulated volume for the three different scenarios. The right hand side figure shows the variation in the relative error *vs* a reference case (considering variation in temperature and pressure as well as removal of the water vapour content). It can be seen that the introduced error varies for the scenario where the pressure is assumed constant. The errors introduced by fixing the temperature or including water vapour are more constant in time.

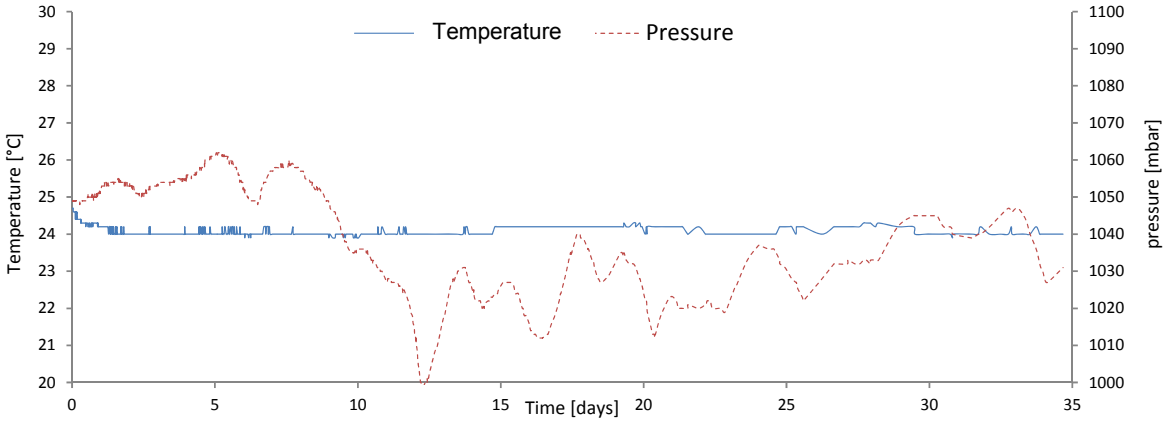

Figure 2 Dynamic profile of temperature and pressure during the biochemical methane potential test.

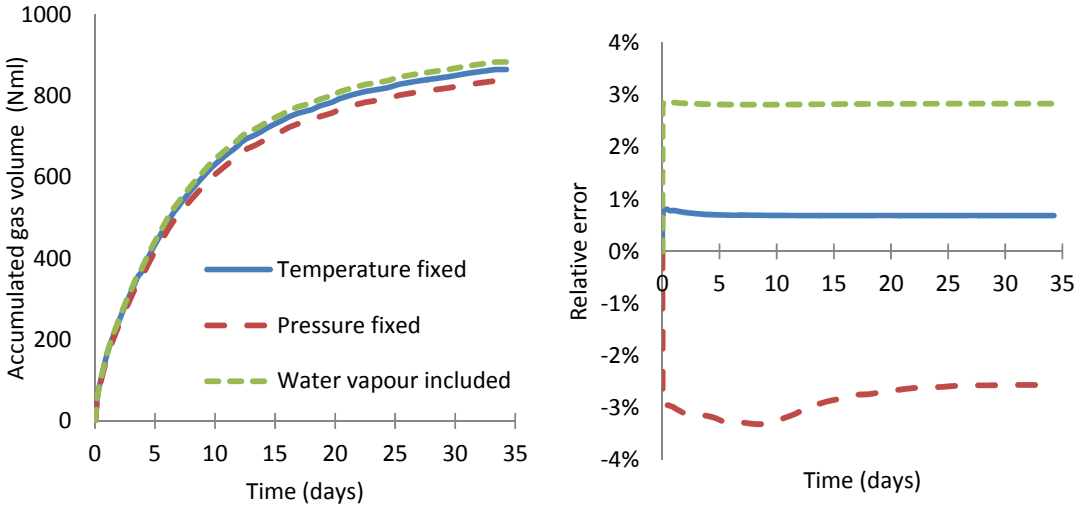

Figure 3 Recorded accumulated volume of a biochemical methane potential test when different factors have been assumed constant, and the resulting error percentage over time for each situation.

The *in vitro* digestibility test was performed together with the Feed Science Division, Department of Animal Nutrition and Management, SLU, Uppsala. The accumulated gas volume was monitored over time, and pH was measured at the end of the incubation, after *circa* 14 hours. The average results are plotted in Figure 4.

Figure 4 shows that the standard deviation of measured gas volumes within triplicates was in general very low, with exception for the highest concentrations of starch and urea (respectively, 9 g and 600 mg). This suggests that an accumulation of volatile fatty acids and the resulting low pH of 5.5 was limiting fermentation. This could also be a sign of substrate overload in the test vessels, as well as the reason for the higher standard deviation within triplicates with high concentrations of starch and urea. A clear correlation can be seen between starch level and gas production. Fermentation of the grass resulted in a relatively low final gas volume but a rapid onset of fermentation, probably as a result of easily fermentable sugars present in the grass.

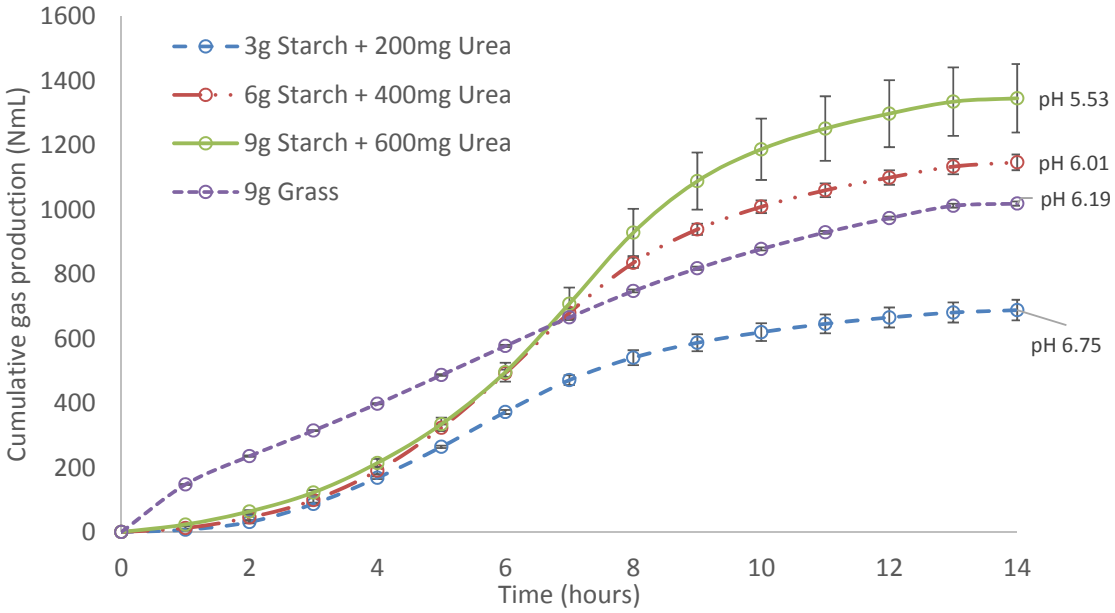

Figure 4 Accumulated gas volume in normalised mL over time, with final pH value.

To further validate the instrument for *in vitro* feed digestibility tests, a long term incubation of 96 hours was also performed. The method was compared to the gravimetric standard Swedish in vitro analysis (VOS). The Gas Endeavour resulted in a slightly higher remaining organic matter amount whereby the relative error compared to the VOS analysis was circa 3% (results not shown), except for the blank sample where the relative error was higher. Overall, the results from the two methods were well correlated.

Conclusions

In this work, results from various biochemical methane potential tests are reported, highlighting the importance of a correct adjustment of quantitative gas measurements. It was shown that variable ambient pressure and temperature can have a significant effect on the measured accumulated gas volume. Some preliminary result of an *in vitro* feed digestibility test was also performed with the Gas Endeavour, showing a clear correlation between the measured accumulated gas volume and starch concentrations used. In general, the variation between triplicates was minimal. More long and short term incubations are currently performed to further validate the instrument for feed digestibility tests.

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Evaluation of bio-forage feeds developed through gene silencing, modification and inserting techniques for ruminant livestock systems: progress update

A. Jonker, M.Y. Gruber, Y. Wang, R. Heendeniya, Y. Lei, A. Hannoufa & P. Yu*
Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, Canada; Agriculture and Agri-Food Canada, Saskatoon Lethbridge and London Centers, Canada
Correspondence: peiqiang.yu@usask.ca

Introduction

Alfalfa (*Medicago sativa L.*) is one of the most important forages in the world (Wang et al., 2006). Alfalfa is a relatively winter hardy and drought tolerant legume with good longevity (Popp et al., 2000), high nutrient levels, high digestibility, unique ratio of structural to non-structural components (Yu et al., 2003a) and high dry matter intake (Thornton and Minson, 1973). A major disadvantage of alfalfa is its excessively rapid initial rate of ruminal protein digestion and degradation (Boderick, 1995; Yu et al., 2004). This results in digestive disorders like frothy bloat (Popp et al., 2000; Wang et al., 2006) and low protein use efficiency with consequent release of up to 25% of alfalfa nitrogen from the ruminant into the environment (Boderick, 1995). Proanthocyanidins (PA) are oligomeric and polymeric secondary plant products which share the early and middle steps of the flavonoid pathway with the plant pigments anthocyanins. The flavonoid pathway arises from the phenylpropanoid pathway (4-coumaroyl CoA + 3 malonyl CoA) via naringenin chalcone (Marles et al., 2003). The building blocks of most PA are the monomer flavan-3-ol (e.g. (+) catechin and (-) epicatechin) and a flavan 3,4-diol (Winkel-Shirley, 2001). The composition of PA varies in linkage between the flavan monomers (C4 to C6 or C4 to C8), stereochemistry at carbons 2, 3 and 4 and the number of hydroxyl groups on the A and B rings. These differences in PA composition affect its molecular structure and influence the capability to interact with other molecules like protein. PA can form complexes not only with protein but also with starch, essential amino acids, carbohydrates and digestive enzymes (Aron and Kennedy, 2008). It provides a strong defense mechanism to plants, especially protecting them against herbivores (Zucker, 1983).

PA-protein complexes are formed in the rumen, reducing protein degradation and consequently increasing ruminal escape protein (Aerts et al., 1999; Broderick, 1995) and reducing foam stability (Fay et al., 1980; Tanner et al., 1995). PA-protein bonds are broken in the abomasum due to low pH (Jones and Mangan, 1977), which may result in increased amino acid (AA) digestion in the small intestine (Aerts et al., 1999). PA are also found to reduce the production of methane (Woodward et al., 2004). Flavonoid structural gene chalcone synthase (CHS) is active in alfalfa leaves but there is no expression of flavanone 3-hydroxylase (F3H) and dihydroflavanol 4-reductase (DFR). These latter genes stimulate intermediate steps in the flavanoid pathway leading towards the accumulation of PA. Instead of PA, alfalfa leaves accumulate upper pathway flavones. In western Canada Three Lc alfalfa genotypes were crossed with hardy Western Canadian varieties to facilitate the movement of the Lc gene into a broader spectrum of alfalfa breeding germplasm. To our knowledge, no forage analysis has been conducted on these crossed progeny of transgenic Lc alfalfa. Transgenic alfalfa germplasm has also been developed by expressing a PA regulating bHLH from lotus uliginosis and with a bHLH gene from alfalfa. This material has not been analyzed yet for the presence of PA. There is a need to develop and evaluate these populations of PA

containing alfalfa, which are able to adapt to the winter hardy climate of Western Canada, improve N efficiency, and bloat safety.

Project objectives: to determine forage quality characteristics of a winter hardy proanthocyanidin- accumulating alfalfa variety with PA in the forage and to contribute to the development of PA optimized alfalfa breeding material. Alfalfa with optimized PA will be bloat safe under grazing conditions in all growing stages and under all climate conditions. This will also significantly increase protein efficiency and reduce N and greenhouse gas pollution to the environment. The final proanthocyanidin accumulating germplasm will be universally adopted by high quality beef and dairy feeding programs in Canada and around the world. In the long term, it is estimated (in a report by Agriculture and Agri-Food Canada) that a $2-4 billion annual cost savings will be realized for the $8-12 billion Canadian beef cattle and dairy industry through the development of 'proanthocyanidin accumulating alfalfa.

Specific objectives: to determine (1) the effect of transgene expression in the cross genotypes alfalfa on the accumulation of PA; (2) N metabolism and fermentation/degradation characteristics of genotypes of crossed Lc alfalfa and parental (control) alfalfa germplasm; (3) nutritional chemical profiles; (4) nutrient utilization and availability; and (5) bloat characteristics of the crossed population in ruminants.

Completed research
Program 1: Characterization of anthocyanidin accumulating Lc-alfalfa for ruminants

Results from five studies conducted within this program are summarised here.

Study I. Nutrient composition and degradation profiles of anthocyanidin accumulating lc-alfalfa populations (Jonker et al., 2010).

The objective of the study was to determine the effects of the Lc-transgene on survival, anthocyanidin, condensed tannin and chemical profiles in crossed populations of western Canadian-adapted Lc-alfalfa. These were compared with their non-transgenic (NT) parental varieties, Rangelander, Rambler, and Beaver.

Lc-alfalfa forage accumulated enhanced amounts of anthocyanidin, with an average concentration of 197.4 μg/g dry matter (DM), while condensed tannins were not detected. Both of these metabolites were absent in the NT parental varieties. Lc-alfalfa had a lower crude protein (CP) content (24.8 vs. 27.3% of DM) and higher total carbohydrate (CHO) concentration (58.3 vs. 55.5% of DM), which resulted in a decreased N:CHO ratio (68.1 vs. 79.2 g/kg) compared with NT-alfalfa. Slowly degradable N:CHO ratio was decreased by 5.9 g/kg and total rumen degradable N:CHO ratio was decreased by 12.9 g/kg in Lc-alfalfa compared with NT-alfalfa. In conclusion, Lc-gene transformation resulted in the accumulation of anthocyanidin, decreased total protein content, increased total carbohydrate content and improved the balance between nitrogen and carbohydrates in the crossed transgenic populations of western Canadian-adapted alfalfa compared to their NT western Canadian parental alfalfa varieties.

Study II. Modeling degradation ratios and nutrient availability of anthocyanidin accumulating Lc-alfalfa populations in dairy cows (Jonker et al., 2011).

Ruminal protein degradation of alfalfa might be reduced by introducing a gene that stimulates the accumulation of mono/polymeric anthocyanidin in alfalfa. The objectives of this study were to fractionate protein and carbohydrates by in situ and chemical approaches, to evaluate

in situ ruminal degradation characteristics and synchronization ratios, to determine protein availability to dairy cattle using the Dutch 2007 DVE/OEB protein systems and to determine net energy for lactation using the VEM energy system for three newly developed transgenic winter hardy anthocyanidin accumulating T1 Lc-alfalfa populations.

These T1 Lc-alfalfa populations, called T1BeavLc1, T1RambLc3 and T1RangLc4, had an average anthocyanidin accumulation of 163.4 μg/g DM while AC Grazeland (selected for a low initial rate of degradation) did not accumulate anthocyanidin. The basic chemical composition of the original samples, soluble and potentially degradable fractions and degradation characteristics of crude protein and carbohydrates were similar in T1 Lc-alfalfa and AC Grazeland. The undegradable in situ crude protein and neutral detergent fiber fraction were, respectively, 1.3 and 4.8% lower in T1 Lc-alfalfa, compared with AC Grazeland. T1 Lc-alfalfa had a 0.34 MJ/kg DM higher calculated net energy for lactation and 1.9% higher buffer soluble protein content, compared with AC Grazeland. By the protein evaluation model, it was predicted that T1 Lc-alfalfa tended to have a 11.9, 6.9 and 8.4 g/kg DM higher rumen degradable protein, rumen degraded protein balance and intestinal available protein compared with AC Grazeland, respectively. The hourly rumen degraded protein balance was highest in T1RangLc4 and lowest in T1RambLc3. The hourly rumen degraded protein balance at 4 and 24 h was similar and balanced for all four alfalfa populations. In conclusion, T1 Lc-alfalfa accumulated anthocyanidin, tended to have a higher predicted intestinal protein availability and a higher predicted net energy of lactation availability for dairy cattle compared with AC Grazeland.

Study III. Fermentation, degradation and microbial nitrogen partitioning for three forage colour phenotypes within anthocyanidin accumulating Lc-alfalfa progeny (Jonker et al., 2012a).

Introducing a gene that stimulates the accumulation mono/polymeric anthocyanidins might reduce ruminal protein degradation rate (by fixing protein and/or direct interaction with microbes) and reduce methane emission. The objectives of this study were to evaluate in vitro fermentation, degradation and microbial-N partitioning of three forage colour phenotypes [green, light purple-green (LPG) and purple-green (PG)] within newly developed Lc-progeny and to compare them to parental green non-transgenic (NT) alfalfa. Purple-green-Lc accumulated more anthocyanidin compared with Green-Lc with LPG-Lc intermediate.

Cumulative gas production incubations were performed in three in vitro runs. Strained rumen liquor was combined with a mineral buffer (1:2 v/v) and ^{15}N labeled ammonium sulfate (1.67 g L^{-1}, 10 atom % excess (APE) and four replicates of each substrate were incubated in 125 mL vials containing substrate (0.5 g) and inoculum (40 mL) in a manually operated *in vitro* system for 48 h. Gas production (measured using a water replacement device) and methane concentration within the gas (collected with a gas-tight syringe) were measured at 4, 8, 12, 24 and 48 h of incubation. The vial contents were processed to determine true DM degradability (TDMD) and true N degradability (TND), microbial-N (MN) accumulation, volatile fatty acids (VFA) accumulation and NH_3 concentration. Sample residues after incubation were freeze dried, weighed for DM determination, and analyzed for total N content and ^{15}N APE.

Filter bags for in situ incubation, ANKOM F57 filter bags (ANKOM Technology, Fairport, NY, USA) were soaked in acetone for 5 min to remove surfactant that may negatively affect microbial activity. Filter bags with 0.5 g sample and empty bags (blank control) were transferred into 2.5 L jars (one *Lc*-progeny × phenotype or NT-alfalfa cultivar per jar)

followed by addition of 1.6 L of pre-warmed (39ºC) mineral buffer and ^{15}N ammonium sulfate) and 0.4 L rumen liquid (prepared from the same animals described above). Jars were incubated in DAISY II incubators (ANKOM Technology, Fairport, NY, USA) at 39ºC under continuous rotation. Triplicate bags with sample residue and duplicate blank bags were withdrawn from the fermentation jars at 4, 8, 12 and 24 h of incubation and processed as described by Wang et al. (2006) to determine DM, total N and ^{15}N APE in N. True DMD, TND, MN firmly attached to feed particles and MN loosely attached onto feed particles were calculated. At the same time points, inoculum (12 mL) was sampled from each fermentation jar and processed to determine liquid associated MN. The 0 h bags were washed in plastic stomach bags with deionized water and processed in laboratory blender (Stomacher 400; Seward Medical Limited, London, UK). The washable fraction was fractionated into a washable truly soluble fraction (SW) and a washable insoluble fraction which was added back to the residue left in the bag.

The study showed that volatile fatty acids (VFA) and potentially degradable dry matter (DM) and N were similar among the four phenotypes. Gas, methane and ammonia accumulation rates were slower for the two purple-Lc phenotypes compared with NT-alfalfa, while Green-Lc was intermediate. Effective degradable DM and N was lower in the three Lc-phenotypes compared with NT-alfalfa. Anthocyanidin concentration was negatively correlated with gas and methane production rates and effective degradability of DM and N. In conclusion, The Lc-alfalfa phenotypes accumulated anthocyanidin. Fermentation and degradation parameters indicated a reduced rate of fermentation and effective degradability for both purple anthocyanidin accumulating Lc-alfalfa phenotypes compared with non-transgenic alfalfa.

Study IV. Foam stability of leaves from anthocyanidin accumulating Lc-alfalfa and relation to molecular structures detected by FTIR vibrational spectroscopy (Jonker et al., 2012b).

Foam stability related to pasture bloat from alfalfa pastures might be reduced by introducing a gene that stimulates the accumulation of mono/polymeric anthocyanidin. The objective of this study was to determine foam formation (at 0 min) and stability (at 150 min) from aqueous leaf extracts of three transgenic Lc-alfalfa progeny (BeavLc1, RambLc3, RangLc4), parental non-transgenic (NT) alfalfa and AC Grazeland (bloat reduced cultivar) harvested at 07:00 or 18:00 h.

Initial and final foam volumes at 07:00 h were lower for AC Grazeland compared with all other treatments and lower for RangLc4 compared with the other two Lc-progeny at 0 min and NT-alfalfa at 150 min. At 18.00 h, initial foam volume was larger for NT-alfalfa and final foam volume was larger for RambLc3 compared with AC Grazeland, BeavLc1 and RangLc4. Spectroscopic vibration associated with carbohydrates increased initial foam volume and stability. More amide I relative to amide II, less α-helices relative to β-sheets and leaf extract ethanol-film and protein content increased initial foam volume but did not stabilize it. Spectroscopic vibration associated with all carbohydrates other than structural carbohydrates were more important than vibration from protein structures for foam formation and stabilization. In conclusion, Lc-alfalfa accumulated anthocyanidin and had reduced foaming properties compared with parental non-transgenic alfalfa but AC Grazeland and RangLc4 had the lowest foaming properties.

Study V. Molecular basis of protein structure in proanthocyanidin and anthocyanin enhanced Lc-transgenic alfalfa in relation to nutritive value using synchrotron-radiation FTIR microspectroscopy (Yu et al., 2009).

To date there has been very little application of synchrotron radiation-based Fourier transform infrared microspectroscopy (SRFTIRM) to the study of molecular structures in plant forage in relation to livestock digestive behaviour and nutrient availability. Protein inherent structure, among other factors such as protein matrix, affects nutritive quality, fermentation and degradation behaviour in both humans and animals. The relative percentage of protein secondary structure influences protein value. A high percentage of β-sheet usually reduce the access of gastrointestinal digestive enzymes to the protein. Reduced accessibility results in poor digestibility and as a result, low protein value. The objective of this study was to use SRFTIRM to compare protein molecular structure of alfalfa plant tissues transformed with the maize Lc regulatory gene with non-transgenic alfalfa protein within cellular and subcellular dimensions and to quantify protein inherent structure profiles using Gaussian and Lorentzian methods of multi-component peak modeling.

Protein molecular structure revealed by this method included α- helices, β-sheets and other structures such as β-turns and random coils. Hierarchical Cluster Analysis and Principal Component Analysis of the synchrotron data, as well as accurate spectral analysis based on curve fitting, showed that transgenic alfalfa contained a relatively lower percentage of the model-fitted α-helices (29 vs. 34) and model-fitted β-sheets (22 vs. 27) and a higher percentage of other model-fitted structures (49 vs. 39). Transgenic alfalfa protein displayed no difference in the ratio of α-helices to β-sheets (average: 1.4) and higher ratios of α-helices to others (0.7 vs. 0.9) and β-sheets to others (0.5 v. 0.8) than the non-transgenic alfalfa protein. The transgenic protein structures also exhibited no difference in the vibrational intensity of protein amide I (average of 24) and amide II areas (average of 10) and their ratio (average of 2.4) compared with non-transgenic alfalfa. Cluster analysis and principal component analysis showed no significant differences between the two genotypes in the broad molecular fingerprint region, amides I and II regions, and the carbohydrate molecular region, indicating they are highly related to each other. The results suggest that transgenic Lc alfalfa leaves contain similar proteins to non-transgenic alfalfa (because amide I and II intensities were identical), but a subtle differences in protein molecular structure after freeze drying. Further study is needed to understand the relationship between these structural profiles and biological features such as protein nutrient availability, ruminal protein escape and digestive behaviour of livestock fed with this type of forage.

Ongoing research programs

The following two programs are ongoing:
Program 2. Lc and C1 gene stacking in alfalfa affects physico-molecular structure, phytochemical composition, and ruminant N metabolism, microbial protein synthesis and nutritive value (Ravindra Heendeniya, PhD student)

Program 3. Molecular basis of structural studies, phytochemical characterization, and nutritional evaluation of new modified alfalfa developed through different gene transformation and gene modification techniques in ruminant livestock systems (Yaogeng Lei, PhD student).

Conclusions

All Lc-alfalfa progeny and phenotypes accumulated anthocyanidin. Lc-alfalfa progeny had lower protein and higher carbohydrate content which improved the nitrogen to carbohydrate balance compared to their parental non-transgenic cultivars. Rate of fermentation and

effective degradability in vitro were reduced for both purple anthocyanidin-accumulating Lc-alfalfa phenotypes compared with NT alfalfa. Intestinal protein availability tended to be higher and net energy for lactation was higher from Lc-alfalfa progeny for dairy cattle compared with AC Grazeland. Foaming properties were reduced in Lc-alfalfa progeny compared with parental non-transgenic alfalfa but not compared with AC Grazeland. Further increases in mono/polymeric anthocyanidin accumulation in alfalfa are required in order to develop an alfalfa cultivar with superior nutritional and bloat preventing characteristics compared to currently available alfalfa cultivars. The findings from Program 2 (double inserting gene) and 3 will be reported soon after completed.

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The effect of pelleting hay upon feed intake, digestibility, growth rate and energy utilization of Icelandic lambs

Th. Lárusson & J. Sveinbjörnsson
Agricultural University of Iceland, Faculty of Land and Animal Resources, Keldnaholt, Árleyni 22, IS-112 Reykjavík, Iceland
Correspondence: jois@lbhi.is

Introduction

In the late 20th century a number of portable hay pellet factories were run in Iceland, producing 2000 tons or more of hay pellets each year. Often the hay was mixed with fishmeal and some vitamin- and mineral premixes. The interest in utilizing hay pellets as feed for Icelandic sheep, cows and horses was greatly influenced by intentions to increase production level per animal but at the same time minimize the need for imported concentrates. In the last decades, the production of barley in Iceland has increased and partly replaced imported concentrates, so the interest in hay pellets has decreased. There is, however, still one mobile hay pellet factory active.

To evaluate the effect of grinding and pelleting hay upon feed intake, digestibility, growth rate and energy utilization of lambs, two trials were conducted in 1985 and 1987 and a smaller trial in 2005. The results of these trials have only been presented in informal reports in Icelandic (Lárusson & Sveinbjörnsson, 2007; Lárusson, Sveinbjörnsson & Árnadóttir, 2006). As some of the data from these trials could be useful for improving the current or developing new feed evaluation systems for sheep, we consider it worthwhile to present them here in English and at the same time try to explain or refresh the theories about the effects of grinding and pelleting course feed materials.

Materials and Methods

Animals. Castrated male lambs, 7-9 months old were used in the trials. In all the three trials, relatively small ram lambs (27-30 kg) were selected at weaning at 4-5 months of age in September/October and fed indoors on hay until the trials started in January/February. The lambs were castrated in November and shorn in December. In January, their feed intake was tested, all lambs were weighed and thereafter, the lambs were assigned to treatments so that the average live weight of each group at start was as equal as possible. Here, at the initiation of the trial, control groups were slaughtered. The first week of the trials was an adaption period, thereafter the formal trial started, which lasted around 10 weeks.

Experimental treatments. In the first trial (**HT 85**), there were three feeding levels (maintenance, 1.5 x maintenance and *ad lib*) with only hay *vs.* 200 g/d hay + pellets from the same hay. The second study (**HT 87**) had the same arrangement of treatments except that there was an additional comparison of two types of hay from the same sward but cut at two different dates. In the third trial (**VB 05**), there was only one feeding level (*ad lib*) and only one type of hay, which was either fed as a) whole hay, b) 300 g whole hay + pellets *ad lib* or c) 300 g pellets + whole hay *ad lib* The experimental treatments are listed in more detail in Table 1.

Table 1 Experimental treatments and feed plan

Experiment/ treatments	No. lambs	Feed[a]	Feeding level
HT 85			
A	4		Slaughtered at initiation of experiment
B	4	Hay	Slightly over maintenance
C	4	Pellets+200g hay	Slightly over maintenance
D	4	Hay	1.5 x maintenance
E	3	Pellets+200g hay	1.5 x maintenance
F	4	Hay	*Ad lib.*
G	4	Pellets+200g hay	Pellets *ad lib*
HT 87			
A	4		Slaughtered at initiation of experiment
BI	4	Hay c1	Slightly over maintenance
BII	4	Hay c2	Slightly over maintenance
CI	4	Pellets + 200 g hay c1.	Slightly over maintenance
CII	4	Pellets + 200 g hay c2	Slightly over maintenance
DI	4	Hay c1	1.5 x maintenance
DII	4	Hay c2	1.5 x maintenance
EI	4	Pellets + 200 g hay c1.	1.5 x maintenance
EII	4	Pellets + 200 g hay c2	1.5 x maintenance
FI	4	Pellets + 200 g hay c1.	Pellets *ad lib*
FII	4	Pellets + 200 g hay c2	Pellets *ad lib*
VB 05			
V	2x8		Slaughtered at initiation of experiment
A	2x8	Hay	Hay *ad lib*
B	2x8	Pellets + 300 g hay	Pellets *ad lib*
C	2x8	Hay + 300 g pellets	Hay *ad lib*

[a]c1=cutting date 1 (early); c2= cutting date 2 (late)

Animal measurements. In the first two trials, the lambs were individually fed and *in vivo* dry matter digestibility was measured by a 1-week collection of feaces (4 lambs in each treatment). Live weight was measured regularly. At the end of the experiment (and control group in the beginning), the lambs were slaughtered. Carcass weight and weight of fleece, skin and gut contents were measured. In the first trial, three different methods were tested to evaluate the energy content of the whole lamb at slaughter. Based on methods selected from these comparisons, the energy content of all animals at slaughter were measured by collection and chemical analysis of different parts of the animals. In the third trial, lambs were group fed. Feed intake was measured on group basis and live weight was measured regularly. Carcass weight and weight of omental fat was registered.

Feed analysis. The analysis presented in Table 2 were done on the whole hay that was used as such in the experiments and also as a raw material for the hay pellets. *In vitro* dry matter digestibility was analysed by a modified method of Tilley and Terry (1963) and crude protein was measured using the Kjeldahl method.

Results and Discussion

The development in feed intake over the experimental period in the first trial (HT-85) is presented in Figure 1. Due to poor hay quality, the *ad lib* hay-fed lambs (Group F) were only able to reach similar feed intake as those fed 1.5 x maintenance (Group D). However, pelleting increased the maximum feed intake by >50% as can be seen if results for Group F and G are compared.

Table 2 In vitro dry matter digestibility and crude protein content of the hay used in the experiments.

Experiment[a]	Dry matter digestibility %	Crude protein g/kg DM
HT 85	63.5	130
HT 87 – c1	68.5	170
HT 87 – c2	58.5	105
VB 05	67.0	137

[a]c1=cutting date 1 (early); c2= cutting date 2 (late)

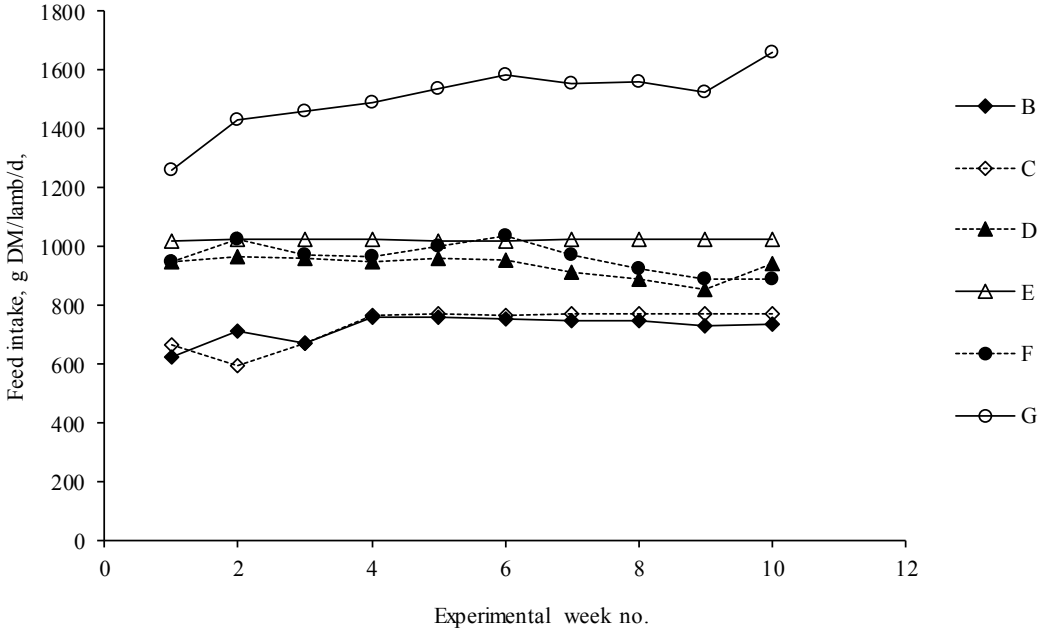

Figure 1 Feed intake development over time in trial HT 85. Capital letters A to G are treatments as described in Table 1.

Tables 3 and 4 summarise the most important results from trials HT-85 and HT-87, respectively. From Figure 3, it is clear that when the hay is ground and pelleted, intake increases and *in vivo* digestibility decreases. This effect increases as the quality (*in vitro* digestibility) of the hay is lower.

In the treatments with *ad lib* feeding, there was a dramatic increase in feed intake due to pelleting, in the range 50 to 65%, greatest with the least digestible forage (HT-87 c2,

treatment FII vs. EII). This increased intake due to pelleting resulted in higher carcass weight and greater amount of total tissue energy, in spite of the lower *in vivo* digestibility. In general (see both Table 3 and Table 4), there are clear indications that digestible energy is more efficiently transferred into net energy when the hay is pelleted. For example, the B and C groups in trial HT-85 (Table 3) are kept at the same dry matter intake; the digestibility is 4 % units lower in the pellet (C) group, but that group has gained 0.8 kg carcass weight compared to the control group (A), but the whole hay (B) group has lost 1.16 kg of carcass weight at the same time. Comparing groups CII (pellets) and BII (hay) in trial HT-87 (Table 4) also shows beneficial effect of pelleting upon carcass weight in spite of decreased digestibility and no significant difference in intake.

The present results agree with earlier findings about the effect of grinding and pelleting hay upon *in vivo* digestibility, feed intake and growth of lambs. According to a review by Minson (1963), pelleting increased *in vivo* digestibility in 5 studies but decreased it in 21 studies. The average decrease in *in vivo* digestibility due to pelleting was 3.3% units on average in all these studies. In trials HT-85 and HT-87, the decrease in *in vivo* digestibility due to pelleting was in the range 4 to 12% units, greater at the higher feeding levels. As found here, the studies summarised by Minson (1963) agree that the effect of pelleting are greater for low quality forages, and that the positive effects of pelleting upon growth rate are substantial at *ad lib* feeding. When feed intake is limited, the effects of pelleting are more variable, according to Minson (1963). The most obvious effect of pelleting are: increased rate of passage and that at *ad lib* feeding has more beneficial effects through greater intake than negative effect through lowered digestibility. Blaxter and Graham (1956) showed that lowered digestibility due to pelleting was counterbalanced by decreased energy loss as heat and methane. Decreased heat loss (heat increment of feeding) can be explained by lower energy use by the digestive tract per unit digested organic matter. Decreased production of methane due to pelleting can be explained by changes in rumen fermentation pattern, as faster digestion results in higher ratio of propionate and lower ratio of acetate, butyrate and methane (Sveinbjörnsson, 2006). As passage rate and intake is increased by pelleting, the *in vivo* digestibility of the cell wall is decreased, but the digestibility of the cell contents remains very high (Blaxter and Graham, 1956). The ratio of the rapidly degrading carbohydrates found in the cell contents, to the slower degrading carbohydrates in the cell wall, will therefore be higher.

Table 3 Trial HT-85: Carcass weight, average total daily feed intake, gross energy in all tissues and in vivo digestibility. Capital letters A to G are treatments as described in Table 1

	Carcass weight, kg	Intake, kg DM/d	Tissue energy, MJ	In vivo digestibility %
A	12.31		14.21	
B	11.15	0.725	10.36	63.54
C	13.11	0.732	14.06	59.29
D	13.34	0.935	14.87	65.91
E	15.28	1.023	17.55	57.22
F	13.75	0.963	14.46	63.33
G	17.01	1.506	21.21	51.70
Least significant difference[a]	1.89	0.054	3.59	3.03

[a]at $p<0.05$

It is well established that increased feed intake/passage rate and an optimal amount of easily digested carbohydrates has a positive effect upon microbial protein efficiency. At the same time, increased passage rate will increase the amount of protein escaping the rumen.

Therefore, it is evident that pelleting, through its positive effect upon passage rate, would be expected to increase the supply of metabolisable protein (AAT) to the ruminant (Volden & Larsen, 2005).

Table 4 Trial HT-87: Carcass weight, average total daily feed intake, gross energy in all tissues and in vivo digestibility. Capital letters A to G are treatments as described in Table 1

	Carcass weight, kg	Intake, kg DM/d	Tissue energy, MJ	In vivo digestibility %
A	13.65		16.65	
B I	14.88	0.657	17.46	71.88
B II	13.60	0.664	16.23	65.90
C I	14.98	0.674	17.90	67.90
C II	14.20	0.695	16.15	59.63
D I	17.00	0.928	22.36	71.13
D II	14.50	0.802	16.88	65.65
E I	17.15	0.968	22.53	66.23
E II	14.73	0.854	19.42	60.40
F I	19.40	1.435	28.12	62.08
FII	17.55	1.407	24.32	55.30
Least significant difference[a]	2.53	0.053	5,00	3.53

[a]at $p<0,05$

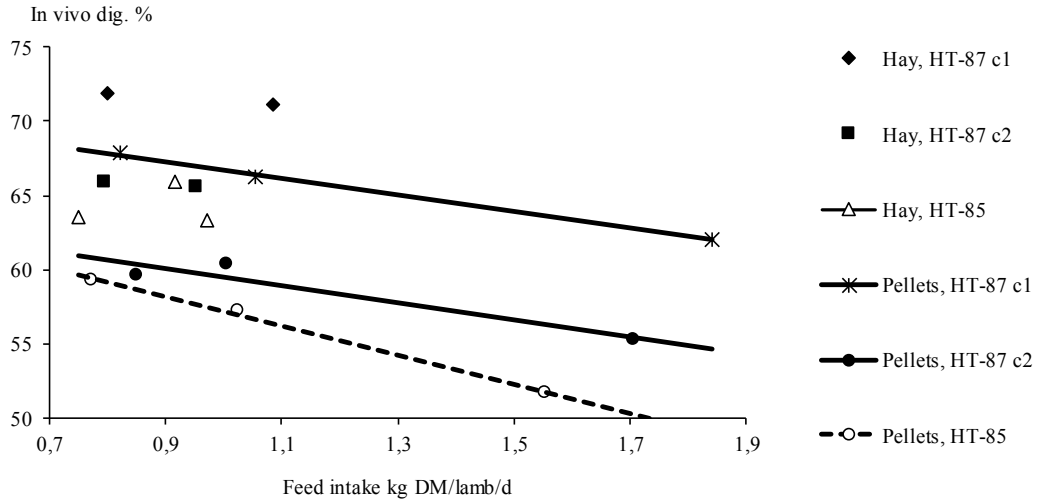

Figure 3 Trials HT 85 and 87: The effect of daily feed intake upon *in vivo* digestibility of hay and hay pellets with different *in vitro* digestibilities as presented in Table 2.

In the most recent study (VB 05, Table 5), the lambs were heavier at the start of the trial than in HT-85 and HT-87. Pelleting had similar positive effects upon feed intake and live weight

gain as found in the earlier studies. The increase in omental fat was highest when pellets were fed at lib (Group B).

Table 5 Trial VB-05: Dry matter intake (DMI), body weight on the day before slaughter, weight of carcass and mesenteric fat and the daily gain of total body, carcass and mesenteric fat. Capital letters V, A, B and C are treatments as described in Table 1

	Average	At slaughter			Average daily gain (g/d) of :		
Group	DMI, kg/d	Live weight	Carcass weight	Omental fat	BW	Carcass	Omental fat
V		37.3	15.0	1.20			
A	1.239	49.9	18.7	1.38	148.4	51.3	2.6
B	1.652	56.1	21.4	2.79	218.8	88.8	22.2
C	1.311	51.1	19.7	1.83	153.6	64.1	8.9

Conclusions

It can be concluded that pelleting is an efficient way of making hay of variable digestibility a more productive feed for growing lambs. Pelleting increases feed intake and decreases digestibility, more for the poorer quality hay. There are clear indications that digestible energy is more efficiently transferred into net energy when the hay is pelleted, and this counterbalances the lowered digestibility with respect to forage utilisation. As the climate in Iceland makes production of cereals for animal feeds unreliable, it is important to evaluate other choices for increasing the production value of the diets. Pelleting is clearly an interesting way of making the most out of the forages.

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Can in vivo methane production be predicted using the in vitro gas system?

M. Ramin[1], R. Danielsson[2], J. Bertilsson[2], P. Lund[3] & P. Huhtanen[1]
[1]Swedish University of Agricultural Sciences (SLU), Department of Agricultural Research for Northern Sweden, BOX 90183 Umeå, Sweden. [2]Department of Animal Nutrition and Management, BOX 7024, 750 07 Uppsala, Sweden. [3]Aarhus University, Department of Animal Science, 8830 Tjele, Denmark.
Correspondence: mohammad.ramin@slu.se

Introduction

Methane (CH_4) is a potent greenhouse gas with a global warming potential 28 times that of carbon dioxide (CO_2) according to IPCC (2013). Ruminants constitute the single most important source of anthropogenic CH_4 emission. Within the agricultural sector, livestock CH_4 emission contributes about 51% of total agricultural CH_4 emission in which the agriculture sector contributes about 60% of the total anthropogenic emission of CH_4 (Moss et al., 2000). Methane emission from ruminants depend on several factors such as dry matter intake (DMI), type of feed, feed quality and digestibility (Johnson & Johnson, 1995, Ramin & Huhtanen, 2013). There are some common in vivo measurement techniques which all have advantages and disadvantages with respect to e.g. accuracy and cost; the most consistent is respiration chamber techniques where concentrations of CH_4 and CO_2 in air flux (L/min) are measured (Johnson & Johnson, 1995, Yan et al., 2010). However, the chamber techniques are very costly and not suitable for measurement on many animals at the same time. In addition, in vivo studies are expensive and laborious. To reduce costs and impact on the animal, in vitro systems have been used. In vitro methods would be useful for screening the effects of diets and additives on CH_4 production. Two types of in vitro methods have been used: continuous culture experiments, as described by Czerkawski & Breckenridge (1977) and batch culture experiments, reported by Van Nevel & Demeyer (1981). Recently, Ramin & Huhtanen (2012) developed an in vitro method for prediction of cow ruminal CH_4 production using the kinetic parameters from an automated in vitro gas production (GP) system and a two-compartment rumen model. This approach takes rumen dynamics into account and may have advantages compared with single time point batch culture systems. However, in vitro techniques are most often used to explain what happens in vivo and it is therefore important that the technique is reliable and validated.

The aim of the current study is to evaluate the in vitro gas production system in predicting CH_4 emission from different diets when observed in vivo data is available.

Materials and Methods

Forty nine diets from 13 different in vivo studies with dairy, beef, growing cattle and sheep were selected and formulated. The majority of the diets were taken from a larger dataset in which CH_4 was measured by the respiration chamber technique (Ramin & Huhtanen, 2013). The diets were selected to include different dietary compositions, feeding levels, proportion of concentrate, carbohydrate composition of concentrates, protein and fat supplementation, forage type and maturity of forages.

The in vitro study was performed at the Swedish University of Agricultural Sciences in Umeå, Sweden. All handling of animals was approved by the Umeå Ethical Committee for Animal Research, Sweden. Three dairy cows of the Swedish Red breed at late lactation, fed a total mixed ration (grass silage/concentrate ratio 600/400 g/kg on DM basis) were used as

donor animals of rumen inoculum. 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 (CO_2). Rumen fluid was strained through four layers of cheesecloth and mixed with buffered mineral solution supplemented with peptone (pancreatic digested casein) at 39°C under constant stirring and continuous flushing with CO_2. Prior to the in vitro incubation, 1000 mg of substrate with ingredient proportion in accordance to the respective diet 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. Each diet was randomly distributed among minimum of three of totally five runs. Incubations were performed in five consecutive runs. Two bottles with no substrate were included as blanks in each run. Predicted in vivo CH_4 production was measured as described by Ramin & Huhtanen (2012).

The relationship between predicted and observed CH_4 emissions were assessed by using the linear regression technique (FIXED model). The performance of the in vitro technique to predict CH_4 emission in vivo was further evaluated by using the MIXED regression model procedure of SAS (Littell et al., 1996) with random study effect. The relationship between independent and dependent variables was estimated by using the following model:

$Y = B0 + B1X1ij + b0 + b1X1ij + eij,$

Where B0, B1X1ij, are the fixed effects (intercept and effects of independent variables), and b0 (intercept), b1 (slope), and eij are the random experiment effects (i = 1 . . 49 studies and j = .. ni values). Root mean square prediction error (RMSPE) was calculated as follows:

$RMSPE = \sqrt{[\Sigma (Observed - Predicted)^2/n]}$

To centre the predicted values, the overall predicted mean value was subtracted from each predicted value. This makes the slope and intercept estimates orthogonal and thereby independently assessable. Residual analysis was conducted as described by St- Pierre (2003) for CH_4 emissions, by a regression of the centred predicted values against the residuals (observed–predicted CH_4 emissions).

Results and Discussion

Diets used in the study were assumed to cover different dietary compositions. The means of DMI in the in vivo studies was 13.4 kg DM/day and the concentration of neutral detergent fibre (NDF) and crude protein (CP) were 396 and 182 g/kg DM, respectively (Table 1).

The in vitro system slightly underestimated CH_4 emission compared to the observed in vivo values 398 L/d compared with 418 L/d (Table 1). The relationship between predicted and observed CH_4 emissions is shown in Figure 1.

The R^2 was 0.94 and a RMSPE of 51.6 L/d (12.3 % of observed mean).

The R^2 for mixed model was 0.96 and RMSPE decreased to 40.1 L/d (9.5 % of observed mean). When predicted values were centred the mean value of slope was significant (P = 0.03) with the fixed model but not (P = 0.19) with the mixed model. Slope of the residual analysis was significant with both fixed and mixed models (P = 0.03 and P = 0.01, respectively).

Table 1 Statistical description of diet and methane production in observed and predicted data

Item	n	Mean	SD	Minimum	Maximum
Diet composition, g/ kg of DM					
Crude protein	45	182	48.2	115	405
Ether extract	41	40.1	19.34	19	109
Starch	41	121	85.2	3.7	324
NDF	49	396	80.4	254	585
Feed intake, kg DM/d	49	13.4	6.92	0.87	21.3
OMD, g/kg	49	767	53.1	591	910
Methane emissions, L/d					
Observed	49	418	207.6	35.8	664
Predicted	49	398	216.2	21	733

Ramin & Huhtanen (2012) assumed a gross energy (GE) concentration of 18.5 MJ/kg DM, for the diets used in their study. The predicted proportion of CH_4 energy as a proportion of gross energy decreased from 7.83 to 5.98% with increased sample size from 300 to 1200 mg in their predicted in vitro CH_4 productions (Ramin & Huhtanen, 2012). These values are close to observed in vivo values at maintenance and production levels of intake in dairy cows (Yan et al., 2000). The reasonable predictions of CH_4 production presented by Ramin & Huhtanen (2012) with the in vitro gas production system can be attributed to taking into account passage rate and digesta kinetic parameters in the rumen model.

The RMSPE prediction error was smaller by using mixed model 40.1 L/d (9.5 % of observed mean) compared to fixed model 51.6 L/d (12.3 % of observed mean). Further, the mean bias was not significant (P = 0.19) when the mixed model analysis was used but with fixed model the mean bias effect was significant (P = 0.01). This can probably be explained by the random effect of study that mixed model analysis takes into account, which fixed model does not. The effect could be due to different types of variations; calibration can vary and type of chamber may have an influence as well. The slope bias can be due to increased efficiency of microbial protein synthesis with increased DMI that was not taken into account in predicted values of CH_4 emission. Simulations with the Karoline model (Ramin & Huhtanen, 2015) indicated that increased efficiency of microbial protein synthesis markedly contributes to decreased CH_4 yield with increased DMI. When evaluating the predictions based on diets formulated from the original feeds used in the in vivo studies (21 diets) the RMPSE decreased to 9.3% for the fixed model.

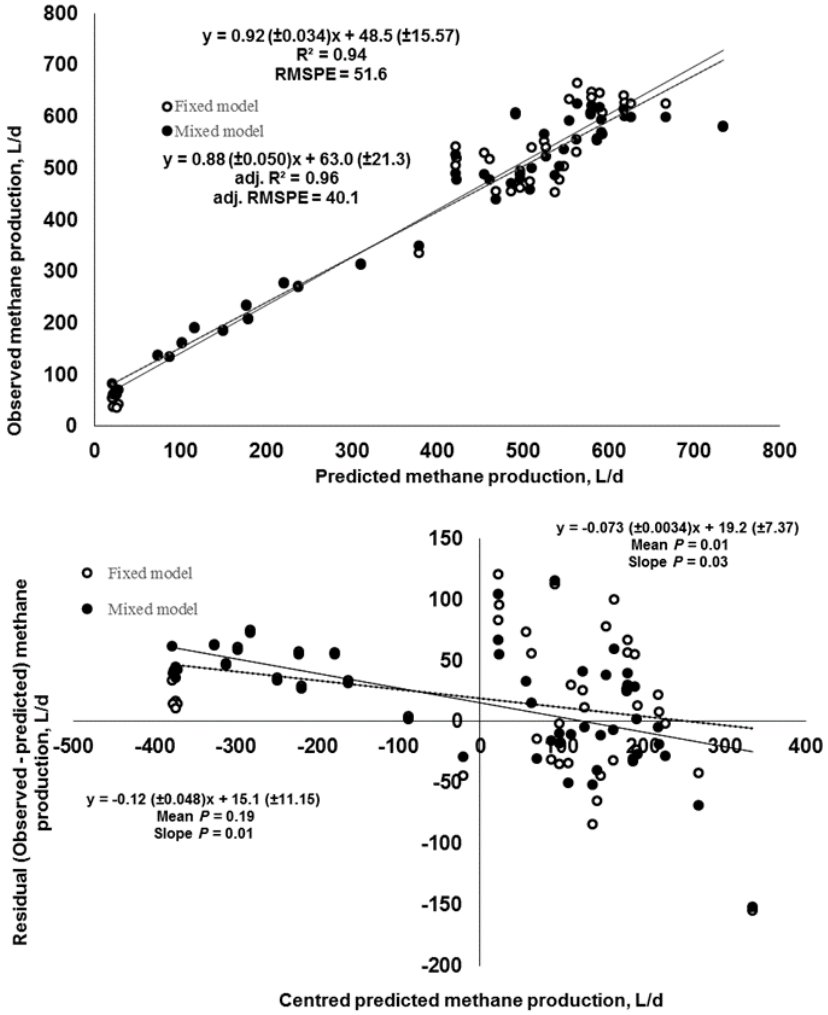

Figure 1 Relationship between predicted and observed methane emissions (L/d) (n = 49) with fixed and mixed model regression analysis (above), and (below) centred predicted values and residuals (observed - predicted) of methane emissions (L/d) (n = 49).

The decrease in CH_4 emission, that occur at higher feeding levels seems to be related to some factors; one of them may be related to that the increase of feeding level that leads to increased passage rate which reduces digestibility of feeds (Ramin & Huhtanen, 2013). Additionally, in a recent modelling exercise (Huhtanen et al., 2016) increased passage rate (shorter mean retention time) decreased predicted CH_4 emission with relation to decreased organic matter digestibility. Further evaluation is needed that relates the most important parameters e. g. DMI and digestibility to the residuals of CH_4 emission in order to detect the biases and weaknesses of the in vitro system in predicting in vivo CH_4 emission.

Conclusions

Across the diets used, the in vitro system seems to predict CH_4 emission with a reasonable accuracy and precision, and might be a useful tool for screening different mitigation strategies related to the diet.

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Effect of adjusting the dietary chewing index for intake of forage NDF on the performance of a net energy intake model

L. M. Jensen & P. Nørgaard
Department of Large Animal Science, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegaardsvej 3, 1, 1870 Frederiksberg, Denmark.
Correspondence: pen@sund.ku.dk

Introduction

The performance of high-yielding dairy cows is closely related with the intake of energy, which depends on both animal and dietary characteristics (Friggens et al., 1995). The intake of net energy (NEI, MJ NE/d) in lactating dairy cows has been found to decrease linearly at increasing corrected dietary chewing index value (CI_{cor}, min/MJ NE): NEI = NE_0 -(NE_0^2 / (4 x CT_{max})) x (625 / BW) x CI_{cor}, where NE_0 (MJ NE/d) represents the metabolic intake capacity and the maximal daily chewing time (CT_{max}) was been given a value of 819 min/d (Jensen, 2015). The NE_0 value was parameterized from a linear function of $BW^{0.75}$ ($kg^{0.75}$), days in milk (DIM), DIM^2 and yield of energy corrected milk (ECM, kg/d). The CI_{cor} value has been corrected for intake of forage NDF (NDF_f) relative to body weight (BW). However, a validation of the NEI intake model by Jensen (2015) showed that the residual NEI (NEI_{resid} = observed - predicted) were negatively related with the intake of NDF_f/BW and the CI_{cor} values. The aim of this investigation was to study effect of different adjustments of the CI_{cor} value for NDF_f intake on the performance of the linear NEI model, and to analyze the relationship between residual NEI and selected animal and dietary characteristics.

Materials and Methods

The study was conducted on two independent data sets, where the NEI model was parameterized with different CI_{cor} values using intake data from 14 experiments including 986 primi- and multiparous lactating dairy cows of different breeds fed 136 different rations *ad libitum* (Exp. I); see details given by Jensen (2015). Evaluation of the NEI model predicting NEI based on different CI_{cor} values were analysed on 19 experiments including 812 primi- and multi-parous lactating dairy cows of different breeds fed 80 different rations *ad libitum* (Exp. II; Jensen, 2015). The daily intake of DM ranged between 13 to 24 kg and 15 to 29 kg in data I and II, respectively. The NDF_f content (g/kg DM) ranged between 106 to 445 and 137 to 409 in data I and II, respectively. The content of metabolizable protein (AAT, g/MJ NE) and starch (g/MJ NE) in Exp. II ranged between 12 to 18 and 5.1 to 55, respectively. The intake of NE was estimated by the NorFor digestive kinetic model. The NEI values were estimated by use of the NorFor digestive kinetic model including values from predicted digested nutrients. The dietary $CI_{cor}(k)$ values were estimated according to the NorFor chewing index system and corrected by k *x* 45 min per kg NDF_f for intake of NDF_f deviating from the NorFor chewing index standard of 0.007 kg NDF_f/BW:

$CI_{cor}(k)$(min/MJ NE) = (625/BW(kg)) x [CI_{DM} + (0.007 - NDF_f(kg) / BW(kg)) x (52 x k)] / (MJ NE/DM)

In Exp. I, the $CI_{cor}(k)$ values were estimated for k = 0.9 to 1.25 by steps of 0.05. Net energy intake were predicted by use of the NEI intake model: NEI = NE_0 -(NE_0^2 / (4 x 819)) x (625 / BW) x $CI_{cor}(k)$ and the NE_0 (MJ NE/d) values for the individual experiments were estimated as: -48 + 0.48 x $BW^{0.75}$ + 0.65 x DIM - 0.002 x DIM^2 + 4.48 x ECM using a k value of 05.

The prediction accuracy of the models were evaluated by mean square prediction error (MSPE): MSPE $= \sum_{i=1}^{n}(A_i - P_i)^2 / n$, where A_i is the observed NEI for group i, P_i is the predicted NEI for group i, and n is the number of pairs of A and P being compared (Bibby and Toutenberg, 1977). The MSPE quantifies the prediction across experiments and it can be considered the sum of error due to central tendency (ECT), error due to regression (ER), and error due to disturbance (ED), as described by Bibby and Toutenberg (1977). This decomposition of the MSPE can be recovered from a simple linear regression of the difference between A and P on the difference between P and its mean. To decompose the error due to disturbance into variation between and within experiments the simple linear regression may be extended by a random effect of experiment.

Relationships between residual NEI (observed - predicted, NEI_{resid}) and daily ECM yield (kg/day), BW (kg) of the animals, the supply of metabolizable protein expressed as AAT (g/MJ NE), the concentration of starch (g/MJ NE), and the CI_{cor} (min/MJ NE) of the diets, were analyzed using linear mixed modelling with random effect of experiment.

Results and Discussion

The ER decreased with increasing *k* value, whereas the ECT and ED increased with increasing *k* value. The minimum MPSE value of 122 was found at a *k* value of 1.2, while higher and lower *k* values lead to increasing MPSE values between 130 at a *k* value of 0.8 and 125 for *k* value of 1.4. The equation for predicting the NE_0 value was re-parameterized to: $-46 + 0.39 \times BW^{0.75} + 0.73 \times DIM - 0.002 \times DIM^2 + 0.39 \times ECM$ using a *k* value of 1.2.

Table 1 Results from parameterization the metabolic capacity (NE_0, MJ NE/d) of dairy cows based on a corrections factor on chewing index of 23 min/NDF_f/BW: $NE_0 = a + b \times BW^{0.75} + c \times DIM + d \times DIM^2 + e \times ECM$

Animal characteristics	Estimate	SE	95% confidence interval	
			Min	Max
Intercept (a; MJ NE)	-47.6	29.9	-104	12.6
Body size (b; $BW^{0.75}$; $kg^{0.75}$)	0.48^{T}	0.25	-0.34	0.96
Days in milk (c; DIM, d)	0.65^{***}	0.13	0.41	0.90
DIM^2 (d; d)	-0.002^{***}	3.9×10^{-4}	-2.5×10^{-3}	1.0×10^{-3}
Energy corrected milk (e; ECM; kg/d)	4.48^{***}	3.4	3.41	5.57

$^{***}P < 0.001$; $^{T}P < 0.1$

The re-parameterized NEI model in combination with CI_{cor}(k = 1.2) were used for predicting of NEI of dairy cows in exp. II. The linear relationships between the NEI_{resid} values and the BW, ECM (kg/d), AAT (g/MJ NE), dietary starch (g/MJ NE) and CI_{cor}(k = 1.2) values were analyzed. The intercept value for ECM was 11 MJ NE/d (P = 0.09) and the slope for increasing AAT value was 1.3 MJ NE/g AAT (P = 0.07). The NEI_{resid} values were neither significantly related to the BW, AAT, starch or CI_{cor}(k = 1.2) values of the diets. The residual plots of the two models ($NEI_{original}$ and NEI_{new}) in Figure 1 demonstrate only minor effect of the correction (k = 1.2) across experiments (black line) as also seen in Table 3. However, the grey line in Figure 1, demonstrating the residuals modelled with random effect of experiment, describing the performance within experiments, shows that the corrected model (NEI_{new}) describes intake within experiments better than the original NEI model.

Table 2 Evaluation criteria, regression estimates, and significance level of the two models predicting net energy intake (NEI) in dairy cows, evaluated across 19 experiments

		Evaluation criteria				Regression estimates[a]	
Correction	*N*	MSPE[b]	ECT[c]	ER[d]	ED[e]	Intercept (MJ NE/day)	Slope (MJ NE/day)
$NEI_{original}$	80	124	5.15	13.5	105	2.27 ^{T}	-0.19 **
NEI_{new}	80	122	6.30	8.59	107	2.51 *	-0.16 *

[a]The regression estimates are retrieved from simple linear modelling.

$^{**}P < 0.01$; $^{*}P < 0.05$; $^{T}P < 0.05$

The intercept and slope of the original model is 1.85 ($P = 0.44$) and -0.11 ($P = 0.06$), respectively, where the intercept and slope of the corrected model is 2.19 ($P = 0.37$) and -0.03 ($P = 0.59$), respectively. The use of CI_{cor} by the original model leads to increasing under prediction of NEI at increasing CI_{cor} values ($P = 0.03$), whereas residuals were not significantly related to the CI_{cor} value corrected by 52 x1.2 min/kg NDF_f by the new model. However, this greater correction of the CI_{cor} value for increasing intake of NDF_f abolished the effect of dietary starch content and resulted in only a tendency of under prediction of NEI at low AAT per MJ NE, in contrast to a significant under prediction of NEI at low AAT content in the original model.

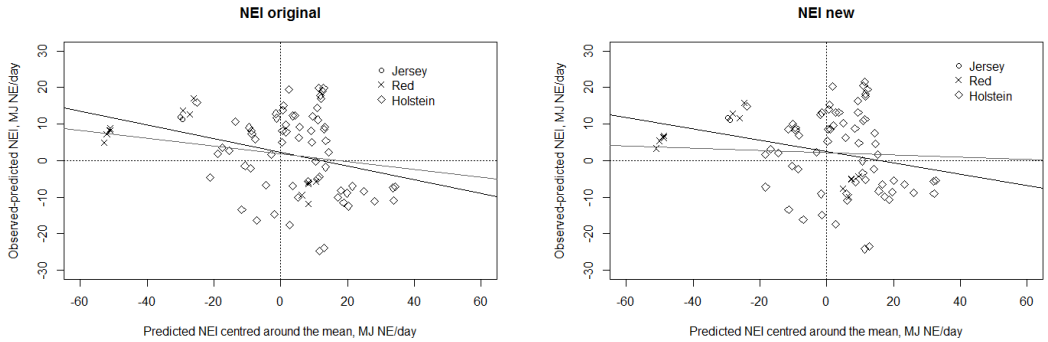

Figure 1 Centralized residual plots of the original and new NEI model evaluated on 80 treatment means from dairy cows of different breeds fed different diets. Error of regression (ER) is the deviation of the slope from zero, the smaller the ER, the better prediction of both low and high NEI. The black line corresponds to simple linear regression across experiments, whereas the grey line corresponds to mixed linear regression with random effect of experiment, evaluating performance within experiments.

Table 3 The relationships between body size (BW), daily energy corrected milk (ECM), content of metabolizable protein (AAT/MJ NE), and the dietary chewing index (min/MJ NE) and the residual NEI (observed-predicted) depending on the correction of chewing index (CI_{cor}) for increased intake of NDF_f/BW detected

Correction of CI_{cor}, min/kg NDF_f	Previous[a]				New[b]			
	Intercept	SE	Slope	SE	Intercept	SE	Slope	SE
BW (kg)	3.99	22.8	-3.2 x10^{-3}	0.04	5.26	23.6	-5.0 x 10^{-3}	0.04
ECM (kg/d)	9.99	6.42	-0.25	0.19	10.7^{T}	6.20	-0.27	0.18
Protein (g AAT/NE)	-23.7*	10.8	1.73*	0.71	-17.3	10.6	1.31^{T}	0.69
Starch (g/MJ NE)	5.26^{T}	2.95	-2.83^{T}	1.47	4.20	2.90	-1.74	1.44
Chewing index (min/NE)	-9.03	5.52	1.98^{*}	0.89	-0.37	5.89	0.47	0.96

[a]$CI_{cor}(k)$(min/MJ NE) = (625/BW(kg)) x [CI_{DM} + (0.007 - NDF_f(kg) / BW(kg)) x (52 x *k*)] / (MJ NE/DM), k=1

[b]As above[a], but with k=1.2

*$P < 0.05$; $^{T}P < 0.1$

Conclusions

A greater correction of the CI_{cor} value for intake of forage NDF appears to lead to a minor improvement of the performance of the NEI model in terms of a shift against more error due random disturbance and less error due to regression, as well as no under estimation of NEI at low AAT content and at high CI_{cor} values.

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Replacing cereals and soybean meal with sugar beet pulp and rapeseed meal or distiller's grain in grass silage diets to dairy cows

J. Karlsson, R. Spörndly, M. Patel & K. Holtenius
Department of Animal Nutrition and Management, Swedish University of Agricultural Science, Box 7024, SE-750 07 Uppsala, Sweden.
Correspondence: johanna.karlsson@slu.se

Introduction

Large volumes of human-edible products as cereal grains and soya beans are fed to dairy cows in intensive production systems (Eisler *et al*., 2014). Given the predicted increase in demand for livestock products and competition of land for food and feed, human inedible feeds are becoming increasingly important. Ruminants have a unique ability to produce high quality food, as milk and meat, from fibrous feed and other products not suitable for human consumption. Substituting human edibles with by-products as feed for ruminants substantially increase the net food production (human edible output minus human edible input) (Ertl *et al*., 2016).

The aim of this study was to investigate the effects on feed intake and milk production when human-edible feeds as cereal grain and soy beans was completely substituted with different blends of by-products as sugar beet pulp, rape seed meal and distillers grains in a high quality forage diet.

Materials and Methods

The feeding trial was carried out at the Swedish Livestock Research Centre, Uppsala (59°50'N; 17°48'E) and was approved by the Uppsala Ethical Committee for Animal Research (Uppsala, Sweden).

Twelve multiparous and twelve primiparous dairy cows in mid-lactation (70-125 days in milk at start), of the breeds Holstein (n=8) and Swedish Red (n=16), were housed in a loose housing system and milked in a single station automatic milking system (VMS^{TM}, DeLaval International AB, Tumba, Sweden).

The cows were used in a change-over experiment with four different concentrates as treatments and in three week periods. The first two weeks in each period were used for adaptation of the feeds and the last week was used for data collection and sampling. Milk was sampled during 24 hours in the middle of the third week in each experimental period.

The cows were randomly assigned to one of the four groups based on breed, parity and milk yield. All cows received grass silage *ad libitum*. The silage was a blend of 2/3 first cut and 1/3 second cut of a perennial grass lay of mainly timothy, perennial rye grass and tall fescue hybrid ensiled in round bales. The silage blend had a dry matter (DM) content of 43.7% and contained 132 g/kg DM of crude protein (CP) and 460 g/kg DM of neutral detergent fibre (NDF). The silage was feed individually and feed intake recorded (CRFI, BioControl Norway As, Rakkestad, Norway).

Concentrates were fed individually in concentrate dispensers (FSC400, DeLaval International AB, Tumba, Sweden) restricted to10 kg DM/day. The four different concentrates were based on: (1) cereal grains and soybean meal (CG-SBM), (2) sugar beet pulp (SBP) and rapeseed meal (RSM), (3) SBP and distiller's grain (DG), (4) SBP, RSM and DG, Table 1. All

concentrates had similar predicted content of crude protein (167 g/kg DM) and energy (11.6 MJ ME).

Table 1 Ingredient of the four different concentrates

Ingredient, % DM	CG-SBM (Control)	SBP-RSM	SBP-DG	SBP-RS-DG
Wheat	23.0	-	-	-
Barley	23.0	-	-	-
Oats	23.0	-	-	-
Soybean meal	20.2	-	-	-
Sugar beet pulp	-	53.0	50.6	50.1
Rape seed meal ExPro ®	-	30.2	-	16.8
Distiller's grain	-	-	36.0	15.0
Wheat bran	-	7.24	3.68	8.00
Limestone, ground	3.03	-	0.30	-
Ako Feed Cattle AK LN45[1]	2.10	4.22	3.68	3.98
Molasses	2.00	2.00	2.00	2.00
NaCl	1.00	-	-	-
Palm kernel expeller	0.97	3.00	3.00	4.00
Green meal pellet	0.81	-	-	-
Lipitec Bovi LM BB[2]	-	-	0.22	-
MCP Yara[3]	0.38	-	0.17	-
Magnesium oxide	0.36	0.09	0.21	-
Premix[4]	0.20	0.20	0.20	0.20

[1] Fatty acids (99% fat; 45% C16:0, 37% C18:1)
[2] Fatty acids (99% fat; 40-55% C16:0, 40-55% C18:0, max 8% C18:1)
[3] Contains 23% P, 16% Ca and 1% Mg.
[4] Containing minerals, vitamins and trace elements.

Table 2 Chemical composition of concentrates

	CG-SBM	SBP-RSM	SBP-DG	SBP-RSM-DG
DM, % of fresh matter	88.2	87.7	87.2	87.7
CP, g/kg DM	177	178	183	179
Crude fat, g/kg DM	51.2	67.8	75.5	67.4
NDF, g/kg DM	137	323	305	322
Ash, g/kg DM	71.7	54.8	58.4	53.6
Starch, g/kg DM	394	36.0	38.3	32.5
ME, MJ/kg of DM[1]	~~11.7~~ 13.3	~~11.6~~ 13.2	~~11.6~~ 13.3	~~11.6~~ 13.2

[1] Predicted, not analysed value.

Table 3 Proportion of feed components in total diet

	Diets			
	CG-SBM	SBP-RSM	SBP-DG	SBP-RSM-DG
CP, % of DM	15.0	15.0	15.2	15.0
Crude fat, % of DM	3.43	4.10	4.41	4.04
NDF, % of DM	33.1	40.5	39.8	40.6
Ash, % of DM	8.27	7.59	7.73	7.57
Starch, % of DM	15.7	1.45	1.54	1.27
Not analysed, % of DM	24.5	31.3	31.3	31.5

The 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 variable.

Results and Discussion

Total dry matter intake (DMI) and silage DMI was not affected by substituting a concentrate made of cereal grains and soybean meal with concentrates entirely based on these by-products. This is in accordance with previous studies comparing CG to SBP and wheat bran (Ertl *et al*., 2016; Dann *et al.*, 2014), RSM to DG (Mutsvangwa *et al*., 2016), SBM to DG (Anderson *et al*., 2006) or SBM to RSM and DG (Maxin *et al*., 2013)**.**

Table 4 Mean treatment effects on daily intake and milk production between the four different concentrates

	Diets					P-value
	CG-SBM[1]	SBP-RSM	SBP-DG	SBP-RSM-DG	SEM	Treatment
Intake (kg/d)						
Silage, DMI	14.3	14.3	14.0	14.9	0.61	0.59
Total DMI	23.8	23.9	23.4	24.5	0.74	0.34
Yield (kg/d)						
Milk	32.0^{ab}	32.5^{a}	30.5^{b}	32.0^{ab}	1.02	0.01
ECM[2]	33.8	35.0	33.0	34.2	0.99	0.08
Composition (%)						
Fat	4.37^{a}	4.57^{ab}	4.68^{b}	4.53^{ab}	0.12	0.04
Protein	3.46	3.47	3.39	3.46	0.05	0.32
Lactose	4.84	4.75	4.81	4.75	0.04	0.10

[1] Control diet.
[2] ECM calculated according to Sjaunja *et al.* (1990).

As expected, the starch content of the by-product based concentrates was much lower. Cows fed a by-product diet consumed less than 400 g starch per day, and the NDF content was much higher compared to the control with cereal grains (Table 2 and Table 3). The by-product based concentrates had a sugar beet pulp content of 50-53% of DM (Table 1), which provides ruminants with rumen-fermentable carbohydrates other than starch, mainly from the NDF fraction of the feed (Chase, 2007). When fed the by-product diets, the cows consumed around 40% NDF of total DM and produced equally well as the ones fed the control diet (33% NDF of total DM, Table 3). Around 31% of the DM in the by-product based concentrates were not

accounted for by the chemical analyses, while this fraction was about 25% of the DM in the control concentrate (CG-SBM). Most likely a large part of the unknown compounds in the by-product based concentrates were pectin and other carbohydrates sources that are not included in the NDF fraction of the feed.

No significant differences were observed in yields of energy corrected milk (ECM) or for the content of protein and lactose in the milk just as in previous studies (Ertl *et al*., 2016; Dann *et al*., 2014; Maxin *et al*., 2013). Anderson *et al*. (2006) got similar results for protein and lactose content when feeding DG as primary protein source compared to a diet with SBM. In another study, comparing RSM to DG as protein sources in feed similar results were obtained for ECM and protein as this study, but significantly higher lactose content in the milk was reported (Mutsvangwa *et al*., 2016). The overall level of ECM yield in this study (33-35 kg/d) was somewhat higher compared to the study by Maxin *et al.* (2013) who reported 30.0-30.9 kg ECM/d even though the forage proportion was only 38% in that study and 60% in the present study. Ertl *et al.* (2016) had a production levels of only 22.5-22.7 kg ECM/d and fed their cows a 75% forage diet on a DM basis.

In the control diet, soybean meal was the main protein source in the concentrate, while in the by-product based concentrates, it was the rape seed meal and/or distiller's grain that mainly contributed to the protein content. In this experiment, higher milk production was observed when feeding SBP-RSM compared to SBP-DG. Huhtanen *et al.* (2011) and Martineau *et al*. (2013) performed meta-analyses and concluded that milk production was usually greater in cows fed a diet containing RSM compared to other protein sources as SBM. However, in the present study no difference between SBP-RSM or SBP-RSM-DG and the control (CG-SBM) was observed.

Milk production was higher among cows fed the diet with SBP and RSM (32.5 kg/d) compared with the diet containing SBP and DG (30.5 kg/d). On the other hand, Anderson *et al.* (2006) reported higher milk and ECM yields on a diet with DG compared to SBM. In other studies, no effect of different by-products was observed on in milk yield (Ertl *et al*., 2016; Dann *et al*., 2014; Maxin *et al*., 2013).

The milk fat content was higher in cows consuming the SBP-DG diet than in cows that got the control diet with CG-SBM (table 4). The present result contrasts with those of many other studies in which no effect on milk fat content was observed when replacing CG or SBM with different by products (Ertl *et al*., 2016; Mutsvangwa *et al*., 2016; Dann *et al*., 2014; Maxin *et al*., 2013; Andersson *et al*., 2006). In the present study, the more fat that was added to the concentrate, the higher content of fat was found in the milk (table 2 and table 4). Adding C16:0 fat to the feed increases milk fat content (Lock *et al*., 2013), which could contribute to the relationship between fat content in concentrate and in milk. The higher fat content in milk might also be explained by higher NDF and lower starch proportions in the by-product feeds (table 2), which is accordance to studies comparing different forage to concentrate proportions (Aguerre *et al.,* 2011; Argov-Argaman *et al*., 2014; Patel *et al*., 2012; Sterk *et al*., 2011).

Feeding high quality grass silage and by-products to dairy cows may be a way to increase the total production of human edibles without lowering the efficiency of intensive milk production systems. Future studies will evaluate the economic and environmental aspects of a dairy production system based on forage and by-products.

Conclusions

In conclusion, replacing concentrate based on cereal and soybean meal with concentrates based on human inedible agricultural by-products in a diet to dairy cows in mid-lactation did not impair feed intake or milk production when combined with a high quality grass silage.

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Feed intake and live weight gain of Hereford bulls offered diets based on whole crop barley, whole crop wheat and grass silages with or without protein supplementation

M. Pesonen, A. Huuskonen & E. Joki-Tokola
Natural Resources Institute Finland (Luke), Green Technology, Tutkimusasemantie 15, FI-92400 Ruukki, Finland.
Correspondence: Maiju.Pesonen@luke.fi

Introduction

Different whole crop cereal silages are increasingly being used for growing cattle feeding in temperate climates due to their potentially lower costs compared to grass silages (Huuskonen, 2013). In Finland, barley (*Hordeum vulgare*) is the dominant small-grain species utilized for whole crop production, but oats (*Avena sativa*) and wheat (*Triticum aestivum*) are also used. Digestibility of whole crop silages is highly dependent on the proportion of straw and is often lower than that of good quality grass silage but the lower digestibility is largely compensated for by higher dry matter (DM) intake (DMI) (Huhtanen et al., 2007). In a review of seven experiments with finishing beef cattle Keady et al. (2013) concluded that the inclusion of whole crop wheat silage in grass silage-based diets increased forage intake by 1.4 kg DM/d, but did not alter animal performance.

Rapeseed meal (RSM) is the most important supplementary protein feed for cattle in Finland. Huuskonen et al. (2014) concluded that because of limited production responses, high prices of protein supplements and increases in the nitrogen and phosphorus emissions, there is generally no benefit from using protein supplementation for growing cattle fed grass silage based diets. However, whole crop silages typically contain less crude protein (CP) than grass silage, and therefore Finnish protein feeding recommendations for growing cattle are not usually fulfilled if whole crop silage based rations are fed without protein supplementations. The present experiment was conducted to study feed intake, live weight (LW) gain (LWG) and diet digestibility of growing Hereford bulls offered diets based on whole crop barley (WCB) and whole crop wheat (WCW) silages relative to moderate digestible grass silage (GS) based diet with or without RSM supplementation.

Materials and Methods

A feeding experiment was conducted in the experimental barn of Natural Resources Institute Finland (Luke) in Ruukki. The experiment comprised in total 30 Hereford bulls. The bulls were placed in an insulated barn in adjacent tie-stalls. A 3×2 factorial design was used to study the effects of (1) forage type, (2) inclusion of RSM and (3) their interactions. At the beginning of the experiment the bulls with average LW of 438 kg were divided into five blocks of six animals by LW. Within the block, the bulls were randomly allotted to one of the six treatments:

1) Grass silage plus rolled barley without RSM supplementation (GS RSM-)
2) Grass silage plus rolled barley with RSM supplementation (GS RSM+)
3) Whole crop barley silage plus rolled barley without RSM supplementation (WCB RSM-)
4) Whole crop barley silage plus rolled barley with RSM supplementation (WCB RSM+)
5) Whole crop wheat silage plus rolled barley without RSM supplementation (WCW RSM-)
6) Whole crop wheat silage plus rolled barley with RSM supplementation (WCW RSM+)

Experimental silages were produced at the experimental farm of Natural Resources Institute Finland in Ruukki (64°44'N, 25°15'E). The grass silage used was the regrowth from a timothy

(*Phleum pratense*) and meadow fescue (*Festuca pratensis*) sward, cut at heading stage of timothy using a mower conditioner, wilted for 24 h, and then harvested using a precision-chop forage harvester. The whole crop silages were harvested at early dough stage (growth stage Z83 on Zadoks scale) (Zadoks et al., 1974) of the cereal using a direct-cut flail harvester. All silages were ensiled in bunker silos and treated with a formic acid-based additive (AIV-2 Plus; Kemira Ltd., Oulu, Finland: 760 g formic acid/kg, 55 g ammonium formate/kg) applied at a rate of 5 litres/tonne of fresh forage.

During the feeding experiment, silage and concentrate were fed separately. The bulls were offered silages *ad libitum* (proportionate refusals as 5%), and in all three forage diets the concentrate used was either rolled barley alone or rolled barley plus RSM. The amount of the concentrate supplementation was 37 g/kg of metabolic LW/animal/day for all treatments, and the target for average concentrate level during the experiment was 400 g/kg DM. The CP content of rolled barley was 126 g/kg DM and RSM was given so that the CP content of the concentrate was raised to 155 g/kg DM. Thereby, the CP content of the concentrate increased 23% with RSM supplementation. The daily ration for the bulls included also 150 g of a mineral-vitamin mixture.

During the feeding experiment, silage sub-samples were taken twice a week, pooled over periods of four weeks and stored at –20 ºC prior to analyses. The samples were analysed for DM, ash, CP, neutral detergent fibre (NDF), starch, silage fermentation quality (pH, lactic and formic acids, volatile fatty acids, soluble and ammonia N content of total N) and digestible organic matter (DOM) in DM (D-value) as described by Pesonen et al. (2013). Concentrate sub-samples were collected weekly, pooled over periods of 8 weeks and analysed for DM, ash, CP, NDF and starch. The metabolizable energy (ME) concentration of the grass silage was calculated from the concentration of DOM using equation ME (MJ/kg DM) = 16.0 (MJ/kg DM) × DOM (kg/kg DM) (MAFF, 1984). For whole crop silages a coefficient of 15.5 instead of 16.0 was used (MAFF, 1984). The ME concentrations of the concentrate feeds were calculated based on tabulated concentrations of digestible crude fibre, CP, crude fat and nitrogen-free extract described by Luke (2016). Amino acids absorbed from small intestine (AAT) and protein balance in the rumen (PBV) values were calculated according to the Finnish Feed Tables (Luke, 2016).

The feeding experiment lasted 168 days, and the bulls were weighed on two consecutive days at the beginning and at the end of the experiment. The LWG was calculated as the difference between the means of the initial and final live weights divided by the number of growing days. Apparent diet digestibility was determined for all animals when the bulls were 525 kg LW, on average. Feed and faecal samples were collected twice a day (at 0700 and 1500 hours) during the 5-day collection period and stored frozen prior to analyses. The samples were analyzed for DM, ash and CP as described above. The diet digestibility was determined using acid-insoluble ash (AIA) as an internal marker.

The data were subjected to analysis of variance using the SAS GLM procedure (version 9.4, SAS Institute Inc., Cary, NC). The statistical model used was $y_{ijkl} = \mu + \gamma_k + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + e_{ijkl}$, where μ is the intercept and e_{ijkl} is the random error term associated with l^{th} bull. α_i and β_j are the fixed effects of i^{th} forage type (GS, WCB, WCW) and j^{th} protein supplementation (RSM-, RSM+), respectively, while γ_k is the random effect of the block (k=1,...,5). Differences between the treatments were tested using orthogonal contrasts: (1) RSM+ vs.

RSM-, (2) GS vs. whole crop silages, (3) WCB vs. WCW, (4) interaction between contrasts 1 and 2, and (5) interaction between contrasts 1 and 3.

Results

Chemical composition and feeding values of the experimental feeds are presented in Table 1. The DM content of WCB and WCW silages was 37 and 29% higher compared to GS, respectively. The grass silage had a clearly higher CP concentration (173 g/kg DM) than WCB (84 g/kg DM) and WCW (64 g/kg DM) silages. Further, GS had a 3 and 13% higher ME content compared to WCB and WCW silages, respectively, and a clearly higher PBV value (Table 1). Barley grain and RSM used in the experiment had typical chemical compositions and feed values.

Table 1 Chemical composition and feeding values of the experimental feeds

	Grass silage	Barley silage	Wheat silage	Barley grain	Rapeseed meal
Number of samples	6	6	6	3	3
Dry matter (DM), g/kg feed	253	347	326	860	890
Organic matter (OM), g/kg DM	918	948	946	982	921
Crude protein, g/kg DM	173	84	64	126	386
Neutral detergent fibre, g/kg DM	591	494	563	199	284
Starch, g/kg DM	8	312	88	672	10
Metabolisable energy, MJ/kg DM	10.5	10.2	9.3	13.3	11.6
AAT, g/kg DM	82	76	68	98	170
PBV, g/kg DM	51	-30	-38	-20	159
Digestible OM in DM, g/kg DM	655	655	602		
Fermentation quality of the experimental silages					
pH	3.97	4.26	4.07		
Volatile fatty acids, g/kg DM	21	7	15		
Lactic + formic acid, g/kg DM	51	23	25		
In total N, g/kg					
NH_4N	62	70	82		
Soluble N	442	518	594		

AAT = Amino acids absorbed from small intestine, PBV = Protein balance in the rumen

In the GS based diets, 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) being 22 and 29 g/kg DM for GS RSM- and GS RSM+ diets, respectively. In the whole crop silage based diets the PBV values were lower than recommended being -26, -18, -30 and -22 g/kg DM for WCB RSM-, WCB RSM+, WCW RSM- and WCW RSM+ diets, respectively.

There was an interaction between forage type and RSM supplementation for silage and total DMI (Table 2). Rapeseed meal supplementation increased both silage and total DMI in the whole crop based feedings but not in the GS feeding. Silage intake increased 22 and 15% in the WCB and WCW feedings, respectively, as a result of the RSM supplementation. Whole crop silage based feedings decreased both silage and total DMI compared to the GS ($P<0.05$) but this effect was evident only in the RSM- treatments (Table 2). There was no difference in silage or total DMI between WCB and WCW treatments.

Replacing GS over whole crop silages decreased ME, CP, AAT and PBV intakes of the bulls. Further, CP, AAT and PBV intakes were higher and ME intake tended to be higher in the WCB based feeding compared to the WCW based feeding. The inclusion of RSM increased CP, AAT and PBV intake and tended to increase ME intake of the bulls. The interactions between the forage type and RSM supplementation were not statistically significant for nutrient intake. Replacing GS over whole crop silages decreased DM, OM and CP digestibilities (Table 2). In addition, the digestibility coefficients were higher in the WCB based feeding compared to the WCW based feeding. The CP digestibility was higher in the RSM+ diets compared to the RSM- diets, but RSM supplementation had no effect on the DM and OM digestibilities (OMD).

Average daily LWG for the GS, WCB and WCW feedings were 1411, 1331 and 1181 g/d, respectively, and replacing GS over whole crop silages decreased LWG of the bulls ($P<0.05$). The LWG tended to be higher ($P=0.06$) in the WCB based feeding compared to the WCW based feeding. Rapeseed meal supplementation tended to increase ($P=0.10$) daily live weight gain 7 and 17% in WCB and WCW feeding, respectively, but only 2% in GS feeding. The inclusion of RSM also improved DM and energy conversion rates ($P<0.05$) (Table 2). Instead, replacing GS over whole crop silages impaired DM and energy conversion ($P<0.05$).

Discussion

In accordance with Huuskonen (2013) apparent digestibility of the whole crop based diets was lower compared to the GS diet which probably partly explain the differences in LWG among the forage types. The higher energy and protein content and higher DMI of the GS diet compared with the whole crop based diets was reflected also as larger daily ME and protein intake of the bulls. The difference in ME intake is probably a crucial explanation for the improved growth rate of the GS bulls compared to the bulls fed with the whole crop diets. For example, a recent meta-analysis by Huuskonen & Huhtanen (2015) demonstrated that energy intake is clearly the most important variable affecting LWG of growing cattle. The observed lower OMD and ME intake were probably the main reasons for the lower LWG of the WCW bulls compared to the WCB bulls.

As expected, RSM supplementation increased feed and nutrient intake and improved gain in the whole crop based feedings in which the PBV values were clearly lower than recommended. Also a meta-analysis of the data from feeding trials in growing cattle (Huuskonen et al., 2013) indicated that increasing the concentration of CP in the concentrate by replacing energy supplements with protein supplements had positive effect on DMI. However, intake response to protein supplementation reported by Huuskonen et al. (2013) was minimal with maximum predicted response less than 2% that is much smaller than the corresponding response in lactating cows (Huhtanen et al., 2008) or the responses in the whole crop feedings in the present experiment. Intake responses to protein supplementation in ruminants have been discussed to be associated with improved OMD related to: (1) overcoming deficiency of rumen degradable N, (2) higher intrinsic rate and potential extent of fibre digestion of protein supplements, (3) better rumen conditions for fibre digestion due to reduced dietary starch content and (4) stimulation of cellulolytic bacteria by amino acids (AA) and peptides derived from supplementary protein. However, RSM supplementation did not affect OMD in the present experiment which does not support any of these early mentioned mechanisms. Increasing intake responses to supplementary protein observed in the present experiment with whole crop silage based diets support the concept that other factors,

such as improved AA to ME balance at the tissue level, can also be involved. Huuskonen et al. (2013) suggested that a possible explanation could be an increased and/or more balanced supply of AA with protein supplementation which improves the performance and as a result of increased energy demand DMI is increased. Such a "pull effect" could explain intake responses in the present experiment as supplementary protein increased LWG in the WCB and WCW based diets.

Conclusions

Replacing grass silage over whole crop silages decreased LWG of growing bulls due to lower energy intake and poorer feed conversion rate. However, the fairly high growth rates measured in the present study indicate that grass silage could be totally replaced over whole crop silages in the diet of growing bulls. If production costs of whole crop cereals are lower than those of grass silage and including them in crop rotation brings benefits, using them may increase the overall profitability of the farm. Rapeseed meal supplementation increased intake and improved feed conversion and growth performance in whole crop based diets when the diet PBV values were lower than -20 g/kg DM.

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Table 2 Effects of forage type and rapeseed meal (RSM) supplementation on daily feed intake, apparent diet digestibility, growth performance and feed conversion of growing bulls

Forage type	GS		WCB		WCW		SEM	Polynomial contrasts				
RSM supplementation	-	+	-	+	-	+		1	2	3	4	5
N	5	5	5	5	5	5	-	-	-	-	-	-
Duration of the experiment, d	168	168	168	168	168	168	-	-	-	-	-	-
Dry matter (DM) intake, kg DM/d												
Silage	5.88	5.64	4.86	5.91	4.79	5.49	0.267	0.02	0.04	0.37	0.02	0.52
Barley grain	4.00	3.76	3.89	3.49	3.80	3.50	0.193	0.06	0.21	0.84	0.74	0.81
Rapeseed meal	0.00	0.44	0.00	0.44	0.00	0.45	0.001	<0.001	0.62	0.23	0.62	0.23
Total	9.88	9.84	8.75	9.84	8.59	9.44	0.338	0.03	0.02	0.41	0.09	0.71
Metabolizable energy intake, MJ/d	114.9	114.4	101.1	111.6	95.3	103.0	3.79	0.07	0.001	0.07	0.15	0.71
Crude protein intake, g/d	1513	1614	904	1115	784	961	35.1	<0.001	<0.001	<0.001	0.13	0.63
AAT intake, g/d	874	907	751	867	699	792	28.1	0.002	<0.001	0.03	0.14	0.69
PBV intake, g/d	220	283	-224	-177	-258	-208	11.0	<0.001	<0.001	0.006	0.43	0.88
Initial live weight, kg	436	456	434	429	432	434	28.4	0.80	0.57	0.96	0.66	0.89
Final live weight, kg	670	696	650	660	615	648	26.2	0.28	0.08	0.37	0.93	0.65
Live weight gain, g/d	1394	1428	1286	1376	1087	1274	75.7	0.10	0.02	0.06	0.42	0.53
Feed conversion												
Kg DM/Kg live weight gain	6.96	6.47	8.18	6.77	8.52	7.25	0.514	0.02	0.03	0.44	0.93	0.89
MJ/Kg live weight gain	78	74	93	77	96	83	6.1	0.04	0.04	0.44	0.94	0.82
Apparent digestibility coefficients												
Dry matter	0.754	0.741	0.699	0.668	0.610	0.625	0.013	0.37	<0.001	<0.001	0.84	0.10
Organic matter	0.771	0.756	0.716	0.687	0.628	0.646	0.014	0.44	<0.001	<0.001	0.69	0.10
Crude protein	0.719	0.754	0.632	0.673	0.546	0.664	0.016	<0.001	<0.001	0.007	0.09	0.02

Forages: GS = grass silage, WCB = whole crop barley silage, WCW = whole crop wheat silage, SEM = standard error of mean, polynomial contrasts: 1= RSM+ vs. RSM-, 2 = GS vs. whole crop silages, 3 = WCB vs. WCW, 4 = interaction between contrasts 1 and 2, 5 = interaction between contrasts 1 and 3, AAT = amino acids absorbed from small intestine, PBV = protein balance in the rumen

Mixed ration or separate feeding in automatic milking systems – does it matter in free cow traffic?

M. Patel & E. Spörndly
Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, P.O. Box 7024, 750 07 Uppsala.
Correspondence: Mikaela.Patel@slu.se

Introduction

The number of farms with automatic milking (AM) has steadily increased in Sweden and other Scandinavian countries over the last years and approximately one third of the milk produced in Sweden is produced from cows in AM systems (Landin & Gyllensvärd, 2012). A prerequisite for successful AM is a well-functioning cow traffic. The most important factor that drives cow traffic in an AM barn is the feed, both the concentrates that is supplied in the milking unit and the feed supplied at the feed table and in some cases also the feed offered in the concentrate stations (Rodenburg, 2011). The feed can be distributed as a mixed ration or else, forage and concentrates can be fed separately. Offering the feed as a mix (total mixed ration; TMR or partial mixed ration; PMR) has proved to be a rational way of achieving a high feed intake. The benefits of feeding a mix, compared to separate feeding, are: *a* more even intake of fibre and starch over a 24-hour period, it facilitates the transition between feed batches, it provides an increased feed intake and cheap by-products can be used in the mixture (Rodenburg & Wheeler, 2002; Spörndly, 2003). However, mixed feed with a high nutrient density, combined with AM is claimed to lead to low milking frequencies with low milk yields as a result, often termed "lazy cow syndrome". In total mixed rations with high quality grass/clover silage, it is not unusual to mix in straw to reduce the concentration of nutrients in the feed mix (Lundborg, 2014). This must be regarded as counterproductive when farmers are striving for early harvests of herbage to obtain a high nutritional value of the silage.

The relationship between milking frequency and milk yield is uncertain in dairy farms with AM. In several studies, no difference in milk yield has been shown between systems with free or controlled cow traffic even though the number of milkings per cow per day were less in the free system (Melin *et al.*, 2007; Forsberg, 2008; Bach *et al.*, 2009). Although the low milking frequencies associated with the provision of mixed feed in AM barns is regarded as a problem, controlled experiments comparing mixed feed with separate feeding of silage and concentrates are scarce in the literature (Rodenburg & Wheeler, 2002). These studies, also, do not cover AM systems or the feeds and cow traffic systems that are common in Scandinavia. Hence, the aim of this experiment was to investigate how mixed silage and concentrates affects feed intake and milk production compared with separate feeding, in an AM barn with free cow traffic.

Materials and Methods

The experiment was conducted at the experimental farm Lövsta at the Swedish University of Agricultural Sciences in Uppsala. Thirty-eight cows, 10 primiparous and 28 multiparous, of the Swedish Red Breed and Holstein with an average of 70 ± 30 days in lactation, were randomly allotted to either a mix of grass/clover silage with crushed concentrates (MIX) or a ration of silage and pelleted concentrates (SR) fed separately. The concentrates used in the MIX and SR treatments contained the same ingredient proportions and, thus, had the same nutrient content as well. The experiment consisted of an adaptation period of four weeks and a measurement period of six weeks in a free cow traffic system with AM (DeLaval, Tumba, Sweden). The experimental feeds and feeding rations were determined according to the regulations of organic production (KRAV, 2015), i.e., the proportions of concentrate were

limited to 50% (on DM basis) during the first 90 days of lactation and to 40% during the remaining part of the lactation. The feeds originated from the same silos/batches and the mix and silage were fed *ad libitum.* The chemical composition of the silage and concentrates is presented in Table 1. The mix contained 35% concentrates and 65% silage on dry matter (DM) basis and the concentrates ration in the SR treatment was continuously adjusted in relation to silage intake to ensure that the silage/concentrates ratio was the same for both treatments. All cows received pelleted concentrates in the milking unit and the allotted daily ration was calculated according to silage intake in order to ensure the predetermined silage/concentrates ratios. The cows had permission to visit the milking unit five hours after previous milking and they were fetched and brought to the milking unit if 13 hours had passed since the last milking. Daily feed intake, milk yield and milking frequencies were recorded automatically and the milk constituents analysed fortnightly.

The statistical model (PROC MIXED, SAS 9.3) included treatment, parity, breed and days in milk using a mixed model with repeated measurements and 'Cow' as random variable. All interactions were included in the model, but were excluded when not significant ($P > 0.05$).

Table 1 Chemical composition of the grass/clover silage and concentrate, means ± standard deviation

Item	Silage, n = 3	Concentrate, n=3
Dry matter (DM), %	32.1 ± 0.23	88.2 ± 0.8
Metabolizable energy, MJ/kg DM	11.4 ± 0.1	13.4[1]
NDF, g/kg DM	444 ± 26	169.5 ± 8.7[2]
Ash, g/kg DM	84.0 ± 3.5	58.1 ± 2.1
Crude protein g/kg DM	138 ± 5.6	192 ± 6.5
Starch, g/kg DM	NA	398.9 ± 17.1
OMD, %[3]	78.5 ± 0.4	NA
pH	4.07 ± 0.1	-
Am-N, % of total N	7.1 ± 0.2	-

[1]Determined from manufacturer
[2]analyzed as aNDFom
[3]OMD, organic matter digestibility
NA, not analyzed

Results and Discussion

The average daily feed intake was higher in the MIX group compared with the SR group, 26.8 kg and 24.0 kg DM, respectively ($P = 0.005$). This difference was shown both in intake of silage and of concentrates (Table 2). Feed intake was also higher in multiparous cows compared to primiparous cows, kg 28.7 and 22.1 kg, respectively ($P < 0.001$). There was no difference in total DM intake in cows < 90 DIM and cows > 90 DIM, although there were differences both in intake of silage and concentrates. This was an effect of the experimental plan since the concentrate ration was adjusted from 50% to 40% of total DM intake at this time point. However, when the concentrate ration was decreased, the cows compensated by increased intake of silage so the sum of the two feeds remained equal regardless of the proportion of concentrate.

The results revealed no differences in milk production or milk constituents, with 35.0 and 35.4 kg ECM/cow and day in the MIX and SR groups, respectively (Table 3). Milking frequency was higher in the MIX, compared with the SR group with 2.6 and 2.3 milkings per day, respectively. This was unexpected since nutrient dense mixed feeds are often claimed to lower milking frequency ("lazy cow syndrome"). The same pattern remained when fetched visits were excluded from the analyses, giving 2.0 and 2.4 voluntary milkings per day in the

SR and MIX group, respectively. The reason for the higher milking frequency in the MIX group may have been that the milking unit was the only place where cows received concentrates, whereas for the SR group, concentrates were also available in the concentrate stations. However, the higher milking frequency in the MIX group did not result in a higher milk yield, which may have been expected since earlier studies have shown increased yield with increased milking frequency (Svennersten-Sjaunja & Pettersson, 2008). The higher feed intake in the MIX group may have resulted in an increased deposition of adipose tissues. These data are still not completely analysed, but will add valuable information to the final conclusions of the experiment. The differences in milking frequency did not result in differences in somatic cell count (SCC) and overall, the SCC was low during the experiment.

Table 2 Feed intake (kg dry matter; DM) by cow and day during the measurement period of six weeks. Figures presented as Least square means and standard error of the means (SEM) by treatment, lactation and days in milk (DIM) in dairy cows fed either grass/clover silage and concentrate separate (SR) or mixed (MIX)

	Treatment				Lactation				DIM			
	SR	MIX	SEM	Sign.[1]	1	≥2	SEM	Sign.[1]	0-90	>91	SEM	Sign[1]
Silage	13.9	15.5	0.47	*	12.7	16.7	0.36	***	13.9	15.5	0.35	***
Concentrate, milkning unit	2.9	3.4	0.13	**	2.8	3.5	0.10	***	4.5	1.9	0.09	***
Total concentrate	10.1	11.3	0.31	**	9.4	12.0	0.24	***	11.5	9.9	0.23	***
Total DM intake	24.0	26.8	0.74	**	22.1	28.7	0.57	***	25.4	25.4	-	ND

[1]Sign., level of significance: NS = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$
ND, not determined

Table 3 Average daily milk production during the measurement period of six weeks in dairy cows fed either grass/clover silage and concentrate separate (SR) or mixed (MIX). Energy corrected milk yield (ECM) and figures for fat, protein, lactose and somatic cell count (SCC) are means of four sampling days (fortnightly sampling). Figures presented as Least square means and standard error of the means (SEM)

	Treatment			
Item	SR	MIX	SEM	Sign[1].
Milk, kg	35.7	34.6	1.37	NS
ECM, kg	35.4	35.0	1.24	NS
Fat, kg	1.39	1.39	0.06	NS
Protein, kg	1.20	1.17	0.04	NS
Lactose, kg	1.68	1.66	0.07	NS
Fat, %	4.05	4.12	0.10	NS
Protein, %	3.47	3.44	0.05	NS
Lactose, %	4.78	4.82	0.21	NS
SCC, $\times 10^3$/ml	31.28	50.43	0.09	NS

[1]Sign., level of significance: NS = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Conclusions

This study showed increased feed intake and increased milking frequencies when dairy cows were fed a nutrient dense mixed ration in a free cow traffic system compared with cows fed the same proportions silage and concentrates separately. Nevertheless, no difference in milk yield was shown between the treatments.

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Effect of grass silages from regrowth on dairy cow performance

D. Pang[1], P. Huhtanen[1] & S.J. Krizsan[1]
[1]*Swedish University of Agricultural Sciences (SLU), Department of Agricultural Research for Northern Sweden, S-901 83 Umeå, Sweden.*
Correspondence: degong.pang@slu.se

Introduction

Currently grass represents 40-60% of the total dry matter intake (DMI) in ruminant diets, especially grass preserved as silage (Jordbruksverket, 2015). The grass harvesting time for silage production is a major factor affecting the supply of digestible nutrients and subsequent milk production (Kuoppala *et al*., 2008). Earlier studies have been restricted in evaluating the effect of harvest time of primary growth silage on milk production and quality (Randby *et al*., 2012). The effect of harvest time of the subsequent regrowth (Kuoppala *et al*., 2008) and primary growth vs. regrowth grass silage (Khalili *et al*., 2005) has been documented. However, no study has evaluated dairy cow performance from diets based on grass silages from regrowth where harvesting strategies have been varied. The objective of the present study was, therefor, to evaluate the effect of five grass silages made from 2- and 3-cut regrowth herbage on performance of lactating dairy cows.

Materials and Methods

The study involved 30 lactating Swedish Red cows fed experimental diets during four 21-d periods in a 5 × 4 Latin square design. The experimental cows were divided in six blocks according to milk yield and parity. Experimental diets were formulated with different regrowth silage sources to meet energy and protein requirements of 35 kg ECM yield (Luke, 2014). Each diet included 580 g/kg of regrowth silage, 340 g/kg of crimped barley and 80 g/kg of rapeseed meal on dry matter (DM) basis. The diets were fed ad libitum as a total mixed ration (TMR). The five leys, dominated by timothy grass (*Phleum pratense sp*.), were harvested in 2015 from three different 2- and one 3-cut system. Three regrowth silages were prepared after an early (E) first cut (June 10th) on July 22nd (early second cut; EE), August 5th (late second cut; EL) and September 3rd (third cut; TC). The other two regrowth silages were prepared after a late (L) first cut (June 24th) on August 6th (early second cut; LE) and September 2nd (late second cut; LL). The silages were ensiled in bunker silos using a commercial acid-based additive (PromyrTM XR 630, Perstorp, Sweden). Daily feed intake was recorded by a roughage intake control system (Insentec B. V., Marknesse, the Netherlands) and DM values of all feed ingredients were updated every week. Fresh silage samples were collected on day 16, 19 and 21 during each experimental period and stored at -20℃ for later analysis of fermentation quality. 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 milkings from the afternoon of day 19 until the morning of day 21 in each period. All samples were analysed for fat, protein and urea. Faeces were collected from 15 cows during the morning and afternoon milking on Day 15-17 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. Crude protein (CP) was analysed by using a 2020 Digester and a 2400 Kjeltec Analyser Unit. The neutral detergent fibre (NDF) analysis was conducted in 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). Total tract digestibility of the diets was determined using iNDF as an internal marker. The 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) during the whole experiment.

Results and Discussion

The chemical composition and fermentation quality of regrowth silages are in Table 1. Postponing harvest of the primary growth (EE and EL vs. LE and LL) increased concentrations of DM and iNDF but decreased CP concentration in silages made from the second regrowth. With progressing regrowth (EE and LE vs. EL and LL), the concentrations of DM and iNDF increased with a reduction in CP concentration. Third cut silage had lower concentrations of DM and NDF, but higher CP concentration than all silages made from the second regrowth. The variation in chemical composition among diets reflected the differences in silage quality since this was the only difference among dietary ingredients used. All silages were well-preserved as evidenced by high lactic acid concentrations and relatively low levels of acetic acid, butyric acid and ammonia N. The LL silage was higher in ammonia and acetic acid, probably because it was slightly harder to compact and it took longer time for anaerobic conditions to establish. Third cut silage had higher concentrations of lactic acid compared to the second regrowth silages, which could be explained by a lower DM content.

Table 1 Silage chemical composition and fermentation quality (g/kg DM)

	Silages[a]				
	EE	EL	LE	LL	TC
Dry matter, g/kg	262	265	261	311	220
Composition					
Crude protein	178	125	137	111	197
Neutral detergent fibre (NDF)	523	557	556	524	459
Indigestible NDF	76	148	143	177	77
Fermentation quality					
pH	3.8	3.6	3.7	3.7	3.8
Ammonium N, g/kg total N	31.6	37.2	33.5	74.0	41.9
Lactic acid	79	102	110	83	117
Acetic acid	18.2	15.2	19.5	27.1	16.3
Butyric acid	<0.3	<0.3	<0.3	<0.3	<0.4

[a] EE and EL, early second cut harvested July 22nd and late second cut harvested August 5th, which were prepared after an early first cut (June 10th); LE and LL, early second cut harvested August 6th and late second cut harvested September 2nd, which prepared after a late first cut on June 24th; TC, third cut harvested September 3rd.

Postponing the primary growth harvest significantly decreased total DMI and nutrient intakes (P<0.01) of the regrowth silages (Table 2). With progressing regrowth, the intakes of DM and CP decreased (P≤0.01). Feeding third cut silage decreased NDF intake (P<0.01), but significantly increased CP intake (P<0.01) compared to feeding second cut regrowth silages. Likely, the reason for the lower intake of late second cut regrowth silages was physical constraints of the rumen that limited intake. Digestibility of grass silage will greatly affect the production performance of dairy cows. In the present study, postponing the harvest of the primary growth decreased apparent digestibility of dietary components (P≤0.01). With progressing regrowth, digestibility of chemical components decreased (P<0.01). The second cut silages were less digestible than the third cut silage (P<0.01). A higher milk yield is often caused by higher DMI, or improved utilization of the feed. Postponing the primary growth harvest also decreased yields of ECM, milk fat and protein (P<0.01). With progressing

regrowth, the effects were similar. Cows consuming third cut silage had higher yields of ECM, milk fat and protein (P<0.01) than those consuming second cut regrowth silages. The reduced intake potential of late regrowth silages explained the lower milk production compared to early second cut silages. However, it's still not clear why feeding third cut silage diets improved milk production without a significant change on DM intake compared to cows consuming second cut regrowth silage diets. It's possible that intake was limited by the lower DM concentration of the third cut silage and that milk production was improved by a better allocation of nutrients for lactation.

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

	Silage source[a]						*P*-value[b]		
	EE	EL	LE	LL	TC	SEM	C1	C2	C3
Intake, kg/d									
Dry matter	22.4	21.2	20.3	20.6	20.8	0.19	<0.01	0.01	0.20
Crude protein	3.9	3.0	3.1	2.8	3.8	0.03	<0.01	<0.01	<0.01
Neutral detergent fibre (NDF)	8.5	8.4	8.0	7.8	7.2	0.09	<0.01	0.09	<0.01
Digestibility, g/kg									
Organic matter	747	694	702	660	738	6.8	<0.01	<0.01	<0.01
Crude protein	716	662	674	606	699	11.6	0.01	<0.01	<0.01
NDF	674	553	573	481	645	9.5	<0.01	<0.01	<0.01
Milk yield and composition									
ECM, kg/d	31.3	28.6	28.7	27.6	31.2	0.35	<0.01	<0.01	<0.01
Fat, g/d	1284	1162	1172	1114	1247	21.6	<0.01	<0.01	<0.01
Protein, g/d	1068	966	947	937	1068	14.2	<0.01	<0.01	<0.01
MUN, mM	4.58	4.24	4.43	3.78	5.17	0.085	<0.01	<0.01	<0.01
Production efficiencies									
ECM/DMI	1.40	1.35	1.43	1.35	1.50	0.025	0.45	0.01	<0.01
N[c], g/kg	273	310	299	323	273	5.3	<0.01	<0.01	<0.01
CH_4, g/kg ECM	14.1	15.4	14.8	15.7	13.7	0.46	0.29	0.02	0.01
CH_4, g/kg DMI	20.2	20.7	21.1	21.5	21.3	0.33	<0.01	0.17	0.27

ECM, energy corrected milk; MUN, milk urea.

[a] EE and EL, early second cut harvested July 22nd and late second cut harvested August 5th, which were prepared after an early first cut (June 10th); LE and LL, early second cut harvested August 6th and late second cut harvested September 2nd, which prepared after a late first cut on June 24th; TC, third cut harvested September 3rd.

[b] C1 = probability of the effect of harvest time of the first cut (EE and EL vs. LE and LL); C2 = probability of the effect of growth stage in second cut regrowth (EE and LE vs. EL and LL); C3 = probability of the effect of second cut vs. third cut.

[c] Calculated as N in milk/N intake.

Nitrogen efficiency was improved for cows fed regrowth silage harvested after the late first cut (P<0.01) due to lower CP intake. With progressing regrowth, feed efficiency decreased (P=0.01), but N efficiency improved (P<0.01). The utilization of DM was more efficient in third cut silage diet but the N efficiency was poorer than second cut silage diets (P<0.01). Enteric CH_4 stands for a loss of energy and is influenced by dietary chemical composition (Brask *et al*., 2013). In the present study, the progressing regrowth of silages made from second cut material increased CH_4 emission per kg of ECM (P=0.02). When feeding diet based on third cut silage, CH_4 emission per unit ECM was lower than for cows fed diets based on silages made from second cut regrowth material (P=0.01). Postponing the primary

growth harvest increased CH_4 emission level per kg of DMI ($P<0.01$). Protein fermentation produces approximately 30-50% less CH_4 than carbohydrate fermentation (Bannink *et al*., 2006), which can partly explain the reduced CH_4 emissions level in cows consuming CP-rich early second cut silage diets. On the other hand, cows feeding on third cut silage diet consumed more CP from intake and produced more milk, which led to a lower CH_4 emission per kg ECM production compared to cows feeding on second cut silage diets.

Conclusions

Postponing the harvest time of primary growth decreased milk production, feed intake and feed efficiency of dairy cows, but improved N utilization. With progressing length of the regrowth period, production performance and digestibility decreased, and more CH_4 was released. Feeding third cut silage didn't improve DM consumption by the cows, but promoted higher milk production and feed efficiency than when silages made from second cut regrowth material were fed. The present study has generated information on production potential of regrowth silages from different harvesting strategies but the practical implication of the results should be based on the individual aim and limitation of each particular farming situation.

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Metabolizable energy in grass and red clover silage fed to sheep

S.J. Krizsan[1], F. Fatehi[1, 2], D. Pang[1], M. Rinne[3] & P. Huhtanen[1]
[1]*Swedish University of Agricultural Sciences, Department of Agricultural Research for Northern Sweden, S-901 83 Umeå, Sweden*
[2]*University of Tehran, Campus of Agricultural and Natural Resources, Department of Animal Science, Karaj, Tehran, Iran 31587-77871*
[3]*Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland*
Correspondence: Sophie.Krizsan@slu.se

Introduction

Grasslands are an important land use in Europe covering more than a third of the European agricultural area (FAO, 2012). In Nordic countries forage crops and temporary grasslands dominate due to a livestock production characterized by long indoor feeding periods. At low levels of concentrate supplementation, silage feeding value has had a major influence on milk production of dairy cows, while at higher levels of concentrate supplementation, its importance is reduced (Ferris *et al*., 2001; Randby *et al*., 2012). In light of a growing global demand for food, animal productivity per unit land area should be optimised by minimising the use of cereals in the diet of ruminants. The feasibility to compensate lower animal performance from a decreased cereal use through improvements in forage quality demands proper evaluation of forage quality. Milk production responses to early harvested grass silages have been lower than could be expected from predicted metabolizable energy (ME) values and intake potential (Rinne *et al*., 1999; Ferris *et al*., 2001; Randby *et al*., 2012). In addition to increased urinary N excretion, one possible reason could be elevated urinary energy output in terms of phenolic acids and their metabolites in ruminants fed early cut grass silage (Martin, 1969). We hypothesize that lignin in grasses of different stage of maturity will to a varying degree solubilize in the rumen, mainly be excreted in the urine, and thereby affect the energy feed value evaluation. The objectives of this study were to discuss ME value of grass and red clover silages fed to sheep at a maintenance level of feeding, and to analyze urine for N and phenolic compounds that could be derived from the solubilization of lignin.

Materials and methods

The samples used in this study were collected from digestibility studies with sheep fed at maintenance levels of feeding. This study comprises samples of a total of 25 primary and regrowth silages of timothy (*Phleum pratense)* and meadow fescue (*Festuca pratensis*) grass mixtures, and red clover (*Trifolium pratense*) from the Natural Resources Institute Finland (Luke) in Jokioinen (60°48´N, 23°29´E), Finland. The grass silages were harvested in two different years. Primary growth grass was harvested at different growth stages between June 4 and July 8 across years and regrowth harvests took place between 41 and 52 days later with an average of 44 days. One regrowth grass silage sample was a third cut harvested August 30, 40 days after previous harvest. Red clover silages were from two different years. Primary growth of red clover was harvested at different growth stages between June 7 and July 16 across years. Regrowth harvests were done between 36 and 56 days later with an average of 47 days. The forages were ensiled as direct cut and preserved with formic acid. The treatment mean data comprised six experiments conducted as balanced (3 × 3 or 4 × 4) or imbalanced (6 × 4) Latin Squares. In all *in vivo* digestibility trials, the total amount of urine and faeces were collected, and representative samples taken for analysis. Details of animals and silages and most experimental and analytical procedures are summarized and described by Huhtanen et al. (2006).

The N concentration of urine was determined in fresh samples using the Kjeldahl method (Official method AOAC-984.13; Association of Official Analytical Chemists, USA)

using Cu as a catalyst and a Foss Kjeltec 2400 Analyzer Unit (Foss Tecator AB, Höganäs, Sweden). The urinary dry matter (DM) was determined from freeze drying and the freeze-dried samples were used for gross energy (GE) determination in urine. Freeze-dried silage samples were used for the determination of GE concentration of the silages. Gross calorific values of freeze dried silage samples, the freeze dried urine and oven dried faecal samples were determined with a Parr 6200 Oxygen Bomb Calorimeter (Parr Instrument Co. Moline, IL 61265, USA) using benzoic acid (CAS 65-85-0, Cat No 3415, Parr Instrument Company) as a standard.

A liquid chromatography-tandem mass spectrometry application was used to determine the concentration of phenolic compounds in the urine samples (1290 Infinity system and Agilent 6490 Triple quadrupole mass spectrometers; Agilent Technologies, Santa Clara, CA, USA). The urine samples were prepared based on the procedure described by Olthof et al (2003). To release conjugated phenolic acids 40 µl β-glucuronidase (Merck, Darmstadt, Germany) was added to each tube. All detected compounds were identified and quantified with reference compounds purchased from Sigma-Aldrich (St. Louis, MO, USA).

The digestible energy (DE) value of the experimental silages was calculated based on the determined silage GE value and daily DM intake by the sheep and DE was calculated by subtracting faecal GE output. Urinary GE determinations were corrected for energy from the H_2SO_4, used to preserve the urine during the total collection. The density of 10 N H_2SO_4 was 1.230 kg/l and 100 ml/d of the acid was used to preserve the urine. A value of 9.41 J/mg S in the sample was used for the correction.

Results and discussion

The chemical composition, *in vivo* OMD and estimated energy values of the grass and red clover silages is presented in Table 1. There was a strong and uniform relationship between urinary GE and silage N concentration for both grass and red clover silages (Figure 1). To illustrate the strong relationship for grass silage samples when relating urinary GE to daily urinary N output, a separate regression was generated for the red clover silages (Figure 2). However, intercepts and regression coefficients were not significantly different when comparing the 95% confidence intervals of the parameters for the two equations. The weaker regression between urinary GE and urinary N in red clover silages could be explained by the polyphenol oxidase system in red clover that inhibits proteolysis in the silo as well as in the rumen. This, together with improved efficiency of microbial N synthesis, has increased protein flow from the rumen with diets based on red clover rather than grass silage (Dewhurst et al., 2003; Vanhatalo et al., 2009). It has also been suggested that more energy rich non-N compounds are excreted in the urine from ruminants fed red clover compared to grass silage, which could explain the poorer correlation for red clover compared to grass silage in Figure 2. In Figure 3, the relationship between urinary GE and silage DE is displayed. The regression for early first cut grass silages was different from the regressions of the other grass and red clover silages ($P<0.05$). The results indicate that the ME/DE ratio was different for these early first cut grass silages compared to the other silages.

Table 1 Chemical composition (in g/kg of dry matter (DM) unless otherwise stated), silage DM intake (DMI), organic matter digestibility (OMD) and energy values of primary and regrowth grass and red clover silages in this study

Forage type	Grass silage				Red clover silage				All			
	Primary growth (*n* 9)		Regrowth (*n* 4)		Primary growth (*n* 5)		Regrowth (*n* 7)		(*n* 25)			
Item[1]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Min	Max
DM, g/kg	212	29.8	257	37.0	233	58.6	253	74.6	235	53.0	175	414
Organic matter	921	7.4	900	6.6	895	10.9	894	10.7	905	15.3	878	934
Crude protein	159	42.0	183	17.7	185	28.5	203	34.3	180	37.1	112	256
NDF	574	84.4	488	17.7	381	80.9	352	74.5	460	120.8	274	669
iNDF	76	46.4	98	23.0	117	57.3	110	53.3	97	48.4	17	211
WSC	42	17.7	83	23.5	45	26.8	45	20.5	50	24.8	14	112
Ethanol	10	4.0	3	1.0	4	1.1	4.0	1.0	6	4.0	2	18
Lactic acid	46	8.6	38	11.0	60	18.0	55	15.4	50	14.4	25	87
Acetic acid	26	11.2	12	3.7	19	8.5	22	7.0	21	9.5	8	49
Propionic acid	0.1	0.18	0.0	0.05	0.2	0.15	0.2	0.25	0.1	0.19	0	0.7
Butyric acid	0.5	0.80	0.2	0.05	0.0	0.06	0.1	0.08	0.3	0.51	0	2.1
NH_3, g/kg total N	59	28.3	51	12.8	59	29.2	70	26.0	61	25.3	29	116
sCP, g/kg total N	668	34.4	544	40.9	461	114.8	403	37.6	533	126.5	339	706
OMD, g/kg	730	73.7	704	42.1	710	71.1	710	65.5	716	63.7	581	840
Silage GE, MJ/kg DM	19.4	0.40	19.5	0.17	18.9	0.16	19.3	0.53	19.3	0.42	18.6	20.2
Silage DMI, g/d	-	-	-	-	-	-	-	-	932	70.6	814	1013
Fecal GE, MJ/kg DMI	-	-	-	-	-	-	-	-	5.7	1.13	3.6	8.2
Urine GE, MJ/kg DMI	-	-	-	-	-	-	-	-	1.2	0.33	0.5	1.6
DE, MJ/kg DM	-	-	-	-	-	-	-	-	13.6	1.37	10.6	16.6

[1]NDF, neutral detergent fibre; iNDF, indigestible NDF; WSC, water soluble carbohydrates; sCP, buffer soluble crude protein; GE, gross energy; DE, digestible energy.

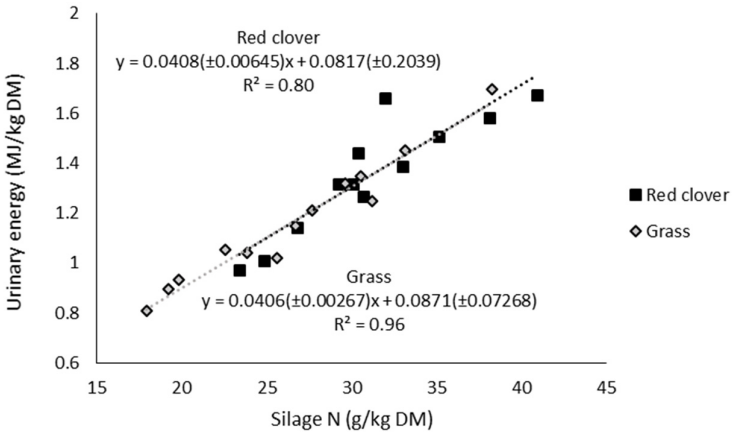

Figure 1 Relationship between urinary energy concentration and silage N concentration.

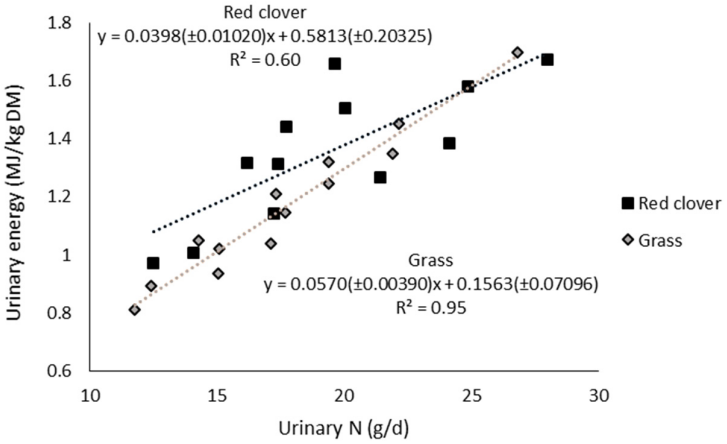

Figure 2 Relationship between urinary energy concentration and urinary N concentration.

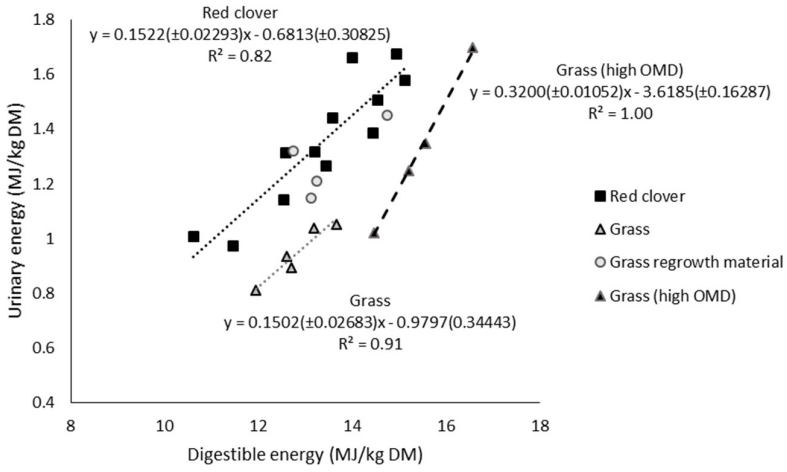

Figure 3 Relationship between urinary energy and silage digestible energy concentration.

In Table 2 are the monophenolic compounds presented that could be derived from rumen metabolism of lignin precursors (Martin, 1982) and that were possible to quantify in the samples of this study. Benzoic acid, 4-OH-benzoic acid and N-benzoylglycine (hippuric acid) were the three phenolic compounds that were excreted in largest amounts. Both daily amounts and standard deviations of these three phenolic compounds were larger in the urine of sheep fed primary growth grass silages compared to the other silages.

Table 2 Phenolic compounds detected in urine of sheep fed the different silages (mg/d)

Forage type	Grass silage				Red clover silage			
	Primary growth (*n* 9)		Regrowth (*n* 4)		Primary growth (*n* 5)		Regrowth (*n* 7)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Benzoic acid (BA)	595	364.2	262	86.9	134	38.2	108	16
4-OH-BA	18.2	10.09	8.5	1.85	17.6	6.23	15	2.32
2,4-diOH-BA	1.8	0.98	2.1	0.92	7.0	1.93	9.2	1.71
3,4-diOH-BA	1.7	1.06	1.0	0.39	3.2	1.26	4.8	1.08
3-OCH_3-4-OH-BA	3.0	0.92	4.1	1.02	9.5	2.45	9.2	0.86
N-benzoylglycine	4753	1433.4	4323	1326.5	3926	962.6	3696	501.3
4-OH-cinnamic acid	0.3	0.14	0.2	0.04	0.3	0.08	0.3	0.03
3,4-diOH-cinnamic acid	1.3	0.57	1.0	0.27	2.8	0.65	3.4	0.49

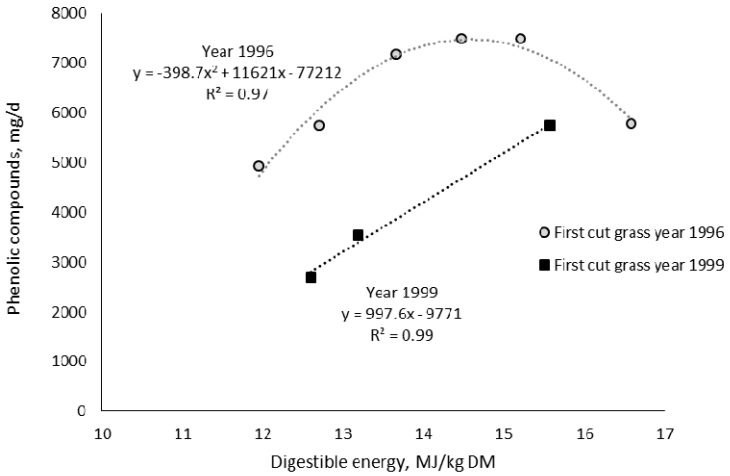

Figure 4 Sum of monophenolic compounds (see Table 2) in urine from sheep fed first cut grass silages harvested in two different years.

The differences observed for grass vs. red clover silages can be explained by higher levels of hemicellulose in grasses than in legumes. Phenolic compounds that cross-link the cell wall carbohydrates can be divided into two groups: cinnamic acids and cinnamyl alcohols. Further, grass lignin contains much more of the ferulic and p-coumaric acids, and legume lignin consist more of the alcohols. This results in ester-linked rather than ether-linked lignin

in grasses compared to legumes (Van Soest, 1994). Phenolic compounds are released from the lignin molecule during ruminal cell wall degradation by fungal and bacterial esterase activity. They are further metabolized in the rumen, absorbed into the blood and may be taken up by other peripheral tissues before being excreted in the urine (Soberon *et al*., 2012a, b). It has also been suggested that lignin in less mature grasses is more prone to solubilisation than in later harvested material (Van Soest, 1994). There was a difference between daily amounts of phenolic compounds excreted in the urine with grass maturity within the first cut grass silage samples. However, the relationship between daily amounts of phenolic compounds excreted in the urine to the silage digestible energy value was curvilinear for grass silages harvested in 1996, but clearly linear for those harvested in 1999 (Figure 4).

Conclusions

The results of this study imply that ME/DE ratio is not constant across first cut grass silages of different maturities. The ME/DE ratio will be lower in highly digestible first cut grass silages, but not due to urinary excretion of monophenols derived from solubilization of lignin. Further, urinary energy was not as clearly explained for red clover silages as for grass silages by dietary and urinary N concentrations.

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The effect of dietary carbohydrate source on ruminal digestion and nutrient utilization in lactating dairy cows

E. H. Cabezas-Garcia[1], S. J. Krizsan[1], K. J. Shingfield[2] & P. Huhtanen[1]
[1]Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences (SLU), SE-901 83, Umeå, Sweden. [2]Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, SY23 3EB, Aberystwyth, United Kingdom.
Correspondence: edward.cabezas.garcia@slu.se

Introduction

Different carbohydrate sources can be fed to dairy cows depending on their availability and prices. Cereal grains can be used directly as human food or more efficiently by simple-stomach animals, but substitution of starch for fibre may lower methane production (Moss *et al.,* 2000). An experiment in lactating cows demonstrated that milk production and total methane production were not influenced when medium-quality late harvested grass silage and barley was replaced with high-quality early harvested grass silage (RuminOmics EU project, 2015). The aim of this experiment was to explain the efficiency of digestion and nutrient utilization of dietary carbohydrate source for production by replacing rapidly digestible carbohydrate (starch) with highly digestible forage fibre in the diets to lactating dairy cows.

Materials and Methods

Four multiparous rumen cannulated Swedish Red cows averaging (mean ± standard deviation) 676 ± 79 kg of BW; 90 ± 19.1 days in milk, and yielding 30.9 ± 6.27 kg of milk at the start of the experiment were used in a balanced 4 x 4 Latin Square. Experimental diets were formulated to meet energy and protein requirements of 35 kg ECM yield according to the Finnish Feed Evaluation System (LUKE, 2016). Late-cut silage (LS) and crimped barley (B) were gradually replaced with early cut silage (ES; 0, 33, 67 and 100%) in order to obtain four diets with the same energy and protein contents named as follows: L, LE, EL and E. The proportion of forage in the diet increased from 42 to 64% and rapeseed expeller was used as a protein supplement. Primary growth herbage for production of ES and LS was harvested from the same third year ley dominated by timothy grass (*Phleum pratense sp*.) at 2-week intervals. The crops were wilted to a DM around 300 g/kg and ensiled in bunker silos using a commercial acid-based additive. The cows were fed experimental diets as TMR ad libitum and milked twice daily. Total tract digestibility was determined by faecal spot sampling using indigestible neutral detergent fibre (iNDF) as an internal marker. Omasal samples were taken by an omasal pump thrice daily on d 17 to d 20, and divided into large particles (LP), small particles (SP) and fluid phase (FP) according to Ahvenjärvi *et al*. (2000). Two rumen evacuations, before morning feeding and 4 h after feeding, were conducted to give a representative estimate of rumen digesta pool sizes and digestion kinetics. Ruminal digestibility was calculated using the reconstitution system based on triple marker technique (Cr-EDTA, Yb-acetate and iNDF; France and Siddons, 1986). Microbial protein synthesis was determined using ^{15}N as microbial marker (Broderick and Merchen, 1992). Samples of rumen fluid (n = 8) were collected on d 21 at 1.5 h intervals starting before the morning feeding to measure pH and volatile fatty acids concentrations. Enteric methane was calculated based on stoichiometric equations (CH_4VFA; Wolin *et al*., 1960). Digestion kinetic variables were calculated by compartmental flux method. Experimental data were

analyzed by ANOVA for a 4 × 4 Latin square design and orthogonal polynomial contrasts were used to evaluate linear and quadratic effects of treatments.

Results and Discussion

Dry matter intake (DMI) decreased linearly (P = 0.02) when LS + B were gradually replaced with ES (Table 1). Feed efficiency in terms of ECM yield related to DMI tended (P = 0.08) to improve with increased inclusion of ES. Minor differences in rumen fermentation pattern between the diets were detected. The molar proportions of isovalerate and valerate decreased ($P \leq 0.03$) when the proportion of ES increased in the experimental diets. CH_4VFA molar concentration was not influenced by the addition of ES in the diet. The results in this study are consistent with the findings of a meta-analysis based on 108 treatment means from studies in cattle fed grass silage based diets that indicated that molar proportion of propionate did not increase with increased dietary starch content (Huhtanen *et al*., 2013).

Table 1 Effects of graded replacement of late-cut silage and crimped barley with early-cut silage on the performance and rumen fermentation parameters

	Diet[1]					*P*-value[2]	
Item	L	LE	EL	E	SEM	L	Q
Animal performance							
DMI, kg/d	20.2	19.2	19.1	17.9	0.83	0.02	0.83
Milk, kg/d	22.1	21.3	22.8	22.4	2.03	0.46	0.79
ECM , kg/d[3]	23.5	21.6	22.8	23.0	2.31	0.93	0.31
ECM/DMI, kg/kg	1.16	1.11	1.18	1.31	0.11	0.08	0.16
MNE, g/kg[4]	266	245	247	256	15.9	0.54	0.17
Milk yield							
Fat, g/d	948	856	897	916	107.8	0.81	0.30
Protein, g/d	828	760	801	801	59.5	0.70	0.19
MUN, mmol/L	3.51	3.03	3.71	3.90	0.117	<0.01	<0.01
Rumen fermentation							
pH	6.04	6.15	6.05	6.15	0.083	0.13	0.70
Ammonia N, mmol/L	32.3	42.0	46.1	46.1	4.83	0.08	0.35
Total VFA, mmol/L	109	103	108	103	4.3	0.27	0.71
Molar proportion, mmol/mol							
Acetate	684	688	686	687	6.4	0.82	0.80
Propionate	167	170	181	178	5.9	0.14	0.57
Butyrate	106	98	94	98	5.6	0.14	0.20
Isovalerate	30.5	30.6	26.8	27.7	0.70	0.01	0.60
Valerate	12.5	12.8	11.4	10.1	1.38	0.03	0.28
CH_4VFA, moles/ mol VFA[5]	0.369	0.367	0.359	0.362	0.0046	0.17	0.56

[1] Diet LC silage: EC silage: L = 100:0, LE = 67:33; EL = 33:67, E = 0:100.
[2] L= Linear and Q = Quadratic effects.
[3] ECM = Energy corrected milk, calculated according to Sjaunja et al. (1990).
[4] MNE = Efficiency of N utilization for milk production defined as milk N/N intake.
[5] CH_4VFA = 0.5 × C2 - 0.25 × C3 + 0.5 × C4 (Wolin, 1960).

In a parallel production trial (RuminOmics EU project, 2015) conducted with 16 lactating cows fed with the same diets, no differences in *in vivo* total CH_4 emissions were found using the GreenFeed system (C-Lock Inc., Rapid City, SD). However when *in vivo* CH_4 was expressed per unit of intake (CH_4, g/kg DMI), CH_4 emissions increased linearly ($P < 0.01$) with the inclusion of ES in the diet (L= 19.4; LE= 21.1; EL= 21.9; E= 22.7). According to empirical model based on meta-analysis 298 treatment means (Ramin and Huhtanen, 2013) differences in DMI (5.0 g/kg BW) and OMD (42 g/kg) accounts for about 2/3 of CH_4 losses as a proportion of gross energy (GE) between diets based on late or early cut silages. The digestibility of the neutral detergent fiber fraction (NDF) increased linearly ($P < 0.01$) as the ES proportion in the diet increased and the differences of this fraction accounted for most of the observed differences between diets in OM digestibility (Table 2). Reduced NDF digestibility with increased proportion of LS + B in the diet can be attributed to the differences in potential digestibility of dietary NDF (pdNDF) and to the adverse effects of increased dietary starch concentration on ruminal NDF digestibility. Both apparent ($P = 0.02$) and true ($P < 0.01$) ruminal organic matter digestibility and contribution of ruminal digestion increased with dietary inclusion of ES silage in the diet.

Table 2 Effects of graded replacement of late-cut silage and crimped barley with early-cut silage on total tract and ruminal nutrient digestion

	Diet[1]				SEM	*P*-value[2]	
Item	L	LE	EL	E		L	Q
Apparent total tract digestibility, g/kg							
OM	681	677	692	722	8.5	<0.01	0.10
CP	651	638	685	708	12.8	<0.01	0.22
NDF	484	502	584	647	13.4	<0.01	0.14
pdNDF	654	659	737	787	17.1	<0.01	0.23
NDS	806	801	777	785	8.7	0.07	0.47
Ruminal digestibility, g/kg							
Apparent OM	432	412	506	498	19.4	0.02	0.77
True OM	626	638	683	701	12.5	<0.01	0.81
NDF	410	458	531	602	14.8	<0.01	0.35
pdNDF	554	601	671	732	18.7	<0.01	0.62
Rumen: total OM digestion	630	607	728	690	21.7	0.02	0.74
Rumen: total NDF digestion	845	911	909	932	26.0	0.04	0.37
EMNS, g/kg OMTDR[3]	28.6	32.2	23.7	26.2	1.91	0.12	0.76
Digestion kinetics, 1/h							
pdNDF passage rate	0.0150	0.0179	0.0157	0.0137	0.0014	0.35	0.13
pdNDF digestion rate	0.0287	0.0347	0.0448	0.0500	0.0031	<0.01	0.87
iNDF passage rate	0.0332	0.0354	0.0355	0.0332	0.0025	1.00	0.41

[1] Diet LC silage: EC silage: L = 100:0, LE = 67:33; EL = 33:67, E = 0:100.
[2] L= Linear and Q = Quadratic effects.
[3] EMNS = Efficiency of microbial N synthesis per kg of organic matter truly digested in the rumen (OMTDR).

Differences among the diets in their contribution to post-ruminal OM digestion can be associated with differences in amounts of starch and NDF digested post-ruminally. Increased contribution of post-ruminal digestion with increased proportion of late-cut silage can contribute to the lower CH_4 production per kg DMI. Differences in ruminal turnover time of NDF were mainly related to digestion rate of pdNDF, whereas differences in passage rate of NDF fractions were small. Indeed, the digestion rate of pdNDF ratio increased linearly ($P < 0.01$) as the proportion of ES increased.

Conclusions

Replacement of late-cut silage and barley gradually with early-cut silage lowered DMI, but had no effect on milk or ECM yields. Feed efficiency in terms of ECM yield per kg DMI improved with increased inclusion of early-cut silage in the diets. No difference in the efficiency of N utilisation was observed. Ruminal and total tract NDF digestibility improved with increased inclusion of early-cut silage in the diet, reflecting differences in intrinsic characteristics of fibre and negative effects of higher starch content in diets with increased proportion of late-cut silage.

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Ruminal pH and volatile fatty acid concentrations in Swedish dairy cow feeding trials

T. Eriksson, J. Bertilsson, R. Spörndly & M. Patel
Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Box 7024, SE-750 07 Uppsala.
Correspondence: torsten.eriksson@slu.se

Introduction

Volatile fatty acids (VFA) from the fermentation of feed carbohydrates in the rumen is the main supply of energy for the dairy cow. A large VFA production is therefore necessary for maintaining energy balance in high yielding cows, but at the same time without causing negative effects associated with acid overload. Those effects range from mild impairments of fiber digestion and microbial efficiency over lowered milk fat concentration to sub-acute (SARA) and even acute ruminal acidosis. Given those implications for the productivity and wellbeing of the dairy cow, measurements of ruminal pH and VFA production are essential elements in dairy cow experiments and have been carried out extensively in research. This paper presents data from feeding trials involving ruminally cannulated cows at the Uppsala research facilities of the Swedish University of Agricultural Sciences. The aim was to provide a description of recorded ruminal pH values and the occurrence of pH below 5.8. A second aim was to examine the relationship between ruminal pH and volatile fatty acid concentration.

Materials and Methods

The feeding trials are listed in Table 1. They had all change-over designs and were in most cases performed with a sub-group of 2 to 6 cannulated cows within a production experiment. Rations generally consisted of grass-legume silages and grain-based concentrates were fed separately with the first meal at 05:00 or 06:00 h. All cows were fitted with ruminal cannulas with 100-mm i.d. (Bar Diamond Inc., Parma, ID, USA). Ruminal liquid sampling was done in each measurement period according to a schedule with a 3 to 6 h period between samplings to cover 17 to 23 of the possible 24 hours during 4 to 5 days. Sampling was done by inserting a 50-ml plastic test tube approx. 20 cm below the ruminal surface, where after it was filled with rumen liqour within a seconds. After removal from the rumen, pH was measured promptly in the tube with a portable pH meter (see original publications for specification) and the sample was immediately frozen without preservatives. After thawing, samples were analyzed for VFA by gas chromatography (Murphy et al., 2000) or HPLC (Ericson & André, 2010). In experiments 1 to 5 and 11, VFA was analysed in every sample, but in the other experiments only in samples pooled within cow and period. In this compilation, "VFA" refers to the sum of the reported VFA:s which were acetic, propionic, butyric, valeric, iso-butyric and iso-valeric acid. For some of the trials, the calibration also included lactic acid.

The experiments included in this compilation spanned 3336 possible sampling hours (24 × cows × periods) and 2533 of these were actually covered. Period means of pH and VFA:s were in the original publications calculated as arithmetic means or according to the trapezoid rule to correct for sampling hours not covered. For the current compilation, a pH value was calculated by linear interpolation for each hour on the hour for all cows in all experimental periods if no sample was available. For experiment 11 with scheduled sampling 30 min past the hour, interpolation was used throughout. From these hourly values, overall arithmetic mean and percentiles for 0.025 and 0.975 (approx. a 95% confidence interval) were calculated. Duration of pH < 5.8 as well as the area of the curve < 5.8 (pH × min) were also

calculated for each cow × period mean. The pH results are only presented with descriptive statistics and numerical differences referred to were not tested for significance.

Comparisons of pH and VFA concentration were done experimental wise by linear regression across cow × period means and with a mixed model with random intercept for cow. Adjusted Y:s (St-Pierre, 2001) were then used for obtaining R^2. Treatment was not taken into account.

Table 1 Experiments included in the compilation of rumen pH data

	n[1]	Treatments	DMI, kg/d	Conc. proportion[2]	Reference
Trial 1	15	Different clover and grass silage	17 - 26	0.35	Bertilsson & Murphy, 2003
Trial 2	9	Different clover and grass silage	18 - 26	0.32	Bertilsson & Murphy, 2003
Trial 3	12	Silage from different ryegrass varieties	17 - 22	0.34	Bertilsson et al., 2015
Trial 4	12	Ryegrass silage with or without sucrose addition	17 - 24	0.33	Bertilsson et al., 2015
Trial 5	12	Fodder beets, potatoes or barley as concentrates	16 - 23	0.29	Eriksson et al., 2004
Trial 6	12	Grass silage mixed with either birdsfoot trefoil or white clover	18 - 21	0.36	Eriksson et al., 2012
Trial 7	8	Lupins or pea as protein concentrate	15 - 18	0.36	Eriksson, 2010
Trial 8	12	Grass silage mixed with either birdsfoot trefoil or white clover	17 - 24	0.28	Eriksson et al., 2012
Trial 9	4	Grass silage from either bags or round bales	21 - 27	0.47	Spörndly & Eriksson, 2012
Trial 10	9	Chopped or cut grass silage or maize silage	18 - 26	0.44	Spörndly & Eriksson, unpublished
Trial 11	18	Incremental forage proportions	12- 18	0.31	Patel et al., 2011
Trial 12	18	Different $KHCO_3$ addition to common grass silage-concentrate diet	17 - 24	0.58	Eriksson & Rustas, 2014

[1]Number of cow × period means in each experiment. Number of pH samples is 17n-23n
[2]Mean of actual consumption. DMI differences were entirely due to different forage intake in Trials 1-4 and Trial 5, with flat rate concentrate allowances of 7 and 5 kg DM/d, respectively. Trials 6-8 and 11-12 had fixed concentrate proportions (in Trial 11 three levels: 0.10, 0.30 or 0.50 of DM) and Trials 9-10 had individual allowance.

Results and Discussion

The diurnal pH fluctuation (Figure 1) followed a general pattern with a peak of 6.54 at 05:00 h and then a drop initiated by the first meal at 05:00-06:00 h and a nadir of 5.80 at 20:00 h. The range of pH units within the 0.025 and 0.975 percentiles varied between 0.90 at 07:00 and 1.30 at 02:00 h. Results were from different experimental treatments and many factors could affect the outcome. However, the narrowing of the pH range at 07:00 h is still logical, considering the logarithmic pH scale. When VFA production is initiated by the morning meal, an increase in VFA concentration would cause a larger pH drop the higher the pH is and, hence, narrow the range of pH values.

The range of experimental pH means (Table 2) was also of considerable magnitude, where Trials 1 and 2 may be most notable. They were actually two replicated years within an experiment with identical protocols but with different cows and different harvest years (Bertilsson & Murphy, 2003), which lowered mean pH with 0.37 units in Trial 2 compared to Trial 1. The pH difference occurring even under similar experimental conditions illustrates

the difficulties in interpreting absolute pH values. This would be even more exacerbated, if also effects of different sampling methodologies were included, as Falk et al. (2016) recently demonstrated.

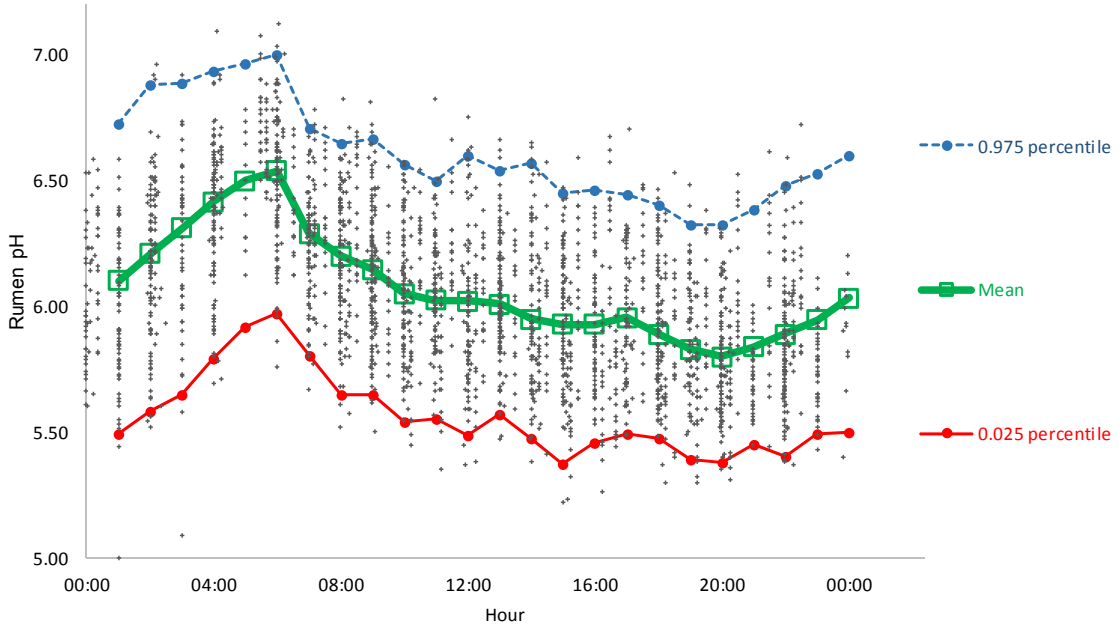

Figure 1 Diurnal fluctuations in ruminal pH from 139 cow × period means in change over experiments at the Uppsala facilities of SLU. Cross sign markers denotes the actual 2533 samples while mean and percentile values were calculated from interpolation to each hour on the hour if sampling was not performed at the actual timepoint.

Table 2 Ruminal pH and extent of pH < 5.8 episodes in change over experiments at the Uppsala facilities of SLU. Data from 139 period × cow means

	pH				Minutes < 5.8[1]		AUC min × pH < 5.8[1]	
	Mean[1]	Min[1]	Max[1]	*pH from.H*$^{+}$ [2]	Mean	Max	Mean	Max
Trial 1	6.23	5.93	6.43	*6.16*	62	287	4	30
Trial 2	5.86	5.65	6.11	*5.81*	652	1416	101	310
Trial 3	6.03	5.87	6.19	*5.94*	287	608	33	80
Trial 4	5.97	5.81	6.36	*5.87*	540	865	72	148
Trial 5	6.04	5.70	6.42	*5.93*	362	1117	52	186
Trial 6	6.10	5.72	6.40	*5.97*	304	1035	38	207
Trial 7	6.34	6.14	6.60	*6.25*	24	84	1	6
Trial 8	6.05	5.86	6.23	*5.99*	201	549	13	36
Trial 9	5.78	5.60	5.97	*5.70*	865	1213	179	328
Trial 10	5.90	5.76	6.02	*5.83*	653	975	71	180
Trial 11	6.24	5.86	6.62	*6.13*	104	701	11	111
Trial 12	6.04	5.74	6.41	*5.89*	416	934	81	272
Overall[3]	**6.07**			***5.95***	**328**		**46**	

[1]Based upon period × cow means in each experiment
[2]Calculated from arithmetic mean of H^{+} concentration in all hourly values from each experiment
[3]Overall means of the 139 period × cow means

The longest duration of pH < 5.8 was also recorded in Trial 2 where one cow maintained rumen pH this low for 1416 (Table 2) of the 1440 diurnal minutes with 19 kg DM ryegrass

silage and 7 kg DM concentrates, providing a total neutral detergent fibre (NDF) intake of 10.0 kg/d. When fed white clover silage, the same cow had pH < 5.8 for 1210 min consuming 18 kg DM silage and 7 kg DM concentrates, supplying 6.4 kg NDF/d.

Murphy (1983) has pointed out the effects on mean calculations if pH values, as is customary, or [H^+] are used to estimate mean pH. The difference will increase with decreasing pH and with increasing range of values being averaged. Mean pH calculated after averaging [H^{+}] from all hourly values in each experiment (Table 2) was 0.06 – 0.15 pH units lower than the arithmetic mean. However, histograms of all data (Figure 2) suggested that pH values rather than [H^+] were normally distributed, questioning the preference of [H^+].

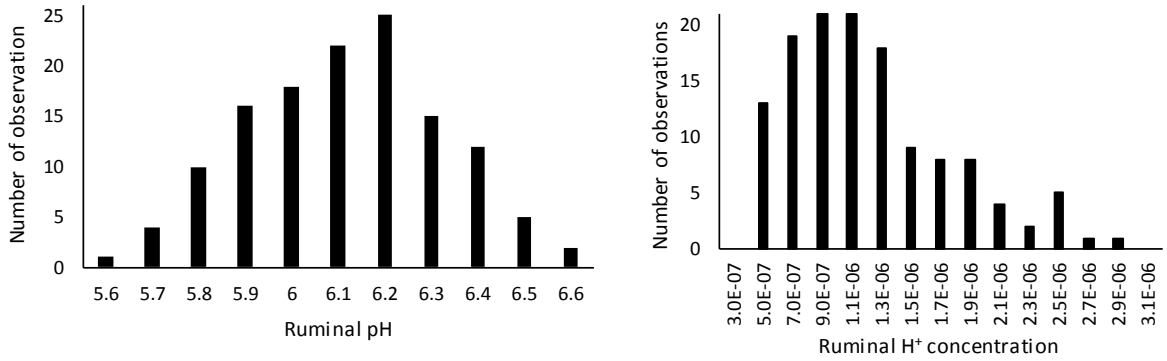

Figure 2 Histogram showing distribution of ruminal pH and ruminal H^+ concentration for the 139 cow × period means.

Regressions of pH against rumen VFA concentrations are shown in Table 3 and Figure 3. The slope for ruminal pH drop against ruminal VFA concentration could be considered a crude measure of how well the actual feeding regimen can increase the energy supply to the cow without causing SARA. Diets where the increased fermentation is accompanied by a large salivation effect and a buffering effect from the feed would hence have a less steep slope (Dijkstra et al., 2012). This is in agreement with Trial 1 to 3 where increased silage intake mediated increased VFA levels. If Trial 9 (with just two cows with different levels) is removed, the steepest slopes occurred in Trial 4, where different sucrose amounts were added and in Trial 5 including either sucrose or different starch sources. There was a relatively good fit for the regression within some of the trials, especially when a solution for a model with random intercept was reached. Regression of pH against VFA across all period means yielded the equations:

(1) pH = 7.26 – 0.0088 VFA (mM), R^2 = 0.44 (simple linear regression)

(2) pH = 7.84 – 0.0129 VFA (mM), R^2 = 0.82 (mixed model, random intercept for each trial)

Equation (1) can be compared to the slope of -0.0076 found by Dijkstra et al. (2012) for a simple linear regression of 104 treatment means from literature.

In Trials 3 to 4 and 11, where VFA and lactate had been analysed on individual samples rather than on pooled samples, lactate became significant as an explanatory variable for rumen pH only in Trial 11, where the mean lactate concentration was 1.5 mM. The slope for lactate was -0.0258, whereas the coefficient for VFA was -0.0108.

Table 3 Regressions for ruminal pH against rumen total VFA concentration from change-over experiments at the Uppsala facilities of SLU. Calculated on period × cow means of pH and VFA, respectively (N = 130).

		Simple linear regression				Random intercept by cow			
	n	Intercept	Slope	R^2	RMSE	Intercept	Slope	R^2	RMSE
Trial 1	15	7.59	-0.0101	0.52	0.11	7.12	-0.0066	0.70	0.05
Trial 2	9	7.50	-0.0105	0.60	0.11	7.45	-0.0102	0.65	0.09
Trial 3	12	7.45	-0.0095	0.53	0.08	7.59	-0.0105	0.64	0.07
Trial 4	12	8.76	-0.0185	0.84	0.07	8.49	-0.0167	0.94	0.04
Trial 5	12	8.71	-0.0185	0.90	0.08	8.72	-0.0186	0.92	0.08
Trial 6	12	8.28	-0.0157	0.86	0.07	8.20	-0.0152	0.95	0.04
Trial 7	8	7.79	-0.0116	0.73	0.09	7.87	-0.0122	0.83	0.07
Trial 8	12	8.18	-0.0157	0.91	0.04	N.R.	N.R.	N.R.	N.R.
Trial 9	4	9.20	-0.0240	0.97	0.03	N.R.	N.R.	N.R.	N.R.
Trial 11	18	7.49	-0.0109	0.59	0.13	7.26	-0.0091	0.63	0.10
Trial 12	18	7.62	-0.0135	0.58	0.13	N.R.	N.R.	N.R.	N.R.

N.R = Not reported because mixed model solution did not result in random parameter estimates

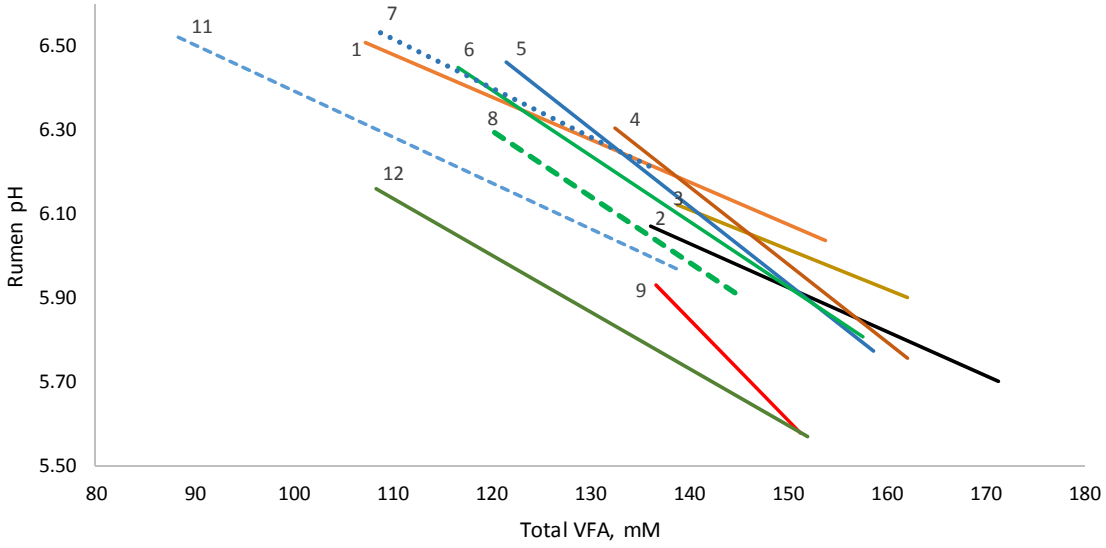

Figure 3 Regression lines for ruminal pH against rumen total VFA concentration from change-over experiments at the Uppsala facilities of SLU as denoted by Trial number referring to equations in Table 3 (No VFA measurements in Trial 10). Calculated on period × cow means of pH and VFA, respectively (N = 130).

Conclusions

Ruminal pH was on average below 5.8 for more than 5 h/d and for individual cows 20 to 23 h/d. This was still combined with dry matter intakes of 25 to 26 kg/d. The effect of cow and harvest year may cause considerable pH level differences even in experiments repeated with the same protocol at the same research station. There was a relatively good agreement between ruminal VFA concentration and ruminal pH within many of the experiments, which suggests plotting those variables as a way of tracing outliers and candidate samples for lactate analysis if not obtained together with VFA results.

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Dry matter intake and water intake and urine excretion in mid-lactating cows on part-time pasture

T. Jansson, H. Kismul, E. Spörndly and T. Eriksson
Swedish University of Agricultural Sciences (SLU), Department of Animal Nutrition & Management, Box 7024, SE-750 07 Uppsala.
Correspondence: torsten.eriksson@slu.se

Introduction

Knowledge about the intake of feeds allocated to dairy cows on a group level is valuable information for management purposes and crucial for interpretation of result in feeding experiments. During the indoor feeding period, individual feed intake can be measured at research facilities, but pasture intake can only be estimated indirectly from production response in practice or with the aid of markers in research (Hellwing *et al.*, 2015). Drinking water intake and urine volume have been correlated to dry matter intake (DMI) in previous research (Murphy et al., 1983; Khelil-Arfa *et al.*, 2012; Fraley et al., 2015). Daily intake of minerals, such as sodium and potassium (Murphy et al., 1983; Eriksson & Rustas, 2014; Fraley et al., 2015) has been more strongly correlated than DMI to water intake and urine volume. The aim of the experiment presented here was to explore the possibilities to estimate dry matter intake from knowledge of drinking water intake or urine volume together with mineral analyses of feeds.

Materials and Methods

The experiment was conducted at the Swedish livestock research centre at Lövsta, Uppsala with three sampling periods of 5 d each during June 9-July 3 2016. A total of 43 lactating cows (102-192 DIM at onset) of Swedish Holstein and Swedish Red breeds were used. The cows were kept in an automatic, voluntary milking system (VMS™, DeLaval International AB, Tumba, Sweden), in a feed first system. The housing system consisted of cubicles with rubber mats and sawdust bedding in the lying area. In the feeding area, the cows where fed grass silage from individual troughs with automatic registration of feed intake (BioControl AS, Rakkestad, Norway). There were in total seven water bowls that were located in the feeding area, equipped with custom-made water flow meters for registration of individual water consumption (BioControl AS, Rakkestad, Norway). Concentrates were distributed from three different stations placed in the lying area as well as in the milking unit (DeLaval International AB, Tumba, Sweden).

The cows were assigned to an ongoing production experiment comparing "exercise pasture" with part-time grazing in a voluntary milking system. The cows were divided into an exercise group (21 cows) fed silage ad lib indoors and a grazing group (22 cows) fed 6 kg DM silage/d. Both groups were fed concentrates (Lantmännen, Stockholm, Sweden) with a maximum allowance (as fed) of: Solid 620, 16 kg/d and Unik 82, 2.5 kg/d according to calculated individual requirement at onset of the experiment with a scheduled weekly reduction based upon an assumed drop in milk yield (NorFor, Aarhus, Denmark). Both groups had access to paddocks for 8.5 h/d, between 06:00-10:30 h and between 16:00-20:00 h, but the grazing group was allowed to pasture with 15 kg DM/cow/d while the exercise group had access to an exercise paddock with virtually no pasture.

Silage and concentrate samples were collected daily and frozen for later analysis. Pasture samples were taken four times daily in connection to when the cows were let out and in from

pasture and dried at 60°C for estimating mean DM concentration in the pasture actually consumed. Pasture samples for chemical analysis were obtained daily before the cows were given access to a new paddock.

Milk yield, drinking water intake, all indoor feed intake and body weight (BW) of the cows were all recorded by the automated systems. A weather station close to the barn registered the ambient temperature and relative humidity outside on an hourly basis. Spot urine samples were taken at 07:00-13:00 h during the three five-d sampling periods with on average 6 samples/cow/period (range 2-10 samples/cow/period). Samples were preserved and diluted ninefold with 0.10 M HCl and freeze-stored at -30°C until being analysed on an AutoAnalyser III (SEAL Analytical GmbH, Nordstedt, Germany) for urea and creatinine with diacetylmonoxime and picric acid, respectively, as reagents. Feed samples were analysed as described by Åkerlind et al. (2011) for DM, ash, CP, NDF and in vitro organic matter digestibility for calculation of metabolizable energy. Minerals (Ca, K, Mg, Na, P and S) were analysed at Agri-Lab, Uppsala by inductively coupled plasma atomic emission spectroscopy (Spectroflame; Spectro GmbH,Kleve, Germany) after digestion with nitric acid. The chemical composition of the feeds is shown in Table

All data including drinking water intake, silage intake, concentrate intake, DMI, urea excretion and creatinine concentration, body weight, urine volume and milk yield was calculated to means for each cow and experimental period. Urine volume was estimated as:

L urine/day = (24.1 × BW) / (mg creatinine/L urine) (Chizzotti et al., 2008).

Table 1 Composition of feeds in pasture experiment

	Pasture		Silage		Concentrates	
	Mean	SD	Mean	SD	Solid 620	Unik 82
DM, %	22.3	0.79	32.3	1.05	89.5	89
Ash, g/kg DM	102	3.43	90	2.92	69	85
CP, g/kg DM	170	17.5	157	3.0	178	296
NDF, g/kg DM	384	37.2	430	9.5	282	243
ME MJ/kg DM	11.07	0.21	11.60	0.20	13.2	14
K, g/kg DM	28.4	4.20	30.1	0.97	8.7	13.3
Na, g/kg DM	0.1	0.06	1.7	0.40	3.7	3.5

Data was analysed with SAS (Ver. 9.3, SAS Institute Inc., Cary, USA). Correlations between different factors were calculated for the exercise group with Proc CORR.

Simple and mixed linear regressions for the control group were done with Proc REG and with a Proc MIXED model with random intercept and slope for individual cows. Results were expressed with "adjusted y:s" (St-Pierre, 2001) to obtain R^2 values. The total daily DMI in the control group was regressed against daily drinking water intake and also against daily urine excretion. Daily K intake was also regressed against daily urine excretion.

The regressions for total DMI intake in the control group were directly applied on water intake and urine excretion, respectively, for the experimental group so that a total DMI could be estimated for each cow in each period. Pasture DMI was then calculated by deducting intake of silage and concentrates. The regressions with K intake on urine excretion were

applied in a similar manner on the experimental group to estimate a total K intake. After deducting K intake with silage and concentrates, the remaining K estimate was divided by pasture K concentration to obtain an estimate of pasture DMI. DMI based on total K intake estimated from urine volume was also calculated by rearranging the simple linear regression equation obtained from previous N balance trials (Eriksson, 2011):

Urinary excretion (L/D) = 1.9 + 0.056 K intake (g/d)

This equation was rearranged and applied on urinary excretion estimates from the experimental group and pasture intake was then calculated after deducting K intake from silage and concentrates as previously described.

Results and Discussion

Silage intake in the pasture group was somewhat lower than the planned 6 kg DM/d (Table 2) while the exercise group consumed more than the expected 12 kg DM. This resulted in a difference of 7.7 kg DM to be covered by pasture, if the groups should achieve equal DMI. Drinking water intake was 8 kg/d lower for the pasture group, while urine volume, as estimated from creatinine concentration, was the same. The difference may be explained by the lower DM concentration in pasture compared to silage and, hence, an increased feed water intake. There was a larger urinary urea excretion for the pasture than for the exercise group, which may be explained by a somewhat higher CP concentration in the pasture than in the silage and possibly also by different diurnal urea patterns.

Table 2 Intake of feeds, drinking water and K, milk yield and urinary excretion in cows

	Exercise group		Grazing group		
	Mean	SD	Mean	SD	Diff.[1]
Silage DM, kg/d	13.6	2.2	5.2	1.0	-
Conc. DM, kg/d	10.2	2.8	10.9	2.9	n.s.
Tot.DM, kg/d (excl. pasture)	23.8	3.9	16.1	3.5	-
K, g/d (excl. pasture)	497	75	253	46	-
Drinking water, L/d	90.5	15.5	82.9	18.6	**
Milk, L/d	35.7	7.8	37.4	9.3	n.s.
Urine, L/d	26.0	4.7	25.8	4.6	n.s.
Urinary urea N, g/d	126	24.6	147	23.0	***

[1]P for overall treatment difference where: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Dry matter intake in the exercise group was strongest correlated to milk yield (Table 3). However, this is not surprising since the ration at onset was adapted to present milk yield. Drinking water intake was better correlated to DMI than was urine volume. This was in spite of that urine volume should be more directly affected by intake than the drinking water, where individual differences in water allocated to milk yield and evaporation may offset the relation somewhat. The feed factor that was most correlated to water intake was CP intake (0.70) and the feed factor most correlated to urine volume was K intake (0.58). However, the correlation between urine volume and K intake was only moderate, compared to previous studies (>0.97) at our facilities (Eriksson, 2011; Eriksson & Rustas, 2014). It is possible that

there was a larger variation than expected in creatinine excretion/kg BW. The concept of a linear response in urine volume to K intake relies on the assumption that urinary K concentration (or probably urinary osmolality) has reached an asymptote so that concentration remains constant and volume increases (Kume et al., 2008; Eriksson & Rustas, 2014). Analysis of urinary K concentration would reveal if there is large variation among the samples that contribute to the moderate correlation.

Table 3 Correlations between different factors obtained from the exercise group

	Total DMI	Water intake	Urine volume	Milk yield	Total DM %	MJ ME	CP	UUN [1]	K
Water intake	*** 0.67								
Urine volume	*** 0.55	** 0.36							
Milk yield	*** 0.79	*** 0.60	*** 0.42						
Ration DM %	** 0.36	** 0.38	n.s 0.04	*** 0.69					
MJ ME	*** 1.00	*** 0.68	*** 0.54	*** 0.81	** 0.39				
CP intake	*** 0.99	*** 0.70	*** 0.55	*** 0.84	*** 0.45	*** 1.00			
UUN, g/d [1]	*** 0.70	** 0.37	*** 0.71	*** 0.55	*** 0.22	*** 0.69	*** 0.70		
K intake	*** 0.90	*** 0.55	*** 0.58	*** 0.52	n.s -0.03	*** 0.87	*** 0.86	*** 0.63	
Na intake	*** 0.88	*** 0.57	*** 0.50	*** 0.84	*** 0.58	*** 0.88	*** 0.90	*** 0.61	*** 0.72

[1]Urinary urea N.

Regression of DMI on drinking water intake and urine volume, respectively, yielded the following simple linear equations (Mixed models did not improve fit):

DMI (kg/d) = 0.167 drinking water intake + 8.66 (R^2 = 0.448) and

DMI (kg/d) = 0.459 urine volume + 11.83; (R^2 = 0.307)

Regression of K intake against urine volume gave the equations:

K intake (g/d) = 9.32 urine volume (l/d) + 255 (R^2 = 0.334) (simple linear model)

K intake (g/d) = 5.04 urine volume (l/d) + 367 (R^2 = 0.468) (Mixed model)

All these equations must be considered highly experiment-specific with their high intercept and moderate fit. They may not be directly applicable in other situations but should be able to give meaningful averages if applied to the pasture group in this experiment. Table 4 shows the estimated pasture intake when those equations and the equation derived from the relationship established in previous trials (Eriksson, 2011) are applied to data from the

pasture group. The overall mean was, for all estimation methods relatively, close to the 7.7 kg DM that the pasture group would have to cover with pasture to get a DMI equal to that of the exercise group. The overall estimate from water intake was lower than that from urine volume, which may be explained by the larger feed water intake with pasture than with silage. Because of this influence from feed DM concentration, the total water intake (from feed and drinking water) should preferably be modelled rather than only drinking water. The range of individual estimates and even a negative value may appear discouraging. However, Hellwing et al. (2015) compared different estimation methods under part-time grazing conditions and found group means spanning from -19.0 to 4.9 kg DM/d for one estimation method.

Table 4 Pasture intake (kg DM/d) estimated from water intake and urinary volume with regressions established on the exercise group with known intake. Estimates for sampling periods 1-3

Regression based on	P1	P2	P3	Overall mean	Min	Max
Water intake	6.62	5.65	7.09	6.46	0.56	10.42
Urine volume	8.04	7.96	6.83	7.61	-0.33	15.46
Urine volume and feed K concentration[1]	8.80	10.45	6.92	8.72	4.00	16.32
Urine volume and feed K concentration[2]	8.73	10.53	7.07	8.78	4.56	14.46
Urine volume and feed K concentration[3]	6.62	7.55	4.62	6.26	0.20	17.31

[1]Simple linear regression on exercise group data
[2]Mixed model regression on exercise group data with random intercept and slope for individual cows
[3]Simple linear regression from previous compilation of feeding trials (Eriksson, 2011)

Conclusions

Drinking water intake and creatinine-based urine volume estimates yielded reasonable results when used for estimating pasture intake on a group level within an experiment. The influence from differing feed water intake with different ration dry matter concentrations suggests that the total water intake including feed water should be taken into account rather than only the drinking water.

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Feed and animal factors related to subacute rumen acidosis

M. Oba
Department of Agricultural, Food and Nutritional Science, University of Alberta, 4-10J Agriculture/Forestry Ctr, Edmonton, AB, Canada T6G 2P5
Correspondence: moba@alberta.ca

Introduction

Subacute rumen acidosis (SARA) is a persistent problem in high producing dairy herds. The SARA should not be considered as a metabolic disorder that occurs just in the rumen. It negatively affects systemic health, productivity of animals, and profitability of dairy operations. We need to feed highly fermentable diets to meet energy requirements of high producing dairy cows although cows have not been evolutionally adapted to grains as the primary energy source. It is important to understand regulatory mechanisms of rumen pH and factors increasing the risk of SARA so that we can mitigate negative consequences caused by SARA. In this article, selected recent research, highlighting feed and animal factors related to SARA, will be reviewed.

Feed factors affecting SARA

Increasing dietary non-fibre carbohydrates (NFC) can increase energy intake of dairy cows, and result in greater milk production. Starch from grains is the primary NFC source in dairy diets, but excess starch fermentation often increases the risk of SARA. Sugar is another NFC source in dairy diets, and its effects on rumen pH are of current research interests. Although sugars ferment very quickly in the rumen, a recent review (Oba, 2011) showed that rumen pH sometime increased when dietary starch was substituted with sugar. In addition, a partial replacement of dietary starch with sugar often increased DMI and milk fat yield (Oba, 2011), which indicates that sugar fermentation might not cause SARA as easily as starch fermentation. Although its specific reasons are not identified, it may be related to greater butyrate production (Gao and Oba, 2016) or greater absorptive capacity (Chibisa et al., 2015; Oba et al., 2015) for animals adapted to a high-sugar diet.

Dairy diets are often formulated for specific level of chemical composition (i.e., starch or NDF), but at a given dietary starch or NDF content, diets greatly vary in their fermentability in the rumen depending on type of grain and its processing. For an example, starch fermentability varies among different type of grain and ranked as oats, wheat, barley, and corn from the fastest to slowest (Herrera-Saldana et al., 1990). Starch granules in the endosperm of corn grain are protected by a protein matrix of prolamin, and starch digestibility is negatively related with prolamin protein content in corn grain (Larson and Hoffman, 2008) while a similar relationship is not observed for barley grain (Oba et al., 2010) or expected for other cereal grains.

Dairy cows fed barley grain often exhibit a decreased DMI and milk yield compared with those fed corn grain (McCarthy et al., 1989; Overton et al., 1995; Silveira et al., 2007) while Grings et al. (1992) did not observe negative effects of replacing corn grain with barley grain. The discrepancy might be attributed to difference in fermentability of the basal experimental diets. McCarthy et al. (1989) and Overton et al. (1995) fed high grain diets containing grain at 47.0 and 44.3% on a DM basis, respectively, and Silveira et al. (2007) used barley silage as the primary forage in diets containing grain at 39.0% of dietary DM, and they reported lower rumen pH for cows fed barley grain. However, Grings et al. (1992) fed experimental diets

containing grain at less than 36% of dietary DM with alfalfa hay and alfalfa silage as the forage sources (less fermentable than corn silage or barley silage). These results indicate that feeding barley grain increases the risk of SARA and may decrease DMI and productivity of dairy cows if it causes excess fermentation in the rumen. When highly fermentable grains are used, it is necessary to pay more attention to fermentability of the forage sources as well as to the dietary inclusion rate of grain to mitigate problems related to SARA.

Grains also vary in their fermentability in the rumen depending on the extent of processing. The Processing Index (PI), expressed as the volume weight of grain after rolling relative to its volume weight before rolling, is an indicator of the extent of processing; smaller numbers indicate more extensive processing. Yang et al. (2000) fed barley grain, steam-rolled to coarse, medium, medium-flat and flat (PI = 81.0, 72.5, 64.0, and 55.5%, respectively) in high grain diets (42.5% of dietary DM), and reported that total tract starch digestibility and milk yield increased linearly as the PI decreased from 81.0 to 64.0%. However, further reduction in the PI from 64.0 to 55.5% (more processing) decreased DMI and milk yield, indicating that excess processing caused SARA and decreased productivity of the dairy cows. It should be noted that the extent of processing did not change chemical composition of the diets, but drastically changed fermentability in the rumen, affecting productivity of the cows.

Rumen pH indicates the H^+ concentration in rumen fluid, and greater fermentation increases H^+ production in the rumen. It is important to note that fermentation acid production is affected by type of grain and its processing extent even though diets look similar in their chemical composition. It is also important to note that H^+ concentration is affected by absorption and neutralization of fermentation acids as well (Allen, 1997). As such, dietary supply of physically effective fibre, sorting, cow comfort, and other unidentified animal factors also increases or decreases the risk of SARA. However, it is beyond the scope of this article to describe all feed factors related to SARA.

Animal factors affecting SARA

For the last several decades, many nutrition researchers have evaluated effects of management strategy to prevent SARA by manipulation of feed ingredients and diet formulation. However, the severity of acidosis greatly varies among animals even if they are fed similar diets. For an example, Gao and Oba (2014) reported that nadir rumen pH varied from 5.16 to 6.04 among 16 lactating cows fed the same diet. Schlau et al. (2012) evaluated acidosis index (area < pH 5.8 divided by DMI) for 17 steers fed a common high-grain diet, and selected 3 steers with the lowest (1.4 ± 1.2 pH·min/kg) and 3 with the highest values (23.9 ± 7.4 pH·min/kg) and noted as low- and high-risk animals, respectively. These six steers were force-fed a diet containing 85% grain at 60% of the expected daily intake within 30 min to induce SARA. Mean rumen pH over the postprandial 6-h period was higher (6.02 vs. 5.55), and mean total VFA concentration was lower (122 vs. 164 mM) for the low-risk compared with the high-risk steers. These differences might be attributed to different microbial activities as the low-risk steers had greater molar proportion of butyrate in the ruminal fluid. Alternatively, the variable responses in rumen pH among animals might be caused by differences in fermentation acid absorption; expression of a gene involved in absorption of fermentation acids (sodium hydrogen exchanger isoform 3) was higher for the low-risk steers (Schlau et al., 2012).

A similar variation exists in lactating dairy cows fed a high-grain diet; some cows experience SARA while others do not, even if they are fed the same diet. Macmillan et al. (unpublished)

compared feeding behaviour of 16 lactating dairy cows that varied in acidosis index, and reported that 7 high-risk cows (acidosis index: 8.03 ± 1.55 pH·min/kg) spent more time eating in the first 8-h period after feeding (186 vs. 153 min) and less in the third 8-h period (19 vs. 43 min) compared to 9 low-risk cows (acidosis index: 0.11 ± 1.37 pH·min/kg). In a follow-up study (Macmillan et al., unpublished), cows were fed TMR once (08:00 h) or three times (08:00, 15:00, and 22:00 h) daily in a crossover design to evaluate effects of feed delivery frequency on feeding behaviour and rumen pH. Frequent feeding (3× per day) reduced eating time between 08:00 and 15:00 h (99 vs. 145 min), immediately after the first feed delivery, and increased eating time between 22:00 and 08:00 h (76 vs. 43 min) regardless of SARA risk category. The severity of SARA (area < pH 5.8) was reduced by 3× feeding compared with 1× feeding (51 vs. 98 pH·min/d) for high-risk cows while it was not affected for lower-risk cows. These data show that the variation in the risk of developing SARA among animals fed the same diet is at least partly attributed to differences in feeding behaviour and that the risk for SARA can be mitigated by management approaches such as frequent feed delivery.

Zhang et al. (2013) reported that a restriction of feed intake to less than 50% (relative to ad libitum feed intake) for a 5-d period decreased rumen pH after end of the feed restriction period, compared with those restricted to 75%. They attributed this to a decreased absorption rate of fermentation acids across the reticulo-rumen caused by the feed restrictions. Their observation has important implications because cows often decrease feed intake for a short period of time due to reasons such as calving, milk fever, displaced abomasum, etc. It is important to note that those animals have greater risk of developing SARA compared with their herd mates even if all are fed the same diet.

Dohme et al. (2008) showed that cows that had experienced SARA previously were more prone to new episodes of SARA. They used an acidosis challenge model, feeding 4 kg ground barley/wheat grain after a 24-h feed restriction (50%) period to evaluate if the severity of acidosis changes with repeated challenges. Nadir rumen pH was 5.19, 5.07, and 4.90 and duration of SARA (pH < 5.8) was 12.2, 13.4, and 15.8 h/d for the first, second and third challenge, respectively (Dohme et al., 2008). Their observations suggest that a bout of acidosis that occurs for any reasons can exert long-term effects on animal health by increasing the risk of future episodes of SARA.

Conclusions

It is not possible to meet energy requirements of high producing dairy cows just from forages even if they are highly digestible, and we need to feed sufficient NFC to maximize productivity of animals. Non-fibre carbohydrates are highly fermentable in the rumen and increase the risk of SARA, but effects on rumen pH greatly vary, depending on type of NFC (starch or sugar), type of grain, and extent of processing. In addition, animals vary in the risk of developing SARA as a result of short-term feed intake depression, previous exposure to SARA, unidentified genetic or microbial factors, and any management factors affecting feeding behaviour. These factors need to be considered to develop management strategies to mitigate severity of SARA and its negative impacts on health and productivity of dairy cows.

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Is monitoring rumen pH a routine tool or a seasonal adjustment to new forage quality?

T.T.F. Mottram
Royal Agricultural University, Cirencester, GL7 6SJ, UK.
Correspondence: toby.mottram@rau.ac.uk

Abstract

This essay reviews field experience of using rumen pH telemetry and points out directions for engineering development in the future. Wireless rumen telemetry addresses directly a long standing problem in nutrient management of knowing what a cow has eaten by measuring pH which is directly affected by nutrient intake and bacterial activity. We are establishing reticulo-rumen pH of 5.8 as the threshold below which the cow should spend less than an as yet undefined percentage of time. My experience highlights two different uses of rumen pH data, one to correct an immediate nutritional problem detected by observation of other data and the second to continuously monitor a rotating subset of cows to provide early warning of deviation from a planned management target. Each method needs a slightly different approach to bolus design. Rumen pH boluses have shown a real benefit to farm management and have a great future.

Introduction

In 2003, I was approached by a major pharmaceutical company to develop a tool to help them monitor the efficacy of a new compound to raise rumen pH to prevent sub-acute rumen acidosis (SARA). Although the company withdrew this request when their compound license was blocked by the regulators, that call started the scientific and engineering adventure that has led me to be able to monitor cows for SARA all over the world. Prior to this invention, invasive methods were the only tool to diagnose SARA (Tajik and Nazifi, 2011) with the definition being extended periods of low rumen pH which was generally taken to be 5.5. Others have also developed boluses (Kahne Animal Health, Smaxtec, Well Cow) and there is a general acceptance in the industry that the bolus approach is a useful tool.

In 2003, we were told that no pH sensor could stay uncalibrated for more than a couple of days, that proteins would coat the probe, that cows would choke or spit out a bolus and that low power radio frequencies could not penetrate tissues. None of these predictions turned out to be true and although we have had to overcome many problems none were insurmountable. We can now routinely monitor rumen pH and temperature of dairy cows in field conditions and it is my opinion that we should immediately outlaw rumenocentesis and oro-rumen sampling as inaccurate, and dangerous to the health and welfare of cows. All veterinarians should use rumen telemetry to diagnose SARA as a routine investigation tool. After only five years since the first commercial system became available we now know that the previous methods often failed to identify the real reasons why some herds have poor performance when rationing appears to be correct.

Description of rumen telemetry

The bolus that I invented is now the eBolus from eCow Ltd. The bolus is 125 mm long by 27 mm diameter weighing 200 g. The sensor end is made of stainless steel which inverts the bolus into a normally sensor down position in cows with a normal shaped reticulum. The electronics are encapsulated with a cold poured resin coat that has proved resilient against rumen liquor in trials and obviates the need for vulnerable seals. The sensor is a combined electrode pH probe routinely used in applications in industry. The temperature probe is

embedded in the stainless steel end cap, which has machined holes to allow rumen liquor to flow past the sensor tangentially without permitting direct impact of stones or grit on the glass sensing bulb.

The density of the bolus (specific gravity greater than 2) allows it to remain in the reticulum for the life of the cow while data is collected wirelessly. The bolus measures pH and temperature every 60 s and takes an average value every 15 minutes and stores up to 2700 lines of data in a text file format of date, time, pH, temp, battery V. If data is not collected within 28 days, the file on the bolus is overwritten starting from the beginning.

The boluses are administered to the cow by mouth with a standard bolling gun, the only restriction on operation is that a period of 2 hours should be allowed before reading to allow it to migrate to the reticulum. The bolus has a temperature switch which causes it only to activate when the temperature is above 31°C, to extend shelf life. The device is calibrated at the factory before use and the calibration is accurate for four weeks in normal storage. The radio frequency used is in the 433 MHz ISM band. We have used the higher frequency of 868-926 MHz but propagation is better through tissue at lower frequencies.

Data is collected wirelessly using an antenna connected to an adapted mobile phone via micro USB. The user collects the data with the adapted mobile phone handset by standing near the cow, usually on the left front side. Fixed antennas can be installed in milking parlours or other loafing areas to collect data without human presence, which is required for routine management. However, due to the attenuation of the radio signal by tissues of other cows around the target, this is not always reliable.

The customer normally administers 3 boluses to 3 normal healthy cows for a feeding group of 100 animals. Cows in the ante-partum period are often used for monitoring through the transition phase and early lactation. Our data is available for comparative analysis on the eCow website where users can compare data between and within their herd.

The accurate life of the bolus is determined by the sensor (batteries can last for years) which becomes contaminated by rumen liquor in a non-linear manner at some time after 90 days. We anticipate that the life of the sensor can be extended by a few weeks but it is extremely unlikely that it can extend to the life of the cow. The main focus of engineering developments is to increase reliability and extend the radio range while making the bolus smaller and cheaper.

Sampling strategies to maintain continuity of representative data from a herd can be achieved by putting boluses into a rotating group of cows every 3-5 months. We anticipate that systems that meet this specification will become common over the next few years.

Results from research herds

The initial requirement for rumen telemetry was to replace the heavy tethered systems with which fistulated cows were monitored. The majority of eBolus sales have been to research scientists needing accurate data and ease of recalibration between experimental phases. Whilst fistulated animals have provided invaluable data to advance the science, they are inherently limited in scale and pose a high health risk to the animal. Various studies (Gasteiner et al. 2010, Mottram 2016) have shown that the reticulum is less dynamic than the ventral sac but that for practical purposes we can use an offset of +0.25 pH units for the reticulum to estimate the ventral sac pH value. This conclusion needs testing in field conditions as other authors (Sato, 2015) propose an offset of 1 pH unit. This is only of

importance when comparing prior publications which used 5.5 pH in the ventral sac as the threshold for SARA. In future, a reticulum threshold value of 5.8 will probably displace the previous values which can only be monitored by invasive procedures. We monitor data coming from the research users when they share this data so that we can help them manage the life of their boluses. We tend to see well-regulated pHs with meals offered at the same time every day and pHs which rarely extend outside a range of 5.8 to 6.5.

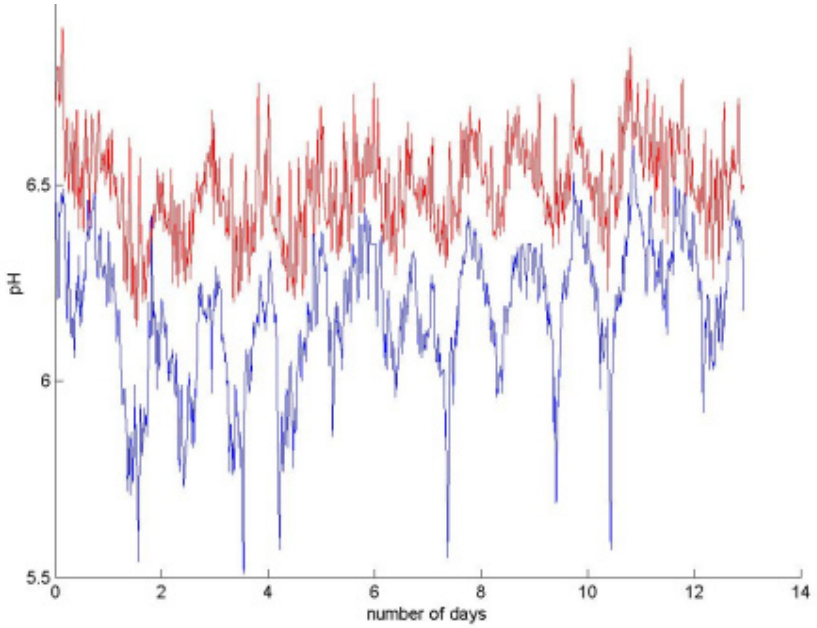

Figure 1 The difference between reticulo-rumen (red) and ventral sac pH (blue) can for herd management purposes be regarded as 0.25 pH units although there are dynamic differences

Results from commercial herds

The data we see from commercial operations is much more varied with wide ranges of pH both daily and seasonally. The mean rumen pH does not vary much across a range of mean annual milk yields of 7-12000 l (Mottram, 2015) and husbandry system has a far greater effect on rumen pH parameters. What the rumen pH data and clinical analysis has shown is that the daily routines to which cows are subjected have a major effect on cow health. The rumen pH data answers the question as to what the cow is eating and when she is eating and ruminating.

In grazed animals, we have seen huge disruptions to rumen pH profiles caused by weather effects and grass variety. In the unstructured environment of the grazing herd, it is more difficult to maintain a stable range of pH and some investment in research is needed to develop management strategies if the societal drive to encourage grazing becomes mandatory. Modern dairy cows may not be suited to the "traditional" systems favoured by consumer groups.

A major benefit of continuous monitoring of rumen pH is that it highlights differences among batches of feed and the immediate effect that a sudden change of feed has on rumen pH. In well managed herds with stable feed supplies and management not pushing for high levels of

milk output, rumen pH profiles are of great value for establishing a base line but are probably not essential for routine monitoring.

The conclusion of Atkinson (2013) from a veterinary field study was that 30% of cows in the UK suffer from SARA and our challenge has recently been to see how that matches our large data set of rumen pH data. If we use an offset of 0.25 pH units, we also find that about 31% of the time, the cows (4000 cow days) have reticulum pH values below 5.8.

Discussion

After five years of making and shipping boluses, I am now of the opinion that rumen pH is a vital indicator of nutrient management in commercial dairy herds. It can be deployed in two ways.

1. A short term veterinary/nutritionist intervention to identify a problem in nutrient management and to observe the effect of correcting an imbalance in feeding. This is particularly important where mycotoxicosis, which has the same clinical symptoms as SARA, is suspected. Most herds will correct the problem and not see a recurrence for months or years, usually when there is a change in bulk forage or grazing area.
2. A continuous monitoring tool to manage constantly changing food sources particularly for herds pushing for high milk yields or precise milk solids targets and using variable quality nutrients (grass and by-products).

These two different strategies require adapting our base technology. I can foresee a need for cheaper short life boluses that can be applied immediately by a veterinarian diagnosing a problem and using a mobile phone to collect data. For the second scenario, we need longer life boluses and a fixed infrastructure to continuously download data throughout the year. Both of these approaches pose engineering challenges for us in the future. Wireless rumen telemetry has shown up the limitations of our previous techniques to detect and manage SARA and has given us a new tool to improve dairy cow nutritional management.

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The difficulties of diagnosing SARA

A. M. Danscher
Boehringer Ingelheim Vetmedica, Strødamsvej 52, 2100 København Ø, Danmark.
Correspondence: anne_mette.danscher@boehringer-ingelheim.com

Introduction

Subacute ruminal acidosis (SARA) has been suggested to be one of the most important problems in modern dairy and feedlot production.

SARA evolves around the fact that cows in intensive dairy production have to eat large amounts of energy dense feed to reach the desired milk yield. When the rumen microbes convert carbohydrate to organic acids at a rate that exceeds the rumen's absorptive and buffering capacity, the pH of the rumen content declines. This leads to a change in the rumen microbial population, causing less effective digestion of fibre and conversion of energy. SARA is also related to decrease in feed intake and milk fat production, inflammation, lowered body condition, liver abscesses, and laminitis related claw lesions which in turn may cause lameness with further negative impact on production and welfare (Plaizier et al., 2008).

The prevalence of SARA in dairy cows ranges between 11% and 26 %. Economic losses due to reduced production alone have been estimated to 400 US$ per lactation (Plaizier et al., 2008).

This indicates that SARA does indeed impact both production economy and animal welfare. However, a very basic problem in studying the pathophysiology and consequences, clinically as well as economically, is the lack of an exact, practical applicable and clinically relevant tool for diagnosis of the condition.

SARA and rumen fluid pH

The SARA diagnosis has previously been based primarily on pH measurements of rumen content, either as point or continuous measurements.

So far the most concrete definition of SARA has been a rumen pH depression below a specified threshold for a specified time period, for example below 5.6 for >3 h/day (Gozho et al., 2005). This definition was developed for research purposes and it is not clear whether it is representative for cases in the field. Obviously, this diagnostic method requires continuous measurement of ruminal pH, via indwelling sensors. This equipment is quite expensive and still most useful for research purposes even though cheaper versions for herd use is being developed. In rumen fistulated animals it is possible to ensure that the sensor is in the desired position (preferably in the ventral sack of the rumen), whereas in intact animals the sensor is administered per os and will in most instances be situated in the reticulum. We found that the pH in the reticulum cannot be taken as direct indicator for rumen pH, as the fluctuations in the reticulum are smaller than in the rumen, at least during experimentally induced SARA. This is in agreement with the results of Falk et al. (2016).

In other studies, single point measurements of rumen pH have been used to define the presence or absence of SARA, and the pH thresholds varies between studies from 5.5 to 6.0 (Plaizier et al., 2008). Rumen content samples are typically retrieved via ruminal intubation or transcutaneous ruminocentesis and both timing of measurements and sampling method implies fundamental challenges. The large diurnal fluctuations in rumen pH related to feeding management and behaviour, as well as variations in pH between different locations in the

rumen and reticulum renders point measurements less accurate and less sensitive for the diagnosis of a longer lasting pH depression indicative of SARA. On the practical side, intubation is an invasive procedure, laborious for the farmer, unpleasant for the cow and the sampling location in the reticulo-rumen is unknown. Ruminocentesis is invasive, though in another way, and comprises a certain, although empirically quite low, risk of infection. None of these methods lends themselves to large-scale herd surveillance.

Additionally, there is reasonable doubt that low ruminal pH alone by definition constitutes a problem for the cow, or if other factors have to be present at the same time to elicit the development of SARA-related signs such as reduced feed intake and inflammation. Prediction of SARA is further complicated by a sizeable individual variation in susceptibility towards low rumen pH. This might be caused by individual differences in genetics, gut micro flora, feeding behavior, capacity for absorption of VFA, and possibly other factors as well (Plaizier et al., 2008). Studies have shown that low rumen pH induced by feeding pelleted, ground alfalfa reduced milk yield and milk fat production but did not induce systemic inflammation and translocation of lipopolysaccharide (LPS, endotoxin), whereas the same level of low pH induced by grain did result in inflammation and translocation (Khafipour et al., 2009). Cows fed only on pasture have been shown to experience low ruminal pH, altered faecal consistency and low milk fat content, but SARA-related signs are not, to our knowledge, commonly reported as a large problem in pasture based production systems. Of cause, this cannot be taken as a proof that SARA problems never develop on pasture. It can, however, be speculated whether the higher level of milk yield, the fact that the feeding is predominantly starch-based, or other factors typical for the TMR based systems in some way interacts with the low ruminal pH to trigger the development of SARA signs more often in this system. It is also very possible that hind gut acidosis, due to spill over of easily fermentable carbohydrate from the rumen to the intestines, plays a key role and that the main problem might not be in the rumen at all. Either way, there is a strong indication that depression of the ruminal pH is just one of several factors involved in development of SARA-related signs and that it is probably not sufficient to use ruminal pH as the only indicator of SARA and ruminal health. Furthermore, it has not yet been clarified if low rumen pH is required, or if SARA related problems can arise even at pH values, we would consider being within normal ranges

Supporting evidence

In practice as well as in research, the SARA "diagnosis" has been supported by clinical observations such as reversible reduced feed intake, decreased ruminal contractions and faecal consistency, and mild depression. These are all signs that are physiologically directly linked to a poor-functioning digestive system but, from a diagnostic point of view, they are also discouraging non-specific.

Alterations inside the rumen

With the emerging of advanced laboratory techniques, such as genomic sequencing, it has become possible to perform detailed investigations of the total microbial flora, the microbiome, of the rumen – and any other compartments of the gastrointestinal canal – under a variety of different conditions. Our studies have, not surprisingly, shown that induction of low rumen pH induces a shift in the microbial population of both rumen and other GI compartments, and we were able to classify and name which groups of microbes were affected to different degrees (Danscher et al., 2014). Studies of the dauntingly complex

microbiology of the GI canal are important and fascinating endeavours and there is a world of knowledge to be uncovered with these techniques, not least in the investigation of the pathophysiology and pathogenesis of SARA. It is, however, very easy to get lost in the myriads of things, we are able to measure and detect, and it is important not to loose track of what it means for the health of the animal.

So, for the sake of this argument, let's forget research and take the approach of a humble veterinarian whose main concern is the clinical health and wellbeing of the animal.

As vets, we are focused on whether the cow is affected of what is going on inside its GI system. The pH may be low and the microbiome and metabolites different from what we perceive as normal, but as long as the body "surrounding the rumen", so to speak, is well functioning and the cow feels good, there might be a very wide range of microbiomes that works. The challenge is to identify the cows that are not functional and not feeling good. To distinguish between the SARA/low rumen pH that are clinically important and the one that are not. Not least in order to target SARA as a possible production problem on a larger scale.

With this in mind, as well as the considerable cost and labor still involved in applying these techniques, the focus will be on searching for SARA indicators outside the rumen.

The ideal indicator

Ideally, an indicator for SARA should be specific (catch only diseased), sensitive (catch all diseased), easy to sample (optimally milk which allows for automatic sampling) and cheap. And optimally, an indicator should be able to detect SARA at an early stage – so the farmer has a chance to act and correct before the cow and production suffers significantly.

Inflammation

Several studies have shown that SARA induced with grain induces systemic inflammation, measured as increased levels of acute phase proteins, as well as localized inflammation in the rumen papillae (Plaizier et al., 2008). Systemic inflammation is a strong indication that the cow's health is affected by the low rumen pH, but as mentioned earlier, it has been shown that feeding alfalfa pellets did not result in systemic inflammation despite the same low level of rumen pH as obtained by feeding grain (Khafipour et al., 2009). Additionally, inflammation is a universal biological reaction which can be caused by many other conditions apart from SARA/low rumen pH, which limits its use as a viable indicator for SARA in the field.

Fecal and urinary pH

Feacal pH has been suggested as an indicator for SARA as high levels of grain feeding can result in carbohydrates bypassing the rumen with subsequent increased fermentation and VFA production in the hindgut causing decreased faecal pH. Results from studies on this are however conflicting. In a study conducted by Danscher et al (2015) the cows that received SARA diet had a lower faecal pH than the control group and Morgante et al. (2009) observed lower faecal pH in herds with an average ruminal pH below 5.8, but Li et al. (2012) showed no effect of SARA induction on faecal pH, and Enemark et al. (2004) concluded that faecal pH was a poor predictor of ruminal pH.

Urinary pH could be considered as an indicator for SARA as decreased dietary cation-anion difference (DCAD) in the SARA diet or increased acid load on the blood bicarbonate buffer system would both result in increased acid secretion by the kidneys. Studies have

nevertheless showed contradicting results. Danscher et al (2015) found that cows on SARA diet had lower urine pH than control cows. In herd studies, Gianesella et al. (2010) also observed lower pH in cows with ruminal pH below 5.5, but Morgante et al. (2009) found no difference in urinary pH between herds with average ruminal pH above and below 5.8, and Enemark et al. (2004) concluded that urinary pH was not suitable for predicting low ruminal pH. In an experimental study by Li et al. (2012), where SARA was induced by pelleted ground alfalfa, urinary pH was increased.

Conclusively, some studies find an association between SARA challenge and decreased fecal and urinary pH, but the decreases are typically small and results conflicts between studies. Serial measurements are not practical in the field, and the diurnal fluctuations in faecal and urinary pH, which reflect physiological variations in feed intake, transit time, fermentation patterns, absorption of metabolites and pH in rumen and hindgut during the day, challenges the use of daily spot samples as indicators of SARA in the herds.

Blood biochemical parameters

Several blood parameters have been investigated as possible SARA indicators. Danscher et al (2015) found slightly decreased ionized blood calcium concentration in SARA-challenged cows compared to control cows, whereas other studies found no difference in blood calcium (Gianesella et al., 2010; Li et al., 2012). Most studies found no difference in packed cell volume (PCV) between diet groups (Brown et al, 2000; Danscher et al., 2015; Ceroni et al., 2012; Gianesella et al., 2010; Li et al., 2012), no or minimal difference in blood pH between groups (Brown et al., 2000; Danscher et al 2015; Goad et al., 1998; Li et al., 2012), where as some studies find an association between SARA and increased blood pCO_2 (Gianesella et al., 2010; Li et al., 2012; Morgante et al., 2009). Even though some of these measures may be helpful to diagnose the SARA syndrome when serial measurements are conducted, results should be used carefully, as the parameters did not change consistently, changes were small, and most parameters remained within normal ranges.

Milk composition

SARA have long been associated with decreases in milk fat content, and also milk fat to milk protein ratio (Plaizier et al., 2008) as well as alterations in the biohydrogenation of unsaturated fat in the rumen and the profile of unsaturated and odd and branch chain fatty acids in the milk fat have been linked to low rumen pH (Colmann et al., 2013). Milk fat depression, however, does not seem to be consistently present in experimentally induced SARA, perhaps due to shorter bouts of low rumen pH. Milk fat and protein can be measured automatically in milk surveillance systems and as such fulfil some of the requirements for a realistic SARA indicator. Nevertheless, the strength of the association to rumen pH and the specificity as a SARA indicator still needs to be investigated.

Proteomics – the new frontier

The science of proteomics is about mapping all proteins in a sample – no matter what form or function. In our search for indicators for SARA, we have used proteomics to look for proteomic markers – one or several proteins that are present in substantially higher quantities in SARA affected cows than in healthy cows or vice versa. Scientifically, the problem in proteomic research as well as in genomic sequencing, is that we do not necessarily know what the result means – what are the functions of the proteins and how they interact with each other. But from the perspective of looking for markers of disease, just identifying a

substance, which is consistently and significantly increased in all cases, would be beneficial – even if we may not yet know the function of the specific proteomic fraction. Li et al. (2015) identified 14 proteins that were present in significant different quantities in plasma from control and SARA periods of which 9 were potential differentially expressed (PDE) proteins LASP1, TMSB4, and the precursors of KNG1, APOE, SERPINA3–8, CRP, SERPINF2, SERPIND1 and CATHL4 reportedly involved in acute phase response, cytoskeletal proteins, blood coagulation, cholesterol transport, liver disease, and innate immune defence molecules. These results suggest that it may be possible to identify proteins that could be used as markers for SARA, and further studies will validate possible candidates. Proteome analyses are, however, still not applicable for large-scale herd surveillance due to the cost and labour involved.

SARA - the "high-concentrate syndrome"

It has not proven easy to find good and reliable indicators for SARA. The problem with all indicators presented so far is that they are either non-specific, with small differences between normal and diseased, have diurnal fluctuations, require sampling from difficult places or advanced laboratory techniques to apply – or suffer from a combination of all of the above.

The difficulties probably reflect that SARA is not a well-defined diagnosis, but rather a syndrome, or a set of signs, associated with low ruminal pH and poor ruminal health. Very central to the problem is that specific pathognomonic signs have yet to be identified.

SARA may be considered simply a clinical phenotype related to high grain feeding. Probably there is a continuum between the well-functioning rumen and physiology of a healthy, high yielding dairy cow in full production and the ecological and physiological breakdown of a cow with full blown acute ruminal and systemic acidosis – with SARA or "high-concentrate syndrome" (Calsamiglia et al., 2012), positioned somewhere between theses extremes.

The complexity of such a continuum may best be described by a combination of known indicators, a risk index for compromised gut health. The main challenge in the development of such an index remains, still, the lack of a "gold standard" for the diagnosis of the SARA syndrome.

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Sveriges Lantbruksuniversitet
Institutionen för husdjurens utfodring och vård
Box 7024
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Margareta.Norinder@slu.se