Proceedings of the 3rd Nordic Feed Science Conference, Uppsala, Sweden

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

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Foreword

The 1st Nordic Feed Science Conference took place in 2010 and received a positive response by the participants. In 2011, it was arranged parallel to the 24th NJF Congress and attracted a somewhat smaller number of participants. This year, we decided to arrange the 3rd conference immediately prior to the XVI International Silage Conference in Finland on July 2-4. The idea was, that overseas visitors would have the opportunity to attend both conferences and spend the weekend in between sightseeing both in Stockholm and Helsinki. This idea seems to have worked out as a number of visitors from as far away as China, Ghana and Congo have registered as participants and also submitted papers to the conference.

It has become apparent that conflicts with other conferences will occur every year. Therefore, the NFSC will have to adapt its theme, not to overlap with these conferences. This year we have therefore modified our program to exclude focus on silage not to overlap with the International Silage Conference.

The contributors of the 2012 NFSC has created a particular focus on forage agronomy, methodology and ruminant metabolism. In the evening session, we are particularly hoping for a good debate on harvest frequency and its effect on forage quality and quantity.

Uppsala 2012-06-13

Peter Udén

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Information fusion of automated recordings and other sources for assessment of dairy cow nutritional status -- transfer of computing principles currently applied in precision crop production

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Introduction

This paper/talk will give a brief input into the research field of Information Fusion. It will describe what anomaly detection and uncertainty management are and exemplify how these kinds of information fusion could help in better understanding the nutrional status of dairy cows.

Information Fusion

Information fusion (Boström et al. 2007) is the synergistic integration of information from different sources about the behavior of a particular system, to ***support decisions and actions*** relating to the system. Information fusion includes theory, techniques and tools for exploiting the synergy in the information acquired from multiple sources, for example sensors observing system behavior, databases storing knowledge about previous behavior, simulations predicting future behavior and information gathered by humans. The resulting decision or action is in some sense better, qualitatively or quantitatively, in terms of accuracy, robustness and comprehensibility, than would be possible if any of these sources were used individually without synergetic exploitation. Note that information fusion not only deals with actual fusion processes, but also with how the results of these processes can be used to improve decision-making. It also involves preprocessing of data to make it ”fusable” and the control of the fusion process algorithms and information sources to acquire additional information.

Anomaly detection

Anomaly detection (Chandola et al.) is one area within information fusion where the aim is to discover the unexpected. When the system is hard to model due to large data volumes, the models are vague or non-existing, but we still want to be able to make well-founded decisions. In Figure 1, we show how anomalies can be described in a set of possible observational data. The approach is to train the system by using recorded historical sensor data to form a notion of normality based on the principle that “*most things are considered normal*”. To this normality notion, **N**, we also describe any known problems that can occur, **P1 – P4**. Giving us a system where our knowledge is described by the smaller circles. If anything is detected by the system to be outside of the known situations we classify this as an anomaly, **A**.

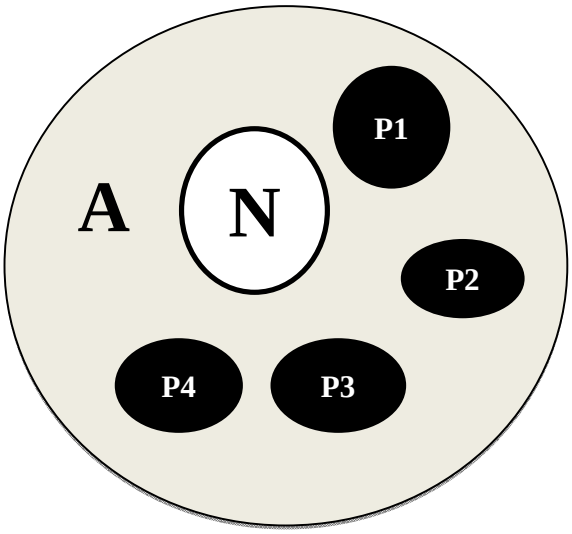

Figure 1 Anomaly detection

In an experiment, where pigs were fitted with radio-frequency identification (RFID) tags to personalize feeding, showed that anomalies occurred due to the fact that some pigs were able to remove their RFID tags (rolling in the dirt) and other pigs picked up these RFID tags and got a double dose of food. The event was anomaly as the system did not consider two RFID tags at the feeding station at the same time. The designers of the feeding system could not anticipate this event.

Uncertainty management

Uncertainty management deals with the notion that data (sensor reading, database knowledge, computations, etc.) has a varying degree of certainty (or uncertainty). Now when we are combining data from different sources with varying certainty, what is the resulting decision support? How much can we rely on our result? Simple problems with faulty or low precision sensors may influence the final decision if care is not taken with how the fusion process deals with the inherent uncertainty.

One of the pillars in Information fusion is to construct "abstract sensors" which is a combination of sensors and other abstract sensors into a new (abstract) sensor with different properties than the constituting sensors. The goal for this is to capitalize on the notion of synergies. For example, if we use one sensor (or even multiple sensors of the same type) helping us to decide a cow's condition regarding heat, we could reach a boarder-line situation. However, if we have multiple sensors of different types that together form an "abstract sensor" (see Figure 2). This can help us decide if a cow is in heat and we can achieve a much better precision and have lowered the uncertainty of sensor reading. Such a sensor is also much more robust since it may be able to single out faulty sensors.

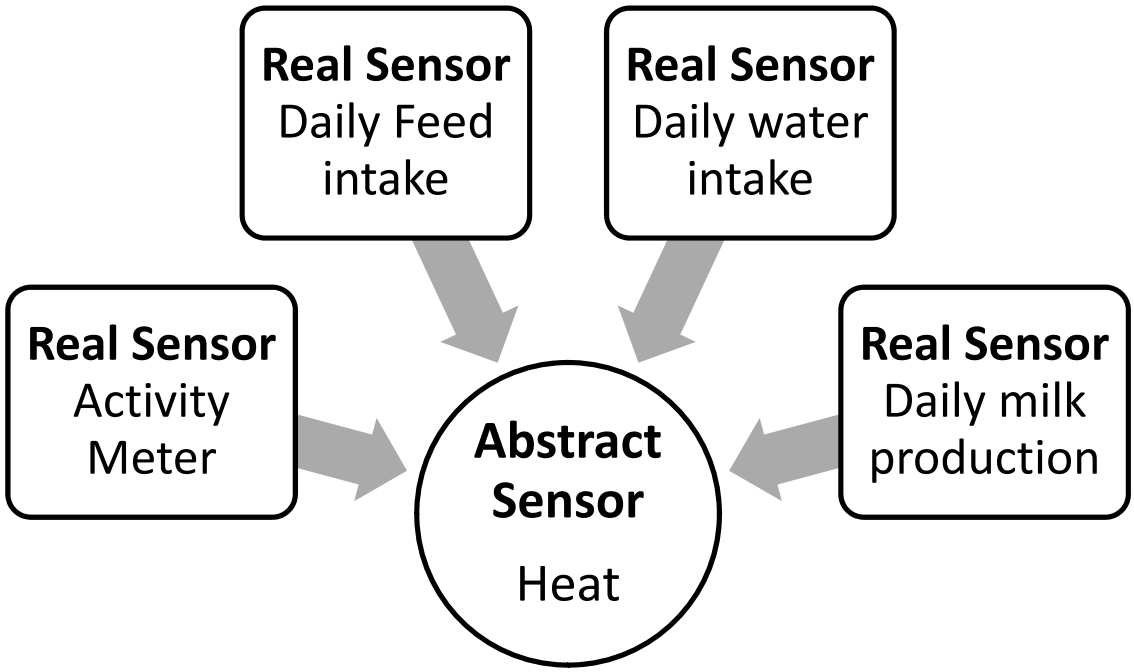

Figure 2 Example of "Abstract sensor"

Also, if we store the individual uncertainties, we can give indications to the farmer on which basis the decision to indicate "cow in heat" was shown, i.e., we can get a trace on which factors that led to the triggering of the abstract sensor, preferably, with known uncertainty.

Conclusions

By applying the two Information fusion techniques anomaly detection and uncertainty management, we can improve the robustness of our sensor system as well as find previously unknown combinations of sensor data to help us identify useful situations with respect to cow health and milk production.

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An overview of laboratory ring tests

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Introduction

Proficiency testing is an important tool for routine laboratories in their attempts to avoid systematic errors or bias in the results that they produce and deliver. In many cases such tests are the only means available for this task. Proficiency tests fall into a category of inter-laboratory comparisons along with collaborative tests and certification studies.

In inter-laboratory comparisons, identical portions of one or more test samples are dispatched to several laboratories for the determination of a certain analyte or analytes. In a collaborative test, an analytical method is under investigation and the participating laboratories are requested to follow the prescribed procedure in every detail. If the results fulfil a pre-defined set of statistical requirements, the method is published as a standard or reference method. A certification study is a vital step in the establishment of new reference materials, where the accurate content of one or more analytes is ascertained by determinations at several different laboratories. In order to exclude method-specific bias, one seeks participants (laboratories) mastering different analytical techniques. In the third category are proficiency tests which are the main topic of the present paper. They are distinguished from the other two categories in being a service to the individual laboratories rather than for the analytical community at large. Proficiency tests are arranged to compare the analytical performance of the participating laboratories against that of the other participants or sometimes against some predefined quality measures. Here, there is usually no obligation regarding what methodology is used, since the main issue is to test the performance of the procedures that are in routine use at each laboratory. The main purpose of a proficiency test is to aid the participating laboratories in locating systematic errors, which is often hard to achieve by other means. All aspects of proficiency testing of chemical determinations are thoroughly treated in the monograph by Lawn et al. (1997).

The three above mentioned categories of inter-laboratory comparisons, along with the therein produced reference methods and materials, respectively, form the backbone of what is termed external quality control of chemical analysis. Here, the main issue is trueness *i.e.* to reduce the magnitude of laboratory bias. The term inter-calibration, which is sometimes used for different types of inter-laboratory comparisons, is ambiguous and should thus be avoided.

Many analytical methods are matrix sensitive. Therefore proficiency tests have the widest applicability of all the external quality assurance measures. A proficiency test can in principle be carried out for any sample and analyte for which there exist a documented analytical procedure and a sufficient number of laboratories willing/wanting to participate. There are, on the other hand, many routinely determined analytes for which there is no reference method available or for which available reference methods are not useful either due to lack of sensitivity or because they have not been tested for the sample matrix at hand. The same holds for certified reference materials. The range of sample matrices covered by available reference materials is limited and in some cases when a material is available, it does not contain the analyte you need to determine at an appropriate concentration level. However, when you have a relevant reference material

available, analysing it along with your unknown samples constitutes the best means for detecting systematic errors.

In the last few decades, the field of Quality Assurance has grown dramatically in importance within the framework of routine chemical analysis. Now, a routine laboratory is required to have a defined quality system with well documented routines for all aspects of the analytical process *i.e.* from the reception of a sample through the actual analytical determination to the reporting and filing of the obtained result. This quality system may be certified or accredited by a relevant external body or it may be defined and approved internally in a less formal way. This development has yielded its own set of parameters and concepts and it may be in order to have a brief look at some of these before proceeding with the actual topic of proficiency testing. The term *precision* refers to the closeness of agreement between independent test results obtained under stipulated conditions and good precision may be characterized by low standard deviation values. Stipulated conditions refer to repeated measurement of the same sample at the same laboratory. *Accuracy* is the closeness of agreement between the result of a (single) measurement and the true value of the measurand. *Trueness,* on the other hand, refers to the closeness of agreement between the average value obtained from a large series of test results and the true value of the measurand. The difference may be explained from Figure 1.

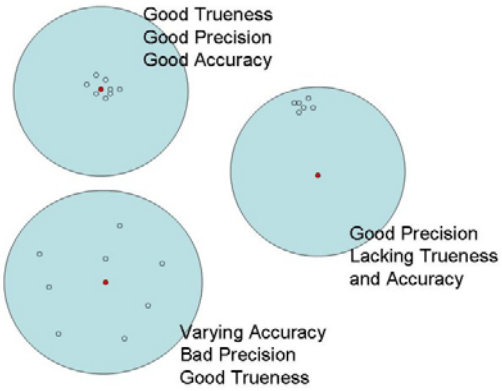

Figure 1 The difference between accuracy, trueness and precision.

Any laboratory strives towards the situation described with the top circle shown in Figure 1, but even a laboratory with good internal quality procedures may often come closer to the situation described by the circle to the right. The seemingly worst situation, depicted in the lowest circle is actually an improvement over the one on the right, since it is easier to improve precision than trueness. Note that the varying accuracy in the lowest circle is actually a result of the bad precision.

In analytical chemistry, accuracy, precision, trueness and errors in general are best quantified through the calculation of standard deviation values, assuming normally distributed errors. When certifying a reference material or collaboratively testing a method, ANOVA is used for dividing the observed variability between different results obtained for the same analyte and sample into within-lab and between-laboratory contributions. In essence, the internal quality assurance at a laboratory deals with the within-laboratory errors whereas the external quality assurance, hereunder proficiency tests, deals with between-lab errors.

In proficiency tests, the between-lab variation evaluated by calculation of so-called z-scores, in accordance with the Harmonized Protocol for Proficiency Testing adopted by IUPAC, ISO and the AOAC (Thompson, et al, 1993). The z-score is defined as follows:

$$z_i = (x_i - X)/s$$

z_i: z-score for laboratory i

x_i: result of laboratory i for a particular analyte and sample

X: assigned true value

s: between-lab standard deviation

Calculating a z-score is in essence a transformation of the reported results from a proficiency test. It yields the deviation of a single result from the accepted true value, quantified in number of standard deviations. For instance, a z-score of +2.6 for a particular measurement, this value has exceeded the true value by 2.6 standard deviations. The z-score is thus a normalized parameter applicable to any type of quantitative analysis. According to WELAC, z-scores values should be interpreted as follows:

- All results producing $|z|<2$ are acceptable
- A result is suspect if $|z| \geq 2$ (=> outside 95% confidence limits)
- A result is an outlier if $|z| \geq 3$ (=> outside 99% confidence limits)
- Several results from one proficiency test with $|z| \geq 2$ constitute a failure of the test
- Same holds for $|z| \geq 2$ for same parameter in repeated tests

For the z-score to be a useful estimator, it is absolutely necessary to pre-treat the data with some sort of an outlier procedure. In general, one is cautioned against using outlier tests in the statistical treatment of experimental data. The reason for this is that a faulty rejection of a suspected outlier may often “improve” your results in the sense that factors or experimental variables may become significant. This may therefore be tempting and it reminds us that there is a reason we’ve been taught to resist temptation. In proficiency testing we are in a way concentrating on outliers. We want to locate outliers for two reasons. Firstly, we want to detect results which are outside the mainstream in order to advice the laboratory in question to look for systematic errors. Secondly and more importantly, we want our estimate to be sensitive and robust. By discarding outlying results, before we calculate our z-scores, we obtain a more strict reference (larger z-scores). A single result grossly in error will numerically reduce the z-score values obtained by all other participants (by producing a larger value for s). Thus, other outlying results may go undetected. On the other hand, should we wrongly discard a result through failed outlier detection, the consequence is by no means as serious as the above mentioned general practice of statistics. In our case, a laboratory will be cautioned to go through its routines in the search for bias. This is hardly a catastrophe, especially not when considering that the result in question would have produced a relatively high z-score anyway.

The North European Proficiency Test (NEPT) scheme for feed stuffs analysis

The frequency is two rounds per year, alternatively dispatching two and three feed samples, totalling five samples per annum. Each round has a compound feed and a roughage sample,

respectively, but the spring round has in addition one raw material. A total of twenty nine analytes can be reported, with no constraints on the analytical methodology used. Participation is open to all, against the payment of a fee. At present, there are about twenty five participating laboratories from all five Nordic countries, Latvia and the Netherlands. Performance is evaluated by calculation of z-scores. Z-score calculations are based on participant consensus. All communication, short of sample dispatch, is by electronic mail. Furthermore, for the z-score calculations, a special outlier test has been designed, based on the z-score itself.

Participating laboratories are requested to perform at least two measurements for each analyte. For this proficiency test, a new parameter, the d-score, has been defined to evaluate individual laboratories with respect to within-laboratory variation, *i.e.*, precision. The d-score is a standardized parameter, analogous to the z-score, which expresses the distance between duplicate results in units of standard deviations. A d-score can be used to compare within-laboratory precision against the other laboratories in much the same way as z-scores are used to compare average results.

A proficiency test is first and foremost a service for the laboratory to help its analysts to locate systematic errors. These same analysts have a tendency to regard this service as an external check or exam. This may evoke a temptation to cheat on this exam and indeed, in early proficiency test, steps were taken to reveal or deal with participants cheating. This idea of being checked or spied on, may totally ruin the benefits from participating in a proficiency test. To counteract this, it is very important to uphold strict confidentiality, regarding the identity of the individual laboratory when results from a proficiency test are reported. In the present test, only the organizer has detailed knowledge of who is responsible for individual results in the test reports and who is getting unsatisfactory z-scores, etc.

If a participant fully realizes the role of the test, it will become evident that a bad z-score is in fact a good thing. The proficiency test did not produce this bad score, it has merely uncovered a problem which might otherwise have been discovered by some part outside the laboratory or even worse, it might not have been discovered at all. The situation is somewhat similar to being stopped from boarding an airplane, after that an inspection has revealed faulty landing gear. You may be annoyed to have to postpone your trip, but you should be glad that the fault was discovered and you would definitely not want to mislead those carrying out the inspection.

As the participants themselves are responsible for data entry, an extra parameter, a pseudo-analyte, if you like, called “reporting” has been included in this proficiency test scheme. This is done in order to make such data transfer errors a test object for the proficiency test. Such errors can be located in obvious cases, such as when dry matter has been reported instead of the water content or protein instead of nitrogen. When such errors are discovered, the corresponding results are corrected by the organizer and the participant in question is given a mark for a reporting error in a table summarizing outlying z-scores. Such results would otherwise be discarded as outliers and the basis for calculating the scores would be correspondingly poorer. Table 1 shows recent proficiency test result for soluble crude protein (sCP). This is an analyte that was introduced through the initiative of NorFor and there was some confusion amongst our participants as to what unit to use. After a few rounds of e-mail correspondence, results reported for this analyte could be converted to a common basis. Both sets of results are shown in the table as well as the corresponding z-scores. The table illustrates two important points. Firstly, we can clearly see the importance of correct reporting of results. The original sCP values seem to indicate that the analytical methodology used for this parameter is rather useless, whereas after

conversion, the agreement between laboratories is quite good. Secondly, the table illustrates the problem of masking in outlier testing. Outlier procedures are not sensitive when there is more than one outlier on the same side. Herein Table 1, there are two suspiciously high or low results causing no outliers to be detected.

Table 1 Values for soluble crude protein (sCP), as reported by participants along with z-scores and corresponding values obtained by a conversion to a common basis

Lab. no.	sCP original g/kg	sCP original z-score	sCP converted g/kg	sCP converted z-score
5	7	-0,7	70	-0,19
6	64	-0,49	64	-1,47
7	71	-0,47	71	0,01
8	55	-0,53	71	-0,11
16	649	1,65	80	1,81
20	7	-0,7	74	0,47
22	540	1,25	69	-0,53

For some analytes, the number of reported results is too small to yield reliable estimates of the statistical parameters. In the present setup, no z-scores or d-scores are calculated for analytes in cases of less than 7 reports remain after outlier rejection. In such cases, the median is reported as it is a better (more robust) estimate of the true concentration than the average.

The author has sensed that many users of analytical results believe that they are more accurate and precise than they actually are. Here, results from proficiency tests may be of great help even if this benefit is not the reason for carrying out the tests. It may therefore be informative here, to take a look at some pooled results from several rounds of the NEPT/NJF test series, shown in Table 2.

Table 2 Relative standard deviation values (percentage) obtained for the last eight roughage samples in the NJF/NEPT proficiency test series, spanning the years 2006-2011

	Water	N (Kj.)	N (D.)	Ash	Fat (h.l.)	Fat (d.e.)	Crude fiber	ADF	NDF
MIN	6.7	1.3	1.8	0.9	13	7	1.8	2.3	3.2
MED	8.3	1.5	2.4	1.4	14.4	11.4	3.5	5.4	4
MAX	17.1	2.3	3.3	1.7	22.2	13	5.8	8.2	5.5
	Starch	P	Ca	Mg	K	Na	Cl	Cu	Zn
MIN	19	4.3	5.5	4.7	4.6	5	0.5	6.9	4.1
MED	64	6.9	10.1	6.9	6.6	12.4	1.9	15.9	20.6
MAX	106	8.6	17.1	10.3	8.6	57.7	3.1	30.8	29.0

Kj.: Keldahl; D.: Dumas; h.l.; hydrolysis; d.e.: direct extraction

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Nordic ringtest on iNDF and NDF contents of ten feed samples

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Introduction

Neutral Detergent Fibre (NDF) and its ruminally undegradable fraction indigestible NDF (iNDF) are key parameters for ruminant feed evaluation. Structural effects and intake potential of feeds have been estimated from the NDF content for decades (Mertens, 1997). With the advent of mechanistic feed evaluation models (Tylutki et al., 2008; Volden, 2011), organic matter digestibility has been replaced by iNDF as the most important measure of available energy in forages to ruminants.

Reliable analyses of NDF and iNDF are crucial considering their huge influence on feed value. The NDF analysis has been thoroughly standardized by Mertens et al. (2002), to include mandatory treatment with heat-stable α-amylase (for starch removal) and sodium sulphite (for protein removal) and to be reported on an ash-free basis as amylase-treated NDF organic matter (aNDFom). This protocol has been adapted in the Norfor system as the reference method (Åkerlind et al., 2011). The corresponding reference method for iNDF is presently a 288–h rumen *in situ* incubation in dry cows using small pore size nylon bags of 12 μm (Åkerlind et al., 2011). Prior to this standardization, a ring test was performed on research laboratories in the five Nordic countries with three samples (Lund et al., 2004). Coefficient of variation (CV) for NDF was then 1, 9 and 19% in grass silage, barley and rape seed meal, respectively, while corresponding CV for iNDF was 23, 18 and 25% on DM basis. Incubation length for iNDF in this ring test varied from 96 to 504 h and bag pore sizes from 17 to 37 μm. A 2006 ring test in the same laboratories, when standardization had been preliminary established, resulted in CV of 5-14% for iNDF in five forages (Norfor, unpublished). The objective of the ring test reported here was to perform a quality assurance of the Norfor reference method, including the possible effect of sample preparation, *e.g* different mills. The ring test was also offered to commercial laboratories analyzing farm samples for iNDF by Near Infrared Reflectance (NIR) or by *in vitro* methods.

Materials and Methods

Ten different samples, including six forage and four concentrate samples, were included in the ring test and sent out to the laboratories dried but not milled. The greatest care was taken when sub-sampling the material in order to maintain homogenity. Two of the samples ('Lucerne 2' and 'DDGS') were, in addition, sent out as replicates. These replicates had been milled on a cutter mill (Fritsch Pulverisette 15, Fritsch, Idar-Oberstein, Germany) to pass a 1.5-mm screen according to the Norfor standard for in situ incubations.

Six research laboratories and four commercial laboratories participated in the ring test. Only forage samples were originally sent to the commercial laboratories, but one laboratory also received concentrate samples on request. The laboratories were instructed to mill the samples according to their standard routines and determine aNDFom, iNDF and DM according to Norfor standards, but without applying DM corrections for volatiles.

The compiled results from the laboratories were tested for outliers (Grubbs, 1969). The results from the six research laboratories were then analysed for laboratory effects in a GLM model of SAS 9.2 (SAS Institute Inc., Cary, NC, USA) with laboratory and sample. Differences between laboratory means were tested with the Tukey option.

Table 1 Neutral detergent fibre (g/kg DM) determined with amylase and sodium sulphite on an ash-free basis (aNDFom[a]) in 10 feed samples by 10 different laboratories.

Lab nr[b]	Method and milling[c]	Maize 1	Maize 2	Grass 1	Grass 2	Lucerne 1	Lucerne 2	DDGS[d]	Barley	Rape-seed meal	Oat hulls	Lab mean[e]
1	F; H, 1.0	383	474	445	585	355	382	245	189	251	665	**397z**
2	O; H, 1.0	376	475	435	589	373	382	227	158	281	591	**389z**
3	A; K, 1.0	383	498	490	652	412	424	261	308	299	624	**435x**
4[a]	A; H, 1.5	391	490	447	601	392	416	251	174	308	627	**410xyz**[a]
5	A; K, 1.0	392	462	453	590	394	377	255	191	306	619	**404 yz**
6	F;H, 1.0	411	492	477	640	404	405	272	239	315	626	**428 xy**
7	A; H, 0.8	371	465	500	562	386	420					
8	A; C, 1.0	351	474	447	599	402	373					
8a	N; C, 1.0	366	447	441	593	415	385					
9	N;	402	448	449	591							
10	A; H, 1.0	365	459	425	540	383	386		173	289	602	
Sample mean		**381**	**471**	**455**	**595**	**392**	**395**	**252**	**204**	**293**	**622**	
Standard dev.		18	17	23	31	18	19	15	52	22	23	
CV[f]		5%	4%	5%	5%	5%	5%	6%	26%	7%	4%	

[a]Laboratory 4 reported values inclusive of ash; [b]Laboratories 1-6 are research laboratories and 7-10 are commercial laboratories; [c]NDF methods are: F= Fibertec type apparatus, O = Oven method (Chai & Udén, 1998), A = Ankom system, N = NIR; milling methods are: H = hammer mill, K = knife (cutter) mill, C = Cyclotec (abrasive) mill; numbers refers to screen apertures; [d]DDGS = distiller's dried grain solubles; [e]Only laboratories 1-6 are compared, values without a common letter differ at $P < 0.05$ for a Tukey test; [f]CV = coefficient of variation.

Results and discussion

There were nine laboratories determining NDF with wet chemistry and two with NIR (Table 1), because one laboratory reported results from both methods. Although the laboratories generally claimed to report results in agreement with the aNDFom determination (Mertens et al., 2002), they used different equipment and procedures: Ankom incubators, Fibertec type apparatus and the oven procedure of Chai & Udén (1998). One laboratory did not use sodium sulphite and compensated for that by deducting 13 g/kg DM from the obtained value while another laboratory used amylase only for starch-rich samples. There was also one laboratory that analysed aNDF inclusive of ash, but the results were still used in the compilation. Hammer mills, cutter mills and abrasive mills were used.

The standard deviation between laboratories was 15-31 g NDF/kg DM with a CV range of 4-7%, excluding barley which had a CV of 26%. For barley, the largest value was almost twice as high as the smallest. Still, none of the values were identified as outliers according to the Grubbs' test. While the outcome for barley was worse than in the pre-standardization ringtest of Lund et al. (2004), rapeseed meal NDF variation was lower in the present study. Laboratory means across samples differed at most by 39 g/kg DM ($P< 0.001$) with laboratories 1 and 2 being lowest and laboratory 3 highest. Laboratory 4, which reported aNDF inclusive of ash, was close to the overall mean. If an assumed residual ash content of 16 g/kg DM (Gustavsson and Martinsson, 2004) is deducted from the results of Laboratory 4, standard deviations across laboratories would not increase.

The NIR determinations of NDF reported for forage samples by two laboratories were at the most 24 g/kg DM (Z score 1.4) from the overall means, which must be considered acceptable.

The six research laboratories determined iNDF *in situ* in two or three cows by somewhat different procedures for handling bag residues, although queries on that were not included in the report form, and neither were details on the different in house bag washing methods. The iNDF protocol of Åkerlind et al. (2011) is detailed regarding sample preparation, bag pore size, donor cow ration composition, etc. and the laboratories were assumed to adhere to this protocol. Moderate deviations were reported in that one laboratory used bags with 17 μm (instead of 12 μm) bag pore openings and another laboratory used a cutter mill with 2.0 mm screen (instead of 1.5 mm) and a concentrate proportion of approx. half of the 0.33 stipulated in the protocol of Åkerlind et al. (2011). Decreasing concentrate proportion from 0.42 to 0.17 has recently been demonstrated to decrease iNDF by 8 g/kg DM across a range of feeds (Krizsan and Huhtanen, 2012). A third laboratory used a hammer mill instead of cutter mill and also reported iNDF inclusive of ash.

All the *in situ* laboratories produced highly correlated results ($r>0.97$). Laboratory 1 and 2 had lowest overall means, 28 g/kg DM less than laboratory 5 ($P< 0.001$, Table 2). Generally, the standard deviation among *in* situ laboratories was of a magnitude similar to the ringtest of Lund et al. (2004) (Table 2), in spite of the fact that the current ringtest also included variation in sample preparation. No result from the *in situ* laboratories was classified as an outlier, even if there was a relatively high CV for many samples. This was partly an effect of the generally higher level in Lab 5. The CV for all samples with low iNDF concentration (< 100 g/kg DM) except for barley was considerably reduced by omitting Laboratory 5. A similar CV reduction could also be achieved if the 'Lucerne 1' and 'DDGS' were replaced by the same samples sent out in their milled forms as iNDF values were reduced by 9 g/kg DM for both these samples at

Lab 5 (data not shown). This suggests that levels can be influenced by the use of a 2.0-mm screen at this laboratory. For the samples of higher iNDF concentration, the relative influence on CV of the higher level in Laboratory 5 was much less or absent. Since the oat hull sample provided by the feed industry had a rather fine structure already before preparation, milling effects may not have manifested itself in this sample. There was no general difference across laboratories between milled and unmilled samples (P = 0.30), which only should have occurred if the already milled samples represented one extreme of particle sizes. Laboratory 4 incubated the two grass samples milled on a hammer mill to pass a 1.0- or a 1.5-mm screen. The iNDF results were 5 and 10 g/kg DM lower for the 1.0- compared with the 1.5-mm screen for 'Grass 1' and 'Grass 2', respectively. If milling effects are mainly caused by differences in particle loss, a coarse milling would be more accurate.

Table 2 Indigestible NDF (iNDF, g/kg DM) in 10 feed samples determined by a 288-h *in situ* incubation, by NIR or by *in vitro* methods, both calibrated with reference samples from Laboratory 1-4. *In situ* results are means of two cows (Lab 1 three cows) presented with between-cow standard deviation.

Lab nr[a]	Method and milling[b]	Maize 1	Maize 2	Grass 1	Grass 2	Lucerne 1	Lucerne 2	DDGS[c]	Barley	Rape-seed meal	Oat hulls	Lab mean[d]
1	Is; K, 1.5	52 ± 1	73 ± 8	40 ± 1	197 ± 8	177 ± 4	199 ± 7	53 ± 1	37 ± 3	100 ± 6	263 ± 6	**119 y**
2	Is; K, 1.5	50 ± 4	70 ± 6	40 ± 0	177 ± 0	209 ± 4	192 ± 12	41 ± 2	32 ± 4	84 ± 4	292 ± 1	**119 y**
3	Is; K, 1.5	65 ± 1	96 ± 1	49 ± 3	206 ± 1	211 ± 11	221 ± 1	69 ± 1	45 ± 4	154 ± 2	263 ± 1	**138 y**
4[e]	Is; H, 1.5	51 ± 3	84 ± 7	44 ± 1	195 ± 4	192 ± 3	212 ± 0	52 ± 2	36 ± 0	158 ± 2	289 ± 0	**131 y**[e]
5	Is; K, 2.0	84 ± 14	121 ± 17	61 ± 3	246 ± 7	238 ± 6	235 ± 5	81 ± 14	32 ± 1	149 ± 0	325 ± 6	**157 x**
6	Is; H, 1.0	49 ± 1	79 ± 2	41 ± 1	215 ± 9	208 ± 2	204 ± 1	54 ± 1	37 ± 4	160 ± 0	325±20	**137 y**
Mean *in situ*		**58**	**88**	**46**	**208**	**209**	**210**	**60**	**36**	**129**	**294**	
SD *in situ*[f]		14	19	8	23	21	16	14	5	33	28	
CV *in situ*[g]		24%	21%	18%	11%	10%	7%	24%	13%	26%	10%	
7	N; H, 0.8	< 25*	79	38	209	191	192					
8	N; C, 1.0	< 25*	42*	< 25*	211	211	198					
9	N	< 25*	34*	< 25*	207							
10	Iv; H, 1.0	54	82	81*	203	162*	171*					
2a	Iv; H, 1.0	64	103	46	187	202	211	95*	44	94	285	

[a] Laboratories 1-6 are research laboratories and 7-10 are commercial laboratories; [b]iNDF methods are: Is = *in situ*, N = NIR, Iv = *in vitro*; milling methods are: H = hammer mill, K = knife (cutter) mill, C = Cyclotec (abrasive) mill; numbers refers to screen apertures; [c] DDGS = distiller's dried grain solubles; [d]Only laboratories 1-6 are compared, values without a common letter differ at P < 0.05 for a Tukey test; [e]Laboratory 4 reported iNDF values inclusive of ash; [f] SD = standard deviation; [g]CV = coefficient of variation; * = the result is an outlier according to Grubbs' (1969) test with z scores based upon standard deviation from the *in situ* laboratories.

The largest variation among *in situ* laboratories, both in absolute and relative values, was for rapeseed meal with the highest value being 90% over the lowest. This was similar to the outcome for the rapeseed sample in the ringtest of Lund et al. (2004), but with a shift towards a higher level, 129 instead of 107 g iNDF/kg DM as overall mean. The laboratories participating in both ringtests actually clustered similarly in both tests with Labs 1 and 2 being considerably lower than the others.

Cow difference was at maximum 28 g/kg DM (*i.e.* a standard deviation of 20 g iNDF/kg DM for determination in two cows). Laboratory 5 reported duplicate iNDF determinations per cow and the variation between cows was the larger part, suggesting true biological differences between individuals as a main source of variation in actual determinations. However, in a recent change-over experiment with six cows (Krizsan and Huhtanen, 2012), cow differences were not significant for iNDF (P = 0.14).

Determinations of forage iNDF by NIR were based upon calibrations developed by the commercial laboratories from reference samples previously analysed at in situ labs 1-4. For the maize sample with lowest iNDF concentration, all three NIR labs reported a value below the detection limit of 25 g/kg DM, as did two of the NIR labs for 'Grass 1'. This resulted in many outliers, emphasizing the need for improved calibrations at the lower range for forage samples.

Two laboratories reported iNDF results calculated from *in vitro* analysis based on a 48-h incubation followed by neutral detergent extraction (Goering & Van Soest, 1970) at Lab 10 and the 96-h *in vitro* organic matter digestibility (VOS) method of Åkerlind et al. (2011) at Lab 2. In both cases locally developed regressions from reference samples were applied to the *in vitro* data. Three of the *in vitro* results from Lab 10 and one from Lab 2 were classified as outliers, but most of the samples were ranked in a similar way by the *in vitro* methods.

Conclusions

There was an acceptable between-laboratory variation in NDF values, except for the very high variation for barley that calls for further action. The variation in iNDF *in situ* was less of a random variation than the result of different levels related to differences in milling as well as to other technical or biological factors. *In situ* results for rapeseed meal differed the most. Forage iNDF values for maize and grass samples were severely underestimated by NIR in the lower range but accurately measured at higher concentrations.

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The effect of analytical method on the content of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) in silage

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Introduction

Silage is a main source of livestock fodder for cattle in Inner Mongolia. Accurate determination of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) in silage plays an important role for the development of animal husbandry. In this study, NDF and ADF contents in silages as determined with different methodologies including three different filter methods were compared.

Materials and Methods

The samples were ensiled in different farms' silage trenches in Linxi (118.02 E, 43.62 N; altitude, 900 m), located in Inner Mongolia of China. There were silages from whole crop corn, dry corn stover, fresh corn stover, beet leaves (*Beta vulgaris)*, alfalfa (*Medicago sativa)* silage, Chinese elymus (*Roegneria turczaninovii*, *Lespedeza (Lespedeza hedysaroides)* and TMR (including corn meal, fresh corn-stove and dregs of beans, etc.). The silages were prepared according to Zhang (2002). Samples were analyzed for neutral (NDF) and acid detergent fiber (ADF), according to a factorial design that included three different filtering methods and an additional treatment that was applied across the filtering methods. For NDF, the additional treatment was a heat-stable α-amylase and for ADF, a neutral detergent extraction was added before acid detergents were applied (Goering and Van Soest, 1970). All extractions were made in beakers either in ANKOM (F57, 30 Fairport, NY; http://www.ankom.com/), in CAU bags (BCRC, Beijing, China; WEI, 2008) or as loose samples according to Van Soest (1991). Filtering was done in either in the bags or in glass crucibles with pore size P2 (loose samples) using a FOSS FIBERTEC^{TM} 2010 Fiber Analysis System (FOSS NIRSystems Inc., Sweden). All analyzes were done in triplicate. The experiments were analyzed with Tukey test of ANOVA by SAS 9.0 (SAS Institute Inc., Cary, NC, USA, 2002).

Results and Discussion

The results showed that the content of NDF measured by CAU bag and ANKOM bag did not differ (P<0.05). The content of NDF measured by P2 glass crucible was generally higher than by the other two filtering methods, especially for TMR silage. Compared with control, the content of NDF of whole crop corn silage and TMR silage decreased with amylase treatment (P<0.05) (Table 1). Compared to ANKOM and CAU bags, the NDF content by P2 Glass crucible treatment was somewhat higher, especially for the TMR silage, there was a significant difference (P<0.05). The NDF content of the eight silage treatments did not differ between ANKOM and CAU bags (Fig. 1).

Table 1 Neutral detergent fiber content (% of DM) in silage samples as determined by three different methods with or without the addition of heat-stable α-amylase

Silage	Treatment	ANKOM	CAU[a]	P2 glass crucible
Whole corn	Control	56.58±0.84a[b]	55.56±0.78a	59.39±3.59a
	Enzyme	53.05±1.35b	53.08±1.01b	53.50±0.44b
Dry corn stover	Control	65.96±0.71a	65.66±0.17a	67.18±1.11a
	Enzyme	65.39±0.72a	65.53±1.07a	66.81±1.54a
Fresh corn stover	Control	59.22±3.45a	58.79±2.22a	60.57±3.23a
	Enzyme	59.38±3.40a	59.00±3.75a	60.78±3.45a
Beet leaves	Control	48.05±1.42a	48.28±2.06a	50.22±2.21a
	Enzyme	47.30±1.12a	47.05±1.91a	49.65±0.74a
Alfalfa	Control	35.70±1.28a	36.19±2.37a	37.48±1.91a
	Enzyme	37.16±2.43a	36.18±3.23a	37.86±3.09a
Elymus	Control	57.83±0.88a	56.76±0.58a	59.36±0.90a
	Enzyme	58.42±0.06a	57.38±0.30a	59.63±0.25a
Lespedeza	Control	40.60±1.34a	40.55±0.38a	43.39±0.64a
	Enzyme	40.44±0.01a	40.21±0.12a	43.73±0.86a
TMR	Control	38.53±0.13a	37.54±0.14a	44.98±0.26a
	Enzyme	31.91±0.06b	30.43±0.07b	41.02±1.63b

[a]CAU: China Agricultural University; [b]Different lower case letters for the same silage between control and enzyme treatment show significant difference ($P<0.05$).

In the treatments of whole-plant corn silage and TMR silage, the content of ADF determined by P2 Glass crucible were higher significantly ($P<0.05$) than that of the ADF content by the other two kinds of filters. Continuous washing was impact on ADF of alfalfa silage and *Lespedeza* silage (Table 2). Influenced by the NDF results, the ADF content of whole corn silage and the TMR silage had a significant difference ($P<0.05$) by using P2 Glass crucible, which was higher than the ANKOM and CAU bags (Fig. 2).

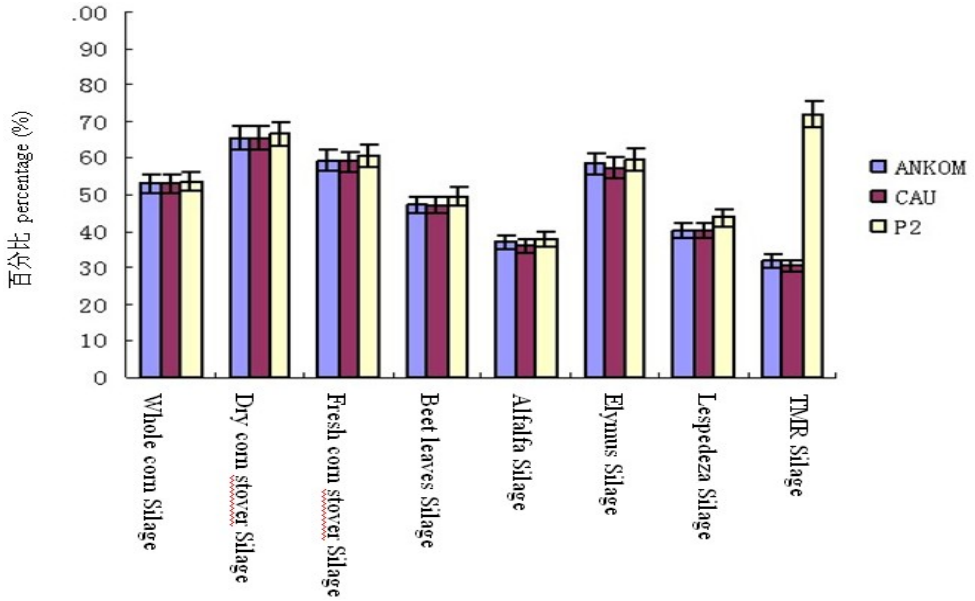

Fig.1 The comparison of silage neutral detergent fiber measured by different filters.

Table 2 Acid detergent fiber content (% of DM) in silage samples as determined by three different methods with (NDF-ADF) or without (ADF) pre-extraction with neutral detergents

Silage	Treatment	ANKOM	CAU	P2 glass crucible
Whole corn	ADF	31.85±1.14a[a]	31.56±0.32a	32.89±1.48b
	NDF-ADF	31.73±0.36a	30.74±0.60a	36.04±1.15a
Dry corn stover	ADF	41.62±1.24a	43.17±2.40a	46.34±1.91a
	NDF-ADF	41.02±1.61a	40.65±1.49a	41.89±1.78a
Fresh corn stover	ADF	38.26±1.77a	39.01±1.55a	41.09±1.83a
	NDF-ADF	35.84±2.12a	37.15±2.95a	40.02±1.27a
Beet leaves	ADF	26.53±1.21a	27.99±1.50a	29.11±0.99a
	NDF-ADF	27.87±1.37a	27.23±1.86a	28.42±0.73a
Alfalfa	ADF	29.75±0.97a	29.69±0.49a	32.60±1.20a
	NDF-ADF	26.07±1.88b	26.44±1.48b	26.71±0.38b
Elymus	ADF	37.95±0.70a	36.48±0.49a	37.87±0.03a
	NDF-ADF	37.44±1.03a	36.02±0.04a	35.58±1.03a
Lespedeza	ADF	39.52±0.36a	45.76±0.23a	37.81±1.65a
	NDF-ADF	31.94±1.16b	31.99±0.57 b	30.75±0.89b
TMR	ADF	18.72±2.16a	16.91±0.24a	22.61±0.18b
	NDF-ADF	17.35±0.08a	17.49±0.62a	27.78±0.40a

[a] Different lower case letters for the same silage between Van Soest and Continuous washing show significant difference ($P<0.05$).

Methods

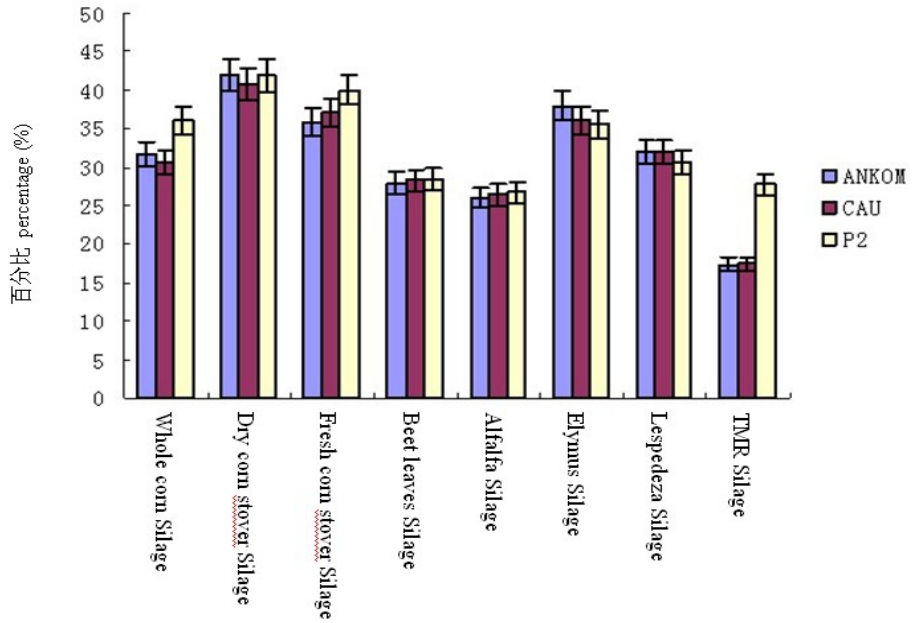

Fig.2 The comparison of ADF of silage measured by different filters.

Conclusions

Enzyme was necessary to improve the accuracy of NDF determination in high-starch silages. Continuous washing was effect in reducing ADF content in alfalfa and Lespedeza silage. Compared to ANKOM and CAU bags, the NDF and ADF contents of TMR silage by P2 Glass crucible had a significant difference ($P<0.05$).

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Can a wireless, magnetoelastic sensor measure pH in the rumen?

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Introduction

Rumen pH is commonly used to describe fermentation patterns and normal pH is a prerequisite for microbial activity in cattle and sheep. There is a cyclic variation where pH drops after feed intake and recovers during rumination (Keunen et al., 2008). High producing ruminants, like dairy cows, are often fed large amounts of highly digestible carbohydrates in order to cover their demand for energy. This leads to extensive fermentation in the rumen resulting in depressed pH. Depressed rumen pH over prolonged time without lactic acid accumulation is referred to as sub-acute rumen acidosis (SARA) and is associated with several health problems, impaired utilisation of feed and production (Plaizier et al., 2009). There is a considerable variation between animals in their ability to tolerate large amounts of highly digestible carbohydrates (Mydland, 2005). Therefore, individual adjustments of feed composition would be desirable in order to meet the energy demand of each cow without risking rumen problems and monitoring of rumen pH might be a tool in this process.

Continuous monitoring of rumen pH is presently done only in research environments and mainly with fistulated animals. There are telemetric techniques with boluses that can be placed in intact animals and transmit recorded pH values. These systems generally rely on battery driven sensors with glass electrodes and therefore their functional life time is limited and cost is relatively high. To be interesting for commercial applications other, less costly and more persistent techniques are needed.

Ruan *et al*. (2003) suggested the use of a passive wireless sensor for measuring pH in the stomach of humans. The sensor was based on a magnetoelastic film coated by a pH sensitive polymer. The cost of this technique is low, making it interesting for commercial applications.

Magnetoelastic sensors are made from amorphous ferromagnetic metal film ribbons (Grimes et al., 2002). A magnetoelastic sensor is activated by a spatially variable magnetic field which makes the film elongate and shrink along its length axis. The magnetic field is created by driving periodical current through a coil. Upon activation, the sensor generates a magnetic flux that can be detected by a pickup coil, either the same that activated the sensor or a separate. If the same coil is used for activation and detection, the response from the sensor is monitored during the periods when the current is switched off (Fig 1.). The frequency of the generated magnetic response depends on the resonance frequency of the sensor which is a function of the sensors size and mass. To understand sensor operation, an acoustic bell can be used as an example. The energy from a clapper that hits a bell deforms the bell and makes it vibrate with its characteristic frequency causing a specific tone. The magnetic field that activates the sensor is analogous to the clapper and the tone of the bell is the frequency of the magnetic flux produced by the activated sensor. Any material deposited onto the sensor will change its resonance frequency, roughly in proportion to the deposited mass (Cai and Grimes, 2000). By coating the metal film with a polymer that swells and shrinks upon pH changes shifts its resonance frequency and can be used to detect pH changes (Ruan et al., 2003). The swelling and shrinking of pH sensitive polymers is

due to functional groups that become ionised relative to pH. The functional groups might be acidic (e.g -COOH) or basic (e.g. -NH_2). With increasing ionisation, repulsion of charged groups causes the polymer to expand, and hence swell, which change the mass load of the sensor.

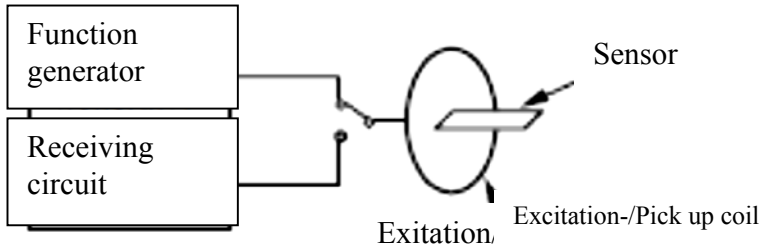

Figure 1 System for excitation and signal reception from a magneto elastic resonance sensor. A switch is used to separate the excitation circuit and the receiving circuit enabling both functions to be carried out by one single coil. Modified after Grimes *et al*. (2002).

Material and methods

Sensors were prepared from thin (29 µm) magnetic film ($Fe_{40}Ni_{38}Mo_4B_{18}$, Metglas Inc., Düsseldorf, Germany) with the size of 2 × 10 mm. The film was covered by a pH sensitive aminated polymer with basic characteristics, prepared according to Ruan et al. (2003). The sensors were covered by nylon bags, attached to a stick and placed in the centre of a covered glass jar. The jar was placed on a shaker in a box at 39°C.

A coil surrounding the jar was used for activation and pick up the resonance frequency of the sensor. Activation and frequency pick up was handled by a laptop computer through a control unit connected to the coil (Figure 2).

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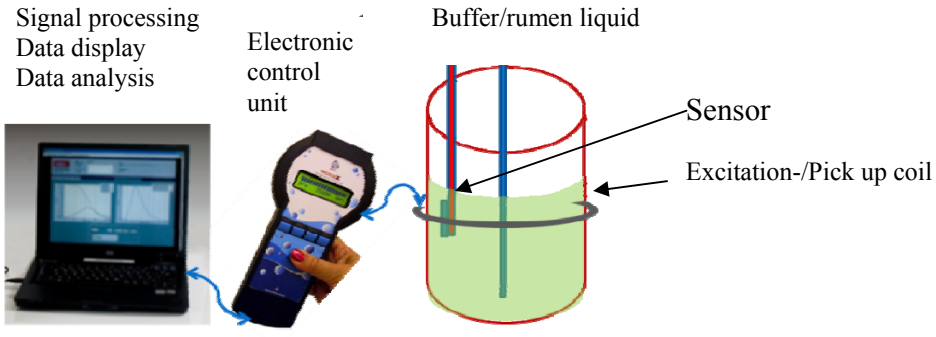

Figure 2 Experimental arrangement.

The sensor was tested in McDougall's buffer and rumen liquid. A mixture of volatile fatty acids (65 % acetic, 20 % propionic and 15 % butyric acid), was used to change pH in the buffer. To change pH in rumen liquid sugar was added. Resonance frequency was recorded continuously and pH was measured at intervals with a handheld pH meter.

We also measured *in vivo*, in a fistulated cow. The sensor was placed within the rumen and connected to a weight of solid rubber to keep it in place.

Results and discussion

Initial results show that the sensor can detect pH changes in buffer and pure rumen liquid. Decreasing pH was detected by decreasing resonance frequency (Figure 3). Bubble formation after addition of VFA disturbed measurements in McDougall's buffer. To reduce the problem buffer was diluted by water. Dilution reduced the problem and it can be seen in Figure 3 that the sensor responded similarly in different concentrations of buffer. This is in agreement with Ruan et al. (2003) who examined the same type of polymer and found measurements to be independent of salt concentration. Bubble formation did not seem to disturb measurements in pure rumen liquid, where gas also would be expected to be formed, and hence it might not be a problem in an *in vivo* application.

Even though the relation between pH and resonance frequency seem linear (Figue 3), the resolution was not satisfactory. After addition of sugar to rumen liquid a decreasing resonance frequency was observed with decreasing pH. The relation between frequency and pH was however not as clear as in Figure 3, probably due to the relatively small pH changes. Also at measurements in buffer small pH changes around pH 6 were not easily detected from the resonance frequency response. This was probably due to that the polymer used in this experiment was made for detecting pH between 4 and 10. As the interesting pH span in the rumen is between 5 and 7, a polymer with a narrower pH detection span would be more desirable. The polymer should also have its best resolution between pH 5.5 and 6.5.

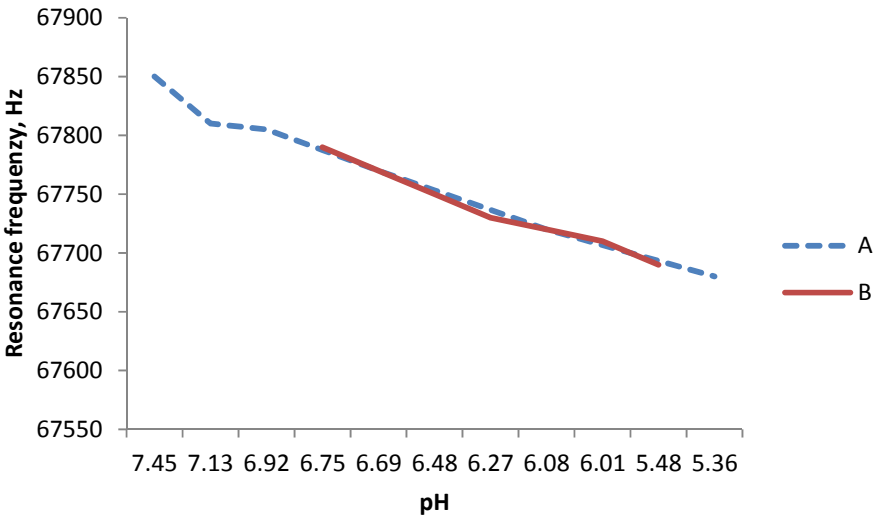

Figure 3 Resonance frequencies from magnetoelastic sensors at different pH in diluted McDougall's buffer. The relationship between buffer and water was 1:7 in A and 1:3 in B.

The measurements were disturbed when the shaker was running. This shows the system's sensitivity to magnetic fields caused by adjacent electrical equipment and this needs to be considered in any future application.

We were able to detect the sensor when placed in the rumen of a fistulated cow. Organic material does not interfere with response signal bur the measurement distance needs to be considered in *in vivo* applications. A sensor of 10 mm size has a detection limit of about 30 cm (Grimes et al., 2002). A larger sensor and a larger coil will increase the detection limit but how this can be accomplished require further attention.

In an *in vivo* application, durability of the sensor is a key issue. The *in vitro* measurements done so far have lasted less than 12 h. Long term effects on the polymer in rumen environment need to be investigated as well as any practical arrangement for protecting the senor mechanically in the rumen.

Conclusions

A wireless pH sensor based on magnetoelastic resonance technique and a pH sensitive polymer was able to detect pH changes in rumen liquid. To be of interest in rumen investigations, measurement resolution at relevant pH interval need to be improved. Durability at long term use and detection limit distance also needs further investigation.

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The variation of control samples in the analyses of crude protein, neutral detergent fibre, in vitro organic matter digestibility and crude fat

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Introduction

The establishment of a quality control system in the laboratory is a part of the verification process and necessary for detection of how well the laboratory can reproduce results over time and under varying operating conditions.

One way to keep control over the repeatability and reproducibility is to use a control sample included in the analysis for monitoring the stability when the analyses are performed. The control sample results are entered in a control card for a specific assay to be used as a tool for analytical laboratories to verify their analyses according to original specifications. When some 10-20 analyses have been performed on the control sample over a period of time and the population is normally distributed, a graph can be constructed with boundary lines, representing upper and lower control limits, *i.e.* standard deviations (SD).Five % of the values are found outside the ±2 SD boundary lines and 0.3% of the values are found outside the ±3 SD boundary lines (Naturvårdsverket, 1991). Rules to apply then are that no result should be: a) outside of ±3 SD, b) outside ± 2 SD two or three results in succession, and c) no more than 7 results in a row are allowed to be on the same side of the mean.

A control sample to be used in an assay should be of similar type as normally analyzed. Repeatability is calculated from the inter-assay variation of analyses performed as SD of replicates of the control samples in the analysis or as the coefficient of variation (CV=SD/control sample mean). Reproducibility can be assessed by calculating intra-assay variation as SD or CV of repeated measurements of control samples on different days.

Material and methods

Amylase treated neutral detergent fibre, excluding ash (aNDFom), was analyzed according to (Chai, W. and Udén, P., 1998). In vitro organic matter digestibility (IVOMD) was determined after 96 h incubation in buffered rumen fluid according to Lindgren (1979). The control sample for these methods consisted of a grass mixture, which was dried and ground to pass a 1-mm screen.

To reduce run variation in IVOMD analyses as influenced by *e.g.* quality of the rumen fluid, we used a system with ‘standard’ samples for bias adjustment. The standard samples consisted of six forage samples with “known” IVOMD values from approximately 20 analyses. These samples had been chosen to cover the normal range of IVOMD from 68 to 90%.The six standard samples were included in each run and water bath. The IVOMD difference between true and observed value for the run was calculated for every separate standard sample. The average difference for the six samples was then used as a bias adjustment of the control sample, as well as for all the samples in that run.

Crude protein (CP) was analyzed by the Kjeldahl method (Tecator-Kjeltec system 2460, Tecator Höganäs Sweden) with Cu as a catalyst and CP was calculated as N x 6.25. Crude fat was

analysed according the EC No. 152/2009. As control sample for these methods was used a concentrate consisting of: 'AkoFeed' 2%, molassed sugar beet pulp 14%, oats 20%, barley 30%, rapeseed meal 'Expro' 16%, soybean meal 14% , wheat germ 4%. The sample was dried and ground to pass a 1-mm screen.

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Results and discussion

For CP (Fig. 1), there is a tendency for values to decrease over time. Since changes in dry matter (DM) were not taken into account, a slow uptake of water from the air might have influenced the CP content of the analysed sample. In addition to the control sample, the Kjeldahl system is checked regularly with recovery tests using ammonium salts and amino acids with known contents of nitrogen.

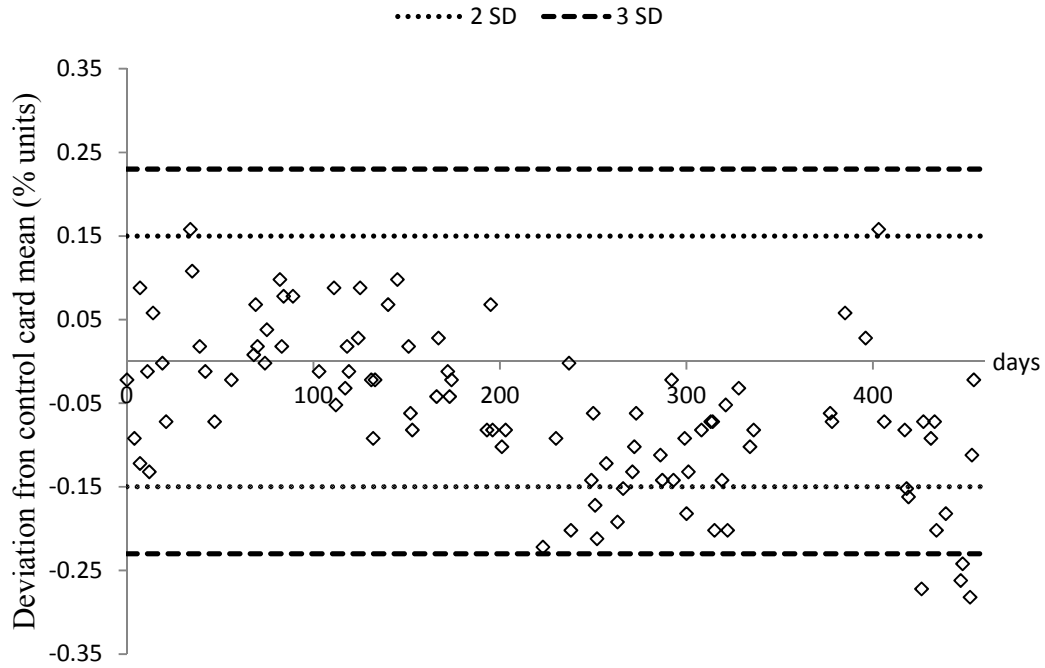

Figure 1 Crude protein: Deviation from the control card mean (20.6%) of a concentrate sample used during a period of 454 days with two and three standard deviations (SD) represented by dotted lines. The first 20 analyses were used for calculation of control card limits.

Values for aNDFom (Fig. 2) are well distributed values around the control card mean even though changes in DM were not taken into account.

Data for IVOMD are shown in Fig 3. Content of DM and ash in the control sample were analyzed regularly in this case and values were used in the calculation of IVOMD. The bias adjustment of the control sample at each run is likely to have contributed to the well distributed IVOMD values. If the difference of an individual standard sample exceeds ± 5% units, that sample is excluded from the bias adjustment calculation. Normal values for the bias adjustments in our laboratory do normally not exceed ±2% units. This rather low bias level is probably due to access to fresh rumen fluid not older than one hour. If bias is higher than ±3% units, the entire run is checked carefully and may be discarded.

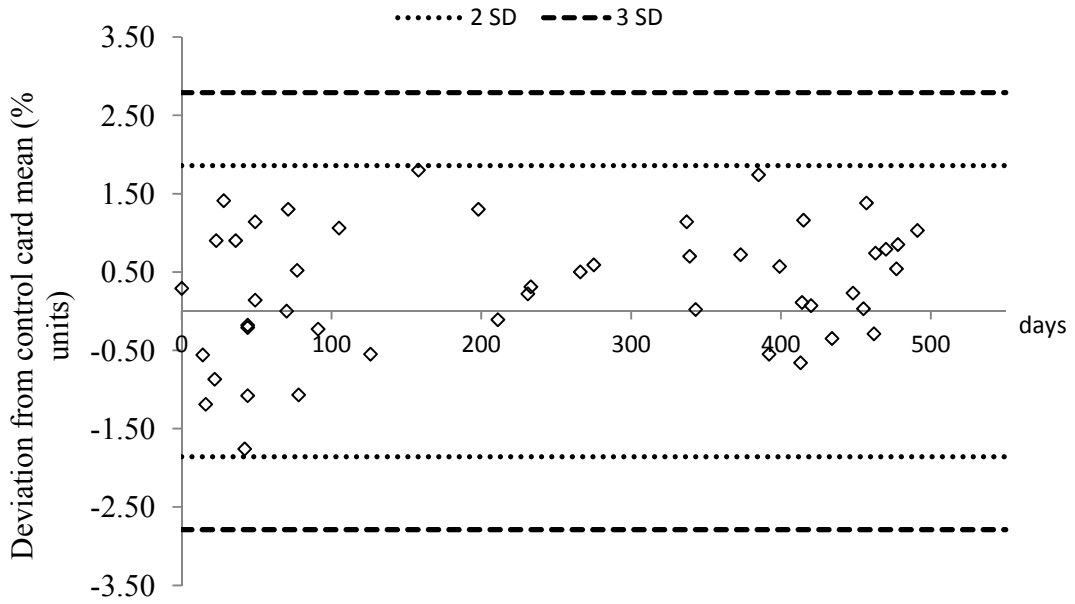

Figure 2 Amylase treated neutral detergent fibre, excluding ash: Deviation from the control card mean (54.1%) of a grass sample used during a period of 491 days with two and three standard deviations (SD) represented by dotted lines. The first 20 analyses were used for calculation of control card limits.

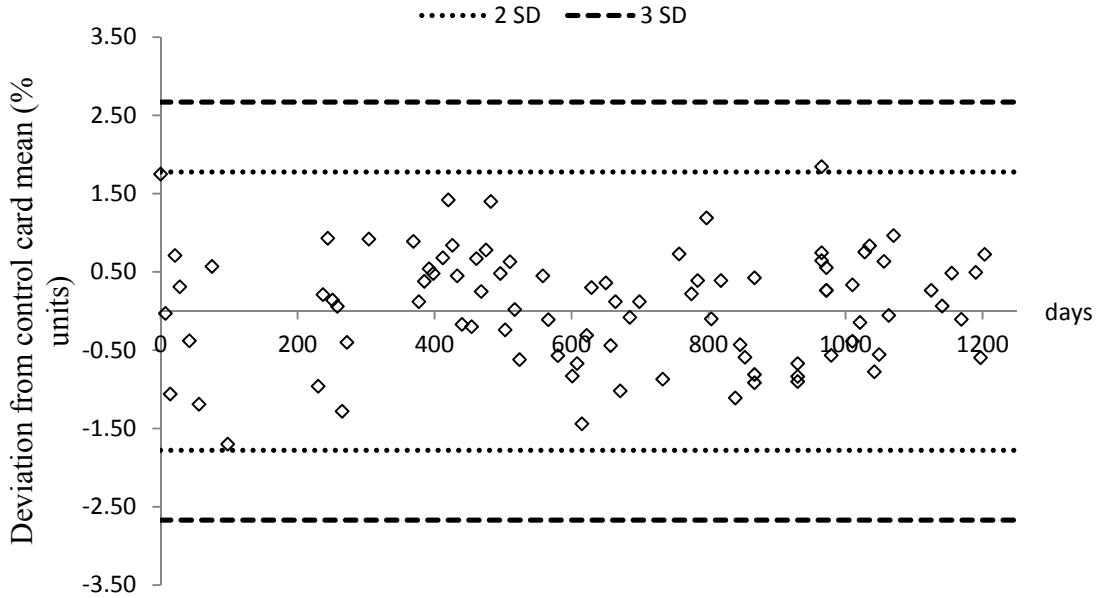

Figure 3 In vitro organic matter digestibility: Deviation from the control card mean (82.8%) of a grass sample used during a period of 1203 days with two and three standard deviations (SD) represented by dotted lines. The first 20 analyses were used for calculation of control card limits.

The crude fat concentration (Fig. 4) of the control sample decreased over time. Over a period of approx 9 years, it has dropped from 4.5 to 4.0%. Changes in DM were not considered but the

drop in concentration was likely due to instability of the crude fat making it increasingly difficult to extract.

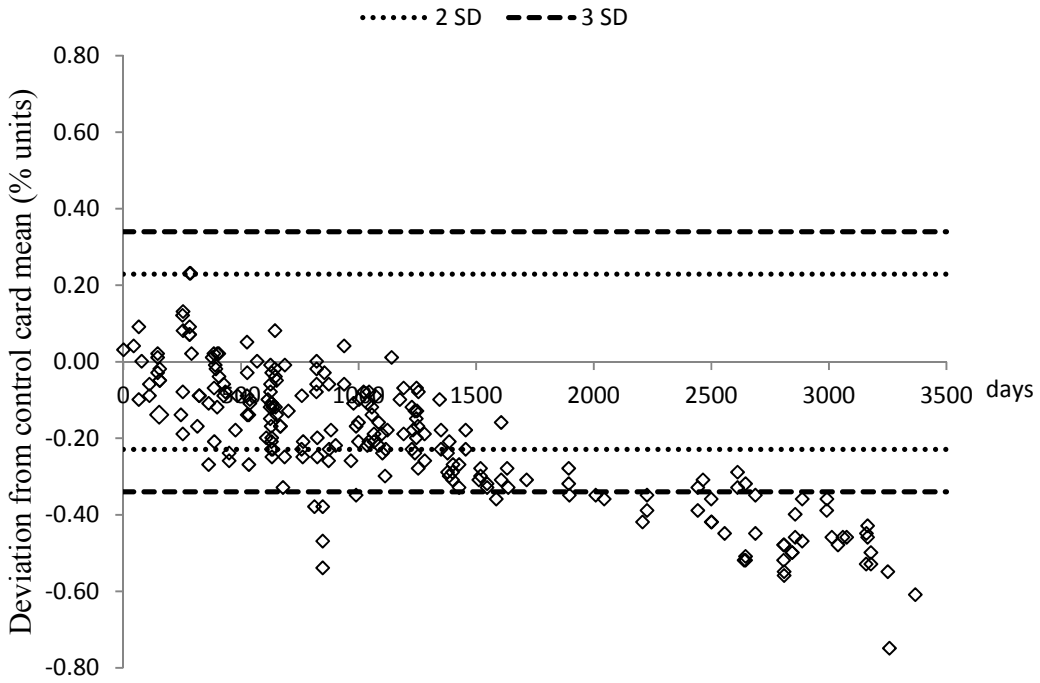

Figure 4 Crude fat: Deviation from the control card mean (4.5%) of a concentrate used during 3367 days with two and three standard deviations (SD) represented by dotted lines. The first 20 analyses were used for calculation of control card limits.

The selection of a control sample is crucial. It should be of similar type as those normally analyzed and drying, grinding and storage until analysis should be identical to ordinary samples. However, storage time is something which cannot be made similar to ordinary samples as laboratories wish to keep control samples over extended periods of time for practical reasons. Crude fat illustrates this problem. In (Fig. 4), the sample values are not stable and decrease noticeably over time. A possible solution to changes in DM and fat over time might be the storage of pre-weighed control samples in a deep freezer for future use.

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BLGG AgroXpertus has been the leading horticultural and agricultural analytical laboratory in The Netherlands for over 80 years. With it`s sampling, analytical and advisory activities BLGG AgroXpertus plays a supportive role in agricultural and horticultural procedures, thereby improving production and lowering production costs. BLGG AgroXpertus offers analyses and advice on nutrient content and quality of soil, manure, water, substrate, crops and cow feed to almost every Dutch farmer and crop grower. The last 12 years, BLGG AgroXpertus, expanded the activities to Europe. 2011 BLGG started up forage analyses in Hörby, Sweden as well.

The company has its own accredited sample collection and transport system, ensuring that samples are collected according to predefined guidelines and ensuring fast overnight transport to the different laboratories. Samples can be transported refrigerated if needed. In total, more than 500.000 samples/year are analyzed in accredited and highly automated BLGG AgroXpertus laboratories.

BLGG AgroXpertus did more than 100 000 analyses of forage 2011. BLGG AgroXpertus has developed a model based on local calibration, that calculates a large number of parameters with very high reliability.
Over the years, BLGG AgroXpertus has collected a large number of calibration sets containing data from samples that are analyzed by both NIR and reference methods. BLGG AgroXpertus has separate databases for all common roughage, raw materials and Total Mixed Ration (TMR) types.
Standard NIRS calculation models can miscalculate the parameter values when the roughage under analysis is contaminated with soil. Therefore, BLGG AgroXpertus, always determines the crude ash content in roughage in the traditional way.
Thanks to a partnership with Masterlab, BLGG AgroXpertus has calibration sets for raw materials as well.

Endophytic fungi in forage grasses – do we need to bother about them?

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In nature, plants are never alone. They are surrounded more or less closely by numerous microbes. As the term indicates, endophytic fungi live symbiotically within a plant. Fungi in the genera *Neotyphodium* and *Epichloë* in the family Clavicipitaceae occur in symbiosis with a number of grasses in temperate regions. Internationally, these symbioses are most well-studied in the forage grasses tall fescue, *Festuca arundinacea,* and perennial ryegrass, *Lolium perenne*.

Neotyphodium lives intercellularly in grass shoots without causing any visible symptoms. When seeds infected with *Neotyphodium* germinate growth of the fungus is synchronized with growth of the tillers so that *Neotyphodium* reaches into inflorescences and further into the developing seeds. When the seeds germinate, the lifecycle of *Neotyphodium* is completed. Thus, these endophytes cannot spread from plant to plant in the field. The same characteristics apply to fungi in the genus *Epichloë.* However, sometimes hyphae of *Epichloë* will grow out to the surface of tillers programmed to flower. The hyphae form a thick envelope, choke disease, beneath the flag leaf of the tiller and flowering and seed production is inhibited. Spores produced from these hyphae can spread by wind or insects to other plants of the same species and infect their flowers so that the developing seeds become infected (Schardl and Clay, 1997).

Detection of endophytic fungi in grasses

Lack of visible symptoms in infected grasses makes laboratory methods necessary to reveal presence of endophytes. Microscopy of seeds, leaves or pseudostems is a useful method alone or as a complement to immunological and molecular biology methods. A drawback with these methods for seed studies is the difficulty to know whether observed fungal hyphae are dead or alive. Seeds need to be germinated so that growing tillers can be tested for presence of endophytes. Immunoblot and PCR-based assays work well on fresh tillers (Dombrowski et al., 2006, Hiatt et al., 1999, Koh et al., 2006). Presence of living endophytes is also possible to verify by properly identified fungal outgrowth from surface sterilized seeds or pieces of tillers on potato dextrose agar plates (Christensen et al., 1991).

Grasses known as possible hosts for endophytes

In the Nordic countries, at least 14 grass species are able to have endophytes, but only three of these species are studied in agricultural environments (Table 1). Within a grass species, the infection rate can vary from 0 to almost 100% of the plants (e.g. Saikkonen et al., 2000). In *Festuca pratensis* cv. Kasper cultivated in Umeå, we found infection rates from 25 to 65% (Puentes et al., 2007). The infection rate was similar in first, second and fourth year leys which suggests that *Neotyphodium uncinatum* can survive winters.

Effects of endophytes on grass and herbivores

Neotyphodium and *Epichloë* may or may not affect growth and production of grasses. For example, in tall fescue, plants with endophytes tolerated drought better than non-infected plants in some studies but not in others (Cheplick and Faeth 2009). Improved drought tolerance could be due to a more extended root system in infected plants, which in turn can alter soil texture and

thereby water holding capacity, so that water and nutrient uptake is improved (Belesky and Malinowski, 2000). Another type of endophyte effect is reduced seed production when *Epichloë* causes choke disease (’kolvsjuka’; Juhlin-Dannfeldt, 1923).

Table 1 Occurrence of endophytic fungi in grasses in natural environments and agricultural environments in Denmark (D), Finland (F), Norway (N) and Sweden (S)

Grass species	*Neotyphodium* or *Epichloë* species	Selected references
Natural environments		
Agrostis capillaris	*E. typhina*	(F) Saikkonen et al., 2000
Calamagrostis purpurea	*E. typhina*	(S) Wennström 1996
Dactylis glomerata	*E. typhina*	(F) Saikkonen et al., 2000
Deschampsia cespitosa	?	(F) Saikkonen et al., 2000
Deschampsia flexuosa	?	(F) Saikkonen et al., 2000, (S) Bazely et al., 2007
Elymus repens	?	(F) Saikkonen et al., 2000
Festuca arundinacea	*N. coenophialum*	(D) Jensen et al., 2007, (F) Saikkonen et al., 2000, Saari et al., 2010a
Festuca ovina	*E. festucae*	(F) Saikkonen et al., 2000, (F&N) Wäli et al., 2007, (S) Granath et al., 2007
Festuca pratensis	*N. uncinatum*	(F) Saikkonen et al., 2000
Festuca rubra	*E. festucae*	(F) Saikkonen et al., 2000, (F&N) Wäli et al., 2007, (S) Bazely et al., 2007, (S) Saona et al., 2010
Festuca vivipara	*E. festucae*	(S) Granath et al., 2007
Phleum pratense	*E. typhina*	(F) Saikkonen et al., 2000
Poa trivialis	*E. typhina*	(S) Bazely et al., 2007
Agricultural environments		
Festuca ovina	*E. festucae*	(S) Koh et al., 2006
Festuca pratensis	*N. uncinatum*	(F) Saari et al., 2010b, (S) Puentes et al., 2007
Lolium perenne	*N. lolii*	(D) Jensen, 2005

Table 2 Examples of alkaloids in some grass *x* endophyte symbioses and toxicity to different herbivores

Grass	Endophyte	Alkaloids (examples)	Herbivore toxicity
Festuca pratensis	*Neotyphodium uncinatum*	Loline	Insects, small mammals
Festuca rubra	*Epichloë festucae*	Ergovaline, lolitrem, loline, peramine	Horses, cattle, sheep, insects
Festuca arundinacea	*Neotyphodium coenophialum*	Ergovaline and many others	Horses, cattle, sheep, insects
Lolium perenne	*Neotyphodium lolii*	Lolitrem, peramine, ergovaline	Horses, cattle, sheep, insects
Lolium multiflorum	*Neotyphodium occultans*	Loline, peramine	Insects

Alkaloids produced in grass-endophyte symbioses are a problem for herbivores. The large number of different alkaloids identified so far is grouped into four categories (alkaloid examples in parentheses): pyrrolizidine alkaloids (lolines), ergot alkaloids (ergovaline),

pyrrolopyrazine alkaloids (peramine) and indol diterpene alkaloids (lolitrem). The kinds of alkaloids produced depend on both grass and endophyte species (Table 2) and also the genotype of grass and fungus. There can be many different alkaloids in a grass species. More than 30 different alkaloids, including ergovaline, were found in tall fescue (Aldrich-Markham et al., 2007). Amounts of alkaloids vary among years, seasons, individual plants and plant parts. In hay alkaloid concentrations can still be considerable although lower than in the corresponding fresh grass (Roberts et al., 2009).

Some alkaloids are deterrent or toxic to herbivores such as insects, birds or small mammals while others are toxic also to large mammals such as horses, cattle and sheep (Table 2). Most of our knowledge about severe illness effects and even deaths of livestock comes from studies of tall fescue and perennial ryegrass introduced from Europe into USA and New Zeeland, respectively. Often horses, especially pregnant mares, are more sensitive to alkaloids than cattle and sheep (Cross, 1997). In tall fescue, ergovaline, loline and other alkaloids contribute to fescue toxicoses, seen as elevated temperature, heat stress, increased vasoconstriction, reduced reproduction and milk production, and to fescue foot or hoof gangrene (Cheplick and Faeth 2009). Infected perennial ryegrass can lead to staggers due to ergot alkaloids and lolitrems. Symptoms are reduced weight gain, decreased fertility and impaired neuromuscular function (Cheplick and Faeth 2009).

In Europe, toxicosis from endophyte-infected tall fescue is noted from France, and some ryegrass staggers are reported from United Kingdom, Germany and The Netherlands (Lewis 1997). As far as we know, there are no reports of proven cases of endophyte related toxicoses among livestock in Sweden. However, there are reports of severe reproduction problems in horses where the symptoms resemble symptoms described for endophyte toxicoses (Darenius et al., 2011).

Alkaloids in endophyte-infected grasses can also be utilized in a positive way. In turfs, such as golf courses, and in grasslands, it is an advantage to have alkaloids that are deterrent or toxic to grass-eating insects. This is well-spread in New Zealand where endophyte-free ryegrass will be completely destroyed by a weevil unless the ryegrass contains *Neotyphodium*. Another example is to use endophyte-infected grass in green areas on airports. Grass-eating birds like geese stay away from the grass minimizing the risk of bird collisions with aeroplanes (Anon., 2012).

Challenges

Seeds with a certified infection rate are available on the market in several countries but, so far, not in Sweden. Today, new grass species, hybrids and varieties of *Festuca* and *Lolium* are introduced into Sweden. From Denmark, there are reports of rather high concentrations of ergovaline in tall fescue (Jensen et al., 2007). This calls for a need to investigate the occurrence of endophytic fungi and alkaloids in Swedish grasses. It is also necessary to develop reliable methods to detect endophytes and to analyse alkaloid content in silage and haylage. Thereby the potential risk of severe suffering by livestock and economic losses for their owners can be avoided. **In conclusion**, yes, we need to bother about endophytic fungi in forage grasses.

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Variation in forage quality and the potential for its management

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Introduction

The development of feeding strategies based on forage quality assessment is a continuous process. Generally, these assessments are carried out as laboratory analyses of bulk samples supposed to be representative of for example a harvest or a silo. Although techniques for weekly or daily quality analysis of fresh forage or silage are emerging, from day to day variations in forage quality are very little considered in practice. This paper aims at indicating what variations that can be found in practice by looking at three different data sets previously gathered and to give a brief insight into the potential use of visible and near infrared (vis-NIR) spectroscopy on fresh forage and silage for on-farm quality assessment. The material cannot be claimed to give a good representation of what can be expected at individual farms, but it shows that variation exist and may have an influence worth considering for future more efficient use of forage.

Materials and Methods

This presentation is based on three datasets. The first was from a consumption study at the research farm of the Swedish University of Agricultural Sciences in Umeå, combined with a feasibility study of predicting quality parameters with vis-NIR spectroscopy (Stenberg et al., 2007). The study consisted of almost daily sampling with 11 and 14 samples taken from two different bunker silos of second and first harvest, respectively, and from big bales from 5 batches of first, second or third harvest with seven or six bales in each batch. Bale sampling represented one bale per day. Batches represented one field harvested within one day. Representative daily samples were taken from silages when fed (in all 59 samples) and analyzed for dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), *in vitro* organic matter digestibility (IVOMD), water soluble carbohydrates (WSC), lactate and acetate. Actual consumption was also registered. The second set was from a study of daily variation of DM, CP and NDF at the experimental farm *Nötcenter Viken* in Falköping (Johansson, 2007). A total of 42 samples, representative of the daily outtake from bunker silos, were taken. The third data set was from a study of quality variation in big bales from a 3-ha field at the experimental farm Rådde of The Swedish Rural Economy and Agricultural Societies in Länghem (unpublished). The field representation of each bale was registered with GPS and each bale was marked with an electronic ship for identification at opening and sampling. Each of the 40 bales was analyzed for DM, ash, CP, buffer soluble CP (BSCP), NDF, metabolisable energy (ME) and IVOMD. All analyses were performed according to standard procedures at the Analyzen/Eurofins laboratory in Lidköping.

Results and Discussion

Variation

Table 1 illustrates the variation of DM, ME, CP and NDF of the individual batches in Umeå. Typically, the standard coefficient of variation ranged between 5 and 10 % of the average DM and CP contents, while the variation of ME and NDF was considerably lower. Looking at the entire data set (not shown) the variation was 11-35% for the different variables and highest for DM.

Table 1 Mean and standard deviation for reference analysis of silage as a part of the intake study in Umeå

Batch	Type	N	Harvest	DM	STDV	ME	STDV	CP	STDV	NDF	STDV
A	Big bale	7	2	56.5	4.7	9.9	0.16	123	5.8	669	9.6
B	Big bale	7	1	32.0	6.7	11.0	0.42	161	17	559	41
C	Big bale	7	3	24.0	2.0	10.2	0.12	193	18	582	22
D	Big bale	7	2	44.0	1.8	10.4	0.08	156	18	630	19
E	Big bale	6	2	57.0	5.0	10.3	0.16	139	8.9	601	8.6
F	Bunker	14	1	27.0	1.2	11.5	0.19	186	11	486	30
G	Bunker	11	2	31.0	1.7	11.0	0.17	153	3.2	558	16

In Figure 1, the geographical representation of the big bales at the experimental farm Rådde is illustrated. As this was a dry cropping season, harvest was low and each bale represented swaths averaging 475 m along the field. This makes each bale covering most of the variation in the N-S direction.

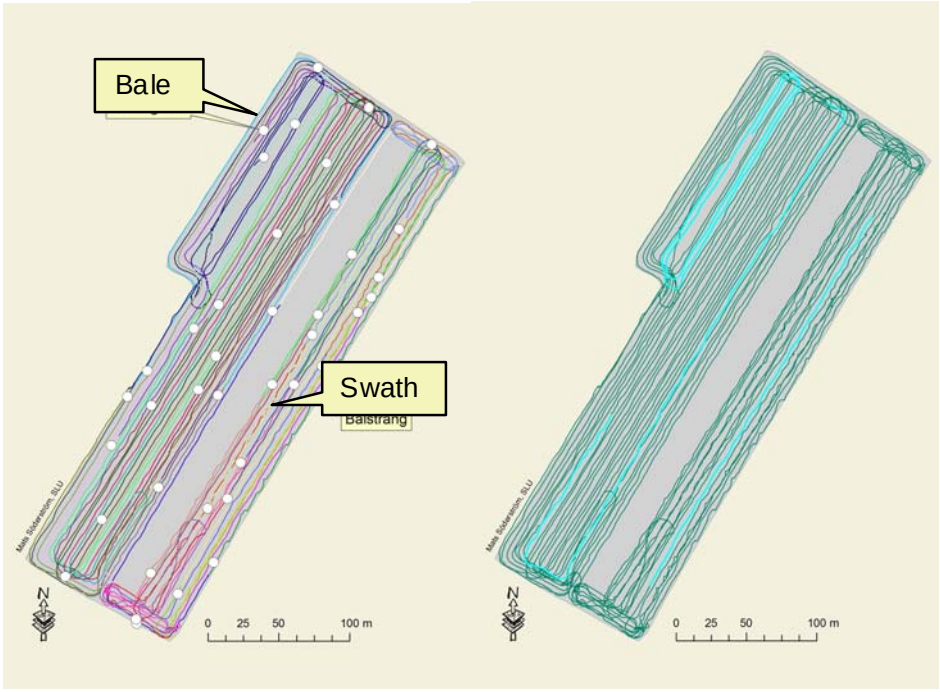

Figure 1 Representation of swaths by big bales at the experimental farm Rådde. To the left, the marked bales are represented by differently colored swaths. To the right, the representation of

three sample bales are marked by a light blue line to make a clearer view of swath length. The empty spot along the centre line is a field experiment not included.

In Figure 2, the distribution of quality parameters at Rådde is illustrated. The scale in the figure represents the total variation of plots in several field experiments at the farm the same year, including fertilization and botanical composition experiments. Considering the long swaths in each bale and the small field size of the present experiment, variation was fairly large. That is, with higher yield and shorter swath length for each bale, the variation would most likely have been larger between bales.

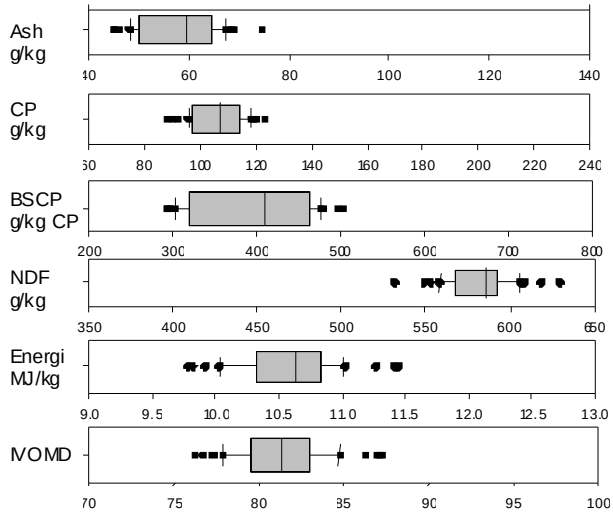

Figure 2 The median, 25/75 and 10/90 percentile and outliers of the different parameters analyzed at the Rådde field (N=40).

Figure 3 indicates that ash, CP and BSCP roughly varies in a similar way over the field, while NDF has a second variation structure and ME a third. The field was located on a slope towards WNW.

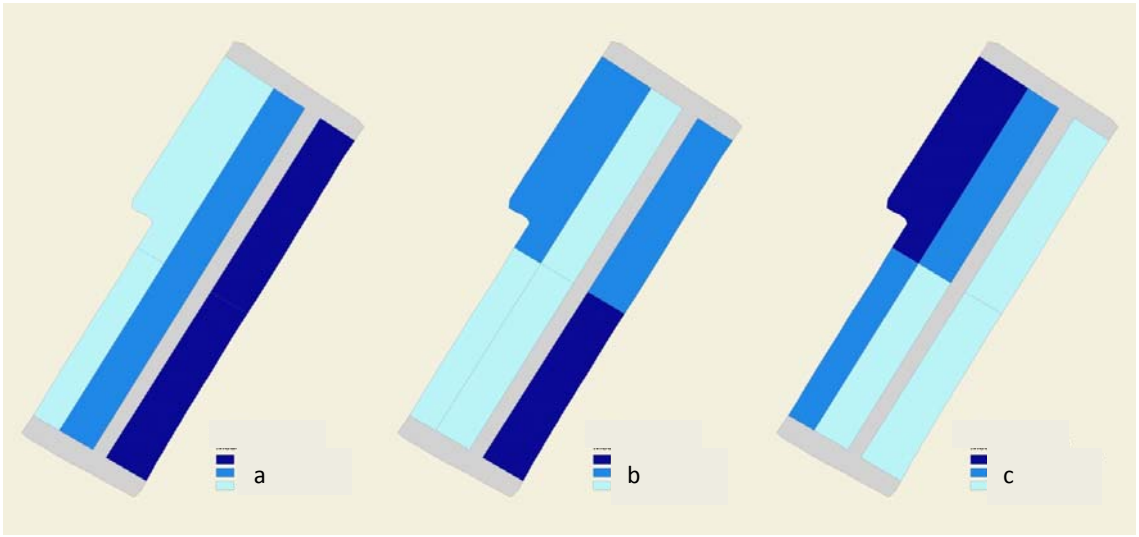

Figure 3 A schematic illustration of high (dark blue), medium (blue) and low (light blue) values in six segments of the Rådde field as calculated from the bale values weighted according to swath length in each segment. From left to right a) Ash, CP and BSCP; b) NDF; c) ME and VOS.

At Viken, daily feed was taken from a bunker silo. In general, a bunker silo was loaded horizontally and silage was taken out vertically. This will most certainly result in an effective reduction of variation between feeding occasions. Especially in a case like Viken, where the larger part of the silo profile was taken out each time. The variation there was small (Table 2) and smaller than in most cases as shown in Table 1, but not always.

Table 2 Average and standard deviation of DM, NDF and CP at Viken (N=42).

Parameter	Average	STDV
DM %	23.5	0.9
NDF g 1000 g^{-1}	578	13
CP g 1000 g^{-1}	157	5

In Figure 4, an attempt is made to illustrate the effect of variation in composition on estimated intake of NDF and CP in relation to the actual intake. The use of average sample values of the quality parameters and DM was compared to daily measured actual DM and average quality parameters. It was assumed that each cow consumed 40 kg of silage per day. Dry matter content accounted for about half of the variation and the other half remained unaccounted for, originating from variations in the NDF and CP content.

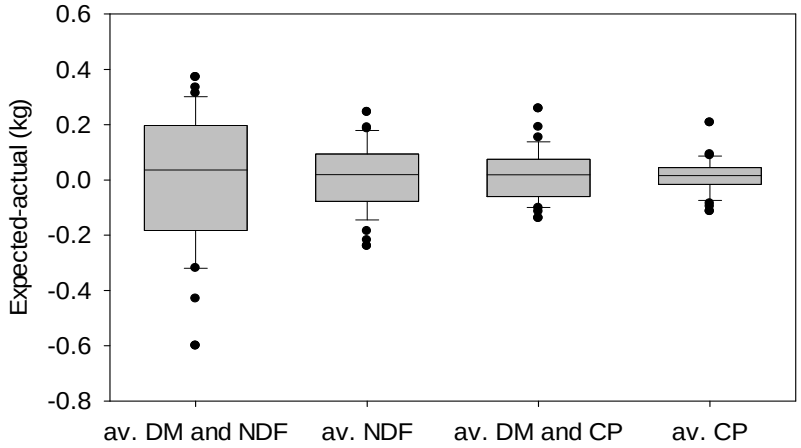

Figure 4 Differences between the daily actual intake of NDF and CP per dairy cow at Viken, and the expected amount based on either average DM and quality parameter or actual DM and average quality parameter. The median, 25/75 and 10/90 percentiles and outliers of the different parameters analyzed are indicated (N=42).

Visible and near infrared for analysis of fresh forage

To be able to account for quality variations in forage, on-line analysis in the daily feeding work flow would be feasible. Visible and near infrared diffuse reflectance spectroscopy is an

established technique for laboratory forage analysis, but is also identified as a very potential technique for on- or at-line analysis for a range of applications (Williams & Norris, 2001). For on-farm analysis, there are emerging technologies.

At the experimental farm in Umeå, we analyzed both fresh and dried and milled silage with a vis-NIR instrument and calibrated prediction models against analyzed quality parameters and DM (Table 3). Calibrations on fresh materials performed almost as good as on dry materials according to a cross validation. It was especially successful for DM and CP, but also NDF, lactate and acetate worked well. Sugars did not perform well, however.

Table 3 Calibration performance with vis-NIR spectroscopy in cross validation at Umeå (N=58)

	Fresh silage			***Dried and milledsilage***		
Parameter	Spectral range	R^2	RMSECV[1]	Spectral range	R^2	RMSE CV
DM	1910 & 2044 nm	0.981	1.72	-	-	-
VOS	NIR	0.793	1.66	Vis-NIR	0.861	1.37
CP	NIR	0.930	6.7	NIR	0.953	5.6
NDF	NIR	0.896	20	Vis-NIR	0.945	15
ADF	NIR	0.761	18	NIR	0.789	17
Sugars	Vis-NIR	0.522	13	NIR	0.487	14
Lactate	Vis-NIR	0.867	9.1	NIR	0.846	10.1
Acetate	NIR	0.818	3.4	NIR	0.821	3.4

[1]Root Mean Squared Error of Cross Validation

The corresponding calibrations at the low-variation data set from Viken resulted in lower R^2; 0.89, 0.64 and 0.52 for DM, CP and NDF, respectively. At low variation, analytical errors can be expected to have a substantial influence on prediction performance as the relative proportion of noise will increase. Still, there was enough actual quality variation for the calibration models to be able to explain between 52 and 89 % of the total variation.

Conclusions

According to data presented here, a coefficient of variation in quality parameters of at least 5-10% can be expected in forages harvested from a single field. As our sampling regimes probably enabled better representativeness than in most real situation, this range is most likely at the lover end. To make better use of sophisticated analytical packages and feed evaluation systems developed, improved control over the variation should be considered. There is a potential in vis-NIR spectroscopy for on-line measurements, or at least at-farm, analyses, of several key parameters with a precision close to what is achieved in the laboratory. This, however, requires further development of strategies and user friendliness of the technique in order to become applicable in practice.

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Methods for reduction of water soluble carbohydrates content in conserved grass forages.

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Introduction

The content of water soluble carbohydrates (WSC) in forages is of importance for feeding horses with decreased insulin sensitivity or equine metabolic syndrome, as both of these conditions may lead to the very painful disease laminitis. It is important to decrease the amount of sugars and starch in the diet for such horses (Frank, 2009). Starch can easily be excluded by removing all grains from the diet, while it may be more difficult to exclude sugars as they may constitute a sizeable fraction of normal temperate grasses. Soaking the forage in water before feeding is a common recommendation, but previous data on the effect of soaking of hay on WSC-content are highly variable (Longland *et al*., 2009). Different methods and/or factors may contribute to a reduction of the WSC content in forages, including conservation form, soaking in water before feeding, storage of longer periods, use of additives etc, and these are studied in an ongoing experiment. Preliminary results from this experiment are presented here, eliciting the effects of conservation method and of soaking on WSC-content in conserved forages.

Materials and methods

A grass crop consisting of 0.4 timothy (*Phleum pratense*), 0.4 meadow fescue (*Festuca pratensis*), 0.1 perennial ryegrass (*Lolium perenne*), 0.06 red clover (*Trifolium pratense*) and 0.04 dandelions (*Taraxacum officinale*) was mowed and left to wilt in 2-m wide rows. When dry matter (DM) content of the wilted crop reached 400 g/kg, approximately one third of the crop was harvested and ensiled in laboratory 25-L silos. The remaining crop was wilted further in the field, and when DM content reached 600 g/kg DM, haylage was harvested as described for silage. When the remaining crop contained 800 g DM/kg, hay was harvested using a high-density baler (Welger AP 730, Wolfenbüttel, Germany) and placed on a barn dryer, where the bales were further dried until DM content had reached 850 g/kg and water activity was less than 0.7. Samples were taken from the forages after 3, 6, 12 and 18 months of storage. At opening of silos after 3 and 12 months of storage, 1 kg DM of silage, haylage and hay was soaked in 17 L of tap water for 12 and 24 hours, after which samples were taken from the soaked forage. Samples were analyzed for glucose, fructose, sucrose and fructans using an enzymatic-spectrophotometric method (Larsson and Bengtsson, 1983), and total WSC content was calculated as the sum of these sugars. Statistical analysis of data was done using SAS 9.3 for Windows and the General Linear Models procedure, examining main effects of conservation method and soaking time, and interactions among conservation method and soaking time.

Results and discussion

Conserving grass as hay resulted in higher contents of all sugars compared to silage with haylage as intermediate except for its sucrose content (Table 1) which was similar in silage and haylage (Table 1).

Table 1 Content of water soluble carbohydrates (g/kg dry matter) in silage, haylage and hay originating from the same fresh crop (N=58)

Variable	Silage	Haylage	Hay	SE	*P*
Glucose	14.0[a]	23.6[b]	40.7[c]	1.75	<0.0001
Fructose	9.7[a]	20.5[b]	34.3[c]	1.55	<0.0001
Sucrose	0.8[a]	1.2[a]	13.3[b]	0.59	<0.0001
Fructans	1.5[a]	0.8[b]	9.9[c]	0.71	<0.0001
Total	24.4[a]	45.3[b]	98.1[c]	2.99	<0.0001

[a,b,c] Values within rows with different superscript letter differs at the P-value presented.

Soaking the forages in water reduced glucose, fructose and total WSC content compared to initial contents (Figure 2a-c). Soaking for 24 h did not result in larger reduction of fructose content in silage (Figure 2a), and there was no difference in glucose, fructose and total WSC content between 12 and 24 h soaking in haylage (Figure 2b). Thus, if soaking practices are applied with haylage, there seems to be little use for a soaking period longer than 12 h. For hay, content of glucose and total WSC was further reduced by soaking for 24 h compared to soaking for 12 h, but contents of fructose, sucrose and fructans were similar after 12 and 24 h of soaking (Figure 2c).

Interactions were present between forage types and soaking time for glucose, sucrose and total WSC content, and for fructan content (P= 0.03). Biologically important interactions were *e.g.* that silage before soaking contained less total WSC than hay that had been soaked for 24 hours (P=0.008). This means that conserving forage as silage may be an effective method to reduce the content of WSC and especially the WSC components giving a glyceamic and insulinaemic response in the horse, i.e. glucose, fructose and sucrose. Considering that the reduction of WSC in all forages in this study was achieved using 17 L of water per kg DM, the total amount of water required for soaking a daily ration for a 500-kg horse is substantial (minimum 5 kg DM * 17 L water = 85 L/d). Other implications from soaking may be decreased hygienic quality of the forage and possible environmental risks from disposal of soaking water containing WSC and other nutrients. It should also be noted, that only one crop was used for the different treatments in this study, and that other crops may produce different results, as Longland *et al*. (2009) reported a high variation in WSC-reduction due to soaking among hays of different origin.

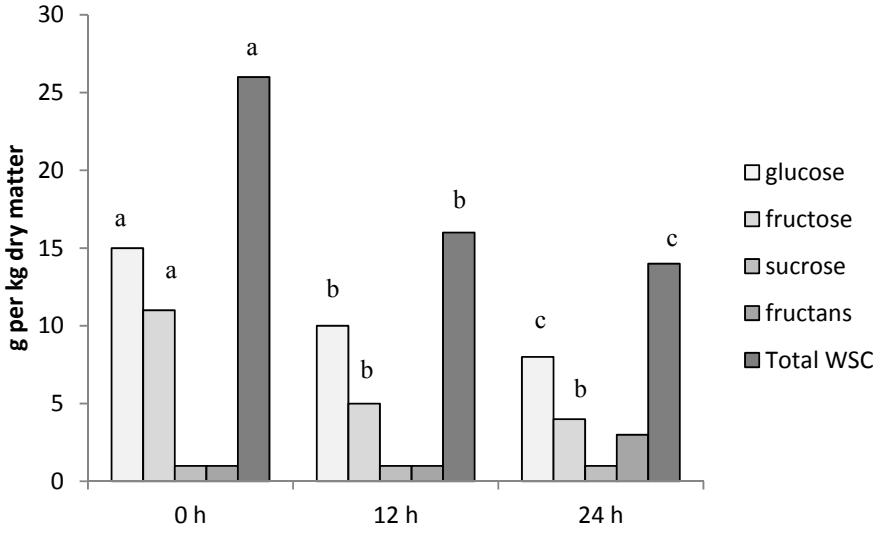

Figure 2 a. Content of water soluble carbohydrates in silage before and after 12 and 24 h soaking in water. Glucose content differed at P<0.04, fructose content differed at P< 0.0006, and total WSC content differed at P<0.007, differences among soaking times are represented with different letters.

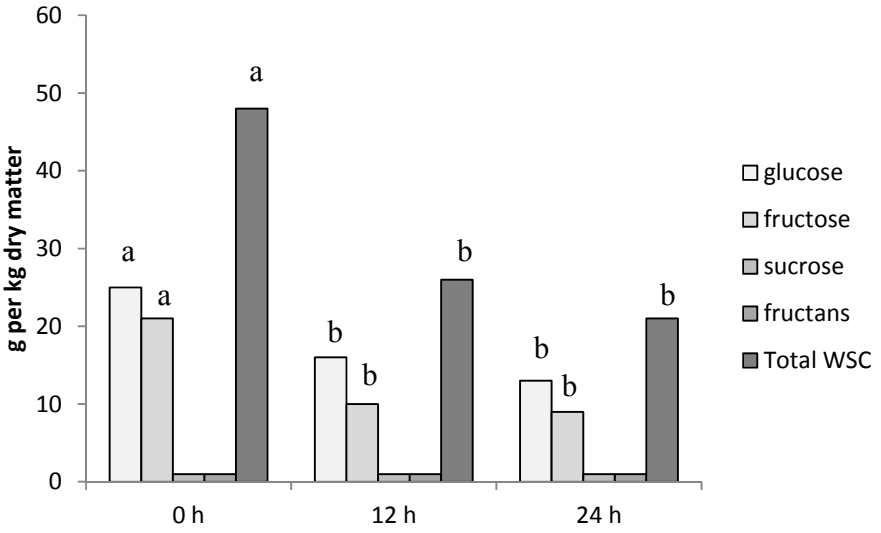

Figure 2 b. Content of water soluble carbohydrates in haylage before and after 12 and 24 h soaking in water. Glucose content differed at P<0.0001, fructose content differed at P< 0.0001, and total WSC content differed at P<0.0001, differences among soaking times are represented with different letters.

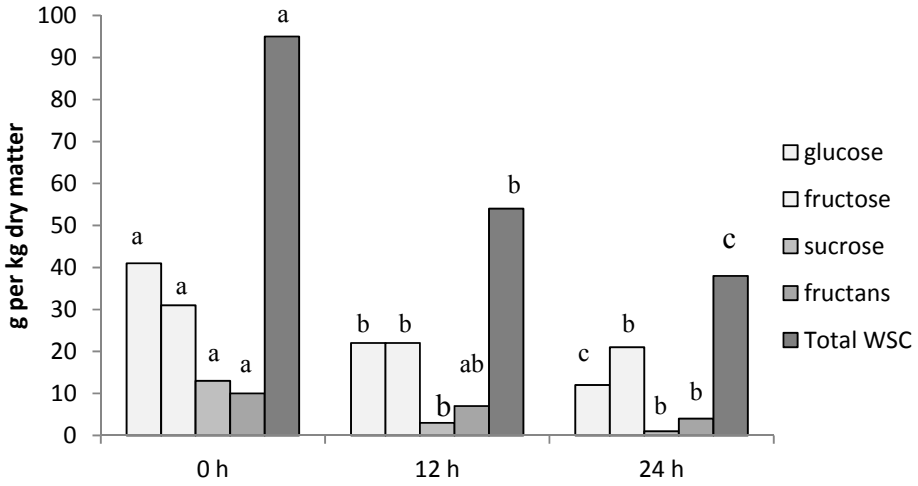

Figure 2 c. Content of water soluble carbohydrates in hay before and after 12 and 24 h soaking in water. Glucose content differed at P =0.0008, fructose content differed at P= 0.0008, sucrose content differed at P<0.0001, fructan content at P = 0.004 and total WSC content differed at P =0.003, differences among soaking times are represented with different letters.

Conclusions

Conservation of grass as silage, haylage and hay resulted in the lowest WSC content in silage, the highest in hay with intermediate levels in haylage. Soaking these forages in water for 12 hours resulted in a reduction of all WSC components and soaking for 24 h further reduced some of the WSC components in silage and hay but not in haylage. Conserving forage as silage may result in a greater reduction of WSC content than soaking hay for 12 or 24 h, and is likely a more convenient method.

Acknowledgements

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SDS-PAGE analysis of seed storage proteins in *Lespedeza hedysaroides* (Pall.) Kitag

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Introduction

Lespedeza Michx. is one of the important eco-economy resources in China. With a variety of excellent features (high content of nutrients, strong resistance to drought and cold, and prevention of soil erosion *et al.*), *Lespedeza* has a very broad application prospects (Lange *et al.* 2006). The aim of this study was to understand the genetic diversity of *Lespedeza* in protein level by SDS-PAGE.

Materials and methods

The materials consisted of three types of *Lespedeza hedysaroides* (Pall.) Kitag., which were named as the 'common type', 'deep green type' and 'tall type'. *L. hedysaroides* were cultivated in Linxi county (118° 01' E, 43° 37' N; altitude, 900 m) located in Inner Mongolia of China in June 3, 2009. The method of SDS-PAGE protocol was in accordance with the Protein Technology Manual (Wang 2008). Water soluble proteins and proteins soluble in alcohol, alkali and salt as well as total seed storage proteins were extracted and analyzed by SDS-PAGE. Protein molecular weights (MW) were calculated as:

$Log_{10}MW = K - bm$, where "m" is protein monomer electrophoretic mobility and "K" and b" are constants. The protein molecular weights (MW) were calculated by using of a known protein marker (D532S). The data was analysis by POPGENE 1.31 (University of Alberta and Center for International Forestry Research, Alberta, Canada and Bogor Barat, Indonesia).

Results and Discussion

The electrophoregrams of SDS-PAGE indicated low proportions of water soluble, alcohol soluble and alkali soluble proteins in *L. hedysaroides*, while salt soluble proteins and seed storage proteins contained multiple bands which could determine the relationships the three *hedysaroides*. Salt soluble proteins and seed storage proteins in the 'common type' and 'deep green type' of *L. hedysaroides* were different from the 'tall type'. The protein molecular weights ranged from 10.375 to 154.53 KDa. Six bands were shared among the three types and 13 bands were specific and polymorphism accounted for 68.42% of total nineteen salt soluble protein bands. In the total seed storage proteins were 19 bands shared and nine bands were specific. Polymorphism accounted here for 32.14% of the total 28 bands (Table 1).

Table 1 Frequency of bands and molecular weight of total seed storage proteins of three *L. hedysaroides* varieties

Loci	Frequency of bands	Rf[a]	Molecular weight (KDa)	Loci	Frequency of bands	Rf	Molecular weight (KDa)
1	0.46	0.17	107.78	15	1.00	0.39	58.07
2	0.73	0.19	101.72	16	0.62	0.4	56.19
3	0.67	0.22	93.69	17	1.00	0.43	51.79
4	1.00	0.25	87.79	28	1.00	0.47	46.56
5	1.00	0.26	84.22	19	1.00	0.52	40.82
6	0.67	0.27	82.14	20	0.81	0.55	37.3
7	1.00	0.28	80.17	21	1.00	0.57	34.98
8	1.00	0.31	76.25	22	1.00	0.59	33.3
9	1.00	0.32	71.96	23	1.00	0.61	31.47
10	0.81	0.33	68.5	24	0.33	0.72	23.27
11	1.00	0.35	66.24	25	1.00	0.75	20.92
12	0.62	0.36	64.05	26	1.00	0.82	17.33
13	1.00	0.37	61.49	27	1.00	0.88	14.79
14	1.00	0.38	60.01	28	1.00	0.94	12.43

[a]Rf = Relative mobility

Applying the genetic distance coefficient clustered the 3 types into two groups - the 'common type' and the 'deep green type' were classified as one group and the 'tall type' as another one (Figure 1). The results of band numbers suggested that polymorphism was greater in salt soluble proteins than in total seed storage proteins, but the ANOVA showed no difference between salt soluble proteins and seed storage proteins. The dendrogram of total seed proteins suggest that the 'common type' and 'deep green type' were clustered as one group as the genetic distance between them was less than 0.02, while the genetic distance was between 0.02 and 0.10 between the 'tall type' and the other types, clustered in one group.

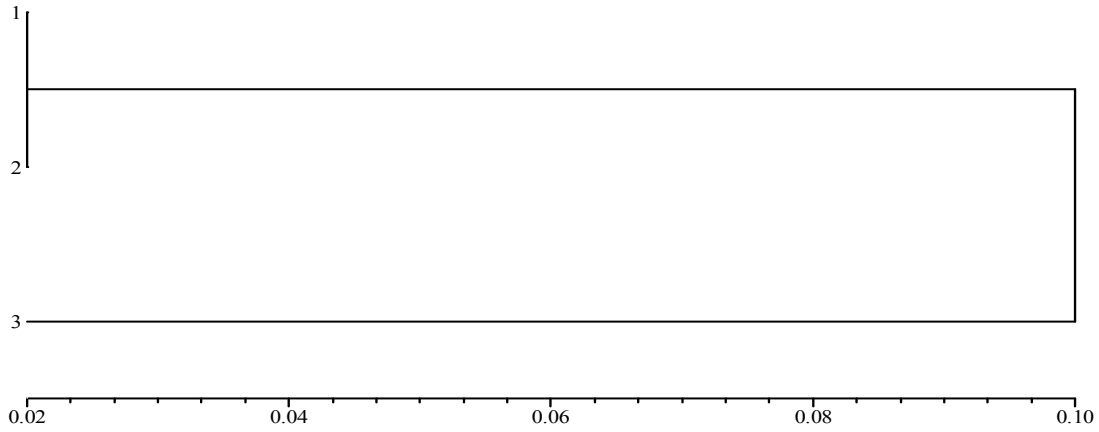

Figure 1. Dendrogram based on Nei's genetic distance of total seed storage proteins where 1: common type 2: deep green type 3: tall type.

Conclusions

The cluster analysis showed that three *L. hedysaroides* could be classified into two categories with the 'common type' and deep green' as one group differing from 'tall type'. The tall type could therefore be regarded as an intra-specific variety of *L. hedysaroides*.

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Effects of different wilting regimes on fatty acid profile of fresh forage

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Introduction

Long chain n-3 polyunsaturated fatty acids (LCFA) have been shown to have beneficial effect on human health (Simopoulos, 2000). A higher proportion of these fatty acids have been found in products from grazing animals than in products from animals fed a diet with conserved forage or high proportion of concentrates (Elgersma et al., 2004a; Fredriksson and Pickova, 2007). The majority of the forage used in beef and milk production is consumed as preserved forage, mainly as silage, due to a limited grazing season. During the ensiling process, it is mainly the wilting procedure that has been shown to have considerable impact on composition and content of LCFA in the forage. In an experiment by Dewhurst and King (1998), extended wilting reduced the content of total LCFA in a crop by 30%, with a reduction of up to 40% of C18:3. The negative effect of wilting on the LCFA profile in the crop was confirmed by Van Ranst et al. (2009). It is assumed that losses of LCFA during the wilting are associated with the activity of lipases, widely present in living plants, during processes of lipolysis (Elgersma et al., 2003). A shortening of the wilting process can thus result in reduction of LCFA losses. Therefore, the aim of the study was to investigate the effect of different wilting regimes on changes in LCFA composition in forage crops.

Materials and Methods

Red clover (*Trifolium pretense)* at early flowering maturity and timothy (*Phleum pretense)* at fully developed heads were used in the study. Forages were harvested manually using a scythe on June 6 2008, nearby Uppsala. Samples were collected in triplicate from each crop and immediately frozen at -18 °C until analysis. The fresh crops were wilted widespread outdoors to four different dry matter (DM) contents. Wilting time varied from 0 and to 48 h, according to the design described in Table 1. The treatments with DM content of 20% were not wilted in the sun but instead placed in the shade and sprayed with water during day time every third hour to simulate unfavorable weather conditions. This combination of wilting times and wilting to different DM contents enabled separation of effect of wilting time and effect of DM content on LCFA content and composition.

Table 1 Experimental design of the study

DM contents	Time of wilting (h)			
(%)	0	12	24	48
20	x	x	x	x
40		x	x	x
60			x	x
80				x

The concentration of DM was analyzed in two steps. First, fresh samples weighing approximately 150 g were dried for 18 h in a ventilated oven at 65°C and milled to pass a 1.0-mm sieve. Final DM concentration was achieved by drying at 103°C for 5 h. Lipids were extracted using the method described by Lourenco et al. (2007). The methylation of samples was performed according to the method described by Appelqvist (1968). Approximately 2 mg of lipids in 0.5 mL of hexane were mixed with 2 mL of 0.01 M NaOH in dry methanol. Samples were incubated at 60°C for 10 min and then 3 mL of BF_3 was added and samples were incubated at 60°C for another 10 min. Afterwards 2 mL of 20% NaCl and 2 mL of hexane were added and lipid phase was separated. The methylated fatty acids in this phase were then chromatographically separated on a BPX-70 fused-silica capillary column (50 m x 0.22 mm x 0.25 μm) in a CP-3800 gas chromatograph.

Results and Discussion

A higher initial DM content and better wilting rate of the timothy crop resulted in higher DM contents during shorter wilting times than planned (Table 2). The opposite was true for the red clover crop, where a lower initial DM content and less good wilting ability of the crop resulted in that planned DM contents were not reached within planned wilting times.

Table 2 Fatty acid (FA) composition (g/100g FAME (fatty acid methyl esters)), FA content (mg/g DM), and crude fat content (g/kg DM) in timothy forage wilted to four DM content levels (%) in four wilting periods (h)

DM contents	Wilting time	C16:0	C18:0	C18:1	C18:2	C18:3	Total FA	Crude fat
32	0	18.2	1.8	3.5	20.1	51.7	17.0	25.8
32	5	18.3	1.9	3.3	19.6	52.1	14.3	26.0
32	21	18.1	1.8	3.4	19.6	52.0	15.3	29.7
32	45	18.4	1.9	3.4	20.6	50.4	12.7	28.1
50	5	17.8	1.8	3.8	20.8	51.1	15.6	26.4
50	21	18.5	1.8	3.3	20.5	51.0	15.5	27.3
50	45	18.2	1.9	2.8	20.0	51.6	11.3	27.2
62	21	16.6	1.9	2.7	19.1	55.5	18.1	34.7
62	45	17.6	1.8	2.8	21.0	51.9	11.5	26.9
77	45	16.2	1.7	2.6	19.7	55.2	15.1	28.5
$LSD_{0.05}$		0.81	0.12	0.68	1.56	2.76	4.93	2.29
Probability	DM	***	*	*	NS	***	NS	***
	Time	NS	NS	NS	NS	NS	**	***
	Int.	NS	NS	NS	NS	NS	NS	***

*, ** and *** at P<0.05, P<0.01 and P<0.001, respectively; NS – not significant; DM – dry matter.

A faster wilting process was probably the reason for limited changes in LCFA composition in timothy forage (Table 2). The increase in forage DM content from 32% to 50% within five wilting hours was not accompanied by any changes in LCFA profile. However, at a DM content of 62% the content of C16:0 and C18:1 was lower in comparison with initial contents.

When timothy had reached 77% DM content, the proportion of C18:0 was reduced (P<0.05). On the other hand, the proportion of C18:3 increased as the crop reached higher DM contents (P<0.001). This result contrasts previous observations (Dewhurst and King, 1998; Van Ranst et al., 2009), where wilting reduced proportion of C18:3 in forage. A possible explanation can be a higher initial DM content of timothy in this study which limited the activity of lipases. In addition, the crop was not mechanically treated, which could contribute to reduce the activity of lipases (Elgersma et al., 2003). There were no differences in FA content in timothy among wilting times within 32 and within 50% DM content, which indicates limited degradation of LCFA due to longer wilting times. A lower initial DM content, and with that an associated higher activity of plant lipases, were probably a reason for large changes in LCFA composition in red clover during wilting compared to timothy (Table 3). Unexpectedly, there were no variation in total FA content in the red clover, but proportions of C18:3 were influenced by an interaction of DM content and wilting time (P<0.01). A reduced proportion of C18:3 was observed due to extended wilting (51 h) or increasing DM content up to 46%. The proportion of C18:3 in the red clover crop containing 62 % DM was however similar to the initial proportion. In the crop containing 16 and 30% DM content, the proportion of C18:1 decreased (P<0.001) during the entire wilting time. Proportion of C18:1 in the red clover crop containing 46 and 62% DM was lower (P<0.03) than the initial proportion. In contrast to timothy, the trend of increased proportions of C16:0 and C18:0 with increasing DM content was observed in the red clover crop, except for C16:0 at 62% DM content. Moreover, extended wilting time (51 h) increased the proportions of C16:0 (P<0.05) and C18:0 (P<0.001), compared to initial proportions. On the other hand, these differences were numerically small, therefore it is uncertain to which extent can these differences can influence the FA composition in animal products.

Table 3 Fatty acid (FA) composition (g/100g FAME (fatty acid methyl esters)), FA content (mg/g DM), and crude fat content (g/kg DM) in red clover forage wilted to four DM content levels (%) in four wilting periods (h)

DM contents	Wilting time	C16:0	C18:0	C18:1	C18:2	C18:3	Total FA	Crude fat
16	0	18.3	2.6	2.1	15.4	57.4	17.9	44.1
16	9	18.4	2.5	1.8	15.2	57.7	20.1	40.7
16	29	18.0	2.5	1.5	14.7	58.9	20.7	43.4
16	51	19.1	2.7	1.6	15.2	56.4	15.9	41.0
30	9	19.3	2.8	2.0	16.3	55.0	21.1	39.8
30	29	18.9	2.7	1.6	15.7	56.4	18.6	41.5
30	51	19.4	2.7	1.8	16.5	54.7	18.1	39.5
46	29	19.4	2.8	1.7	16.3	55.2	17.6	41.0
46	51	19.2	2.7	1.7	16.0	55.8	17.6	41.0
62	51	18.7	2.7	1.6	15.5	56.7	17.0	41.5
$LSD_{0.05}$		0.67	0.12	0.16	0.87	1.42	5.20	3.79
Probability	DM	***	***	***	*	***	NS	NS
	Time	NS	NS	**	NS	*	NS	NS
	Int.	*	***	NS	NS	**	NS	NS

*, ** and *** at P<0.05, P<0.01 and P<0.001, respectively; NS – not significant; DM – dry matter.

Conclusions

Effect of wilting time and DM content influenced the LCFA composition differently in timothy and red clover crops. In timothy forage, extended wilting reduced FA content and a higher DM content caused increase in C18:3 proportion. In red clover, extended wilting reduced the proportion of C18:3 up till 30% DM content. No effect of wilting time or DM content on FA content in the red clover crop was observed.

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Potential of fibre digestion in sugarcane across the harvesting window

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Introduction

Sugarcane is characterized by its high dry matter (DM) yield and high energy value at the maturity point allowing high animal stocking rates. Basically, sugarcane is composed of two principal fractions: a) soluble carbohydrates (mainly sucrose) and b) insoluble fibre (neutral detergent fibre - NDF). True digestibility of the soluble fraction is almost complete (~0.98; Van Soest, 1967), but NDF typically present a low digestibility and has been indicated as the causative factor of low dry matter intake (DMI) of sugarcane based diets (Corrêa et al., 2003).

Thus, the objectives of this study were: 1) to determine the proportion of indigestible NDF on NDF fraction (iNDF/NDF), 2) to estimate the NDF digestibility (NDFD) across sugarcane harvesting window, and 3) to predict sugarcane DM digestibility based on its fibre fractions.

Materials and Methods

Whole plant samples of two modern sugarcane varieties (IAC86-2480 and IAC93-3046) were collected manually during the crop harvesting window in 2007 and 2009, respectively. The IAC86-2480 (3rd cut) was harvested at 150, 180, 210, 240, 270, 300, 330, and 360 days after annual cutting, whereas IAC93-3046 (1st cut) was harvested 300, 360, 420 and 540 days after planting. Fresh cut samples were chopped in a stationary chopper, dried in a forced air oven at 60^{o}C and ground in a Wiley mill through a 1 mm sieve. The *in vitro* true DM digestibility (IVTDMD) and fibre contents (NDF, ADF, iNDF) were determined by near infrared reflectance spectroscopy (NIRS) using specific equations developed for sugarcane. Equations presented R^2 from 0.91 to 0.99 and standard error of cross validation (SECV) from 0.87 to 1.59. Potentially digestible NDF (pdNDF) was calculated as NDF – iNDF, and neutral detergent solubles (NDS) as OM – NDF. The NDF digestibility was estimated as: NDFD = (IVTDMD – 0.98×NDS) ÷ NDF. The same calculation was used for pdNDF digestibility: pdNDFD = (IVTDMD – 0.98×NDS) ÷ pdNDF. The fractional rate of pdNDF degradation (k_d) was calculated from kinetic parameters using the two compartment model that incorporates selective retention of feed particles in the rumen (Allen and Mertens, 1988; Huhtanen et al., 2006) as follows: $k_d = [-(k_p+k_r) + [(k_p+k_r)^2 + 4\times pdNDFD\times k_r\times k_p \div (1- pdNDFD)]^{0.5}] \div 2$, where k_p is the fractional passage rate and k_r is the fractional rate of particle release from the rumen non-escapable pool to the escapable pool. Rumen retention time was assumed as 50 h distributed as 40:60 between the two compartments (*sheep at maintenance*, k_p = 0.033/h and k_r = 0.05/h) (Huhtanen and Krizsan, 2011). The relationship between dependent variables (iNDF/NDF, NDFD, pdNDFD, and k_d) and crop growing period (CGP) were analyzed using the mixed model procedure of SAS, including the random study effect (intercept). Fibre fractions were also tested to predict sugarcane digestibility using the mixed model including the random effect of study (St-Pierre, 2001).

Results and Discussion

There was no close relationship between CGP and iNDF/NDF ratio (Figure 1) and neither between CGP and k_d (Figure 2). Therefore, NDFD ($\bar{x}$ = 0.34, *P* = 0.70) and pdNDFD ($\bar{x}$ = 0.66, Figure 3) remained almost constant during the harvesting window. There is evidence that sugarcane fibre is poorly digestible already at early growing stages (Thiago, 2008). For that reason the most suitable harvesting point should be based on DM digestibility.

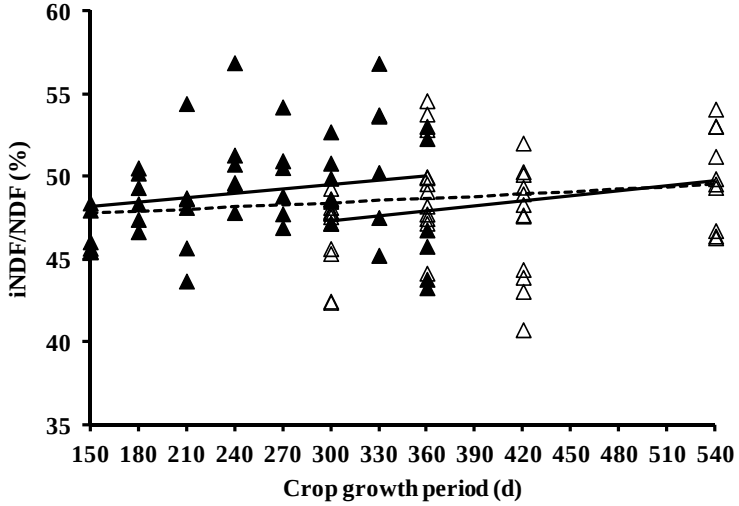

Figure 1 Ratio indigestible neutral detergent fibre /neutral detergent fibre (iNDF/NDF) during sugarcane harvesting window, including random effect of study (▲ IAC86-2480, Δ IAC93-3046). iNDF/NDF = 45.93±1.67 + 0.008±0.004 × GD, R^2 = 0.00, RMSE = 3.12, *P* = 0.28.

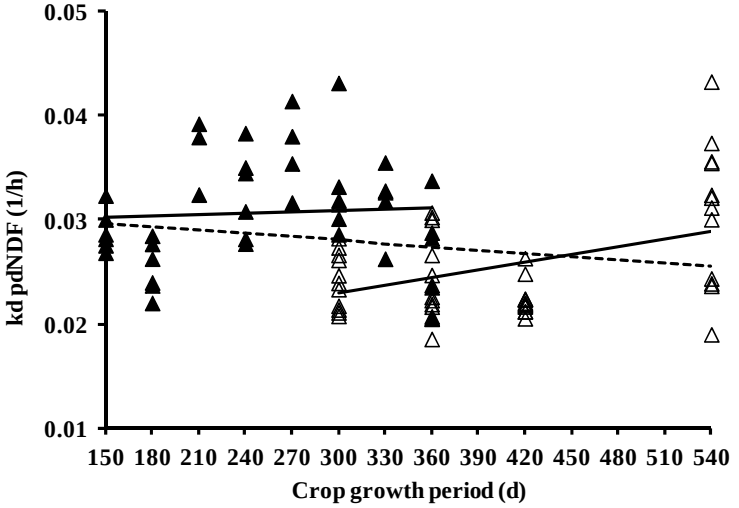

Figure 2 Digestion rate of potential digestible neutral detergent fibre (k_d) during sugarcane harvesting window, including random effect of study (▲ IAC86-2480, Δ IAC93-3046). k_d = 0.023±0.005 + 0.000±0.000 × GD, R^2 = 0.02, RMSE = 0.007, *P* = 0.30.

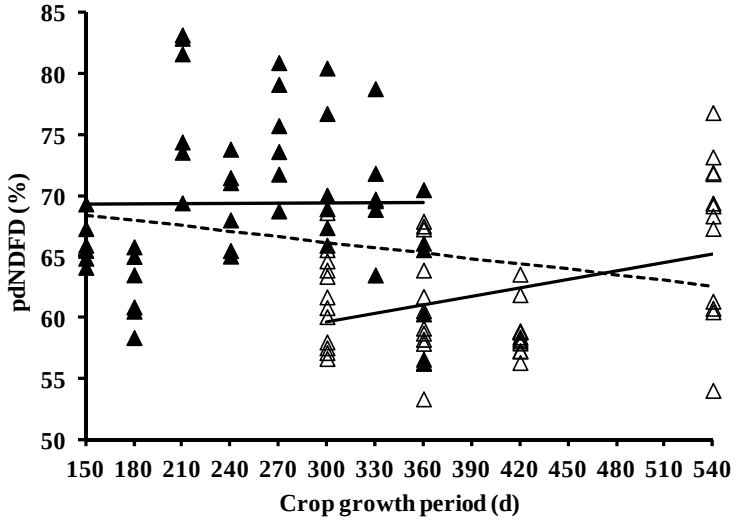

Figure 3 Potential digestible neutral detergent fibre digestibility (pdNDFD) during sugarcane harvesting window, including random effect of study (▲ IAC86-2480, Δ IAC93-3046). pdNDFD = 61.13±5.33 + 0.014±0.008 × GD, R^2 = 0.04, RMSE = 6.01, P = 0.32.

Since the overall digestibility is a key factor in sugarcane nutritive value, insoluble fibre fractions were tested as predictors of IVTDMD (Table 1). Root mean square errors (RMSE) ranged from 2.36 to 1.39 and the adjusted R^2 from 0.56 to 0.81. Across univariate models, ADF showed better fitness rather than iNDF, which is also typically a good predictor of cool-season grass digestibility (Huhtanen and Krizsan, 2011). Teixeira (2004) also defined ADF as the best predictor of DM digestibility in 20 commercial varieties of sugarcane.

When the residuals (observed minus predicted) of IVTDMD estimated by iNDF were checked, they were significantly correlated (R^2 = 0.57, P < 0.01) to the fraction [ADF - iNDF]. This fraction was similar to the proportion of potentially digestible fibre inherently difficult to be digested in ruminants. In cool-season grasses, pdNDF is almost a uniform nutritional entity with digestibility coefficient of 0.85, which pointed out the iNDF as the best predictor of OM digestibility (Huhtanen and Krizsan, 2011). Conversely, that was not confirmed for sugarcane. Obviously, there were some improvements when new variables were included into the model, however benefits were only marginal (Table 1). The simple model based on ADF seems to be the best model to predict sugarcane digestibility and could be adopted as a screening tool for a variety breeding program.

Conclusions

In conclusion, NDF digestibility varies little across the growing period. This is why the sugarcane harvesting point should be based on overall DM digestibility which, in turn, is greatly influenced by sucrose accumulation. Other strategies such as ration formulation might be more successful to reduce the negative effect of NDF on diet DMI. Acid detergent fiber was the best predictor of sugarcane DM digestibility. Including some of the other variables into the model did not lead to significant improvements.

Table 1 Predictions of *in vitro* true DM digestibility from fibre fraction in sugarcane using a regression equation (F) or a mixed model regression (M) with random trial effect ($Y = A + BX_1 + CX_2 + DX_3$)

X_1, X_2, X_3	Model	A	SE†	B	SE†	*P*	C	SE†	*P*	D	SE†	*P*	RMSE‡	Adj-R^2
NDF	F	91.77	1.65	-0.58	0.03	<0.01							1.73	0.78
	M	92.38	1.67	-0.59	0.03	<0.01							1.56	0.78
pdNDF	F	80.36	1.45	-0.71	0.05	<0.01							2.21	0.65
	M	80.47	1.51	-0.71	0.05	<0.01							2.15	0.65
ADF	F	92.07	1.64	-0.98	0.05	<0.01							1.71	0.79
	M	91.98	1.64	-0.98	0.05	<0.01							1.69	0.79
iNDF	F	93.36	2.94	-1.25	0.11	<0.01							2.36	0.56
	M	95.27	2.85	-1.33	0.10	<0.01							2.11	0.56
NDF, iNDF	F	94.09	2.09	-0.47	0.05	<0.01	-0.31	0.13	0.02				1.68	0.78
	M	95.69	2.00	-0.45	0.04	<0.01	-0.43	0.11	<0.01				1.42	0.78
pdNDF, iNDF	F	94.09	2.09	-0.47	0.05	<0.01	-0.78	0.10	<0.01				1.68	0.78
	M	95.69	2.00	-0.45	0.04	<0.01	-0.87	0.08	<0.01				1.42	0.78
ADF, iNDF	F	96.37	2.00	-0.76	0.07	<0.01	-0.44	0.11	<0.01				1.59	0.80
	M	97.20	1.96	-0.69	0.07	<0.01	-0.56	0.11	<0.01				1.48	0.80
ADF, iNDF, pdNDF	F	95.73	1.99	-0.53	0.14	<0.01	-0.51	0.11	<0.01	-0.18	0.09	0.05	1.57	0.81
	M	96.38	1.93	-0.30	0.13	0.02	-0.71	0.11	<0.01	-0.28	0.08	<0.01	1.39	0.81

†Standard error; ‡Root mean square error; NDF: neutral detergent fibre (% DM), ADF: acid detergent fibre (% DM), iNDF: indigestible NDF (% DM), pdNDF: potential digestible NDF (% DM).

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Indigestible NDF (iNDF) in grass and clover mixtures at different harvesting times

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Introduction

The fiber fraction in forages is commonly analyzed as neutral detergent fiber, NDF (Van Soest et al., 1991) and the indigestible part of the NDF is estimated as iNDF. The NDF and iNDF content of feeds are of great importance for ruminants and used in many feed evaluation and ration formulation systems such as the Norfor system in Denmark, Iceland, Norway and Sweden (NorFor, 2011). The content of metabolizable energy (ME) is closely related to the NDF content and particularly to the indigestible part of NDF where high proportions of iNDF are related to low ME values.

As forage mature, the lignin and iNDF contents increase which results in a lower ME. Species and varieties of grasses and legumes have different abilities to retain a high ME content during maturation. Perennial ryegrass (*Lolium perenne* L.) and hybrid ryegrasses are known to be among the better species in this respect (Johansson, 1995; Johansson & Nilsdotter-Linde, 1995; Halling, 2008).

A way to prolong the ”harvest window” can be to choose species and varieties in multispecies seed mixtures so that the iNDF increase is as slow as possible when cutting time is delayed. Such mixtures are available today on the market (Danielsson, 2005).

The aim of the present study was to add information about NDF and iNDF contents in forages harvested at different stages of development in a number of forage species as well as in various multispecies mixtures. An aim was also to test if varieties of Lolium perenne in multispecies seed mixtures, cultivated under Swedish conditions, show slow development of indigestible fiber as maturation of the sward proceeds.

Material and Methods

A total of 199 forage samples of multispecies mixtures were harvested at three sites during three years in 1 to 3 harvests per year. From these mixtures, 49 samples of pure species were identified and retained for further analyses, resulting in a total sample number of 248. These samples were analyzed for NDF and iNDF. The sampling scheme is illustrated in Table 1.

The first harvest was either cut at an early or later stage of development. The “early” cut was aiming at what is considered a high quality dairy feed in Sweden at approximately 11 MJ ME per kg dry matter (DM) and “late” was cut 10–12 days later than “early”. The harvest times of the second and third cut were made with identical periodicity in all cases. The multispecies seed mixtures A–E were designed to produce early or late maturing leys and with or without clover according to the following scheme (and Table 2): commercial standard SW 944 (A), early maturing with clover (B), late maturing with clover (C), early maturing without clover (D) and late maturing without clover (E).

Table 1 Distribution of samples according to Site, Year, Harvest, Stage of development at 1st harvest (1=early; 2=late) and multispecies mixture (A–E). Pure species are all separated from original mixtures at site Jönköping

Site and species	Year	Harvest	Stage of dev. at 1st harvest	Species mixture[1]	Number of samples
Jönköping	2007	1, 2, 3	1 and 2	A–E	30
	2008	1, 2, 3	1 and 2	A–E	30
	2009	1, 2, 3	1 and 2	A–D, A–E	29
Rådde	2007	1, 2	1	A–E	10
	2007	3	2	A–E	5
	2008	1, 3	1 and 2	A–E	20
	2008	2	1	A–E	5
	2009	1, 2, 3	1 and 2	A–E	30
Kalmar	2007	1, 2, 3	1 and 2	A–E	30
	2008	1	1 and 2	A–E	10
Sum mixtures A–E					199
Lolium perenne	07–09	1	1 and 2		12
Phleum pratense	07–09	1	1 and 2		14
Festuca pratensis	07–09	1	1 and 2		7
Trifolium pratense	07–09	1	1 and 2		14
Trifolium repens	09	1	1 and 2		2
Sum pure species					49
Total					248

[1]For details on species in the multispecies seed mixtures A–E, see text and Table 2.

Table 2 Seeds and amounts used

Species	Variety	Ripening character	Seed mixture (kg/ha)				
			A	B	C	D	E
Phleum pratense	Ragnar	Moderate	4		4		5
	Grindstad	Early	2	6		7.5	
	Tundra	Late			4		5
Festuca pratensis	Sigmund		3	6		7.5	
	Tyko		3				
Lolium perenne	Helmer	Moderate	4				
	Baristra	Early		4		5	
	Tivoli	Late			2.5		3
	Herbie	Late			1.5		2
	Condesa	Late			2.5		3
	Cancan	Late			1.5		2
Trifolium pratense	Sara		2		2		
	Titus			2			
Trifolium repens	Ramona		2	2	2		

Samples were first dried at 60°C for 16 hours in a ventilated oven and milled on a knife mill (Brabender OHG, Duisburg, Germany) to pass a 1.5-mm sieve and analyzed *in sacco* (288 h) for iNDF according to Lund *et al.* (2007) and recommended by NorFor (2011). Samples for

neutral detergent fibre analysis (aNDFom) were milled on a beater cross mill (Kamas, Malmö, Sweden) to pass a 1.0-mm screen according to ISO 16472:2006 IDT, recommended by NorFor (2011). Both amylase and sodium sulphite was used and values were expressed exclusive of ash. Dry matter was determined by drying the samples at 103°C for 5 h and ash was determined after a 3-h combustion at 550°C.

Statistical analysis of the seed mixtures was made by analysis of variance and the GLM procedure of SAS (SAS, 2010). The effect of harvesting time (early or late) and seed mixture (A–E) on aNDFom and iNDF content was analysed with site, year and harvest number as class variables. Interactions between seed mixture and site and between year and harvest number were included when significant.

Results and Discussion

In Table 3 the aNDFom and iNDF content of the multispecies mixtures at different cutting times are shown. Mixture C had a lower and D had a higher aNDFom content compared with the other mixtures at both harvest times which was in line with expectations. The iNDF content, expressed as a proportion of the total aNDFom, was significantly lower for mixture D and E, compared with the other mixtures at the early cutting time, in contrast to what was expected of an early developing seed mixture. For E, this was in line with the expectation for a late maturing seed mixture, particularly when the grass species consist of *Lolium* varieties. However, the difference between the early and late maturing forages was more pronounced at the late harvest time where mixture E retained a low iNDF content while D became equal to the other mixtures. In Table 3, the change in aNDFom content and iNDF proportions between the two cutting times are also shown.

Table 3 Least square means of neutral detergent fibre (aNDFom) and indigestible aNDFom (iNDF) in forages from five different seed mixtures harvested at three sites during three years at three cuts per year

	aNDFom, g/kg DM			iNDF, g/kg aNDFom		
Seed mixture	Early cut	Late cut	Change	Early cut	Late cut	Change
A – standard	414^{ab}	459^{a}	+ 45	158^{a}	181^{a}	+ 23
B – early with clover	436^{ac}	459^{a}	+ 23	160^{a}	188^{a}	+ 28
C – late with clover	389^{b}	411^{b}	+ 22	165^{a}	183^{a}	+ 18
D – early without clover	495	515	+ 20	126^{b}	160^{ab}	+ 34
E – late without clover	447^{c}	462^{a}	+ 15	124^{b}	144^{b}	+ 20

^{a,b,c}Means in the same column sharing the same superscript do not differ significantly (P>0.05).

Table 4 presents aNDFom and iNDF contents of the pure species from the mixtures at site Jönköping. The aNDFom content of both red clover (*Trifolium pratense* L.) and white clover (*T. repens* L.) were significantly lower than the grasses timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* Huds.) and perennial ryegrass (*Lolium perenne* L.) but the proportions of iNDF in total aNDFom was higher in both red and white clover.

Table 4 Least square means of aNDFom and iNDF in pure species from seed mixtures A–E

Species	aNDFom, g/kg DM	iNDF, g/kg DM	iNDF, g/kg aNDFom
Lolium perenne	487[a]	70[ab]	137[a]
Phleum pratense	556[b]	72[ab]	132[a]
Festuca pratensis	566[b]	73[ab]	125[a]
Trifolium pratense	307[c]	81[a]	254[b]
Trifolium repens	223[c]	47[b]	203[b]

[a,b,c]Means in the same column sharing the same superscript do not differ significantly (P>0.05).

Conclusions

Late maturing grasses such as perennial ryegrasses (*Lolium perenne*) produced the ley with the least increase of iNDF between the early and late harvest and, thereby, had the longest "harvest window". A ley with a high content of clover also resulted in a long "harvest window". However, the high iNDF proportion in clover generally tends to decrease the ME value of clover.

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Harvest system and the effect on forage quantity and forage quality

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Introduction

The timing of cutting for silage is one of the most important factors affecting yield and quality. There is an increasing desire for high digestibility (high energy concentration) forage resulting in earlier harvests of both first and second cut. This often results in a fall growth of a "third" crop. The impact of changes in harvest systems on total production, feed value and economy is not clear. The varieties used today are different than in previous studies of the effects of harvest strategy. No systematic studies on long- and short-term effects of two- and three-cut systems have been conducted in northern Sweden. Also in other parts of the country, complementary studies are required.

A preliminary study at Röbäcksdalen, in 2003 and 2004, showed that a two-cut system with early cuts, did not exploit the growing season as well as a three-cut system or a two-cut system with later cuts. The yield for the early two-cut system was about 2000 kg less of dry matter (DM) per hectare than for the three-cut system. The following year, when all systems were cut at the same time, the three-cut system had the lowest yield. The difference to the two-cut system with early cut, was again about 2000 kg of DM per hectare. The results from this preliminary study were based on limited data, but the trends were nevertheless interesting. The result indicated, that in a two-cut system, where the aim was a high feed value, we failed to exploit the growing season, but it also indicated that there may be an after effect of the three-cut system on harvest in the following year. Thus, there is a lack of information on different harvesting systems and their impact on feed value, yield and plant winter survival. The objective of the present study was to examine both short- and long-term effects of different harvesting systems (two or three cuts per season) over three years and at two locations in leys composed of red clover, timothy and meadow fescue.

Materials and methods

Field trials were established at two sites, Röbäcksdalen, Umeå, and Riddersberg, Jönköping. The trials were placed in fields with a first year ley of a red clover, timothy and meadow fescue mixture. Two- and three-cut systems were evaluated by measuring DM yields at all harvests. Feed value was analyzed plot wise at all cuts. The effect of litter in spring on silage quality was studied in harvests from Röbäcksdalen. Results from the field experiment were then used in ration calculations and in an economic evaluation. Ration calculations were performed according to NorFor (2011). The seed mixtures applied (kg/ha) were: Röbäcksdalen: 5 kg red clover (Betty), 12 kg timothy (Grindstad) and 8 kg of meadow fescue (Kasper). Riddersberg: 3 kg red clover (Ares), 16 kg timothy (Raymond) and 6 kg of meadow fescue (Sigmund). The trials were fertilized in spring with P and K according to the area and corrected for soil analyses. Nitrogen was given to each crop and was adapted to the clover content. As there were rather low amounts of clover and the difference between treatments were low, all plots received 60 kg N/ha in spring and 50 kg N/ha after first and second cut respectively.

The following harvesting systems were compared in the study:

A. Two cuts with the aim to get high quality forage, re-growth after 2nd harvest left uncut.

B. Three cuts with the aim to get high quality forage

C. Two crops, with a somewhat later harvest.

For treatment A and B, first harvest was determined by sampling the crop to estimate optimal time for high quality. The first cut of treatment C was done about 1 week later. The second cut of treatment B was made five weeks after the first cut and in treatment A, six weeks after the first cut. The second cut of treatment C was made about 10 days after the second cut of treatment A. The third cut of treatment B was harvested about 6 weeks after the second cut in that treatment.

The field plan included four plots for each treatment. Each plot was divided into three sub-plots. The first year, all sub-plots were harvested according to the treatments A, B and C respectively. The second year, one sub-plot in each of the main plots was harvested at the same time in all treatments in two cuts to measure the effect of the different treatments after one growing season. These sub-plots were then omitted from the experiment for the coming years. The other two sub-plots were harvested according to the initial treatments (A, B and C) as in year one. In the third year, one of the two remaining sub-plots was again harvested at the same time in all treatments in two cuts. The last sub-plot in each main plot was still harvested according to the original treatments. With this approach, the effect of the systems could be monitored throughout the duration of the three years, while also the effects after one and two years could be studied. In treatment A and B we used the harvested crop from the sub-plots, harvested at the same date, to study the effect of litter in the silage, using small experimental silos.

Measurements and analyses

Each year, DM yield as well as botanical composition was determined. Also composition of the harvested green material was determined as well as amounts of residual crops in the autumn.

Year 2 and 3, the effect of cropping systems on the crop the following year was studied in the sub-plots harvested at the same time in all treatments (see above). Fermentation pattern as affected by the quantity of litter in the spring was determined in sub-plots.

Feed analyzes have been conducted on sub-plot wise samples according to NorFor (2011) and included: DM, metabolisable energy (ME), crude protein (CP), neutral detergent fibre (NDF) and indigestible NDF (iNDF).

Results and discussion

Röbäcksdalen

The highest total DM yield over the three years was produced by the three-cut system, treatment B. During individual years, the three-cut system (B) had the highest yield in year 1 and 2, while the two-cut system with later harvests (C) had the highest yield in year 3 (table 1).

Table 1 Harvest date and dry matter (DM) yield for the various systems for each consecutive year at Röbäcksdalen

	1st harvest		2nd harvest		3rd harvest		Total harvest
	DM, kg/ha	Date	DM, kg/ha	Date	DM, kg/ha	Date	DM, kg/ha
Year1							
System A	3168	19-Jun	3435	31-Jul			6603a
System B	3157	19-Jun	3100	26-Jul	3826	06-Sep	10083c
System C	4636	27-Jun	4064	14-Aug			8700b
Year 2							
System A	2857	15-Jun	4789	30-Jul			7645a
System B	3193	15-Jun	3691	23-Jul	2810	05-Sep	9695c
System C	4119	21-Jun	4792	15-Aug			8911b
Year 3							
System A	2553	13-Jun	4711	28-Jul			7264
System B	2562	13-Jun	3268	18-Jul	1721	02-Sep	7551
System C	4146	18-Jun	4688	05-Aug			8834

Different letters (a, b, c) indicates that there is a significant difference between the means (p=0,05; Fishers LSD test).

The late third cut in system B did not affect the first cut the following year negatively, as the first cut in system B was similar to the first cut in system A. Compared with the other systems that were relatively stable over all three years, the yield of system B decreased from 10083 kg DM/ha to 7551 kg DM/ha. A marked decline in yield in the third year indicates that the system can cause problems with the persistence of the ley. Average yield per year for the three years (kg DM/ha) were, System A: 7171 kg DM/ha; System B: 9110 kg DM/ha, and System C: 8815 kg DM/ha,

Table 2 –Crop composition - averages of 3 years

	ME, MJ/kg DM	CP, % of DM	NDF, % of DM	iNDF % of NDF
1st harvest				
System A	11.0	15.6	49.0	13.2
System B	11.2	14.3	51.5	13.7
System C	10.8	12.3	52.7	16.0
2nd harvest				
System A	10.5	12.8	49.1	19.2
System B	10.8	12.8	47.7	16.2
System C	10.3	11.0	50.7	20.7
3rd harvest				
System B	10.4	14.1	50.6	17.1

ME= metabolisable energy; DM= dry matter; CP=crude protein; NDF=neutral detergent fibre; iNDF=indigestible NDF.

System C with two cuts with somewhat later harvests gave higher yields than the system with two early cuts (A) which was in agreement with previous studies. System C also showed a good persistence over the years. The botanical composition indicated that system A retained a higher clover proportion in the third year compared to system B and C (data not shown). Table 2 shows crop composition for the different cuts as an averaged over the 3 years. System C consistently gave lower energy concentrations as compared with system A and B.

Riddersberg

During all three years, the three-cut system (B) gave the highest DM yield, although the two-cut system with later harvests (C) had as high a yield as system B during year 2 (table 3).

Table 3 Harvest date and dry matter (DM) yield for the various systems for each consecutive year at Riddersberg

	1st harvest		2nd harvest		3rd harvest		Total harvest
	DM, kg/ha	Date	DM, kg/ha	Date	DM, kg/ha	Date	DM, kg/ha
Year1							
System A	7283	31-May	3775	16-Jul			11058a
System B	7182	31-May	3059	12-Jul	3771	19-Sep	14012c
System C	8134	07-Jun	3603	26-Jul			11737b
Year 2							
System A	5883	28-May	4742	23-Jul			10625a
System B	5166	28-May	2555	10-Jul	5031	29-Aug	12752b
System C	6917	05-Jun	5411	30-Jul			12328b
Year 3							
System A	5676	01-Jun	3106	14-Jul			8782b
System B	5387	01-Jun	2090	09-Jul			11856a
System C	6924	09-Jun	2706	18-Jul			9630b

Different letters (a,b,c) indicates that there is a significant difference between the means. (p=0,05; Fishers LSD test).

The decrease in DM yield was almost the same, about 2000 kg DM, in all systems over the three years. Across years, the total DM-yield was highest in the three-cut system (B) and lowest in the early two-cut system (A) while the late two-cut system (C) was intermediate. Average yield per year for the three years (kg DM/ha) as well as relative value were, respectively, in System A: 10155 kg DM/ha; System B: 12873 kg DM/ha and System C: 11232 kg DM/ha.

The energy content of the various cuts in the different systems was the desired and expected given the proportion of clover in the sward. However, the energy content of the third cut (system B) was lower than was expected and what is desirable. The relatively low content of NDF was explained by the proportion of clover which also explained the elevated content of iNDF.

The system with two cuts harvested at a later date (C) gave higher yields than the system with early cuts (A), in good agreement with previous studies. In Table 4, crop composition is presented as an average across years. System C consistently gave lower energy concentrations as compared with A and B.

Table 4. Crop composition – ME, CP, NDF and iNDF - averages of 3 years.

	ME, MJ/kg DM	CP, % of DM	NDF, % of DM	iNDF % of NDF
1st harvest				
System A	11.0	14.1	51.0	17.4
System B	11.1	14.1	49.8	17.2
System C	10.1	12.2	54.3	21.0
2nd harvest				
System A	10.6	17.7	43.5	18.4
System B	10.9	21.2	39.3	17.6
System C	10.4	17.1	43.3	21.6
3rd harvest				
System B	9.5	17.1	47.3	30.4

ME= metabolisable energy; DM= dry matter; CP=crude protein; NDF=neutral detergent fibre; iNDF=indigestible NDF.

Based on the yields from the field trials and calculations according to NorFor (2011), feed rations from each harvesting system were calculated. The difference between the systems was evident when calculating the rations. System C gave higher costs for the ration than the others as it required greater amounts and more expensive concentrate feed than the other two systems with earlier cuts. From both economical and environmental points of view, it was interesting that forage in the system A and B could be supplemented with a larger percentage of home produced cereals and less purchased concentrates, compared with system C. The actual cost depends on how much land that is available and the cost to utilize it. The choice of harvesting system then becomes a trade-off between the availability and price of land, the cost to harvest and the cost of concentrate feed. Interestingly, system B combined a low feed cost per cow with a high number of cows per ha. System B is therefore the most interesting alternative in situations with limited land availability in combination with high concentrate prices.

Conclusions

The three cut-system had the highest DM yield over three years. Harvesting systems with early cut silage resulted in cheaper feed rations with less concentrate. The overall conclusion was that the three-cut system is combining a lower cost of the ration with a higher DM yield and a more efficient land use.

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The utilization of multidimensional scaling to evaluate preferences of goats for different grass silages

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Introduction

Ensiling of forages is a complex process where a multitude of compounds is generated as a consequence of fermentation. These fermentation end products in silage have long been believed to impact silage dry matter (DM) intake (DMI) through an effect on preference (Krizsan et al., 2007). Until now, it has not been fully understood which fermentation products lead to preference or avoidance of silages by ruminants. Forage preference is generally difficult to assess but is likely an important factor in determining DMI (Buntinx et al., 1997). Since feeding behaviour is more sensitive to the different characteristics of feeds in a choice situation (Baumont, 1996), preference trials should be performed in choice situations. The aim of this study was to evaluate the preference of goats for grass silages after different lengths of aerobic storage using the SAS procedure Multidimensional Scaling (MDS).

Materials and Methods

Eight grass silages were produced differing in DM content (25% and 33%), cut length (short- and long-cut) and packing density in the silo (high and low). Each factor combination, i.e. treatment, was ensiled in six 110-L plastic barrels for at least six months. At the day (d) of silo opening (d0) and then at two-day intervals (d2, d4, d6, and d8) the following measurements were conducted: temperature, sensory evaluation, chemical composition, fermentation products and microbiological testing. Chemical analysis consisted of proximate constituents, fibre fractions and also 24-h *in vitro* gas production was measured. Furthermore, pH, lactic acid (HPLC), volatile fatty acids and alcohols (GC) as well as esters (ethyl lactate and ethyl acetate) and water soluble carbohydrates were determined after coldwater extraction. The DM was corrected for the loss of volatiles during drying according to Weissbach and Strubelt (2008). For use in preference trials, samples of silages (1.5-2.0 kg fresh matter per portion) were taken and stored anaerobically in evacuated and vacuum-sealed polyethylene bags in a dark, dry and cool room. Storage time ranged between 5 and 26 days depending on the day when the silage was fed in the preference trial.

Afterwards, eight preference trials (Buntinx et al., 1997) with goats (n = 5) were carried out, each one lasting 21 days. All trials were conducted with castrated male Saanen type goats (German Improved White Goat breed, mean body weight 88.5 kg ± 12.8 kg). During the experimental phase, each possible two-way combination of the five silages (d0, d2, d4, d6, and d8) and one standard lucerne hay (n = 15), was offered as free choice for 3 h in the morning.

Intake data from every experiment was used to calculate the following ratio for each combination:

d = (Intake of preferred forage - Intake of less-preferred forage)/Total intake

In this way, preference was expressed as a difference ratio ranging between 0 and 1. These ratios were analyzed using the SAS procedure MDS, which develops a spatial arrangement

representing the differences expressed as selective forage intake by the animals. Silages with coordinates that are similar in the dimensional space can be seen as similar in preference and, conversely, coordinates far apart from each other indicate forages that differ in preference (Buntinx et al., 1997). Each experiment was also tested by analysis of variance after averaging intake of each forage by each animal. Within the silages, means were separated using the minimum significant difference (MSD) from the Waller-Duncan k-ratio t-test (Burns et al., 2001).

Results and Discussion

Composition of the grass silages at the day of opening and a lucerne hay, which was used as a standard forage in the preference trials, is given in Table 1.

Table 1 Composition of lucerne hay (standard forage) and silages at the day of opening

	25% DM silages		33% DM silages		
	Mean (n = 4)	SD	Mean (n = 4)	SD	Lucerne hay
DM* (g/kg)	25.1	2.12	32.8	0.26	90.8
Composition (g/kg DM)					
Ash	100	10.0	93	4.4	91
Crude Protein	133	5.9	127	4.8	153
Crude Fat	37	3.2	33	3.8	27
Crude Fibre	273	6.5	258	16.8	287
NDFom	449	13.1	451	10.8	464
ADFom	291	13.8	275	2.5	346
Lactic Acid	68.6	8.9	55.6	4.9	
Acetic Acid	26.6	3.0	22.5	3.3	
Butyric Acid	20.9	10.5	3.5	1.4	
Propionic Acid	0.4	0.6	0.0	0.0	
Ethanol	14.8	3.8	11.6	3.2	
Propanol	2.7	1.5	0.9	0.5	
pH	4.4	0.1	4.6	0.1	

*Dry Matter corrected according to Weissbach and Strubelt (2008); 0 = below detection limit (0.03% of fresh matter); NDFom = neutral detergent fibre expressed exclusive residual ash; ADFom = acid detergent fibre expressed exclusive residual ash

Concerning the proximate constituents, all silages were within normal ranges of good quality silages found in the literature. The 25% DM-silages were not well-fermented with concentrations of butyric acid and alcohols exceeding typical ranges (Kung and Shaver, 2001).

Goats showed a decrease in preference and DMI of silage with 33% DM after four or six days of aerobic exposure ($p < 0.05$). Silage from d8 was strongly avoided in each case. The

DMI of 25% DM silages was generally lower and fewer differences could be observed between silages with different lengths of aerobic storage (see Table 2).

Table 2 Dry matter (DM) intake (g/3 h) of grass silages after 0 to 8 days of aerobic exposure and lucerne hay in eight experiments with goats, n = 25

	Length of aerobic storage (days)							
Silage	0	2	4	6	8	Lucerne hay	Mean d0-d8	MSD
S-25-1	305^{a}	312^{a}	333^{a}	354^{a}	323^{a}	379^{a}	325	151
S-25-2	418^{b,c}	479^{a,b,c}	537^{a}	396^{c}	379^{c}	536^{a,b}	442	119
L-25-1	343^{a}	388^{a}	401^{a}	377^{a}	324^{a}	409^{a}	367	131
L-25-2	312^{b}	493^{a}	513^{a}	409^{a,b}	322^{b}	473^{a}	410	130
S-33-1	496^{a,b}	557^{a}	545^{a}	564^{a}	399^{b,c}	359^{c}	512	126
S-33-2	566^{a}	561^{a}	630^{a}	509^{a}	272^{b}	289^{b}	508	124
L-33-1	492^{a,b}	492^{a,b}	587^{a}	406^{b}	378^{b}	433^{b}	471	130
L-33-2	658^{a}	549^{a,b}	550^{a,b}	492^{b,c}	354^{d}	412^{c,d}	521	129

S = short chopping length; L = long chopping length; 25 = 25% DM; 33 = 33% DM; 2 = high packing density; 1 = low packing density; MSD = Minimum significant difference (Waller Duncan k-ratio t-test); $^{a-d}$ = Means within a row with different superscripts differ ($p < 0.05$)

Table 3 shows DMI and stimulus coordinates for the two-dimensional solution to the preference among goats in one of the experiments. In this case, mean DMI was highest for silages d2 and d4 as well as the lucerne hay. There was a lower DMI of d0 and d8 silages; in the case of d0 the high content of acetic acid (30.4 g/kg DM) likely was a contributing factor, which has frequently been shown to reduce intake (see review by Dulphy and Van Os, 1996).

Table 3 Dry matter intake and stimulus coordinates for the two-dimensional solution to the preference among goats in one preference experiment (Silage with long chopping length, 25% DM and high packing density), n = 25

	Length of aerobic storage (days)						
	0	2	4	6	8	Lucerne hay	MSD
Dry matter intake (g)	312^{b}	493^{a}	513^{a}	409^{a,b}	322^{b}	473^{a}	130
Dimension 1	0.77	0.77	0.14	-0.28	-1.63	0.24	
Dimension 2	-2.04	0.74	1.57	0.61	-0.62	-0.26	

MSD = Minimum significant difference (Waller Duncan k-ratio t-test)
$^{a-d}$ = Means within a row with different superscripts differ ($p < 0.05$)

The two-dimensional solution to the preference trial is shown graphically in Figure 1. Treatments d2, d4, d6 and hay are situated quite closely together with d0 and d8 at a greater distance. Generally, for a given forage, a positive rank in both dimensions would represent preference whereas, a negative rank in both dimensions would indicate avoidance. In this case, treatments d2 and d4 were preferred, while d8 silage was strongly avoided. A small distance between treatments would indicate that they were similar in preference while forages located far from each other can be seen as different in preference, such as silages d2 and d8.

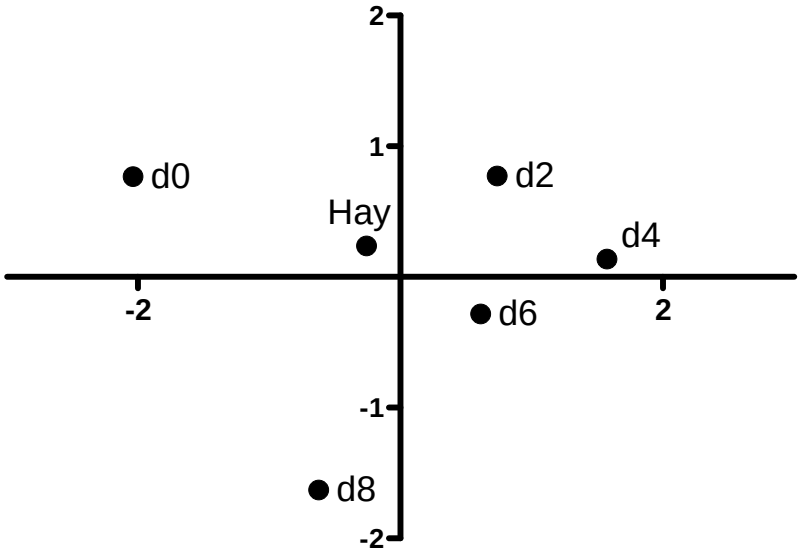

Figure 1 Multidimensional scaling of the mean preference shown by goats for five silages (d0 - d8 = length of aerobic storage of grass silage in days) and one lucerne hay (Hay).

Conclusions

Multidimensional Scaling turned out to be a suitable tool for evaluating feeding preferences for grass silages by goats. The graphical solution helps to get an impression of preference and avoidance of different forages. It is helpful to conduct it together with an analysis of variance to separate means.

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Investigation and comparison of nutritional value and forage quality indicators in some rangeland's species

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Introduction

Study of chemical compounds in rangeland plants and information on effects of the environmental conditions on these compounds is important in rangelands management (Biondini et al., 2006Graza and Fulbright, 2008;). On the other hand, the nutritional requirements of the animals varies with different environmental conditions (McDowell, 2005; Norton and Waterfall, 2003;). Researchers believe that several factors affect the forage feed value. White (2003) reported that the forage plants have different feed values at various phenological stages. Larbi et al. (2011) stated that the movement of plant nutrients from the leaves and stems to roots and seeds are important for the changes in forage feed value. Different rangeland plants have been studied by several researchers, and all report that the differences in forage feed values result from differences in plant metabolism (Davidson and Milthorpe, 1995).

Several factors that affect forage feed values such as crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and metabolizable energy (ME), have been studied (Menke and Trlica, 1985; Moore and Biddingscomb 1994;). Information on the compounds that provide food reserves in plants is very important for rangeland management. The knowledge of how these compounds are produced in plants and in which plant parts they are located can be a great help in identifying the appropriate grazing time, number of grazing livestock, and the length of the grazing period. Physiological changes in plants vary as a result of growth rate, germination, type of leaves, stems, roots and height. The knowledge of carbohydrate production, translocaton, storage and use in plants can help rangeland managers to manage pastures (Mikic et al., 2010). The most important information for determining stocking rate and rangeland capacity is probably the knowledge of forage quality and pasture productivity. It is essential to determine forage nutritive value, because it is strongly related to animal performance during the grazing season. This information helps the rangeland managers to balance the available forage and the animal's nutritional requirements, and using these factors enable them to obtain maximum animal performance.

Materials and Methods

Plant materials and sample collection

Six plant species were harvested from the Natanz rangelands (province Isfahan, Iran). The species included two grasses (*Secale montanum* and *Festuco ovina*), two forbs (*Lotus corniculatus* and *Sanguisorba minor*), and two shrubs (*Kochia prosterata* and *Salsola rigida*). The species were harvested from natural rangeland habitats in three vegetative stages (first vegetative, flowering and seedling) . As the respiration and photosynthesis in cut plants continue after clipping for several hours and this affects the content of soluble carbohydrates, the samples were frozen using a mobile freezer. Frozen plant samples were then used for chemical analysis after oven-drying at 80°C for 24 hours and used for the forage quality analysis.

Analytical methods

For the measurement of soluble carbohydrates, the phenol-H_2SO_4 method was used. In this method, 0.5 g dried plant sample was taken and 15 ml ethanol 80% was added to it, heated at 75°C for 5 minutes by a heater, then centrifuged at 3000 rpm for 10 minutes. Then, the centrifuge was turned off and the clear solution in the flask was separated. This was repeated twice. The aliquots taken from these two replications were mixed and put in an oven at 70-80 °C. After 1 hour, volumes were raised to 100 ml by adding distilled water. Then, 4.7 ml $Ba(OH)_2$ was added to it. After 3 minutes, 5 ml $ZnSO_4$ was added to it and thoroughly mixed. A 35-ml of this thoroughly mixed solution was centrifuged at 3000 rpm for 10 minutes and 2 ml of this aliquot was used for spectrophotometry at 485 nm. In this study, 2 ml H_2O and 2 ml H_2SO_4 were used for control. Data obtained with this method were expressed in mg L^{-1}. Tthe following formula (McDowell, 2005) was used to convert the data to carbohydrate content in plant dry matter.

$$\%C = V/106 * DM * 100$$

To measure the acid detergent fiber (ADF) content of the plants, the Fibertec method was used (Norton and Waterfall, 2003)

Estimates of digestible dry matter and metabolizable energy

To estimate the digestible dry matter content (DDM), the following formula was used (Fonnesbeck and Davidson, 1985):

$$DDM\% = 88.9N - 0.779ADF$$

After the DDM content was calculated, the following formula was used to calculate the content of ME in MJ/kg DM (McDowell, 2005):

$$ME = 0.17DDM\% - 2$$

Statistical analysis

Plants were sampled at random in full flowering stage in a completely randomized (design with three replications and analyzed by SPSS software. The results of the analysis of variance (ANOVA) Differences among mean values of content of CP, ADF, ME, and DDM were regarded as statistically significant when $P<0.01$..

Results and Discussion

Results of the forage quality indicators

The results of the analysis of variance (ANOVA) showed the mean values of the four important indicators of the forage quality included CP, ADF, ME, and DMD differed among species and phenological stages ($P<0.01$), except for ME in 2009 (Table 1).

Table 1 Analysis of variance of crude protein (CP), dry matter digestibility (DMD), metabolizable energy (ME) and acid detergent fibre (ADF) contents

Year	Variable sources	df	Mean squares			
			CP%	DMD%	ME	ADF%
2009	SP	4	247.7**	735.5**	7.6**	495.8**
	Ps(SP)	11	144.9**	211.4**	5.8**	169.5**
	Error	70	0.431	1.02	0.731	1.12
	CV%	-	4.91	2.54	6.49	1.51
2010	SP	3	140.6**	520.6**	14.4**	266.5**
	Ps(SP)	10	145.2**	122.7**	2.6**	66.9**
	Error	42	0.124	0.281	0.107	0.169
	CV%	-	2.66	1.62	2.24	0.48

**P<0.01; SP: Species, PS: Phenological stage, CV%: Coefficient of Variation.

Crude Protein content

As shown in Figures 1 and 2, 2009 and 2010, respectively, forbs had the highest CP and shrubs the lowest.

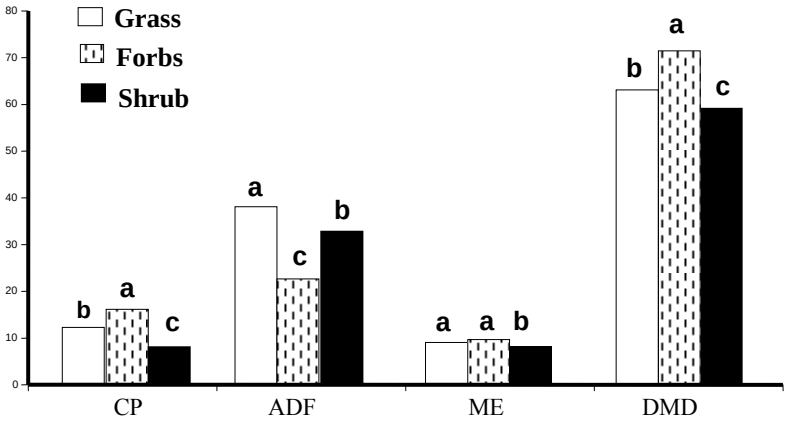

Figure 1 Average crude protein (CP; %), dry matter digestibility (DMD; %), metabolizable energy (ME; MJ/kg dry matter) and acid detergent fibre (ADF; %) contents in forbs, grasses and shrubs in 2009.

Metabolizable energy

Mean values of the metabolizable energy (MJ/kg DM) in 2009 and 2010 showed that these values were the same for grasses and the forbs and that the mean values of shrubs was less than that of grasses and forbs in 2009 (Figure 1). However, in the second year (2010), forbs had higher ME than shrubs (Figure 2). Since the grasses were annual, there was no grass cover in the second year (2010) and as a result, no data were recorded for the grasses in the second year.

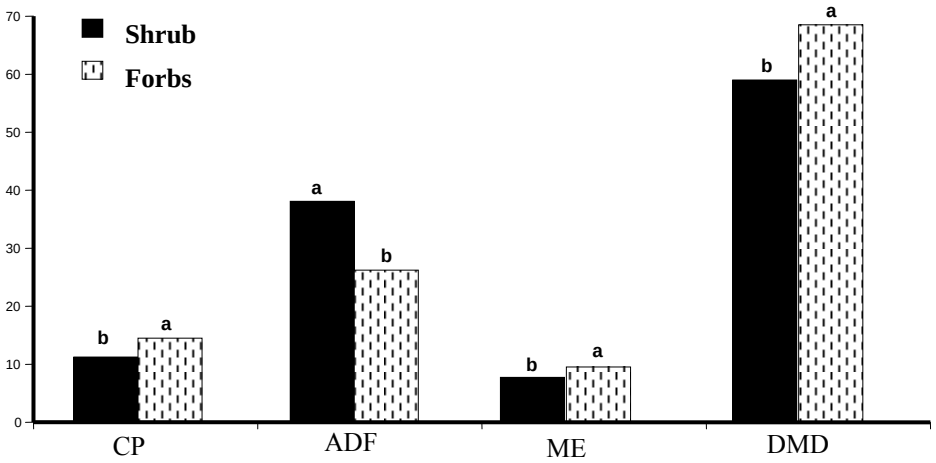

Figure 2 Average crude protein (CP; %), dry matter digestibility (DMD; %), metabolizable energy (ME; MJ/kg dry matter) and acid detergent fibre (ADF; %) contents in forbs, grasses and shrubs in 2010.

Dry matter digestibility

The results of the analysis of variance indicate that the mean values of DMD differed between 2009 and 2010. Forbs had the highest DMD in both years and shrubs had the lowest (Figures 1 and 2).

Acid detergent fibre

In 2009, grasses had the highest ADF mean values and forbs had the lowest (Figure 1). However, since there were no data for the grass species in 2010, only shrubs and forbs were analyzed in the second year. Shrubs had higher ADF than the forbs (Figure 2).

Soluble carbohydrate reserves in vegetation cover types

Soluble carbohydrates values differed among the 6 studied species ($P<0.01$; Figure 3). *Sanguisorba minor* in the first year and *Lotus corniculatus* in the second year had the highest soluble carbohydrate contents, while *Salsola rigida* had the lowest in both years.

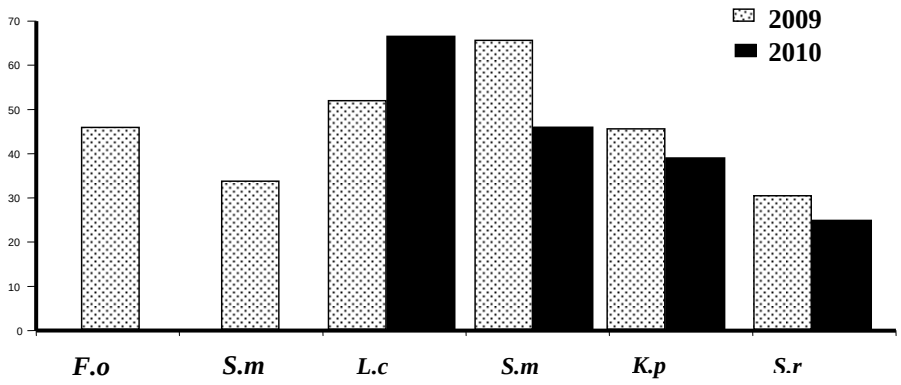

Figure 3 Soluble carbohydrates (g kg^{-1}) in rangelands species in 2009 and 2010.

Study of the vegetation cover types showed that forbs, grasses, and shrubs have different carbohydrate reserve contents. Therefore, management of the rangelands that contain these three types of vegetation covers should be done with close attention. The forage quality indicators, including DMD, ME, ADF, and CP in the 6 species also differed.

Changes in the chemical compounds in these 6 rangelands species showed that vegetation cover type is the most important effective factor on forage quality. Therefore, in order to improve the rangelands conditions and selecting suitable grazing system and grazing time, the following two factors are essential as well as their nutritive value during the growth period in order to provide the nutritional needs of the animals and to ensure the re-growth of the rangelands' plant species.

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Impact of invasion of *Eragrostis plana* Nees on feeding choices by cattle and sheep in native vegetation

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Introduction

The heterogeneity of rangelands of southern Brazil has been reduced by the annual expansion of the area invaded by *Eragrostis plana* Nees. This tufted warm-season (C_4) perennial grass, originating from South Africa, is avoided by grazing animals, principally by sheep; as a consequence, this grass becomes dominant. Carvalho and Batello (2009) consider that among the many threats posed to the rangelands of southern Brazil, one of the most relevant in this moment is the invasion process of *E. plana,* with expansion rate of 14000 ha per year. For a sustainable management in rangelands, it becomes important to understand the interactions between the vegetation and the foraging strategies by animals. Herbivores have distinct behavioral strategies to overcome constraints on preferences imposed by spatial heterogeneity (Dumont et al., 2000), and thereby, cattle and sheep perceive and respond to heterogeneity in their environments in different scales of perception (Laca et al., 2010). According to these authors, the differences in scales of perception between animals of different body sizes, that might affect their selectivity of one food option over another remain poorly understood. Our study focuses on the effects of interactions between sward structure and foraging strategies of two herbivores - cattle and sheep - in order to understand the factors influencing the impact of different proportions of tussocks of *E. plana* on foraging patterns.

Materials and Methods

Experimental design

The experiment was carried out between November and December 2009, at the Research Station of the Federal University of Rio Grande do Sul, Brazil (30º05'27''S, 51º40'18''W), in a rangeland area invaded by *Eragrostis plana* Nees. The treatments consisted of different proportions of tussocks: 0, 25, 50 and 75% of *E. plana*, considered as the non-preferred item of the diet due to its low palatability (Carvalho and Batello, 2009). The inter-tussocks areas (INTER) was predominantly composed of grass - *Andropogon lateralis* (30.7%), *Paspalum notatum* (21.3%), *Axonopus afinnis* (18.5%), *Paspalum umbrosum* (4.7%), *Cynodon dactylon* (3.5%), *Coelorachis selloana* (3.5%), *Setaria parviflora* (2.9%), *Paspalum nicorae* (2.5%), *Kyllinga odorata* (2.5%) and *Dichanthelium sabulorum* (1.0%) - and the legume *Desmodium incanum* (9.1%). Tussock (T) vegetation was predominantly (>95%) consisting of *E. plana*. The vegetation found inside the tussocks (intra-tussocks; INTRA) was predominantly composed of *Desmodium incanum*. A randomised block experimental design with four replicates (n = 16) was used. Independent of the percentage of tussocks, all paddocks were 100 m^2 of inter-tussock area, considering that all treatments had the same forage allowance and sward structure of the inter-tussock stratum, according to Gonçalves et al. (2009). This area was defined by previous grazing tests. During grazing tests of 45 min, the sward height of the inter-tussock area was measured every ten min using a sward stick (Bartham, 1986) to define the minimal area necessary so that the post-grazing sward height would not be reduced more than 10% of the pre-grazing sward height.

Forages

Determination of the actual percentages of tussock cover
Estimation of the percentage tussock cover in the area was performed by a previously trained evaluator. The cover was visually estimated by systematic sampling in a quadrat of a known area (1m x 1m) subdivided into four equal areas of 0.25 m^2. Therefore, the total percentage of the area covered by *E. plana* was mapped, as was the total percentage of area covered by plants belonging to the inter-tussock sward stratum. After obtaining the actual percentage of tussock cover in all of the experimental area, the position of each experimental unit area was designed to obtain proportions of tussocks as close as possible to 0, 25, 50 and 75%. The actual proportions of the total area represented by tussocks in the four treatments were 2.5 ± 0.3, 26.6 ± 1.7, 45.8 ± 1.6 and 69.8 ± 1.2, respectively.

Sward characteristics
In the experimental units, the sward height of the preferred inter-tussock vegetation was maintained at predetermined heights throughout the grazing periods (11.4 cm for cattle and 9.5 cm for sheep), following Gonçalves et al. (2009). During the experimental period, the sward height of the inter-tussock vegetation was estimated based on 100 pre- and 100 post-grazing measurements using a sward stick (Barthram, 1986). The mean sward height was calculated based on the average between the pre- and post-grazing values.

Animals
A total of four crossbred heifers (Angus x Brahman) weighing 286.7 ± 1.2 kg and 12 adult Suffolk ewes weighing 51.0 ± 0.72 kg were used. Evaluations of the heifers were conducted first, with the same group of four "tester" animals being used in all experimental units. After completing the heifer's evaluation, evaluations with the ewes were conducted. The ewes were separated in two groups of six animals, consisting of four "tester" animals and two additional animals, to preclude any effect of group size during grazing (Penning et al., 1993). During the pre-experimental period, the animals were trained to become familiar with the presence of observers, behavior equipments and the experimental procedure.

Selectivity patterns
The animals were submitted to grazing periods of 45 minutes, during peak grazing times at 08:00 and 18:00 hours (the first and the last grazing meals, respectively; Hodgson, 1990). At one-minute intervals during grazing activity, instantaneous records were taken whether the animals were grazing on T, INTER or INTRA strata. Two observers recorded the position and the grazed stratum by the animals on a scaled map of the plot (sampling quadrats of 1m^2). Selectivity was defined as the ability to exhibit selection of a preferred forage (INTER and INTRA strata) when given more options of contrasting vegetation (percentages of tussock cover). According to Senft (1989), selectivity is defined as the relative proportion of selected forage divided by the relative availability of that forage on the landscape. Partial selectivity at a particular level (i.e. pasture, path and feeding station) was the percentage of preferred forage (INTER + INTRA strata) selected, divided by the percentage available at that level. The calculations were based on Laca et al. (2010):

i) Partial Selectivity at pasture level: proportion of INTER stratum in path (square visited of 1m^2 and it's 8 neighbors) divided by proportion of INTER stratum in paddock
ii) Partial selectivity at path level: proportion of INTER stratum in squares visited divided by proportion of INTER stratum in path
iii) Partial selectivity at feeding station level: proportion of observed bites in INTER and INTRA strata divided by proportion of INTER stratum in squares visited.

Under random selection, the expected percentage of preferred forage in the diet would be equivalent to that encountered and would be equal to 1.0. Values different from 1 indicates that the preferred forage was selected more or less than expected by random encounter (Laca et al., 2010). During the experimental period, the animals remained in an adjacent area of natural grassland close to the experimental area. One hour before grazing tests, the animals were removed from this area to be fitted with bags for the collection of faeces and urine and with an IGER Behaviour Recorder. Thus animals were non-fasted to ensure their normal behavior activities (e.g. diet selection; Newman et al., 1994), according to the experimental procedure (Bremm et al., submitted).

Statistical analysis

Statistical tests for selectivity levels included the fixed effects of animal species (heifers vs. ewes), tussock percentage of cover and time of evaluation (blocking factor) and the random effects of interactions and animal. Statistical tests were carried out using JMP version 8 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

No differences (P>0.05) between treatments were observed for sward height of both INTER and T strata. The mean sward heights of the INTER and T areas grazed by heifers were 10.3 ± 0.3 and 40.4 ± 1.1 cm and for ewes were 10.5 ± 0.3 and 44.2 ± 1.3 cm, respectively. Sward heights of the inter-tussock stratum were 10.3 and 10.5 for beef heifers and ewes, respectively and can be considered non-limiting to the intake rate of both cattle and sheep. According to the equation defined by Gonçalves et al. (2009), in relation to the potential herbage intake rate for sheep, the sward height observed should provide 99.1% of the potential intake rate. For cattle, the estimated herbage intake rate to the sward height of 10.3 cm would represent 99.0% of the potential values defined by Gonçalves et al. (2009).

At the higher selectivity levels (pasture and path), no effects of animal species, percentages of tussock cover and of the interaction between both effects (P>0.05) were observed (Fig. 1).

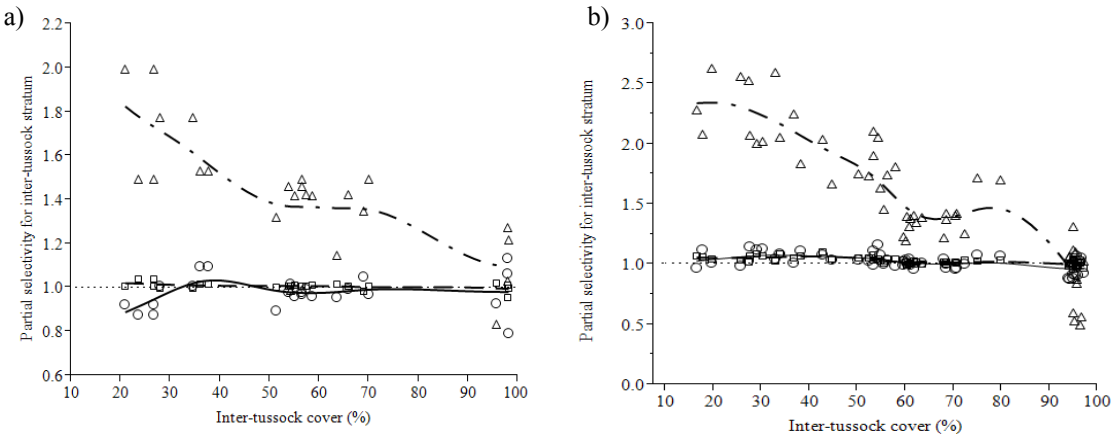

Figure 1 Partial selectivity levels for inter-tussock stratum (○ ——pasture level; □- - - path level; Δ - · - feeding station level) of beef heifers (a) and ewes (b) under distinct percentages of inter-tussock cover (%).

As the INTER stratum became more finely interspersed, both ewes and heifers increased their partial selectivity at the feeding station level. However, the partial selectivity at feeding station level differed between the two animal species as the tussock percentage of cover

increased (P = 0.050). Ewes were more selective than heifers at the smaller INTER level (P=0.0337), when the inter-tussock stratum were limited (up 60% of tussocks). This indicates that differences in selectivity at higher percentages of tussocks were due to the limited selectivity ability by heifers at smaller scales of perception. In a situation of limiting preferred forage, heifers may have adopted the strategy of reducing diet quality (in case of availability of lower quality species – *E. plana* - that allow rapid ingestion) in order to maintain high level of daily intake without increasing the grazing time. Ewes showed on average higher (P = 0.0149) partial selectivity at feeding station level (mean ± SE = 1.57 ± 0.04) than heifers (mean ± SE = 1.34 ± 0.07). Laca et al. (2010), evaluating each level of the grazing hierarchy - patches, paths, feeding stations, bites and intake, observed that smaller herbivores (sheep) exhibit higher selectivity than larger ones (cattle) at all scales of observation. Foraging theory predicts that selectivity increases with increasing difference in profitability (e.g. nutrient intake) among options (e.g. preferred *vs.* non-preferred plant species; Utsumi et al., 2009). This was also observed in this study for both ewes and heifers at the smaller scale of perception (Fig. 1). Finally, both heifers and ewes reject tussocks of *E. plana* at smaller scales of perception.

Conclusions

Our results confirm that animals exhibit grazing behavioural signals which could provide a basis for interpreting the richness of a particular pastoral environment and direct management actions (Carvalho et al., 2008). The selection strategies for cattle are more difficult to perform than for sheep in heterogeneous vegetations, with complex structure and a mixture of preferred (inter-tussock) and avoided (tussocks) strata.

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Characteristics of soil weed seed bank in alfalfa fields among different forage crops

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Introduction

Alfalfa (*Medicago sativa* L.) is an important legume forage species in China, which is seriously harmed by weed at seedling stage. Weed seed bank is dynamic and consists of seeds retained on the soil surface and in the soil (Roberts 1981; Qiang 2001). The purpose of this study was to understand the influence of weed seed bank changes in alfalfa fields. Of particular interest was to investigate the weed seed quantity and its distribution in the soil to provide a further theoretical basis for weed management in alfalfa fields.

Materials and methods

Materials were cultivated in Linxi (118.02 E, 43.62 N, 900 m altiude) located in Inner Mongolia of China. Each test plot was 2.5 m×32 m, repeat three times. The forage crops *Lespedeza hedysaroides* (Pall.) Kitag. and *Astragalus adsurgens* (H.C.Fu) were cultivated in June 25, 2004, and maize (*Zea mays*) was cultivated in May 18, 2007. The alfalfa was cultivated in June 30, 2009; drill seeding of 50 cm. The soil was ploughed in the autumn of 2008 before planting alfalfa (Table 1). Soil samples were collected in the second year alfalfa field in 2010 by diagonal five point sampling using a cylindrical soil sampler (diameter = 5 cm). Sampling was repeated three times within each plot. Soil samples were divided into three layers (0–5, 5–10 and 10–15 cm depths). The method of inducing germination was used to investigate the presence of viable weed seed in the samples. All data were processed by Microsoft Office Excel 2003 and analyzed by a simple analysis of variance which included Forage crop and Month in the model using SAS9.0 (SAS Institute Inc., Cary, NC, USA, 2002).

Table 1 Plot history from 2004 to 2010.

Species	2004-2006	2007-2008	2008 -autumn	2009	2010
L. hedysaroides	*L. hedysaroides*	*L. hedysaroides*	Ploughed	Alfalfa	Alfalfa
A. adsurgens	*A. adsurgens*	*A. adsurgens*	Ploughed	Alfalfa	Alfalfa
Maize		Maize	Ploughed	Alfalfa	Alfalfa

Results and Discussion

From the research of Wei *et al*. (2005), we know that density of weed seed banks generally ranges from 0 to 1.0×10^6 grains m^{-2}. The density of the alfalfa weed seed bank ranged from 1247 to 6190 grains m^{-2} in the present study. Density of the soil weed seed bank was affected by previous crops grown. Weed seeds were higher in *L. hedysaroides* than in the other two forage crops ($P < 0.05$). Also, the density of the soil seed bank differed between months ($P < 0.05$). April was lower than July, August and September and August was higher than October ($P < 0.05$). The results suggest that both forage crop and sampling time influence density of the soil weed seed bank. Density of the seed bank differed between *maize* and the other two forage crops in July and *A. adsurgens* and the other two forage crops

in October (Table 2). The mechanisms affecting density of weed seed banks among forage crops were not clear. Moreover, the change of the weed seed quantity in soil had close relation with weed quantity and species, but may also be influenced by the environment, something which needs further research.

Table 2 Density of weed soil seed bank of alfalfa (grains m^{-2}, 0–15 cm)

Forage crop	Sampling time					
	April	July	August	September	October	Mean
Lespedeza hedysaroides	3639±2185^{abB}	5794±188$^{a\ A}$	6190±1178$^{a\ A}$	5799±1642^{aA}	4643±531$^{ab\ A}$	5213±571^{a}
Astragalus adsurgens	1247±207$^{b\ C}$	4598±505^{abAB}	6088±943$^{a\ A}$	3997±529^{abB}	1395±328$^{b\ C}$	3465±545^{b}
Zea mays	2018±301$^{b\ C}$	2211±455$^{b\ C}$	4303±1807^{abAB}	4048±1328$^{ab\ B}$	4354±940$^{ab\ AB}$	3387±505^{b}
Mean	2302±730^{C}	4201±565^{AB}	5527±745^{A}	4615±694^{AB}	3464±612^{BC}	

Different lowercase letters indicate differences among forage crops, uppercase letters indicate difference among months ($P < 0.05$).

Conclusions

The results showed that the density of soil weed seed bank differed among forage corps and sampling time. The density of the soil seed bank was higher in *L. hedysaroides* than after the other two forage crops. April differed from July, August and September.

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Effect of dietary energy level during the dry period on insulin resistance in dairy cows

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Introduction

Voluntary dry matter intake (DMI) of dairy cows decreases during the final 2 to 3 weeks before parturition (Ingvartsen and Andersen, 2000). This is accompanied by the gradual increase of lipid mobilisation from adipose tissue and elevation of plasma free fatty acid concentration. In order to reduce lipid mobilisation and the consequent hepatic lipid accumulation, there has been interest to maximize prepartum feed intake and to prevent the decrease of prepartum energy intake. However, high-energy intake increases plasma insulin concentration and may increase insulin resistance of peripheral tissues during late pregnancy (Holtenius et al., 2003), thus promoting tissue mobilisation during periparturient period. Our aim was to study the effects of high and restricted energy allowances during dry period on whole-body insulin resistance in dairy cows during periparturient period.

Materials and Methods

Two studies with 16 multiparous Finnish Ayrshire cows in each were conducted, using randomized complete block design. In the first experiment (EXP 1), the treatments were restricted feeding (RESTR) according to the energy requirements of pregnant cow (MTT 2011) or high energy feeding (150% of energy requirement, HIGH). In the HIGH group decreasing feed allowance was implemented during the last three weeks of pregnancy to study the combined effect of high energy intake during the early dry period and the diminishing feed intake with approaching calving. Both groups were fed grass silage (D-value 664 g/kg DM) during the dry period, and grass silage supplemented with commercial concentrate (2-3 kg DM/d) during the last three weeks of pregnancy. In the second experiment (EXP 2), treatments were *ad libitum* feeding of grass silage (D-value 665 g/kg DM) (HIGH) or a mixture of grass silage (D-value 667 g/kg DM), wheat straw and rapeseed meal (55%: 40%: 5%) (RESTR). Before allotment to treatments, cows were divided into pairs, taking into account expected calving date, parity and body condition score (BCS). After calving, all cows were offered wilted grass silage *ad libitum* and an increasing amount of commercial concentrate. Blood samples were collected from tail blood vessels for analyses of plasma glucose, insulin and NEFA.

Intravenous glucose tolerance tests (IVGTT; 0.25 g of glucose/kg of BW) were performed at 7 ± 1 d before expected calving and 10 ± 1 d after parturition in EXP 1, and 11 ± 1 d before the expected calving day and 8 ± 1 d after parturition in EXP 2. Feeding was suspended one hour (EXP 1) or two hours (EXP 2) prior to and during IVGTT. Blood samples were collected between -10 and +180 min relative to glucose infusion in EXP 1 and between -10 and +240 min in EXP 2. Plasma glucose, insulin and NEFA responses to IVGTT were calculated as net incremental areas under the response curve (AUC).

Adipose tissue and liver biopsy samples were collected on days -8, +1 and +9 relative to parturition in EXP 1 and on days -12, +1 and +7 in EXP 2. Total RNA was extracted from adipose tissue samples using RNeasy Lipid Tissue Kit and from liver tissue samples using

RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany). The following genes were examined: adiponectin, adiponectin receptor 1 (AR1), adiponectin receptor 2 (AR2), resistin and leptin in the adipose and mitochondrial carnitine palmitoyltransferase 1A (CPT1), glucose-6-phosphatase catalytic subunit (G6P), pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase 1 (PCK1, cytosolic) in the liver. Quantitative real-time PCR analyses were conducted using LightCycler 480 instrument (Roche Diagnostics GmbH, Mannheim, Germany). The internal control gene was eukaryotic translation initiation factor 3 subunit K (EIF3K). In order to avoid negative digits, ΔCt values were subtracted from the arbitrary value 10 (10-ΔCt) before statistical analyses.

Data for milk production, blood samples and gene expression were analyzed as repeated measures ANOVA using the Mixed procedure of SAS version 9.2 (SAS Institute, Cary, NC, USA). The statistical model included fixed effects of treatment, day relative to calving, interaction between treatment and day, and a random effect of block and interaction between block and day. Body weight and BCS data and the results of the IVGTT were analyzed with the PROC MIXED procedure with a model including fixed effect of treatment and random effect of block. Treatment effects were declared significant at $P<0.05$, and tendencies for treatment effects were declared at $0.05\leq P<0.10$.

Results and Discussion

In the EXP 1, ME intake of HIGH group was 42% higher than in the RESTR group during 6 to 4 weeks prepartum, and 18% higher during 3 to 1 weeks prepartum. In EXP 2, cows in HIGH group had approximately 35% higher ME intake than the cows in RESTR group during the dry period. Average live weight gains during the dry period tended to be higher in HIGH groups: 1.3 vs. 1.1 kg/d in EXP1 and 1.4 vs. 1.0 kg/d in EXP 2. There were no differences between treatments in average BCS or BCS change before or after calving. Milk yield (44.3 vs. 40.5 kg/d, $P<0.10$) tended to be higher in RESTR group in EXP 1, whereas no difference (40.0 vs. 42.6 kg/d, $P>0.10$) was observed in EXP 2.

High energy intake during the dry period increased prepartum plasma insulin concentration in both experiments, which is in line with the study by Holtenius et al. (2003). Restricted energy intake increased plasma NEFA before calving in EXP 1, but not in EXP 2. There were no differences in plasma insulin or NEFA concentration after calving. Lack of the differences of lipid mobilization during the transition period was probably due to relatively small differences in live weight gains between treatment groups. Holtenius et al. (2003) and Douglas et al. (2006) observed larger differences in live weight gains and BCS changes during the dry period and an increased lipid mobilization as indicated by higher plasma NEFA concentrations after calving.

During prepartum IVGTT, peak concentration of insulin was higher ($P<0.05$) and AUC of insulin tended to increase ($P<0.10$) in HIGH vs. RESTR in EXP 2 (Table 1), while no difference in insulin response was observed in EXP1. Similarly, Holtenius et al. (2003) found that overfed cows (178 % of energy requirements) had higher insulin response to glucose prepartum than cows fed according to requirements. The larger insulin response resulted in a smaller ($P<0.05$) glucose AUC of the HIGH than that of the RESTR during 240 min. Plasma glucose concentrations exceeded renal threshold for glucose excretion during the first 60 min of IVGTT, and consequently glucose clearance was probably overestimated (van Meirhaeghe et al. 1988). However, the difference of AUC between 60 and 240 min in RESTR and HIGH suggests

increased glucose disposal or alternatively more pronounced inhibition of endogenous glucose production in HIGH. Inclusion of concentrate in the prepartum diet and short feed withdrawal period before IVGTT may have contributed to the lack of glucose responses in EXP 1, and most likely explains the high basal insulin and glucose concentrations.

Table 1 Effect of feeding level during the dry period on plasma glucose, insulin and NEFA responses to intravenous glucose tolerance test (IVGTT) prepartum

	Experiment 1				Experiment 2			
Item	RESTR	HIGH	SEM	P=	RESTR	HIGH	SEM	P=
Glucose								
Basal[1](mmol/l)	4.5	4.4	0.17	0.54	3.9	4.0	0.12	0.13
Peak (mmol/l)	15.9	16.8	0.62	0.38	19.4	19.2	0.37	0.71
AUC_{60}[2]	336	353	14.2	0.36	403	386	19.2	0.31
AUC_{tot}[3]	431	432	29.1	0.98	525	413	47.9	0.02
Insulin								
Basal (µIU/ml)	40.3	44.6	6.14	0.57	13.9	15.6	2.08	0.58
Peak (µIU/ml)	301	378	80.5	0.32	225	382	66.9	0.01
AUC_{tot}[3]	11726	15279	4214.3	0.56	10064	17762	3520.3	0.05
NEFA								
Basal[1](mmol/l)	0.25	0.16	0.029	0.04	0.36	0.25	0.060	0.25
Nadir (mmol/l)	0.10	0.07	0.017	0.29	0.12	0.09	0.017	0.15
AUC_{60}[2]	-3.2	-2.3	0.44	0.11	-2.6	-2.3	1.06	0.85
AUC_{120}[4]	-8.7	-5.9	1.11	0.05	-17.7	-11.2	4.03	0.29

[1]Basal = Average concentration at 10 and 5 min before the IVGTT; [2]AUC_{60} = Area under the response curve during the first 60 min of the IVGTT (mmol/l x 60 min); [3]AUC_{tot} = Area under the response curve during the whole IVGTT (mmol/l for glucose and µIU/ml for insulin x 180 and 240 min in EXP 1 and 2, respectively); [4]AUC_{120} = Area under the response curve during the first 120 min of the IVGTT (mmol/l x 120 min).

High-energy intake during the prepartum period affected plasma NEFA responses during the prepartum IVGTT in both experiments. The restricted energy intake increased the basal NEFA concentration in EXP 1 ($P<0.05$). However, the suppression of plasma NEFA was impaired in HIGH cows in EXP 1, because the absolute value of NEFA AUC tended to be smaller in HIGH vs. RESTR ($P<0.10$). A similar trend was observed in EXP 2, as the HIGH cows needed more insulin to achieve the same suppression of lipolysis (AUC of NEFA) than the RESTR cows. These results suggest that limiting the energy intake during the dry period enhanced insulin sensitivity in the adipose tissue prepartum. Reduced NEFA response to insulin in high-energy fed dry cows was also confirmed recently by Schoenberg et al. (2012).

NEFA responses during the IVGTT after calving were not consistent with the prepartum results. Despite a similar glucose and insulin response in both treatments and experiments,

NEFA response of the HIGH cows was more pronounced in EXP 1 when compared with RESTR, as indicated by the larger absolute value of NEFA AUC (-10.6 vs. -6.0 mmol/l x 60 min, $P<0.05$).

Hepatic expression of PCK1 and PC genes were down-regulated in HIGH group in EXP 1 ($P<0.05$ and $P<0.10$) (Table 2). In line with this, Murondoti et al. (2004) observed decreased activity of PCK1 in the liver of overfed cows one week before calving and during the first lactation week. They attributed the decreased gluconeogenetic capacity to lipid infiltration of the liver. Recently, Hammon et al. (2009) showed that high fat content in the liver did not impair gene expression of enzymes related to gluconeogenesis. Therefore, we speculate that restricted energy intake during the dry period stimulates gluconeogenesis during periparturient period, even when there are no differences in lipid mobilisation between overfed and restrictively fed cows.

Increased expression of PC after calving in both groups ($P<0.05$) is concordant with the results of Murondoti et al. (2004) and Hammon et al. (2009), promoting increased entry of lactate and amino acids in gluconeogenesis. Higher expression of CPT1 in RESTR group ($P<0.01$) may indicate enhanced hepatic capacity for β-oxidation of fatty acids. Similarly, Douglas et al. (2006) observed increased CPT activity in cows with restricted dry period feed intake.

Table 2 Effect of feeding level during the dry period on relative mRNA abundance of the studied genes in the liver and adipose tissue in EXP 1

Item	RESTR	HIGH	SEM	Treatment	Day	Trt * day
			Liver			
PC	12.98	11.61	0.486	0.07	0.01	0.34
PCK1	17.12	15.53	0.428	0.03	0.58	0.23
G6PC	14.66	13.58	0.576	0.21	0.91	0.98
CPT1	14.16	12.38	0.375	0.004	0.38	0.79
			Adipose tissue			
Adiponectin	15.90	15.01	0.504	0.24	0.33	0.47
AR1	8.53	8.57	0.758	0.98	0.48	0.52
AR2	13.08	11.54	0.860	0.16	0.40	0.68
Leptin	11.24	10.61	0.652	0.47	0.01	0.27
Resistin	6.27	5.28	0.426	0.13	0.05	0.57

PC=pyruvate carboxylase; PCK1=phosphoenolpyruvate carboxykinase 1 (cytosolic); G6P= glucose-6-phosphatase catalytic subunit; CPT1=mitochondrial carnitine palmitoyltransferase 1A; AR1=adiponectin receptor 1; AR2=adiponectin receptor 2

In adipose tissue, the expressions of studied genes were not affected by the level of energy intake (Table 2). In concordance with the study by Sadri et al. (2011), no time effect was observed in the expression of adiponectin or its receptors, whereas Lemor et al. (2009) reported down-regulation of AR1 and AR2 expression in subcutaneous adipose tissue postpartum compared with prepartum cows. Leptin expression decreased after calving ($P<0.05$), in line with the well documented decrease of plasma leptin during periparturient period. The tendency

(P<0.10) towards increased expression of resistin after calving may indicate decreased insulin sensitivity of adipose tissue in both groups (Vernon 2005).

Conclusions

These results imply that restricting energy intake during the dry period may improve insulin sensitivity in the adipose tissue during the dry period although this effect does not carry over to the early lactation. Restricted energy intake during the dry period may also enhance gluconeogenetic capacity of cows, potentially affecting early lactation milk production.

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Effects of the inclusion of NaOH-treated wheat on the voluntary feed intake and milk production in dairy cows

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Introduction

Inclusion of grain at the expense of forage, promotes a higher dry matter (DM) intake and milk production. Too high concentration of grains in ruminant diets can, however, cause rumen acidosis and reduce DM intake (DMI). A shift in starch degradation from the reticulo-rumen to the small intestine have been claimed to improve the energy utilization of the diet, due to decreased losses of energy as heat and methane (Reynolds, 2006). Treatment with NaOH of grain is a chemical processing of the kernels that includes a combination of heat and moisture treatment, dissolving the bran and leaving the endosperm naked. Campling (1991) reviewed the effects of processing cereal grains and concluded that NaOH treatment of grains could have positive effects on forage intake in ruminants, attributed to reduced acidic conditions in the rumen. The objectives of the present study were to evaluate the effects of gradual replacement of dried rolled wheat with NaOH-treated wheat and to compare the feeding value of an oat/barley mixtures and rolled wheat in lactating dairy cows fed grass silage-based diets.

Material and Methods

Animals, housing and experimental design
The experiment was conducted at the research farm of the Swedish University of Agricultural Sciences (SLU) in Umeå, Sweden using 24 Swedish red dairy cows 147±51 (S.D.) days in milk (DIM) and a live weight (LW) of 611±66 (S.D.) kg and producing 31±5.6 (S.D.) kg of milk. The animals were assigned to one of six parallel 4 x 4 balanced Latin squares according to DIM. Within each square, the animals were randomly allocated to four total mixed rations (TMR; Table 1). Each experimental period lasted for 21 d, with 14 d for adaptation and 7 d for data recording and sample collection.

Feeds and their conservation
Two different qualities of wheat grain, rolled and NaOH-treated, were produced from one batch of winter wheat (approx. DM concentration 860 g/kg) provided from a commercial farm and rolled using a mobile crimper. NaOH-treated wheat was produced by mixing the whole grain with 3 per cent NaOH granules on fresh weight basis with the addition of 25 per cent water on fresh weight basis. In addition, an oat/barley mixture was acquired, consisting of pelleted whole grains of oat and barley in a 1:1 ratio. Additional protein was provided as rape seed meal pelleted with the addition of fat, vitamins and minerals. Grass silage was prepared by cutting a grass ley dominated by timothy (*Phleum pratense* L) on the 17 June in 2010 with a disc mower and conditioner.

Diets, feeding and animal recordings
The experimental diets were named after their main cereal components, rolled wheat (RW), NaOH-treated wheat (SHW), RW/SHW and oat barley mixture (OBM) fed as total mixed diets rations (TMR) with grass silage and rape seed meal (RSM) are presented in Table 1. Diets were offered *ad libitum* and daily feed intake was recorded for individual animals. Milk production

and LW was recorded daily using gravimetric milk recorders and automatic weighing stations for the animals.

Table 1 Inclusion levels of feed components (g/kg DM) in the four dietary treatments fed as total mixed rations

Components	Diets			
	OBM	RW	RW/SHW	SHW
Grass silage	520	520	520	520
Rolled wheat	-	340	170	-
NaOH-treated wheat	-	-	170	340
Oat /barley mixture	340	-	-	-
Rape seed meal	140	140	140	140

OBM=Oat barley mixture, RW=Rolled wheat and SHW=NaOH treated wheat

Analyses of feed, milk urine and faeces

Analyses of feeds and faeces were conducted accordingly, DM (105^{o}C for 16 h), ash (525^{o}C for 6 h), acid insoluble ash (AIA), crude protein (CP) and neutral detergent fibre (NDF). Concentrations of indigestible NDF (iNDF) were determined following a 288-h *in situ* incubation. The ME of the concentrate feeds, the supply of amino acids absorbed in the small intestine (Metabolisable protein, MP), was calculated according to Spörndly (2003). The grass silage was analysed for *in vitro* organic matter digestibility and metabolizable energy (ME) after a 96–h fermentation. Urinary samples were analysed for concentrations of total N. Total tract digestibility of DM, CP, NDF and starch were determined using AIA as an internal marker. Degradation parameters of the cereal feeds were estimated using an *in vitro* gas production (GP) technique and a modelling approach (Huhtanen et al. (2008) for determination of first order degradation kinetics and rumen digestibility. Concentrations of milk fat, protein, lactose and urea were measured using an IR technique.

Statistical analysis

Data from the production experiment was analyzed using the general linear model procedure, using the following model $Y_{ijkl}=\mu+S_i+P_j+C_k(S_i)+T_l+\varepsilon_{ijkl}$ where Y_{ijkl} is the independent variable, μ the overall mean, S_i the effect of square (i=1-6), P_j the effect of period (j=1-4), $C_k(S_i)$ the effect of a cow within square, T_l effect of treatment (l=1-4) and ε_{ijkl} is the residual error. Multiple effects between treatments are detected by contrasts testing the probability of linear and quadratic responses from increased inclusion of SHW compared to RW and a direct comparison between OBM and RW.

Results and discussions

The diet compositions based on actually intake recordings are presented in Table 2. The results of the GP recordings (data not shown) indicated that increased inclusion of NaOH-treated wheat decreased the effective k_d of the concentrate feeds which is similar to the findings of O'Mara et al. (1997) and Phipps et al. (2001). There was a quadratic response on DMI (Table 3) by inclusion of NaOH-treated wheat as well as on intakes of CP, starch, ME and MP, followed by a quadratic response on the digestion of DM, OM and CP, with maximum responses for the RW/SHW diet. The comparison between the oat/barley mixture and rolled wheat resulted in higher intakes of, NDF, iNDF, for the OBM diet. The RW diet had higher intakes of, starch, and MP compared to the OBM diet. The results of the present study suggest that possible benefits of

increased intestinal digestion and improved starch utilization with NaOH-treated wheat are reduced due to increased starch fermentation in the hindgut.

Table 2 Chemical composition and nutritive values (g/kg DM) of the dietary treatments

	Diets			
Parameters	OBM	RW	RW/SHW	SHW
Composition				
CP	182	185	185	185
NDF	390	354	354	361
iNDF	74	58	53	51
Starch	175	220	222	213
Nutritive values				
ME (MJ/kg DM)	11.7	12.2	12.1	12.0
MP	92	97	97	96

CP=crude protein, NDF= neutral detergent fibre, iNDF=indigestible NDF, ME=metabolizable energy and MP=metabolizable protein. For other abbreviations see Table 1.

Table 3 Least square means of nutrient intake (kg/day), digestibility coefficients of the four different dietary treatments and probabilities (P) for linear (L) and quadratic (Q) effects of increased inclusion of NaOH-treated wheat and comparisons between the oat/barely mixture and rolled wheat (O. vs. R)

Parameters	Diets					P=		
	OBM	RW	RW/SHW	SHW	SEM	L	Q	O vs. R
Intake								
DM	21.7	21.5	22.2	21.7	0.3	0.65	0.07	0.6
CP	3.91	3.94	4.11	3.98	0.06	0.61	0.03	0.69
NDF	8.37	7.49	7.85	7.71	0.11	0.18	0.06	<0.01
iNDF	1.59	1.22	1.18	1.09	0.02	<0.01	0.3	<0.01
Starch	3.75	4.64	4.93	4.56	0.62	0.39	<0.01	<0.01
ME (MJ/d)	251	259	269	258	3.6	0.81	0.01	0.12
MP (g/d)	1982	2065	2151	2064	28.1	0.98	0.01	0.04
Digestibility								
DM	0.714	0.747	0.759	0.754	0.0059	0.35	0.03	<0.01
CP	0.712	0.728	0.734	0.69	0.0101	0.01	0.04	0.24
NDF	0.659	0.675	0.698	0.69	0.0101	0.21	0.15	0.19
Starch	0.944	0.966	0.974	0.971	0.0038	0.44	0.24	<0.01

DM=dry matter, CP=crude protein, NDF= neutral detergent fibre, iNDF=indigestible NDF, ME=metabolizable energy and MP=metabolizable protein. For other abbreviations see Table 1.

The hindgut has lower capacity to digest carbohydrates compared to the rumen and cannot fully compensate for reduction in rumen degradation (Ørskov, 1986), which may explain the limited responses from increased NaOH-treated wheat inclusion in this study. The increased inclusion

of NaOH-treated wheat had few effects on milk production (Table 4) besides quadratic responses on the concentration and production of milk fat. Inclusion of NaOH-treated wheat resulted in a linear decrease in milk protein and urea concentrations and tended to decrease milk protein yield. The shift in starch digestion from the rumen to lower tract may have reduced the amount of fermentable substrate for rumen microbes Reynolds (2006), which could explain the trend towards lower milk protein yield and significantly reduced milk protein concentration in cows fed NaOH-treated wheat. The NaOH-treatment influenced negatively milk nitrogen efficiencies. Milk production and composition generally were not different for the RW diets than OBM diet except that RW resulted in an increase in milk protein concentration but milk urea concentration was decreased compared to OBM. Inclusion of NaOH-treated wheat increased the concentration of faecal CP as well as the faecal total N excretion (Table 5). The increased intake of NaOH-treated wheat resulted in an increase in urinary production with decreasing both the urinary N concentration and urinary total N excretion Faecal CP concentrations of cows fed OBM diet was lower compared to cows fed RW diet.

Table 4 Least square means of milk production (kg/day), composition (g/kg), feed efficiency (kg ECM/kg DM intake), milk nitrogen efficiency (kg N milk/kg N intake) of the four different dietary treatments probabilities (P) for linear (L) and quadratic (Q) effects of increased inclusion NaOH-treated wheat and comparison between the oat/barley mixture and rolled wheat (O. vs. R)

Parameters	Diets					P		
	OBM	RW	RW/SHW	SHW	SEM	L	Q	O vs. R
Production								
ECM	28.6	27.8	28.4	27.6	0.52	0.79	0.28	0.26
Protein	0.97	0.98	0.95	0.94	0.016	0.10	0.47	0.72
Fat	1.20	1.16	1.21	1.15	0.027	0.88	0.04	0.13
Composition								
Protein	38.0	39.0	38.1	37.7	0.20	<0.01	0.39	<0.01
Fat	46.0	45.8	48.7	46.4	0.79	0.898	0.04	0.27
Milk Urea	4.9	4.5	4.2	3.9	0.13	0.01	0.98	0.03
Efficiency								
Feed Efficiency	1.36	1.36	1.31	1.33	0.030	0.55	0.33	0.92
Milk nitrogen	.50	.52	.32	.39	0.0045	0.05	0.01	0.76

ECM=energy corrected milk, OBM=Oat barley mixture, RW=Rolled wheat and SHW=NaOH treated wheat.

Conclusions

The inclusion of NaOH-treated wheat had no marked effects on intake and production, except for a decrease in protein concentration. The increased faecal concentrations and excretion of N and the decreased urinary N excretion as result of the increased inclusion of NaOH-treated wheat indicating that the processing partially shifted the digestion of starch from the rumen to the small intestine and lower tract. NaOH-treatment of wheat cannot therefore be justified.

Table 5 Least square means of faecal and urinary excretion of the four different dietary treatments, probabilities (P) for linear (L) and quadratic (Q) effects of increased inclusion of NaOH-treated wheat and comparison between the oat/barley mixture and rolled wheat (O. vs. R)

	Diets					P		
Parameters	OBM	RW	RW/SHW	SHW	SEM	L	Q	O vs.R
n	12	12	12	12				
Faeces								
DM (kg/day)	6.14	5.35	5.37	5.63	0.17	0.24	0.53	<0.01
CP (g/kg DM)	181	197	203	219	3.7	<0.01	0.25	<0.01
N (g/day)	180	170	175	198	0.01	<0.01	0.24	0.32
Urine								
Volume (l/day)	23.9	25.6	36.7	34.3	2.0	<0.01	0.01	0.34
N conc. (g/l ml)	11.5	11.2	9.1	8.3	0.52	<0.01	0.33	0.64
N (g /day)	272	277	307	272	<0.01	0.72	0.01	0.25

DM=dry matter, CP=crude protein For other abbreviations see Table 1.

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Prediction of methane production from dairy cows

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Introduction

Methane (CH_4) from enteric fermentation in ruminants is a significant source of greenhouse gas. Thus, strategies to reduce the emission of enteric CH_4 are being actively sought with modeling playing a key role in the identification of viable mitigation strategies. Extant models for predicting enteric CH_4 emission are based on antiquated data, and it is questionable if they are still valid given the dramatic advancements in feeding practices that have occurred over the last 20 yr.

The main objective of this study was to elucidate the potential for improving the prediction of enteric CH_4 by using data from more recent experiments.

Materials and Methods

Database
Twenty experiments (comprising 69 dietary treatment means) with data of CH_4 production, diet- and animal characteristics were collected. All experiments were with dairy cows, but in three of them (9 dietary treatment means), they were non-lactating. In 11 of the experiments, the sulphur hexafluoride tracer technique was used while the chamber technique, the hood calorimetry technique and the room tracer approach were used in 6, 1 and 2 of the experiments, respectively. The roughage part of the diets was grass silage and/or corn silage whereas the concentrate was mainly based on barley. Range and mean values for diet characteristics and enteric CH_4 emissions for the database are presented in Table 1.

Table1. Range and mean values for diet characteristics and enteric CH_4 emissions for the database

Variable	Minimum	Maximum	Mean	SD[1]
DMI, kg/d	7.5	27.2	17.3	4.3
Forage,% on DM basis	45.0	100.0	69.5	16.2
Starch, g/kg DM	0.0	394.3	177.2	100.6
Sugar, g/kg DM	12.0	290.2	135.3	71.0
Fat, g/kg DM	17.9	78.6	43.4	13.0
CP, g/kg DM	112.0	266.7	167.8	40.1
Methane, MJ/d	10.1	25.9	19.8	4.5

[1]SD=standard deviation

Statistical analysis
Treatment means were weighted using the reciprocal of the number of experimental units. In order to maintain the original scale of the data, each reciprocal was divided by the mean of all reciprocals, *i.e*., the weighting variable used, has then a mean equal to 1 (St-Pierre, 2001).

Model Development
The dataset was randomly divided into two parts before the development of the models. Half of the dataset (38 observations) was then used for development of prediction equations, while the other half of the dataset (31 observations) was used to evaluate these equations. The correlations between CH_4 production and diet-/animal input variables among others: DMI (kg /d), milk yield (kg/d) NDF, fat, CP, starch (in g/kg DM, intake, g/day and digested, g/day) were determined using PROC CORR in SAS. The backward elimination procedure for multiple regressions in SAS was used to develop the linear models. The PROC MIXED (SAS, 2012) was used to determine individual regression coefficients.

Model Evaluation
Models developed in this study (Table 2) and the models of Mills et al. (2003), Yan et al. (2006), Jentsch et al. (2007) and a stoichiometric equation of Volden (see summary by Volden, 2010) were evaluated using two methods. Firstly, mean square prediction error (MSPE), calculated as the sum of the square differences between observed and predicted values divided by the number of experimental observations. The MSPE was decomposed into error due to disturbance, error in central tendency, and error due to regression (Bibby & Toutenberg, 1977). Root MSPE (RMSPE) was used as a measure of accuracy of prediction. Secondly, concordance correlation coefficient analysis (CCC) was performed (Lin 1989).

Results and Discussion

In general, both our (Eq. A, B, C in Table 3) and the equations of others overpredicted the enteric CH_4 production compared to measured values (μ= - 0.06 to - 0.41). Input variables DMI and metabolizable energy intake in models (Linear 1 and Eq. A, Linear 2 and Eq.B, respectively) gave somewhat higher over predictions than the equation based on digested nutrients (Jentsch et al., 2007) or the stoichiometric balance in the rumen (Volden, 2010). Because the stoichiometric equation is based on sound theory, it is expected that this model would be the most precise one. However, there was a surprisingly high correlation between CH_4 production and other more simple input variables sucah as DMI and milk yield. Increasing the complexity of the models had only marginal effect on the precision. It is well known that fat depresses whereas NDF increases CH_4 production. Therefore, it was not surprising that the equation CH_4 (MJ/d)= 5.84 ($\pm$1.81) + 0.955 ($\pm$0.0873) x DMI – 21.21 ($\pm$6.48) x gFAT/gNDF (Eq. C in Table 3) was the most robust based on having the lowest root mean square prediction error (13.3%, with 80% being random error).

Table 2 Preliminary results: statistical models used to predict CH_4 production from dairy cows[1]

Equation no.	Equation
Equation A	CH_4 (MJ/d) = 4.09(±1.71) + 0.9762(±0.1144) x DMI (kg/d)
Equation B	CH4 (MJ/d) = 3.59(±1.49) + 0.0874(±0.008) x ME intake (MJ/d)
Equation C	CH4 (MJ/d) = 5.84(±1.81) + 0.955(±0.0873) x DMI – 21.21(±6.48) x gFAT/gNDF (%)

[1]Equation parameters are ±SE

Table 3 Preliminary results; Comparison of model performance

Equation no.	MSPE%	ECT%	ER%	ED%	CCC	μ
Equation A	21.3%	12.20	0.02	86.19	0.729	-0.25
Equation B	20.6%	38.44	0.01	60.94	0.730	-0.41
Equation C	13.3%	19.90	0.00	79.83	0.887	-0.19
Jentsch et al., 2007	15.6%	31.71	5.53	62.76	0.867	-0.26
Mills et al., 2003 linear 1	20.7%	39.66	15.16	45.18	0.777	-0.37
Mills et al., 2003 linear 2	20.3%	37.28	6.86	55.86	0.776	-0.37
Volden, 2010	15.3%	1.51	0.48	98.02	0.880	-0.06
Yan et al., 2006 equation 1	20.8%	46.82	7.50	45.68	0.764	-0.41
Yan et al., 2006 equation 2	17.4%	36.79	9.50	53.71	0.837	-0.31

MSPE= mean square prediction error; ECT = MSPE decomposed into error due to overall bias of prediction; ER = error due to deviation of the regression slope from unity; ED = error due to the disturbance or random variation. CCC = concordance correlation coefficient; μ = location shift relative to the scale (squared difference of the means relative to the product of 2 standard deviations).

Conclusions

It is concluded that equation with DMI (kg/d) and fat content in proportion to NDF in the diet as input variables predicted enteric CH_4 production more precisely and accurately than existing models tested.

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Effect of dwarf birch leafs (*Betula nana*) on *in vitro* methane production using a gas production technique

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Introduction

Methane production from ruminant livestock represents approximately one quarter of total anthropogenic greenhouse gas emissions (Beauchemin et al., 2008). The eructed methane represents a dietary energy loss of 0.04 to 0.12 of the gross energy in the rations consumed (Johnson and Johnson, 1995). Methane is produced by microbes during the fermentation process in the rumen and the hindgut of ruminants, where the microbes use hydrogen (H_2) to reduce carbon dioxide (CO_2) to methane (Mills et al., 2001). Removal of H_2 is necessary for efficient fermentation in the rumen. Modification of ruminal fermentation using feed additives such as plant extracts is a suggested strategy to improve the production efficiency in dairy cattle (Kongmun et al., 2010). One group of chemicals that have shown effects in reducing methane emissions from ruminants, are condensed tannins (CT) (Puchala et al., 2005). Leaves of dwarf birch (*Betula nana L.*) contain hydrolysable tannins (Salminen et al., 2002). It could therefore be interesting to evaluate the effects of dwarf birch leafs as an alternative feed additive on ruminal methane production. The aim of this study was to evaluate the effect of dwarf birch leafs on *in vitro* methane production using an automated *in vitro* gas production (GP) system.

Materials and Methods

Dwarf birch leafs (DBL) were collected in Umeå, Sweden during the late spring 2011, freeze dried and crushed in a mortar. The effects of the crushed leaves on *in vitro* gas production (GP) were evaluated by incubating 1000 mg of a mixture of grass silage and barley (GSB) (60:40) in buffered rumen fluid with increasing addition of DBL at five levels: 0, 10, 25, 50 and 100 mg. In addition, 1000 mg of DBL was incubated alone. Prior to the incubations, triplicates of each treatment were weighed into serum bottles (250 ml). The bottles were placed in a shaking water bath at 39ºC, 60 ml buffered rumen fluid (rumen fluid: buffer 1:5) dispensed as an inoculum and the GP was continuously recorded for each vessel during 48 h.

Blank corrected methane production was determined as described by Ramin and Huhtanen (2012) at 24 and 48 h of incubation and reported as ml/g dry matter (DM) incubated. Concentrations of volatile fatty acids (VFA) were determined in the buffered rumen fluid of the control and the pure DBL treatments. Methane concentration at 48 h was predicted according to volatile fatty acids (VFA) stoichiometry equations as described by Wolin (1960) for the control and DBL treatment only. Data for *in vitro* measurements were subjected to the general linear model procedure of the SAS program were the sum of squares of DBL effect was further partitioned into linear and quadratic effects of the increased level of DBL using orthogonal polynomial contrasts. The DBL alone treatment was excluded from statistical analysis. The student t-test was used for the comparisons of VFAs between the control GSB and DBL alone treatments.

Results and Discussion

Total GP (after 48 h incubation) decreased linearly ($P < 0.01$) as the level of birch leafs increased from 10 to 100 mg (315 vs. 285 ml/g DM, respectively). The total GP for the dwarf birch alone treatment was 158 ml/g DM, almost half as compared to the control (340 ml/g DM) (Table 1). Methane also decreased linearly ($P < 0.01$) with increased level of birch leafs from 41.2 ml/g DM for the 0 mg level to 35.0 ml/g DM for the 100 mg level, and surprisingly a very low amount of methane (1.0 ml/g DM) was produced from the dwarf birch alone treatment.

The pH after 48 h of incubation decreased linearly from 6.25 to 6.15 ($P < 0.01$). However, it was greater (6.52) for the birch alone treatment (Table 1). The ratio of methane to total gas was constant at both time points. The quadratic effect was not significant in all cases and therefore it was not reported. When the amount of total gas or CH_4 was calculated based on grass silage-barley DM weight only (total amount of substrate will exceed 1000 mg by supplementing DBL at each level), total gas production was not affected, whereas CH_4 production after 48 h decreased linearly ($P = 0.02$) (Table 1). The latter indicates an inhibitory effect of DBL on CH_4 production from the basal substrate, not only based on the fact that no CH_4 was produced from DBL alone.

Table 1. Least square means of the effect of increased level of DBL on *in vitro* gas production parameters (ml /g DM) after 24 and 48 h incubation.

Time (h)	Parameters	level of DBL[a] (mg)					SEM[b]	P-value[c]
		0	10	25	50	100		
24								
	CH_4	32.2	31.1	31.6	30.1	28.0	0.58	<0.01
	Total gas	298	280	295	283	264	8.3	0.03
	CH_4/gas	0.107	0.111	0.107	0.106	0.106	0.0019	0.22
48								
	CH_4	41.2	38.8	38.8	37.2	35.0	0.67	<0.01
	Total gas	340	315	324	298	285	11.8	<0.01
	CH_4/gas	0.121	0.123	0.119	0.125	0.123	0.0037	0.69
	pH	6.25	6.23	6.21	6.19	6.15	0.007	<0.01
	CH_4[d]	41.2	39.2	39.8	39.0	38.4	0.69	0.02
	Total gas[e]	340	318	332	312	314	12.5	0.18

[a] DBL: dwarf birch leafs, 0: Control with no DBL (60:40, grass silage: barley); [b] SEM: standard error of mean (n = 15); [c] Linear effect of increased level of DBL, quadratic effects were non-significant; [d-e] Calculated based on grass silage-barley DM weight only.

Total VFA concentration was significantly lower for the DBL alone treatment as compared to the control treatment without DBL (94.0 vs. 136 mmol/L, respectively) (Table 2). Molar proportion of acetate was greater for the DBL treatment compared to the control, whereas that of butyrate was significantly lower compared to the control (91.4 vs. 127 mmol/mol). Methane concentration (mmol/mol VFA) was predicted based on stoichiometric equations, but there was no difference between DML alone and control treatments (Table 2), indicating that the changes in methane production can not be explained in terms of rumen fermentation pattern.

Table 2. Least square means of the effect of birch on total volatile fatty acids (VFA) production and molar proportion of net VFA production after 48 h incubation.

		Control	DBL[a]	SEM[b]	P-value
Total VFA (mmol/L)		136	94.0	1.42	<0.01
Molar proportion (mmol/mol)	Acetate	579	599	4.8	0.04
	Propionate	228	245	6.2	0.13
	Butyrate	127	91.4	2.63	<0.01
	Isovalerate	35.7	39.0	0.47	<0.01
	Valerate	29.5	26.1	0.77	0.03
	CH_4VFA[c]	296	284	5.2	0.17

[a] DBL: dwarf birch leaf, incubated as whole substrate (1000 mg); [b] SEM: standard error of mean (n = 6); [C] CH_4VFA: methane concentration (mmol/mol VFA) predicted from VFA stoichiometry (0.5 × acetate – 0.25 × propionate + 0.5 × butyrate).

In earlier studies it has been reported that methane inhibitors could change the fermentation pattern in the rumen favouring the production of propionate rather than acetate (Czerkawski, 1986). In most cases methane inhibitors were associated with the production of free H_2 and formic acid. A low concentration of formic acid (around 1 mmol/L) was also detected in the current study with the DBL alone treatment. However, the production of formic acid could not account for the reduced methane production with DBL alone. Although, based on reduced total gas production and a lower digestibility of DBL compared to control, this cannot explain the difference in methane production.

Other effects than protein binding (Woodward et al., 2004) and reduced protein degradation are not well understood. Perhaps a lowered methane production can also occur. Condensed tannins have been shown to reduce digestion in the rumen, consequently affecting the bacterial species. The reductions in methanogenesis linked to CT reported for sheep fed *Maku lotus* may arise from a reduction in H_2 production, alternative H_2 sinks or through direct effects on methanogens (Woodward et al., 2004). Most likely, the explanation for the lack of methane production in the present study was production of free hydrogen, which unfortunately was not possible to measure in our system.

Conclusions

Dwarf birch leafs (*Betula nana* L.) were tested for its inhibitory effect on methane production *in vitro*. In can be concluded that, increased level of DBL linearly decreased methane and total gas and showed some inhibitory effects on methane production and also some production of formic acid in the current study. The effects of DBL and its chemical components on rumen fermentation and methane production will be evaluated to better understand the mechanisms of reduced methane production with DBL supplements.

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Comparing microbial communities to evaluate methane production in a gas *in vitro* system vs. *in vivo*

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Introduction

Methane (CH_4) production from ruminants has been an issue for several years, both as it is a greenhouse gas and an energy loss for the animal. Methane is an end-product from the microbial fermentation and the microbial community structure in the rumen is one factor that affects the amount of CH_4 produced. *In vitro* systems are common for measuring CH_4; the advantages compared to *in vivo* are reduced impact on the animal and lower costs. Feed additives have been used in previous studies to reduce the CH_4 production. Ramin et al. (2011) have used the same *in vitro* system as in the present study and showed that nut shell extract (NSE) at 10 mg level reduced the CH_4 production by 50%. Glycerol as a feed additive has shown to increase the propionate to acetate ratio which may be able to compete with the methane producing bacteria and less CH_4 will be produced. The aim of the study was to analyze how the microbial population and the CH_4 produced in an *in vitro* system reflect the situation *in vivo* and to evaluate the feed additives' impact on the CH_4 production and the microbial population.

Material and Methods

Prior to incubation, 1000 mg of substrate, a mixture of 600 mg silage and 400 mg concentrate, was weighed into 250-ml serum bottles (Schott, Mainz, Germany). The substrate was the same as the diet given to the cows used as rumen fluid donors. The mixture was supplemented with one of two different treatments: 10 mg NSE from cashew nuts (100 µl NSE dissolved in 400 µl 99.5 % ethanol) or 132 µl glycerol (refined glycerol. 99.5%. AkoGly 100, Aarhus-Karlshamn, Karlshamn, Sweden). Rumen fluid was collected from three lactating rumen cannulated cows, fed 60:40 forage:concentrate ratio, strained through a two-layer cheesecloth and then pooled to one representative sample, where after pH was measured. Subsamples were taken for further VFA and microbial analyzes. Pooled rumen fluid was then mixed with buffered mineral solution (Menke and Steingass, 1988), in proportion 20:80. Buffered rumen fluids (60 ml) were weighed and added to the serum bottles and incubated in a water bath at 39°C for 48 h with constant agitation. Four serum bottles were assigned as blanks without substrate. Incubations were done triplicates for each treatment and control. Gas production was recorded in a fully automated system, described by Cone et al. (1996). Methane and CO_2 sampling and calculations were as described by Ramin and Huhtanen (2012). In brief, gas was drawn from the bottles with a tight syringe at six different time points; 2, 4, 8, 24, 32 and 48 hours of incubation. Methane and CO_2 was determined by injecting 0.2 ml of gas into a star 3400 (CX series) gas chromatograph (Varian Chromatography. USA) with thermal conductivity detector. Fluid samples for analyses of VFA and microbial population were taken at 8, 24 and 48 hours of incubation. *In vivo* rumen samples were taken at the same time points during ongoing incubation. Parameters of

fermentation kinetics were estimated from fitting a two-pool Gompertz model to gas recordings obtained every 12 min by. These parameters were then used in a dynamic, mechanistic rumen model for predicting *in vivo* gas production for a mean rumen retention time of 50 h. The microbial population in the fluid samples obtained in the experiment will later be analyzed by 454-pyrosequencing.

Results and Discussion

The dry matter (DM) of the silage was 916 g/kg and the DM contained (g/kg): ash 84.2 crude protein (CP) 172 and neutral detergent fiber (NDF) 543. The DM of the concentrate was 955 g/kg and the DM contained (g/kg): ash 65.3. CP 188 and starch 293. Preliminary results indicate that CH_4 production was reduced by 20% when NSE was added, but no reduction was observed with the glycerol treatment (Table 1). Assuming a gross energy (GE) concentration of 18 MJ/kg DM, the predicted CH_4 was 7.2%, 8.2% and 5.2% of GE for the control, glycerol and NSE treatments, respectively. Total VFA concentrations were higher in the glycerol treatment due to higher propionate and butyrate production. The microbial population analyses will demonstrate if the difference in CH_4 production between treatments is explained by a shift in the microbial population structure. The microbial structure *in vitro* will be compared with *in vivo* results as a step for evaluating the gas *in vitro* system.

Table 1 Methane and total gas production (ml/g DM) with the different treatments and control.

		Treatment		
		Control	Glycerol	NSE[a]
CH_4				
	Asymptotic CH_4	38.2	45.2	28.2
	CH_4 production rate (/h)	0.078	0.057	0.061
	Predicted *in vivo* CH_4 [b]	33.0	37.4	23.7
Total gas				
	Asymptotic gas	243.8	234.7	212.8
	Gas production rate (/h)	0.092	0.105	0.102
	Predicted *in vivo* gas[b]	221.0	216.2	195.5

[a] Nut shell extract

[b] Methane and total gas *in vivo* was predicted by using a 50 h rumen retention time in the mechanistic model.

Conclusions

The results that we have until now show that glycerol doesn't seem to inhibit CH_4 production, while NSE does. The studies under progress will show the effect on the microbial population structure.

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Effects of moisture content and exposure time on nutritional value and microorganism composition of TMR during aerobic deterioration

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Introduction

TMR can be defined as a mixture of roughage, concentrate ingredients, minerals and vitamins, which is blended thoroughly to supply the ruminant's requirements, with moisture content adjusted to 35~45% commonly (Coppock al, 1981). It has become common practice in domestic large-scale farms that TMR diets are prepared by compound feed, hay, straw, silages and high-moisture food by-products. These TMR diets cannot be stored for a long time and they will deteriorate within approximate 12 hours after preparation. Aerobic deterioration leads to changes of nutritional value. The spoiled TMR diets not only reduce the palatability, cause serious feed waste, but also negatively influence livestock performance.
The purpose of this study was to evaluate the effects of moisture content and exposure time on nutritional value, digestibility and microorganism composition of TMR diets during the process of aerobic deterioration.

Materials and Methods

Preparation of TMR: The TMR was formulated with compound feed, cotton meal, fat powder, corn silage, alfalfa hay, fresh Chinese lymegrass (*Leymus chinensis*), wet brewers' grains and a vitamin-mineral supplement in ratios of 37.0%, 5.2%, 0.8%, 22.0%, 11.0%, 16.0%, 4.5%, 3.5%, respectively, on DM basis. TMR as prepared above was adjusted to moisture contents of about 40%, 45% and 50% by adding water.

Aerobic stability evaluation: About 200 g FM of the TMR was loosely placed into an open polythene bag (24 cm × 18 cm). Twenty-seven bags were prepared for each moisture level and three of them were randomly sampled at 0, 12, 24, 48, 72, 96, 120, 144 and 168 h during the process of air exposure. Ambient and TMR temperatures at each moisture level were monitored by temperature sensors at 1 h intervals. The aerobic deterioration test was performed in a room with temperature maintained at 31±1°C.

Nutritional value, pH value and microorganism composition analysis: The dry matter (DM) and crude protein (CP) of TMR diets with different moisture contents were analyzed according to methods 934.01 and 976.0, respectively, of the AOAC (1990). The acid detergent fiber (ADF) and neutral detergent fiber (aNDF) were analyzed by the methods of Van Soest et al. (1991) with amylase and sodium sulphite, and the results were expressed inclusive of residual ash. Water soluble carbohydrate (WSC) was determined by the method of McDonald and Henderson (1964). In vitro dry matter digestibility at 48h (IVDMD) was measured with ANKOM DAISYII Incubator (USA) following the operating instruction. pH value was measured using a glass electrode pH meter (Mettler Toledo S20, Switzerland) and numbers of LAB, aerobic bacteria, yeasts and *Escherichia coli* were counted by the method of Cai et al. (1999).
Statistical analysis: The data of TMR diets were analyzed by two-way analysis of variance to evaluate the effects of moisture level, time of air exposure and their interaction on the items of nutritional value, digestibility and microorganisms. The means were then compared for significance by Duncan's multiple range method. All statistical procedures were performed using the SPSS package (SPSS 13.0; SPSS Inc., Chicago, IL, USA).

Results and Discussion

The nutritional value, pH value and microorganism composition of TMR diets before air exposure are shown in Table 1. The DM content of TMR diets of 40%, 45% and 50% moisture levels showed the value of 62.51%, 56.78% and 51.67%, respectively. For 40% moisture level the concentrations of CP and WSC were 15.8% and 6.5%, respectively and IVDMD was 70.9%. The parameters of nutritional value and digestibility of other two moisture levels presented no obvious difference. For all moisture levels, pH value was above 5, numbers of LAB exceeded 10^7 colony-forming units per gram of fresh matter (cfu/g FM), and populations of yeasts were 10^5~10^6 cfu/g FM.

WSC is a crucial growth substrate utilized by spoilage-causing microorganism and there is evidence that yeasts are involved in onset of aerobic deterioration (Huisden et al., 2009; Schmidt and Kung, 2010). Silages contaminated with yeasts at a level of >10^5 cfu/g FM are prone to deteriorate in presence of air (McDonald, 1991). In this study, the population of yeasts in TMR diets before air exposure reached approximate 10^6 cfu/g FM, so a short aerobic stability was expected in these TMR diets.

Table 1 Composition (% DM), digestibility (%), pH and microorganism composition (log cfu/g FM) of the TMR diets

Item	Moisture levels of TMR diets		
	40%	45%	50%
DM	62.5 ± 1.07	56.8 ± 0.81	51.7 ± 0.33
CP	15.8 ± 0.50	15.4 ± 0.12	14.8 ± 0.42
WSC	6.5 ± 0.08	6.0 ± 0.14	6.7 ± 0.32
aNDF	38.7 ± 2.93	37.2 ± 0.98	40.6 ± 2.02
ADF	22.4 ± 2.62	20.7 ± 0.42	22.7 ± 0.72
IVDMD	70.9 ± 0.47	69.1 ± 1.30	70.6 ± 0.34
pH	5.2 ± 0.01	5.2 ± 0.02	5.1 ± 0.03
Lactic acid bacteria	7.1 ± 0.24	7.3 ± 0.01	7.2 ± 0.01
Yeasts	5.9 ± 0.38	6.0 ± 0.28	6.4 ± 0.23
Escherichia coli	4.4 ± 0.21	4.4 ± 0.72	4.0 ± 0.19
Aerobic bacteria	6.6 ± 0.10	6.5 ± 0.29	6.7 ± 0.21

Means ± SD, n=3.

The effects of moisture level and exposure time on nutritional value and digestibility of TMR diets are reported in Table 2. All TMR diets deteriorated along with the air exposure up to 168 h. During air exposure, moisture level increased WSC concentrations ($P<0.01$). IVDMD was reduced by exposure time ($P<0.05$) The DM loss at 50% moisture level was much higher than the other two moisture levels ($P<0.01$) and DM losses at all moisture levels increased ($P<0.01$) during the process of air exposure.

Table 2 Effects of moisture level and exposure time on nutritional values (% DM) and digestibility (%) of TMR during air exposure.

	DM	CP	WSC	DM Losses	IVDMD
Moisture level					
40%	68.9^{a}	16.1^{a}	7.6^{c}	5.5^{c}	66.2^{a}
45%	65.1^{b}	15.7^{b}	8.3^{b}	8.5^{b}	65.0^{a}
50%	60.6^{c}	15.8^{ab}	9.0^{a}	12.4^{a}	66.0^{a}
SEM	0.66	0.20	0.24	0.85	0.74
Exposure time (h)					
0	55.7^{f}	15.3^{c}	6.4^{d}	0.0^{e}	70.2^{a}
12	57.1^{f}	15.4^{c}	5.4^{e}	13.1^{e}	68.7^{a}
24	58.2^{ef}	15.4^{c}	5.9^{de}	0.6^{e}	68.3^{ab}
48	59.9^{e}	15.9^{abc}	8.3^{c}	5.2^{d}	66.0^{bc}
72	62.3^{d}	16.5^{ab}	9.8^{ab}	10.9^{c}	63.4^{cd}
96	67.3^{c}	15.9^{abc}	9.5^{ab}	11.7^{bc}	64.7^{cd}
120	72.0^{b}	15.9^{abc}	10.3^{a}	14.2^{b}	64.3^{cd}
144	72.6^{b}	15.8^{bc}	9.3^{b}	17.9^{a}	62.6^{d}
168	76.5^{a}	16.5^{a}	9.8^{ab}	17.7^{a}	63.1^{d}
SEM	1.15	0.35	0.41	1.48	1.29
Significance levels:					
Moisture level	**	NS	**	**	NS
Exposure time	**	*	**	**	**
Interaction	NS	NS	**	**	NS

$^{a\text{-}f}$ means in the same column with different superscripts differ significantly; * P<0.05, ** P<0.01; NS, no significance (P > 0.05).

In the first phase of deterioration, easily oxidizable WSC and lactic acid were quickly depleted, and as a consequence, CP, crude fiber and ash tended to increase on DM basis. With progressing deterioration, CP and fiber might be degraded, increasing the concentration of WSC and offering the possibility to other aerobic microbes to degrade the mass (Pahlow et al., 2003). The process of deterioration and activity of aerobic microorganisms not only leads to nutritive losses but also to DM losses, which reduces silage quality and digestibility. Changes of the nutritional value of TMR diets were considerable during air exposure in this study. The increase of CP and WSC contents during air exposure of TMR diets may be owing to the DM losses associated with deterioration.

The temperature of all TMR diets first increased and then decreased during air exposure (Figure 1a). Although TMRs with high moisture content need more heat energy to warm up, the temperature of the TMR with 50% moisture level increased more rapidly than the other two moisture levels, making it more prone to deteriorate. Regardless the moisture levels, pH values increased with prolonged process of air exposure possibly because of degradation of organic acids and generation of ammonia-N (Figure 1b). Figure 1 (c, d, e, f) showed that high numbers

of LAB (>10^7 cfu/g FM) were sustained during air exposure. LAB numbers increased first and then decreased for all moisture levels. Numbers of yeasts presented a similar trend. In the first 72 h of air exposure, populations of yeasts promptly increased from 10^6 up to 10^8 cfu/g FM for all moisture levels and then populations decreased to 10^7, 10^6 and 10^4 cfu/g FM for moisture levels of 40%, 45% and 50%, respectively. Populations of aerobic bacteria and *Escherichia coli* rapidly increased in the first 72 h and then stayed constant thereafter during air exposure.

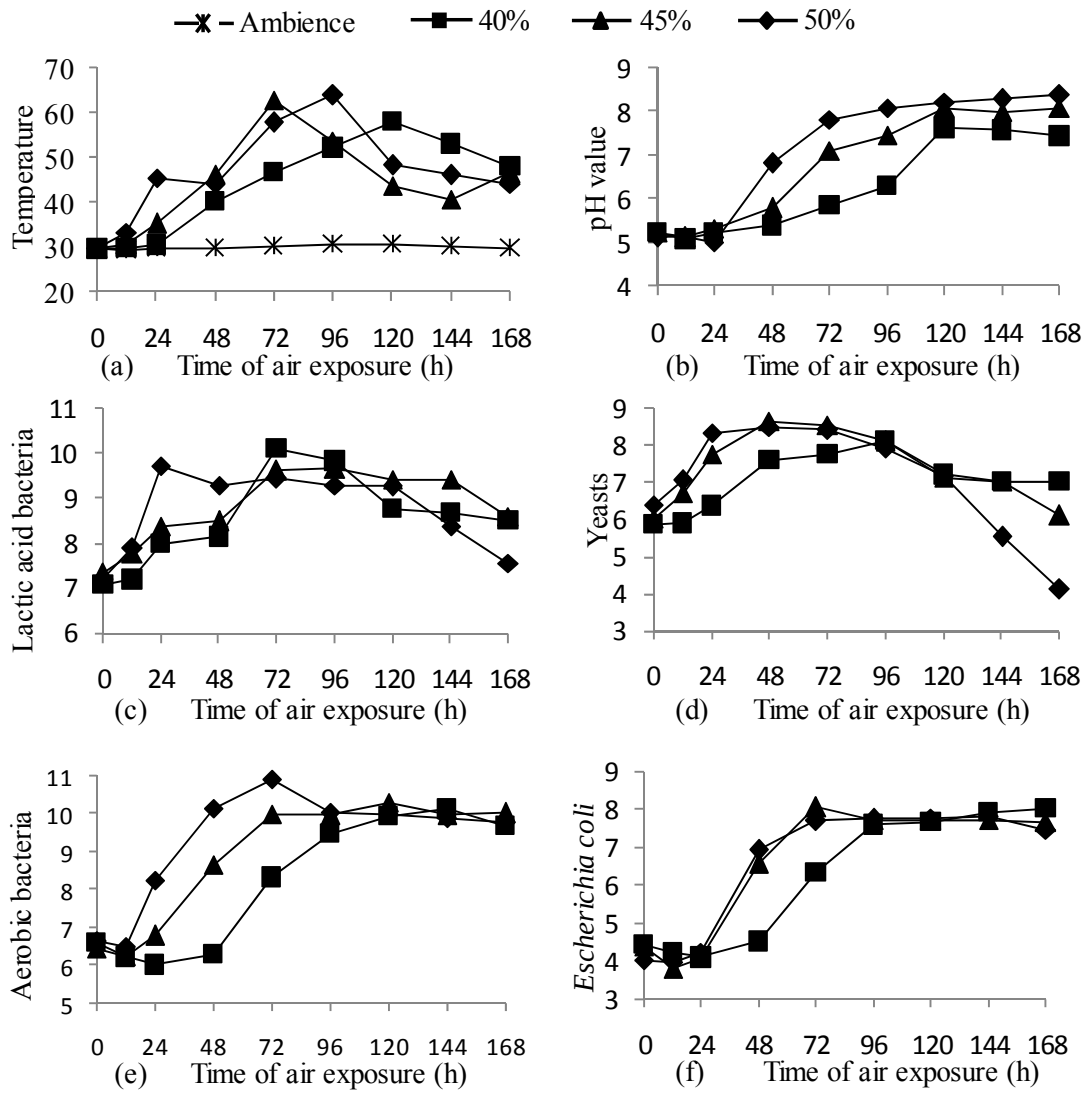

Figure 1 TMR and ambient temperature (a), pH (b), lactic acid bacteria (c), yeast (d), aerobic bacteria (e) and *Escherichia coli* (f) (log cfu/g FM) in TMR diets with 40%, 45% and 50% moisture contents during air exposure.

Many workers had found that aerobic deterioration resulted from the activity of bacteria, yeasts and molds utilizing residual WSC and lactic acid, leading to a rise of pH and the loss of energy (Muck et al. 1991). When the course of TMR temperatures (Figure 1a) is compared with the course of yeast counts (Figure 1d), it can be concluded that yeast growth preceded heating when

TMR deteriorated, suggesting that yeasts might act as a trigger of deterioration in TMR diet. The activity of aerobic bacteria, including *E. coli*, may contribute substantially to the temperature rise in TMR during deterioration process.

Conclusions

The data presented here confirm that TMRs with higher moisture level are more prone to deteriorate. Aerobic deterioration not only leads to the changes of nutritional value, causes DM losses, but also reduces the digestibility. Aerobic bacteria may contribute to the heating of deteriorated TMRs and yeasts might be involved in the onset of aerobic deterioration.

Acknowledgements

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Feed factors affecting iodine content in milk

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Introduction

Iodine is an essential element in human and animal diets. Due to low concentration in natural feed sources, iodine deficiency was common particularly in inland Norway a century ago. To overcome deficiency, iodine has been added animal feed for several decades. Although iodine is added mainly to cover animal needs, high transfer of iodine form feed into milk has resulted in more or less absence of clinical iodine deficiency signs in humans. Iodine content in milk and iodine status in the Norwegian population has been studied several times. On data collected in year 2000, Dahl et al. (2003) found average iodine content in milk of 232 µg/L in winter milk and 88 µg/L in summer milk. Dahl et al. (2003) concluded that the iodine status in parts of the population was low and pointed out people with low intake of dairy products, salt water fish and other sea products as the main risk group. In a recent study at the Norwegian University of Life Sciences (Haug et al., 2011), the average iodine content in winter and summer milk was 125 and 91 µg/L, respectively. Iodine status in humans was not studied, but although milk and dairy products are not the only source of iodine in human diets, the almost halved content of iodine in winter milk in the year 2008 study compared to the year 2000 study is of concern. The content of iodine in milk is so low that even groups with normal intake of dairy products can be in the risk of sub-optimal iodine intake both in respect to nutritional needs and nuclear safety.

Iodine in ruminant feed

In nature, iodine accumulates in sea water whereas most soils are low in iodine. Thus, plant feed sources usually contain low amounts of iodine, whereas feed sources from the sea like fish meal and in particular various sea weeds, contain considerable amounts of iodine. Fish meal and sea weed is not used in ruminant feed today, and iodine is most commonly supplemented as calcium iodate (hydrous or anhydrous), sodium iodine or potassium iodine. In Norway, iodine is mainly supplemented in the compound feed and the addition of iodine in ruminant feed has for many years been 2 mg/kg, whereas according to EU regulations up to 5 mg/kg dry matter is allowed.

Iodine metabolism in ruminants

Iodine is absorbed as iodide. Thus, iodate is transformed before absorption. The main storage of iodine is the thyroid gland where it is an essential part of the thyroid hormones triiodothyronine (T_3) and thyroxin (T_4). In dairy cows, iodine is excreted in milk, urine and faeces. Schöne et al. (2009) reported that 35 to 47 % of supplemented iodine was excreted in milk. They also concluded that excretion in milk and urine was the main routes of iodine clearance from the blood. Iodine excreted in faeces originates from endogenous excretion (e.g. thyroid hormones), indigested iodine or exfoliated intestinal cells. In contrast to humans, where iodine appears to be reabsorbed (cited in Franke, 2009), 24 to 46 % of daily orally administered iodine is reported lost in faeces in ruminants (Miller et al. 1975).

Transfer of iodine from feed to milk

The report by Schöne et al. (2009) that 35 to 47 % of supplemented iodine was excreted in milk indicates a high transfer of iodine from feed into milk. Franke et al. (2009a) confirmed these results with 30 to 56 % carryover of iodine from feed to milk in diets free of rapeseed meal. However, in diets containing rapeseed meal, the carryover of iodine from feed to milk was only 11 to 25 %. Thus a negative effect of rapeseed meal, or a factor associated with rapeseed meal, appears to exist. Miller and Swanson (1973) showed that increased iodine supplementation increased iodine excretion in all three main excretion routes. These results were confirmed by Franke et al. (2009b), but they also showed that urinary excretion of iodine increased more in animals fed rapeseed meal than in those not fed rapeseed meal. This implies that iodine was absorbed, but not extracted from blood to milk. In that respect, both the thyroid gland and the mammary gland use an active transport system (NIS; sodium iodide symporter) for extracting iodine from the blood. This transport system is competitively inhibited by thiocyanate. Thiocyanate is a well known degradation products from glucosinolates that exists e.g. in rapeseeds. In the study of Franke et al. (2009a) transfer of iodine from feed to milk was considerably reduced in the diets containing 16 % rapeseed meal compared to a diet without rapeseed meal, although the rapeseed product used in the study was in the group that can be considered low in glucosinolates. This indicates that either is the NIS mechanism very sensitive, or thiocyanate at higher concentrations were present in the rapeseed meal. Unfortunately, only total glucosinolates were analysed by Franke et al. (2009a).

The Norwegian studies

In the Norwegian studies on data from 2000 (Dahl et al., 2003) and 2008 (Haug et al., 2011), the addition of iodine in ruminant feed was 2 mg/kg dry matter both years. Since iodine is added to compound feeds, daily intake of dietary iodine, and thus, iodine concentration in milk, is related to compound feed allowances. Reduced compound feed allowances in summer compared to winter feeding explain the lower iodine concentration observed in summer milk, but not the reduction in iodine content in winter milk between year 2000 and 2008. Compound feed allowances were unchanged between year 2000 and 2008. As shown by Franke et al. (2009a), transfer of iodine from feed to milk is considerably reduced by inclusion of rapeseed meal in the diet. Since 2000, the use of various rapeseed products originating from production of bio-fuel production has increased considerably in Norway. Thus, although the rapeseed product used in the study of Franke et al. (2009a) can be considered low in glucosinolates, our main hypothesis is that the reduced content of iodine in winter milk is related to the increased use of rapeseed products in ruminant diets. However in addition, teat dipping with iodine containing liquids totally disappeared during the same period. Iodine can be absorbed through the skin and enter the milk (Hemken, 1979) and next to iodine intake, teat dipping probably is the most important factor determining iodine supply to the animal (Flachowsky et al., 2007).

Project on optimal iodine content in milk

Iodine is among the trace elements with a rather short interval between recommended and toxic level. In adult humans, recommended upper intake of iodine is in the range 500 to 1000 µg/day, whereas recommended intake is in the range 150 to 200 µg/day. Thus, producing milk with an optimal content of iodine according to expected daily intake of dairy products is the challenge.

With respect to animal feeding, the challenge is to administer iodine in sufficient amounts irrespective of inhibiting factors like glucosinolates. Based on the work carried out in Germany by Franke and co-workers (Franke, 2009), a three-year research project involving a PhD is presently starting up at the Department of Animal and Aquacultural Sciences. The ultimate goal of this project is to present a description on how to produce milk with an optimal content of iodine. The PhD position is financed by the Norwegian University of Life Sciences and the research work is financed by TINE and Felleskjøpet Fôrutvikling. The project is expected to be completed in 2015.

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Changes in body condition scores during lactation of Norwegian Cattle

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Introduction

Body condition score (BCS) is a subjective mean for estimating fat stores in dairy cows independent of frame size and body weight (BW) (Waltner et al., 1993). It is a valuable tool in predicting productive and reproductive performance in many domesticated animals (Berry et al., 2002). BCS has been shown to be a useful tool in dairy cow management and feed control. Changes in BCS from calving throughout lactation may indicate how cows mobilize and deposit body fat in different periods of the lactation. This information will improve the ability to estimate milk production and feed efficiency at a given stage of lactation. Moreover, it will improve the ability to estimate nutritional requirement for maintenance since BW alone does not account for fatness. The aim of this work was to use BCS curves to develop a mathematic model for mobilisation and deposition of body fat during lactation and incorporate it into the NorFor feed evaluation system (Volden, 2011).

The objective of this presentation is to describe changes in BCS during lactation of dairy cows based on data from the Norwegian heard recording system.

Materials and methods

Individual cow data was collected during seven years (2005 to 2011) from the Norwegian Dairy Herd Recording system. The cows were of the Norwegian Cattle (NRF) Breed. A visual scoring technique, designed for Holstein dairy cattle, was used (Edmondson et al., 1989). It had a five-point scale (1 = emaciated to 5 = severely over-conditioned) with 0.25-unit increments. The BCS time scale was adjusted relative to date of calving to find lactation stage at the time of body condition scoring. Cows with 305-d lactations and BCS records for the same lactation were selected. Data consisted of a total of 108285 BCS from 2323 individual farms, of which 45576 records were from 1st lactation cows, 29635 were from 2nd lactation cows and 33074 were from older cows (>2nd lactation). Information on 305-d milk yield and concentrate supply based on individual cow-lactations are presented in Table 1.

Table 1 Milk yield and concentrate supply based on individual cow measurements

		305 d milk yield		305 d concentrate supply	
	N	Mean	Std	Mean	Std
1st lactating cows	21997	6061	1252	1814	513
2nd lactation cows	14401	6990	1462	2020	565
>2nd lactation cows	16097	7522	1543	2140	588

The dataset was analysed for fixed effects of lactation number (1st lactation, 2nd lactation, >2nd lactation cows), weeks in milk (WIM) at time of BC scoring, calving month, geographical region and the two-way interactions of lactation number x WIM. Effect of year was included in the model as a random variable, and data were analyzed by Proc Mixed of the SAS software (SAS Institute Inc. Cary, USA).

Results and discussion

Least-square means of average BCS estimated for lactation number 1, 2 and >2 are presented in Table 1. The corresponding values during the lactation period (BCS*WIM interaction) are shown in Figure 1. Average BCS differed (P<0.01) between all lactation numbers, and was lowest for 1st lactation cows and highest for cows older than 2nd lactation. However, the numerical difference between 1st and 2nd lactation cows was small, 0.01 point. In general, BCS for all lactation numbers decreased after calving and reached the lowest level 6 to 8 weeks after calving, of which 0.33, 0.31 and 0.45, points were lost for 1st, 2nd and older cows, respectively (Figure 1). Changes in BCS of 1 point in British Friesian cows account for about 115 kg (Chamberlain and Wilkinson, 2000). This is much higher than what is assumed for NRF where changes of 1 point indicate a mobilization of about 60 kg body weight (Åkerlind et al., 2011). Using 60 kg for 1 point of BCS loss as assumed for NRF, the loss of body weight of first, second and older cows would be 20, 19 and 27 kg, respectively. After a few weeks on the lowest level, BCS increased for all lactation numbers. However, the interaction of BCS*WIM showed that the trajectory between the lactation numbers differed (P<0.01). First lactation cows had a numerical higher BCS than 2nd lactation cows at calving, but numerical lower than older cows. The increase after lowest level was reached was slower for 1st lactation cows than for older cows, and they had not returned to initial BCS at the end of lactation which resulted in a numerical lower BCS at 2nd calving. However, at the end of 2nd lactation, the cows had increased BCS above the initial value at calving, resulting in a numerical higher BCS at 3rd calving and onwards. In spite of the numerical highest initial BCS at calving and the highest loss of BCS after calving, cows older than 2nd lactation returned to initial BCS after 42 weeks. These results indicate that cows in 1st lactation are somewhat underfed due to the requirement for growth and milk yield during lactation. This affects the BCS for 2nd lactation cows resulting in the lowest BCS at calving.

Table 1 Least-square means of body condition score (BCS) estimated for 1st, 2nd, and >2nd lactating cows

				Lactation number		Lactation number *WIM[a]	
	1st lactation	2nd lactation	>2nd lactation	F=	P	F=	P
Mean	3.30[b]	3.31[c]	3.39[d]	487	***	13.5	***
SEM	0.0088	0.0089	0.0089				

[a]Weeks in milk; [b,c,d]LSMeans with different superscripts differ; ***P<0.001.

Conclusions

First lactation cows had a BCS close to what is recommended at calving but had problems to return to initial BCS during lactation, resulting in a poorer initial BCS at 2nd calving. However, 2nd lactation cows were able to deposit more body fat as lactation progressed than 1st lactation cows as lactation progressed, probably due to a lower requirement for growth. They also reached a higher BCS at 3rd calving and onwards. These curves show that fat mobilization and deposition differs during lactation and between lactation numbers, indicating that a mathematic model for BCS curves should differ for 1st, 2nd, and >2nd lactation cows.

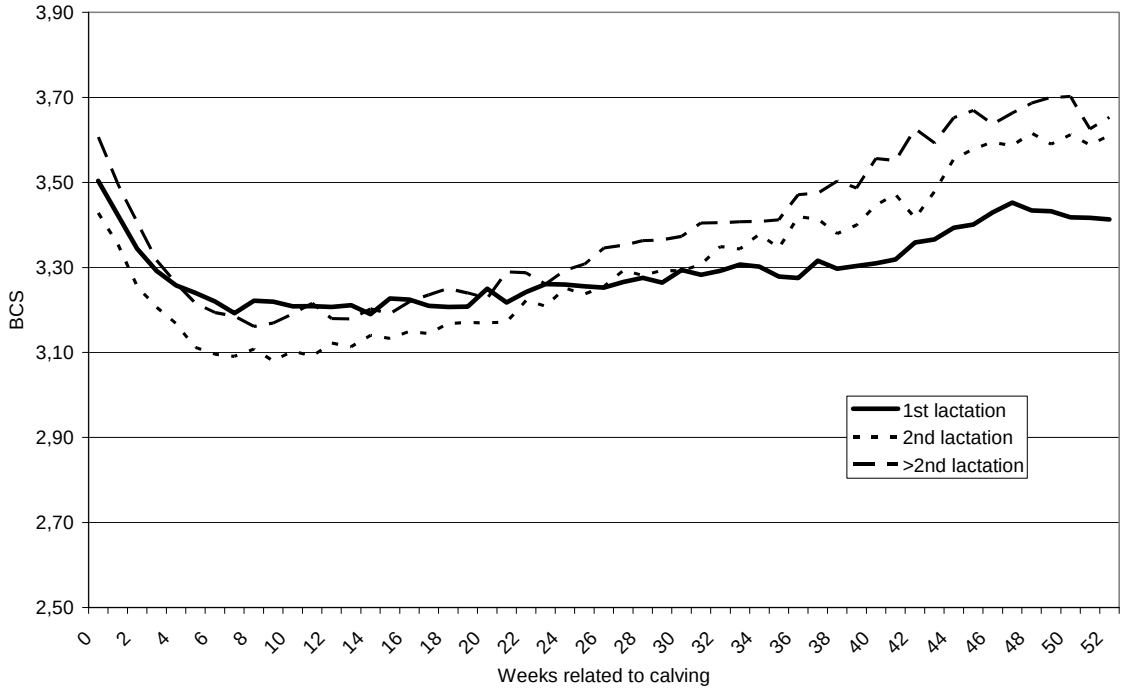

Figure 1 Changes in body condition score during lactation of 1st, 2nd, and >2nd lactation cows.

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Feedout strategy does not affect animal performance under optimal silage management

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Introduction

Corn silage is the major forage source for high producing dairy cows in Brazil. Most of this silage is stored in horizontal silos, which is exposed to air penetration, especially in the upper parts near the walls, which are difficult to pack and seal properly (Ashbell and Lisker, 1988). During storage and feedout phases, deterioration can occur due to aerobic microorganisms which use silage as nutrient source. In tropical conditions, particularly when sealing strategies are not efficient, visually inedible silage typically reach 10% of ensiled mass, whereas with a suitable sealing procedure visual losses might amount to only 3% FM (Amaral et al., 2012).

Aerobic deterioration also decreases the hygienic quality of silages because of the increased risk of proliferation of undesirable microorganisms, including those potentially pathogenic (Lindgren et al., 2002). Thus, silage from upper parts of silo might be of poor quality compared to those from bottom zone, and may have a negative impact on the animal performance.

The objective of this study was to evaluate the performance of dairy cows fed diets base on corn silage unloaded from the top half or bottom half of bunker silo.

Materials and methods

The experiment was carried out at the Department of Animal Science, College of Agriculture Luiz de Queiroz, Piracicaba, Brazil. A corn crop with 32% DM was harvested with a pull-type harvester (10 mm theoretical length of cut, Mentamit®, Cajuru, Brazil) and stored quickly in a bunker silo (3.0 m height × 4.0 m width). Packing extended throughout harvesting period (10 h/d) using a tractor weighing around 50% of forage delivery rate (11 t FM/h). Immediately upon completing filling, silo was sealed with a 200µm black-on-white polyethylene film. Gravel filled bags were positioned along the perimeter of the silo to seal edges. The entire film surface was covered with sugarcane bagasse (0.10 m thick). Sugarcane bagasse protects the film against sun light, which ultimately decreases oxygen permeability. Further, the bagasse layer weighed down the plastic film, decreasing the ingress of air between film and silage. After a storing period of 190 d, the silo was opened and treatments were defined: TOP SILAGE - corn silage from the top half of silo (0 to 1.5 m deep); and BOTTOM SILAGE - corn silage from the bottom half of silo (1.5 to 3.0 m deep) (Figure 1). Corn silage was taken from the silo twice daily and offered as a TMR to the cows. The left half of the working face was unloaded at 6:00 a.m. (i.e., morning feeding) and the right hand side at 4:00 p.m. (i.e., evening feeding). During the trial, actual removal rate was 0.18 m/d.

Sixteen Holstein cows (12 multiparous and 4 primiparous) were housed in a tie-stall barn and assigned randomly to treatment sequence in a crossover design experiment with one 14-d preliminary period and two 21-d experimental periods. During the preliminary period, the first 10 d were allowed for diet adaptation, and samples were collected during the final 4 d. During each experimental period, the first 14 d were allowed for diet adaptation and samples were

collected during the final 7 d. Cows were on average 250 days in milk (DIM), 680 kg body weight and yielded 31.3±8.8 kg milk per day (mean ± SD) at the end of the preliminary period. The two experimental diets contained either 60% of top corn silage or 60% bottom corn silage and 40% concentrates (dried citrus pulp, soybean meal and mineral premix). Diets were formulated to contain 16.5% CP and 30% forage NDF. Ration ingredients were mixed during 7 min in a self-propelled mixer (Data Ranger American Callan®, New Hampshire, USA) and fed twice daily (8:00 a.m. and 6:00 p.m.). The amount of offered feed was adjusted to allow more than 10% as orts. Cows were moved to an exercise lot twice daily before milking in a parlor (6:00 a.m. and 5:00 p.m.). Milk yield as well as DMI was recorded from d-14 to d-21 in each period. Milk samples were collected in flasks containing bronopol from d-15 to d-19 for analysis of fat, protein, casein, lactose, and urea by infrared spectroscopy, and somatic cells count (SCC) by flow cytometry at a local laboratory (Clínica do Leite, Piracicaba, Brazil). Milk energy content (MJ/kg) was calculated according to NRC (2001): milk NE_L = 0.389 × fat % + 0.229 × protein % + 0.165 × lactose %. Daily excretion of milk energy (MJ/d) was defined as milk NE_L × milk yield. Energy efficiencies of diets were estimated by dividing the excretion of milk NE_L by DMI (MJ/kg). Urinary N excretion (g/d) was estimated from milk urea-N (MUN, mg/dL) and milk yield (kg/d) as follows: -127 + 13.1 × MUN + 6 × milk yield (Nousiainen et al., 2004). From this, nitrous oxide (N_2O, kg/cow/year) and CO_2 equivalent (CO_2-eq, kg/cow/year) emissions were calculated using default values from IPCC 2006 methodology (AMEE, 2012).

In addition to the animal responses, silage temperatures across working face were measured in each experimental period immediately after morning feedout. Temperatures of 44 points (4 locations and 11 elevations) of the left half of the working face were monitored at depths of 0.2 m using bulb thermometers. Temperatures of the right half of panel were assumed as mirror of left half. A contour map was generated from temperature data set by the software Surfer 10 (Golden Software®, Golden, USA). The temperature of the central zone of the silo (core) was defined as the reference temperature. The difference between the temperature of the silage samples and the reference temperature was calculated for each point (dT) and used as a heating index associated with aerobic deterioration (Borreani et al., 2010). Areas with dT higher than 5and 10^{o}C were also calculated. Yeast counts of four samples from core zone were measured through serial dilutions using petrifilm plates (3M®, Sumaré, Brazil).

Data from cow's performance were analyzed with the Mixed procedure of the statistical PC package SAS (2001) including fixed effect of treatment, fixed effect of period, random effect of cow, and the interaction between treatment and period effects.

Results and discussion

Feeding top or bottom silage did not affect DMI (P = 0.62), milk yield (P = 0.47) and milk composition (P > 0.15), with exception of MUN. Likewise, energy efficiency to yield milk was not affected by diet (P = 0.37, Table 1), showing similarity in the nutritive value of corn silage from both zones (top and bottom). The bunker silo showed no visibly spoiled areas, so all silage was fed to the animals. Thermal images indicated a high quality of corn silage across the working face (Figure 1). Only 4.8% and 0.4% of panel area presented 'dT' higher than 5 and

Table 1 Performance of dairy cows fed corn silage from top or bottom of bunker silo

	Treatment			
Item	Bottom Silage	Top Silage	SEM	*P*-value
DMI, kg/d	23.94	23.52	1.03	0.62
Milk, kg/d	28.86	28.15	3.60	0.47
3.5% FCM, kg/d	28.56	29.15	3.39	0.59
Fat, %	3.66	3.85	0.18	0.15
Protein, %	3.48	3.54	3.51	0.26
Fat:Protein ratio	1.05	1.09	0.04	0.49
Casein, %	2.72	2.78	0.13	0.15
Casein:Protein, %	78.1	77.9	0.39	0.20
Lactose, %	4.54	4.52	0.06	0.64
Urea, mg/dL	8.92	11.30	1.19	<0.01
SCC, ×1000/mL	134	110	-	-
Log SCC	1.96	1.91	0.13	0.48
Fat, kg	0.99	1.05	1.01	0.29
Protein, kg	0.97	0.95	0.95	0.72
Milk NE_L, MJ/d	82.38	83.60	9.12	0.69
Milk NE_L:DMI, MJ/kg	3.39	3.60	0.38	0.37

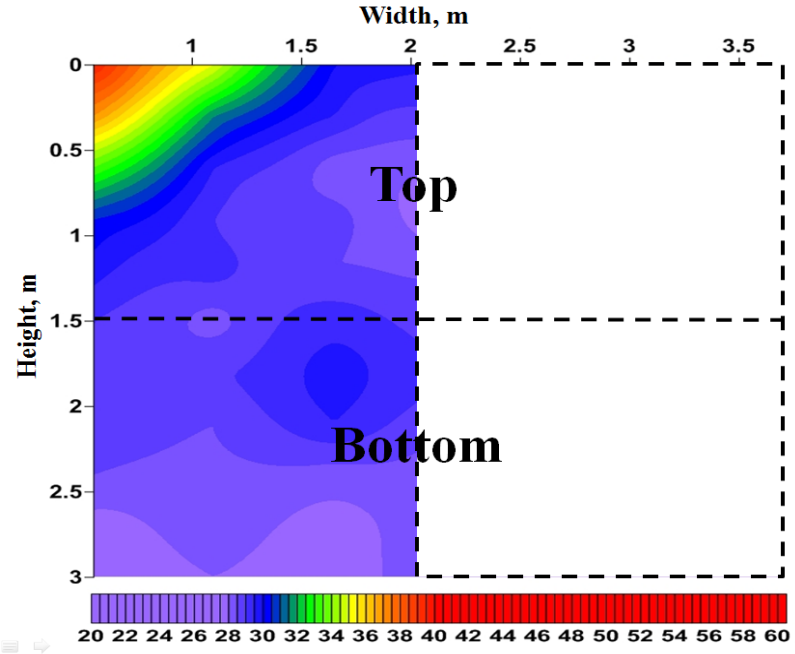

Figure 1 Thermal image of the working face of the silo after daily unloading (the bunker silo showed no visibly spoiled areas). Temperature scale (°C) is showed below the bottom.

10°C, respectively. Borreani and Tabacco (2010) found similar values (3.7 and 0.8%, respectively) for the best managed silos in a survey of northern Italy. All samples showed yeast counts lower than 2 log cfu/g. In contrast, Amaral et al. (2012) reported a range from 3 to 10% FM of inedible corn silage when different sealing strategies were compared.

Milk urea nitrogen concentration was the only variable altered by silage feedout strategy ($P < 0.01$). Silage from the bottom zone (8.92 mg/dL) decreased MUN by 21% compared to silage from the top zone (11.30 mg/dL). Although the values of MUN could be considered low, the high concentration of milk protein ($\bar{x}$ = 3.51%) and the milk fat:protein ratio ($\bar{x}$ = 1.07) indicate an adequate protein supply (Nousiainen et al., 2004; Hutjens and Chase, 2010). Diet CP and metabolizable energy contents are the main factors that affect MUN (Oltner and Wiktorsson, 1983; Nousiainen et al., 2004; Hutjens and Chase, 2010). Because diets were similar in terms of CP (16.5%), ruminally digested energy might have been higher for bottom than top silage, even though this was contrary to energy efficiencies estimates based on milk yield. Energy partition may have been different across diets as changes in body weight and body condition score were not registered. Chemical analyses of silages are underway and might help to understand this issue.

If a more intense proteolysis had occurred in the top zone silage (McDonald et al., 1991), the intake of rumen degradable protein might have increased for cows fed the top silage based diet. Silage NH_3-N has not measured so far, but a great effect of corn silage proteolysis on MUN is not expected (Nousiainen et al., 2004).

From MUN content and milk yield, it was possible to estimate urinary N excretion. Feeding a diet based on bottom corn silage led to a 14% lower urinary N loss, compared to feeding silage from the top zone (163 vs. 190 g/cow/d), and might result in <9.9 kg N losses/cow/year. Under a global warming perspective, it could be translated into <0.3 kg N_2O/cow/year, equivalent to 106 kg CO_2-eq/cow/year (IPCC, 2006). In the present study, MUN seems to have been a side effect response, but it raises the potential of well managed silages to mitigate air pollution and the emission of greenhouse gases.

Conclusions

Under optimal management conditions, corn silage feedout strategy does not seem affect the performance of dairy cows.

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Fermentation quality and nutritive value of total mixed ration silage of green tea grounds at ten or twenty percent of dry matter

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Introduction

In China, consumption of ready-made tea drinks in bottles, packs and cans have been increasing significantly in recent years (Wang et al., 2011). Meanwhile, a large amount of tea leaf grounds are released annually by beverage companies manufacturing various tea drinks. Although a small part of tea grounds is used as compost material, most is generally incinerated. There is increasing demand for efficient use of food by-products due to economic and environmental concerns. These grounds are usually high in crude protein (CP), amino acids, catechins such as epigallocatechin-3-gallate, epicatechin, epigallocatechin and epicatechin gallate, and vitamins (Xu et al., 2003; Kondo et al., 2004), suggesting that tea grounds may have potential as an animal feed.

Because wet green tea grounds (WGTG) has high moisture content, it deteriorates rapidly. The technology of silage preparation for tea grounds was established using Lactobacillus plantarum FG 1 isolated from forage and commercial cellulases from fungi (*Acremonium* genus).The nutritive value of tea grounds silage for wethers was estimated by Xu et al. (2003). However, problems with the use of WGTG as feed were found in this study, such as nutritional imbalance, poor palatability and poor preservation (Xu et al., 2003). If high-moisture by-products were ensiled with dry feeds as a total mixed ration (TMR), the risk of effluent production would be minimized and the time for mixing prior to feeding could be omitted. In addition, unpalatable by-products could possibly be incorporated into a TMR if their odours and flavours were altered by the fermentation. Therefore, there is need for a better understanding of the ensiling characteristics of WGTG in order to better apply current technologies enabling an efficient use of this wet by-product.

The purpose of this study was to evaluate fermentation characteristics of silage prepared from WGTG mixed with various feeds in the form of TMR silages and to estimate the nutritive value of these TMR silages in wethers.

Materials and Methods

Silage preparation of total mixed ration

WGTG was obtained from a commercial beverage factory. The TMR was prepared using a compound feed, WGTG, corn, soybean meal, timothy hay, alfalfa hay, dried beet pulp and a vitamin-mineral supplement (Table 1). Treatments included replacement of soybean meal and alfalfa hay with 0, 10, and 20% WGTG on DM basis. The moisture of all TMR was adjusted with water to 55%. The TMR was treated with Lactobacillus plantarum Chikuso-1 (Snow Brand Seed Co., Ltd, Japan) at a rate of 5 mg/kg to supply 1.0×10^5 cfu of lactic acid bacteria (LAB)

per g of fresh TMR. The ensiled amounts of TMR were 350 kg in polyethylene bag silos. The silages were stored outdoors at 12 to 30°C, and the silos were opened after 42 d.

Digestion trial

Six 2-yr-old Suffolk wethers (74.5 ± 3.8 kg) were used in a replicated 3 × 3 Latin square. The wethers were individually housed in metabolic cages and fed the 3 treatment diets at 18 g DM/kg BW/d. Half of the ration was fed at 09:00 and the other half at 17:00 h. Water and mineral blocks were accessible at all times. A 7-d preliminary adjustment period was followed by a 7-d period during which all faeces and urine were collected.

Chemical analysis

The WGTG, TMR silages and faecal samples were dried in a forced draught oven at 60°C for 48 h and ground to pass a 1-mm screen with a Wiley mill. DM, CP, ether extract (EE) and organic matter (OM), were analyzed according to methods 934.01, 976.05, 920.39 and 942.05, respectively, of AOAC (1990). The acid detergent fiber (ADF) and neutral detergent fiber (aNDF) were analyzed by the methods of Van Soest et al. (1991) with amylase and sodium sulphite, and the results were expressed inclusive of residual ash. Acid detergent insoluble CP (ADICP) and neutral detergent insoluble CP (NDICP) was determined as N × 6.25. Condensed tannins (CT) were analyzed by the method of Makkar and Goodchild (1996). The gross energy (GE) was determined by using an automatic bomb calorimeter (CA-4PJ, Shimadzu, Kyoto, Japan). TMR silage pH, organic acids and ammonia-N were determined using the procedure described by Xu et al. (2003).

Table1 Ingredient proportions (% DM) of total mixed ration silages

Item	Treatments		
	0%	10%	20%
Wet green tea grounds	0	10.0	20.0
Concentrate	30.0	30.0	30.0
Corn	4.0	4.0	4.0
Soybean meal	6.0	3.0	0
Alfalfa hay	14.0	7.0	0
Timothy hay	33.5	33.5	33.5
Beet pulp	11.0	11.0	11.0
Vitamin and mineral supplement	1.5	1.5	1.5

Statistical Analysis

Digestion trial data were analyzed as a replicated 3 × 3 Latin square using the General Linear Model procedure of SAS (AOAC, 1990) and silage, period, and block were included in the model. Polynomial contrasts were used to determine the influence of increasing WGTG inclusion in the TMR silages, and the Tukey test (AOAC, 1990) was used to identify differences ($P < 0.05$) between means.

Results and discussion

Table 2 shows the chemical composition of WGTG, soybean meal and alfalfa hay. WGTG contained more CP (31.0 % DM) than alfalfa hay (21.1 % DM) but less than soybean meal (52.2 % DM). The aNDF and ADF contents in WGTG were higher than in soybean meal, and lower than in alfalfa hay.

Both ADICP and NDICP protein fractions in WGTG were higher than in soybean meal and alfalfa hay. Licitra et al. (1990) defined that the fraction of NDICP degrades slowly, and that of ADICP has a low biological availability. Higher NDICP and ADICP contents may be caused by heat treatment in the dry processing of tea leaves and/or the extraction of tea from the leaves with hot water. Therefore, there is need for a better understanding of the ensiling characteristics of WGTG.

Chemical composition and fermentation quality of the TMR silages are shown in Table 3. The three TMR silages were well preserved, as indicated by low pH and low ammonia-N content, and high lactic acid content. Increasing concentrations of WGTG in the TMR silage resulted in lower lactic acid content ($P < 0.01$). Propionic and butyric acids were not detected. These results could be attributed to adequate water soluble carbohydrates or pectins and hemicelluloses in the dried beet pulp, alfalfa and timothy hay used by LAB, which resulted in favourable lactic acid fermentation (Winters, 1998). The DM, OM, CP, ADF and aNDF contents of all TMR silages were similar. However, the EE content ($P < 0.05$) was higher in the TMR with the highest proportion of WGTG (20% WGTG).

Table 2 Chemical composition of wet green tea grounds (WGTG), soybean meal and alfalfa hay.

Item	WGTG	Soybean meal	Alfalfa hay
Dry matter (%)	25.6 ± 0.26	88.6 ± 0.25	86.1 ± 0.75
Organic matter (%DM)	97.0 ± 0.25	95.9 ± 0.18	93.0 ± 0.66
Crude protein (% DM)	31.0 ± 0.20	52.2 ± 0.11	21.1 ± 0.91
Ether extract (% DM)	4.2 ± 0.02	1.5 ± 0.02	2.2 ± 0.15
ADF (% DM)	21.5 ± 0.35	8.9 ± 0.21	31.1 ± 1.01
aNDF (% DM)	35.8 ± 0.31	14.3 ± 0.18	39.3 ± 1.06
ADICP (% DM)	1.8 ± 0.03	1.2 ± 0.01	0.8 ± 0.01
NDICP (% DM)	9.6 ± 0.04	1.8 ± 0.03	1.6 ± 0.02
CT (% DM)	8.76 ± 0.14	<0.01	<0.01

Means ± SD, n = 3. ADF, acid detergent fiber; aNDF, neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; ADICP, acid detergent insoluble crude protein; NDICP, neutral detergent insoluble crude protein; CT, condensed tannins.

Table 3 Chemical composition and fermentation characteristics of 42-d total mixed ration silages

Item	Treatments			SEM	P-value
	0%	10%	20%		
Chemical composition, % DM					
Dry matter	43.7	43.9	43.4	0.23	0.212
Organic matter	93.2	93.4	93.1	0.14	0.652
Crude protein	15.4	15.5	15.5	0.17	0.743
Ether extract	2.2[a]	2.4[b]	2.6[c]	0.29	0.037
Neutral detergent fiber	38.8	39.2	39.6	0.58	0.164
Acid detergent fiber	24.2	23.9	23.6	0.46	0.230
Energy (MJ/kg)	19.8	19.8	19.9	0.09	0.782
Fermentation profile					
pH	3.91	3.94	3.98	0.007	0.917
Lactic acid (% DM)	6.96[a]	6.72[ab]	6.56[b]	0.062	0.004
Acetic acid (% DM)	0.70	0.91	0.82	0.071	0.142
Propionic acid (% DM)	nd	nd	nd	-	-
Butyric acid (% DM)	nd	nd	nd	-	-
Ammonia-N (% TN)	3.52	3.36	3.64	0.102	0.384

Nutrient digestibility, nitrogen balance and energy density of TMR silages are shown in Table 4. Digestibilities for TMR silage at 10% concentration of WGTG was similar to 0% ($P > 0.05$), but DM, OM and energy digestibility was lower for the 20% compared to the 0% ($P < 0.05$). Increasing concentrations of WGTG in the rations decreased digestibility of CP ($P < 0.01$). The TDN and DE contents of TMR silages at 0% and 10% concentrations of WGTG did not differ ($P > 0.05$). However, TDN and DE contents were higher ($P < 0.05$) in these rations than the TMR silage with 20% WGTG. Intake nitrogen, urinary nitrogen and retained nitrogen was not different between the treatments. However, the faecal nitrogen for TMR silage at 20% concentration of WGTG increased than that of other silages ($P < 0.01$).

Table 4 Digestibility, nitrogen balance and nutrient content of total mixed ration silages

Item	Treatments			SEM	P-value
	0%	10%	20%		
Digestibility (%)					
Dry matter	72.8[b]	72.1[b]	70.3[a]	0.45	0.003
Organic matter	74.3[b]	73.9[b]	71.9[a]	0.51	0.007
Crude protein	74.7[c]	74.1[b]	72.2[a]	1.25	0.002
Ether extract	68.4	68.6	67.9	1.17	0.172
Acid detergent fiber	59.5	59.0	58.5	1.72	0.287
Neutral detergent fiber	63.9	63.6	62.6	1.91	0.357
Energy	68.8[b]	68.8[b]	66.7[a]	0.48	0.008
Nitrogen balance					
Intake nitrogen (g)	32.91	33.32	33.29	0.54	0.076
Nitrogen loss in faeces (g)	8.41[a]	8.64[a]	9.22[b]	0.32	0.003
Nitrogen loss in urine (g)	15.52	15.61	15.11	0.45	0.764
Nitrogen retention (%)	27.3	27.2	26.9	1.83	0.215
Nutrient content					
Total digestible nutrients (% DM)	71.2[b]	71.1[b]	69.2[a]	0.173	0.006
Digestible energy (MJ/kg DM)	13.6[b]	13.6[b]	13.3[a]	0.093	0.012

n = 6; [a, b.c] Values within line with different superscript letters differ ($p<0.05$).

Kondo et al. (2004) used goats to compare whole-crop oat silage rations containing 0, 5 and 20% WGTG and reported that the highest WGTG level showed only a tendency for a lower DM intake than the zero level. Both the 5% and 20% level of additional WGTG in forage had no effect on the digestibility of DM, aNDF and ADF ($P > 0.05$). However, Kondo et al. found that the CP digestibility increased with the addition of WGTG, and was highest in WGTG 20% ($P < 0.05$). In the present study, the DM, OM, CP and energy digestibility for TMR silage at 20% concentration of WGTG was lower than that for 0% ($P < 0.05$). As for the nitrogen balance, with progressive increases in WGTG concentrations, the intake nitrogen and urinary nitrogen of TMR silage did not change, but the faecal nitrogen increased ($P < 0.01$). These results are consistent with the results of previous studies, apart from Kondo et al. (2004), in that they showed decreased digestibility of CP and increased faecal nitrogen excretion in animals fed tannin-rich diets (Ben Salem et al., 2005). Acid detergent insoluble CP includes lignin-bound N, Maillard products and tannin-protein complexes which are highly resistant to mammalian and microbial enzymes (Krishnamoorthy et al., 1983). The WGTG used in this experiment contained high CT content, which might be the reason that the digestibility of CP decreased with increased WGTG concentrations.

Based on the TMR silage fermentation and nutrient characteristics, we have found that WGTG can be well preserved as TMR silage and it is a good potential material for TMR. Increasing the

concentrations of WGTG in the TMR silages decreased the digestibility of CP. Future studies should determine the best mixing ration of TMR silage with WGTG as a diet for lactating cows.

Conclusions

The data presented here confirm that TMR silages in which WGTG was substituted for soybean meal and alfalfa hay were well preserved. The DM, OM, CP and energy digestibility of the silage containing 20% WGTG was lower than that of the control. Therefore, it is suggested that the proportion of WGTG for TMR silages should be approximately 10% of diet DM.

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A comparison of dietary protein evaluations by the NRC-2001 and Nordic Feed Evaluation systems[1]

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Introduction

Microbial protein formation in the rumen allows dairy cows and other ruminants to be moderately productive on diets containing poor quality protein or relatively large amounts of non-protein N (NPN). Older readers may remember the stir created by Virtanen (1966) who reported milk yields of about 2000 to >5000 kg/year in Finnish Red cows fed all of their crude protein (CP) in the form of urea and ammonium salts. Thus, the old dogma in ruminant nutrition was that total CP, rather than protein quality (amino acid (AA) pattern of dietary protein relative to requirement) was our principal concern. This is illustrated by Ferguson (1959) who summarized data showing that wool growth was not influenced by dietary CP content in excess of 8%, or by dietary protein quality, but wool growth was proportional to energy density when sheep were fed diets with ≥8% CP. Microbial action in the rumen "leveled" the protein in Ferguson's diets: high quality supplemental proteins were largely degraded and used to form microbial protein, the amount of which was proportional to energy intake. It was found that abomasal infusion of small amounts of protein, or as little as 1 g/day of methionine or cysteine (wool protein is >10% sulfur-AA), doubled wool growth (Reis and Schinckel, 1964). Beef cattle and dairy cattle responded in much the same way. For example, abomasal casein infusion increased milk protein yield by about 10%, with nearly maximal response occurring within 12 hours of starting infusion (Broderick et al., 1970). A vast amount of quantitative data on ruminant protein nutrition and metabolism has been published in the intervening years; an excellent reference for much of this work is Van Soest (1994). Models applying the quantitative estimates of synthesis of microbial CP (MCP), rumen-degraded protein (RDP) and rumen-undegraded protein (RUP) soon began to appear. One of the first in North America was that of Burroughs et al. (1974), which computed "urea-fermentation potential" in beef cattle and sheep diets. To determine if urea (or other NPN) could be effectively added to the diet, it was necessary to know the dietary supply of RDP and rumen-fermentable energy and how these influenced MCP formation. The early models were very simple, applying static constants for MCP/unit digestible energy as well as RDP and RUP content of feedstuffs. Of course, the models evolved to more complex forms; we in the U.S. were quite proud of our NRC (2001) publication when it first appeared. Although the person principally responsible for the protein section recognized its limitations (C. G. Schwab, personal communication), NRC-2001 has become a sort of standard of comparison. The NorFor (2011) system is a more recent and sophisticated model developed in Scandinavia. It is our opinion that NRC-2001 is beginning to show its age and NorFor may be better alternative for use in the Nordic countries.

[1]Mention of any trademark or proprietary product in this paper does not constitute a guarantee or warranty of the product by the USDA or the Agricultural Research Service and does not imply its approval to the exclusion of other products that also may be suitable.

The NRC-2001 Protein Model

Compared to the previous version (NRC, 1989), the 7th Revised "Nutrient Requirements of Dairy Cattle" (NRC, 2001) had greatly expanded feed composition tables plus somewhat altered factorial equations for computing metabolizable protein (MP) requirements, variable intestinal digestibilities for RUP sources, and substantially revised approaches to estimating MP supply from MCP and RUP. The "new" NRC-2001 publication ran to 381 pages versus 157 pages in the prior edition; under Schwab's leadership, the protein section increased from 10 pages to a 61-page chapter. Details on the estimation of requirements for MP and metabolizable AA (MAA) can be found on pp. 67-74 (NRC, 2001). The general approach was to determine Net Protein (NP) requirement, which was equivalent to the amount of protein in the tissue or product formed and to assume that all MP was used with 67% efficiency for NP (the exception is that an efficiency of 0.33 is applied to the products of conception). For example, the MP requirement for milk synthesis for a cow secreting 1.00 kg/d of protein would be 1.00/0.67 = 1.50 kg/day. This type of static approach has been criticized by workers such as Lapierre et al. (2005), although her group found that application of the NRC constant of 0.67 was about equally reliable to applying a variable efficiency based on experimental measurement of AA utilization. Although acknowledging importance of His as a potentially limiting AA (Korhonen et al., 2000), the committee restricted information on essential AA requirements to Met and Lys (pp. 81-85; NRC, 2001). This approach was used to define Met and Lys "requirements" at, respectively, 2.2-2.4% and 6.6-7.2% of MP, with an "optimal" Lys:Met ratio of 3.0.

Probably the most significant change in the NRC-2001 protein system was the switch from constant RUP values (e.g., solvent soybean meal RUP = 35% of CP in NRC-1989, regardless of feed intake and rate of passage) to relying on in situ kinetics to estimate RDP and RUP. The NRC committee considered adopting the Cornell model, in which RDP and RUP were estimated largely from chemical characterization of feed protein and fixed degradation rates for these fractions; however, it was decided that in situ methodology would be more reflective of the microbial degradative activity found in the rumen (C. G. Schwab, personal communication). Thus, NRC-2001 applied the basic kinetic approach of Ørskov and McDonald (1979); for details, see pp. 58-62 (NRC, 2001). However, this simple in situ model is subject to a number of errors when estimating rate and extent of protein degradation. One major concern is that N present in soluble fractions--intact proteins and small particulates as well as NPN compounds--are assumed to be completely degraded and the observed, single degradation rate is applied only to the remaining CP. Gierus et al. (2005) reported similar degradation rates for fine particles escaping through the nylon mesh (the material used to make in situ bags) and the residue retained on the mesh. Further, Aufrère et al. (2002) showed that between 7 and 13% of the nonammonia N disappearing from nylon bags also escaped degradation in the rumen. Although errors of this type would underestimate rumen escape, a comparison of RUP values computed ones, using the in situ-based NRC-2001 system to RUP values determined using omasal sampling, indicted that NRC (2001) estimates were, on average, 22% greater than omasal values (Broderick et al., 2010). The overestimate of RUP may derive from using inappropriate passage rates and, more importantly, to not accounting for passage lag; actual residence times in the rumen may be substantially greater and, thus, extent of degradation greater, than degradation accounted for by the simple in situ approach (Huhtanen and Hristov, 2009). It should be noted that NRC-2001 does not apply a single rate of passage, but uses a series of equations to compute

different rates of passage, depending on level of intake and whether the feed is forage or concentrate, dry or wet (i.e., silages and other fermented feeds). Microbial protein, the other major contributor of MP, is estimated assuming that 130 g MCP are produced per kg of TDN, discounted for fat contribution to TDN (total digestible nutrients), a measure of total tract digestible energy. Adequacy of RDP is set at 1.18 x MCP. Thus, MCP = RDP/1.18 when RDP supply is inadequate, and MCP = 130 x discounted TDN when RDP supply is adequate. Details of philosophy and methodology are on pp. 55-58 of NRC (2001). Note that the Huhtanen meta-analysis of omasal MCP flow versus NRC predictions of MCP flow indicated that the NRC approach underestimated MCP passage out of the rumen on average by 26% (Broderick et al., 2010). A series of 10 empirical equations predict essential AA supply from intestinal digestion of MCP and RUP (pp. 78-81; NRC, 2001).

The NorFor Protein Model

The Nordic countries have seen a great deal of modeling activity in ruminant nutrition and metabolism. Among the more prominent efforts is "Karoline" (Danfaer et al, 2006), which grew out of the large Nordic modeling project. Karoline is among the most sophisticated mechanistic models on ruminant nutrition and metabolism. The NorFor modeling effort was spearheaded by Harald Volden of Tine in Norway but had contributions from many workers; NorFor attempts to bridge the gap from basic knowledge to practical application. Although in use for 3 to 5 years by the organizations of Tine in Norway, the Knowledge Center for Agriculture in Denmark, the Farmers' Association in Iceland and the Swedish Dairy Association (Svensk Mjölk), including one of us (MÅ), the publication delineating the model appeared last year (NorFor, 2011). We will only briefly describe some of the differences in how protein utilization is handled between NorFor and NRC-2001.

NorFor computations of MP requirements are detailed in Chapter 9 (pp. 93-104; NorFor, 2011). Metabolizable protein is referred to in NorFor as AAT_N--AA absorbed from the small intestine; however, we will use MP as the shorthand descriptor for AAT_N in the present discussion. Maintenance MP requirements are calculated using a factorial approach similar to that applied by NRC-2001. However, MP requirements for pregnancy, growth and lactation apply variable efficiencies of conversion of MP to NP, with declining MP capture as production approaches the maximum. This is consistent with the philosophy of Lapierre et al. (2005) and others that AA incorporation by the mammary gland and other tissues does not follow the "broken-stick" model of constant efficiency until requirement is met. Although NorFor computes AA requirements by applying a similar philosophy to the 10 essential AA, these have not yet been put to use within the model. The methodology used to arrive at MP and MAA supplies are detailed in Chapter 7 (pp. 59-80) and Chapter 8 (p. 83). Relative to NRC-2001, the NorFor approach of computing passage rates is more complex. Passage rates for particulates and liquids are computed as functions of intake per unit body weight and, unlike the simple Ørskov and McDonald (1979) model used in NRC-2001, soluble proteins are assigned degradation rates and do contribute to RUP passing out of the rumen. Formation of MCP is computed using variable microbial efficiency, which alters with intake per unit BW and proportions of starch and "non-starch" in fermented carbohydrates. Lower energetic efficiencies are assigned for protein and lactate (from silages) fermented in the rumen. Metabolizable essential AA are computed by applying variable intestinal digestibilities to all RUP sources as well as MCP, assuming AA availability is in proportion to AA concentration in the protein. However, metabolizable essential AA are not yet

compared to requirements within NorFor. Of additional note is the computation of a requirement for rumen-N balance (PBV_N), which must be more positive as productivity increases, before plateauing at a yield of 30 kg ECM/day. The NRC-2001 model handled this issue by depreciating MCP yield unless RDP supply equaled 1.18 times MCP.

Performance of NRC-2001 versus NorFor

We have applied the two feed evaluation systems to see how each predicted the production of milk and milk protein that was observed in five trials conducted by one author's (GAB) research group. Results are from four published studies [Olmos Colmenero and Broderick, 2006a (Olmos 1); Olmos Colmenero and Broderick, 2006b (Olmos 2); Brito and Broderick, 2007 (Brito); Broderick and Reynal, 2009 (Reynal)] plus one that is unpublished (Nursoy & Gonzalez). Although NorFor predicts both ECM and protein yield directly, NRC-2001 only estimates milk of standard composition that is "allowable" based on NEL and MP predicted to be supplied by the diet. The lower of these two values, which would reflect yield based on the limiting nutrient (NEL or MP), and the composition of the standard milk, were used to compute ECM predictions from NRC-2001. Figure 1 compares these sets of predicted ECM yields to observed ECM yields. The two models both appeared similarly poor; although the NorFor model fit data somewhat better ($r^2 = 0.92$), only the slope from the NRC regression was significant: ECMnrc = 2.06 (±0.33, $P < 0.001$) x ECMobs – 44.4 (±12.5; $P = 0.003$), $r^2 = 0.79$; and ECMnorfor = 0.26 (±0.14, $P = 0.087$) x ECMobs + 26.5 (±5.3; $P < 0.001$); $r^2 = 0.92$. The results suggested that both systems underpredicted ECM yield. The poor relationship for NorFor was due largely to the imprecise predictions for results from Brito and Broderick (2007), in which CP supplementation came all from urea, soybean meal, cottonseed meal or canola meal. The most satisfactory prediction was for urea; the other three predictions ranged from 5 to 9 kg/day too low.

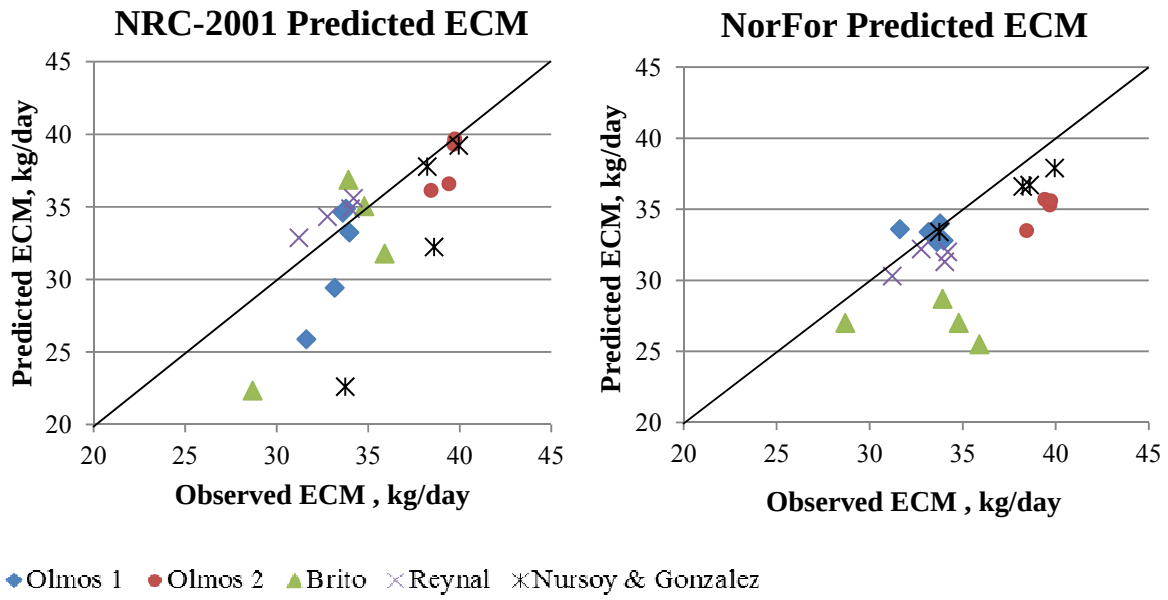

Figure 1 Comparison of yield of energy-corrected milk (ECM; x-axis) observed in 5 different studies (listed in the text) to ECM predicted (y-axes) by NRC-2001 (left panel) and by NorFor (right panel). The ECM values derived from data observed in the 5 trials and from results predicted by both models were computed using NorFor ECM equation 3.2 (p. 28; Norfor, 2011). The solid line is the x = y line.

Figure 2 compares the predicted milk protein yields to actual yields. The NRC-2001 estimates of milk protein yield were computed by multiplying MP-allowable by the standard milk protein concentration used in the model. In NRC-2001, if MP-allowable milk is less than NEL-allowable milk (which was the case in 12 of the 21 comparisons), then the extra energy is assumed to be deposited in body tissues. As can be send in Figure 2, the NorFor model performed better in predicting milk protein yield (MPY); NRC-2001 tended to under-predict protein secretion at lower levels of production. The linear regression parameters obtained were: MPYnrc = 1.625 (±0.359, $P < 0.001$) x MPYobs – 0.838 (±0.439; $P = 0.076$), $r^2 = 0.66$; and MPYnorfor = 0.895 (±0.077, $P < 0.001$) x MPYobs + 0.169 (±0.094; $P = 0.094$), $r^2 = 0.97$. The numerically large slope and lower r^2 from the NRC regression reflected its poorer fit to the observed data. Based on this comparison, NorFor is more accurate in predicting MP protein supply and utilization in lactating dairy cows. Comparisons of other outputs from the two model will be presented at the Nordic Feed Science meeting.

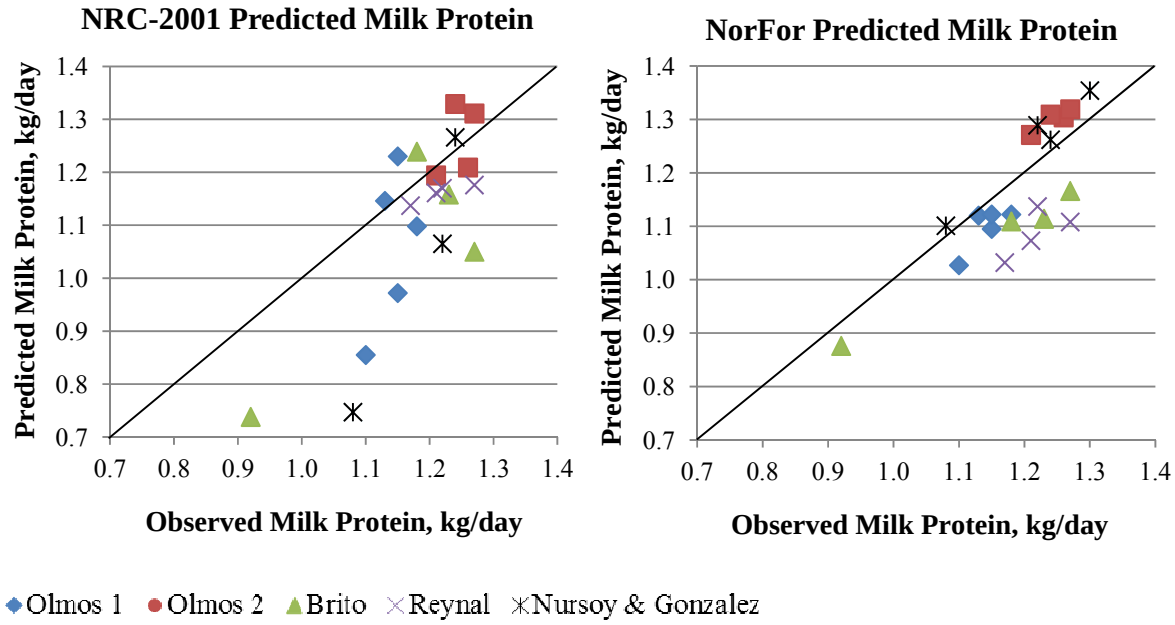

Figure 2 Comparison of protein yield (x-axis), observed in 5 different studies (listed in the text) to protein yield (y-axes) predicted by NRC-2001 (left panel) and by NorFor (right panel). The solid line is the x = y line.

Summary

After a brief presentation of the components making up the NRC-2001 and NorFor protein models, the precision of the two models was compared for predicting yields of ECM and milk

protein observed in five different feeding studies in which 21 different diets were fed. Although the regression of NRC predictions on observed ECM yielded a significant slope, neither model was very precise for predicting observed ECM. However, NorFor was much more precise than NRC-2001 for predicting observed milk protein yield. These results suggest that NorFor will be more reliable for describing supply and utilization of dietary MP in lactating dairy cows.

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Diurnal urinary urea concentration patterns in lactating cows, dry cows and heifers at different dietary crude protein concentrations

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Introduction

Ruminants have evolved a system of N circulation to maintain rumen function in spite of temporary imbalance between ruminally available protein and fermentable carbohydrates in the diet. A surplus of ammonia from ruminally degraded protein enters the plasma urea pool together with ammonia stemming from amino acids oxidized by the liver or gut wall (Lapierre et al., 2005). It may then be recirculated to the rumen via the rumen wall or through saliva and be utilized for microbial growth.The N status can be monitored by milk urea or experimentally by rumen ammonia measurements. Diurnal pattern of rumen ammonia concentration is affected by the properties of the ration, but also by feeding routines. Dairy cows fed a total mixed ration (TMR) once daily (Colmenero & Broderick, 2006) showed an increase within 1 h to 15-20 mg NH_3-N/dL, where the maximum was depending on ration crude protein (CP) concentration. About 10 h later, rumen NH3-N concentration had returned to the baseline, which was approximately 3-10 mg NH_3-N/dL, also depending on ration CP concentration. With different feeding routines, different ammonia patterns will occur. It is common in Sweden that forage (*i.e.* silage) is fed separately from concentrates at two or more occassions per day. In a series of feeding trials at SLU, grass-legume silage was fed at ad libitum or at semi-restricted levels three to four times daily to mid-lactating cows (Eriksson et al., 2004; Eriksson, 2010; Eriksson et al., 2012). This has resulted in typical patterns with a peak two hours after feeding and complete return to a baseline of 5-10 mg NH_3-N/dL four hours post feeding.

Lefcourt et al. (1999) demonstrated a diurnal rhythm with a zenit at 10:30 h for blood urea in dairy cows fed TMR once daily at 09:00 h. The concentration began to rise long before the daily feeding in their study, which suggests that the rhythm was not only caused by the feed itself. Urea from the plasma pool that is not recirculated is excreted in urine. There is often a slope close to 1 for amount of urinary urea vs. total urinary N, *i.e.* the entire urinary N increase is as urea. Also urinary urea may exhibit diurnal patterns similar to blood urea.

The objective of the study reported here was to investigate if such a common diurnal pattern exists for concentration and total excretion of urinary urea N.

Materials and Methods

Experiment 1 (Eriksson, 2003) involved six dairy cows in mid to late lactation (DIM = 205, ECM = 22 kg/d) fed rations with alfalfa-perennial ryegrass silage, rapeseed cake, fodder beets and potatoes at 18.7 kg dry matter (DM)/d. Ration CP concentration was 154 g/kg DM. Total 48-h urinary collection was performed during 48 h with a harness and funnel device (Eriksson et al., 2004). Analysis of urea N and creatinine was made on a Technicon Autoanalyzer.

In Experiment 2 (Eriksson et al., 2009), 18 cows in mid to late lactation (DIM = 217, ECM = 22 kg/d at spot sampling) were fed incremental amounts of fodder beets and potatoes for a 21-d test

period, whereafter individual rations were fixed at 17.0 ± 1.7 kg DM/d. The same feeds as in Experiment 1 were used and ration CP concentration was the same, 154 g/kg DM. Urinary spot-sampling was made during 48 h, yielding a total of 290 samples analyzed as described for Experiment 1.

Experiment 3 (Pelve et al., 2012) was a 3 x 3 Latin square with six dry cows (weighing 563 ±46 kg) and six heifers (weighing 309 ±34 kg) in periods of 21 d. Experimental treatments were three different forages harvested from seminatural grasslands as haylage (DM>770 g/kg) or as hay, all with measured crude protein (CP) concentrations and in vitro organic matter digestibilities (OMD). From vegetation characteristics, growing areas were classified as periodically inundated shore meadow (118 g CP/kg DM, OMD 0.57), naturalised cultivated grassland (76 g CP/kg DM, OMD 0.61) or species-rich naturalised cultivated grassland (80 g CP/kg DM, OMD 0.65). The forages were provided at 15 g/kg BW for cows and 20 g/kg BW for heifers. Urinary spot-sampling was performed during the last 5 days of each period, yielding a total of 597 samples, analyzed as in Experiments 1 and 2.

Data from spot samples in Experiments 2 and 3 were assigned to the 2-h interval when respective sample had been taken. Data were then analyzed by procedure Mixed of SAS 9.2 (SAS Institute, Inc., Cary, North Carolina, USA) with models that included as a minimum: sampling time as a fixed variable and animal as a random variable. If significant ($P<0.05$), sampling day (Exp. 1 and 2) and period and treatment (Exp. 3) were included as fixed variables and their interactions with individual as random variables. Least square means were then calculated for the time intervals.

Results and Discussion

The urinary urea N (UN) concentration in Experiment 1 was constant throughout the day (Figure 1). However, urinary volume increased after the first measurement points in the morning so that total amount of UN peaked 10 hours after first feeding, at 15:00 h (*e.g.* the collection interval from 13:00 h to 17:00 h). The ratio UN/creatinine N followed the curve well. In Experiment 2, UN concentration peaked at 10:00 h, but the ratio UN/creatinine N that should describe total excretion of UN reached its peak value at 20:00 h (Figure 2).

The two forages with lowest CP concentration in Experiment 3 resulted in very low urinary urea concentrations (Figure 3). The ration with 80 g CP/kg DM and highest OMD gave a daily UN excretion of 2.7 g with heifers and 10 g with cows (Pelve et al., 2012). With the forage containing 118 g, UN concentration was similar to Experiments 1 and 2. For heifers, there was a peak with this forage 6 hours after first feeding (14:00 h) for both UN concentration and the ratio UN/creatinine N. For cows on that forage, as well as for both heifers and cows on the diets of lower CP concentration, a single peak was much less obvious or totally absent.

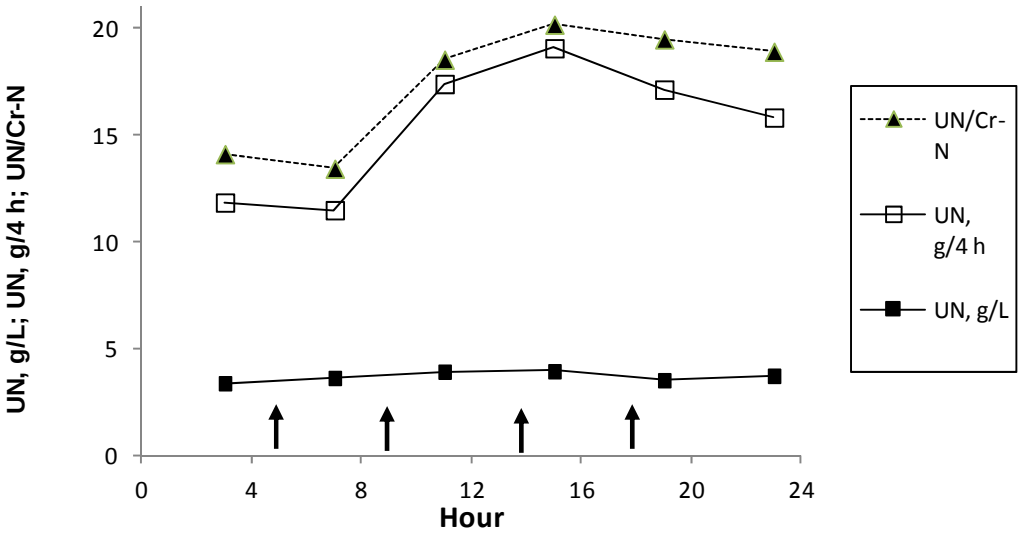

Figure 1 Total excretion and concentration of urinary urea N from quantitative collection on 6 mid-lactating cows. UN/Cr-N = ratio urinary urea N/urinary creatinine N. Arrows indicate meals.

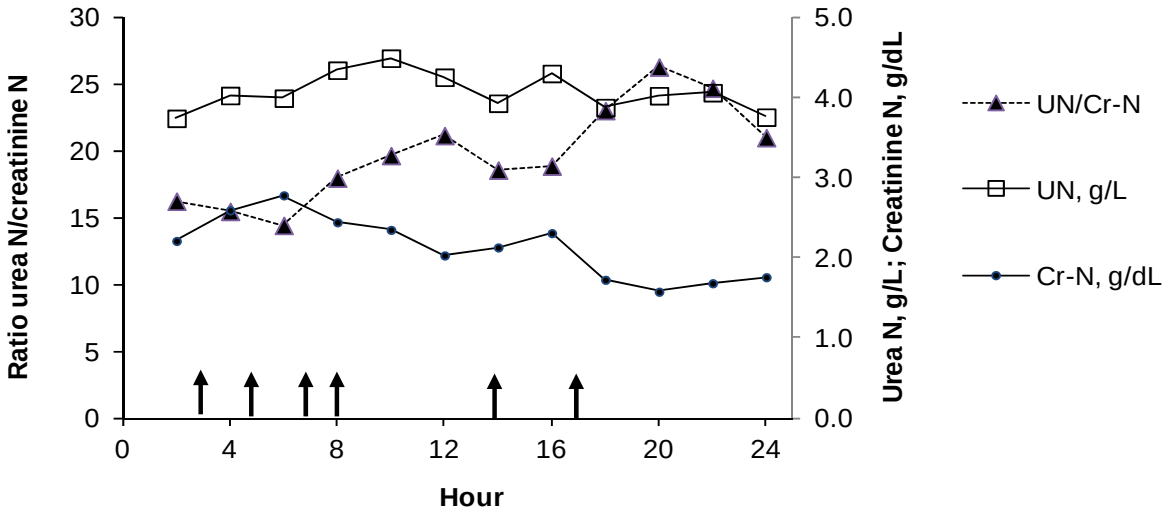

Figure 2 Concentration of urinary urea N (g/L) and urinary creatinine N (g/dL) in spotsamples from 18 mid-lactating cows (N=290). UN/Cr-N = ratio urinary urea N/urinary creatinine N. Arrows indicate meals.

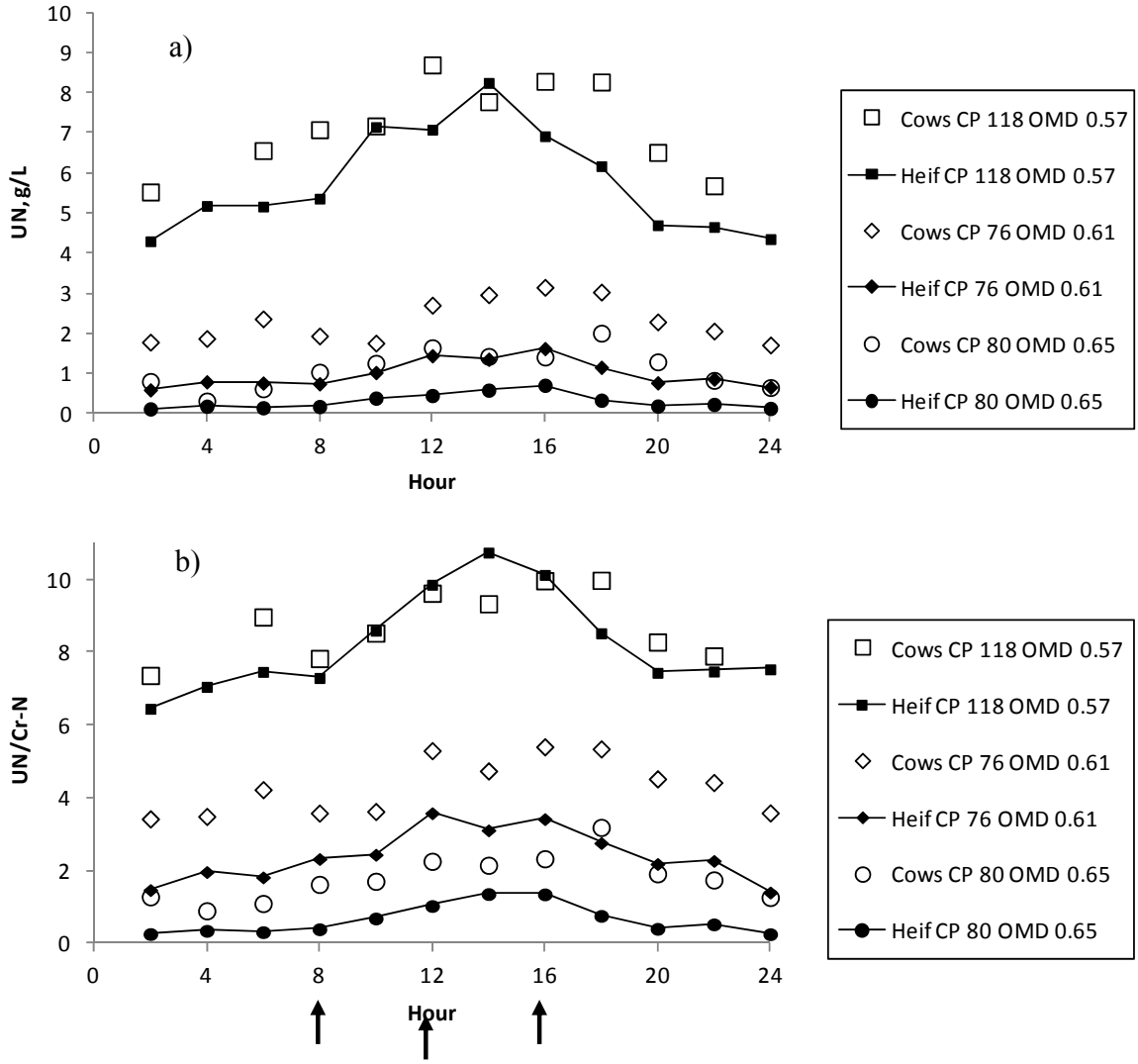

Figure 3 Urinary urea N (UN) concentration (a) and ratio urinary urea N/urinary creatinine N (b) in spot samples (N = 597) from dry cows and heifers stall-fed forages of varying crude protein concentration (CP as g/kg DM) and *in vitro* organic dry matter digestibility (OMD). Arrows indicate meals.

Conclusions

Peak values for urinary urea N concentration occur sometimes and sometimes not in lactating cows. Total urinary urea N excretion from 2-4 h intervals in lactating cows peak but at very different times of the day. Both concentration and total excretion of urinary urea N may peak in growing heifers if ration crude protein concentration allows that.

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Nitrogen and phosphorus excretion in manure from dairy cows calculated by using NorFor

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Introduction

Animal manure is rich in several important plant nutrients and is therefore a valuable fertilizer. However, manure may also represent a potential pollutant and a source of greenhouse gases (GHG) methane (CH_4) and nitrous oxide (N_2O). Optimizing the use of manure as fertilizer and calculating reliable amounts of GHG emission from manure require good estimates on amount and composition of manure in Norway. The data used today is based on results from the 70's and 80's when average milk yield was below 6000 kg/year. Today, the average milk yield in Norway is approximately 7100 kg/year, and both feeding and management have changed considerably during the last 30-40 years. Accordingly, the numbers need to be revised. The main objective of this study was to calculate total excretion of phosphorus (P) and nitrogen (N) in manure from dairy cows by using the Nordic feed evaluation system, NorFor. The present data material will later be used to develop prediction equations for excretion of P and N.

Materials and methods

The excretion of N and P from dairy cows was calculated using the NorFor system (Volden, 2011). The amount of N and P excreted are calculated as the difference between amount ingested and the sum of that secreted in milk and deposited in foetus and tissues.

To cover different feeding and management situations and to be able to develop prediction equations with dietary protein level included in the equation, a range of crude protein contents in roughage (100, 140 and 180 g/kg DM) and concentrate (140, 170, 200 and 230 g/kg DM), milk yields (5000, 7000, 9000 and 11000 kg energy corrected milk (ECM)/year) and animal weights (600 and 650 kg) were included. The concentration of P in the concentrate and roughage was set to 5.0 and 2.8 g/kg DM respectively, which is equal to the average values from concentrate manufacturers and farm samples collected in Norway. The roughage:concentrate ratio was optimized for energy balance with maximum roughage proportion. Excretion of P and N was calculated by the NorFor system, for each combination of feeds, milk yields and animal weights. Accordingly, there was an imbalance between protein and energy in some of the situations. However, all combinations were included to get more variation in the data material, and to be able to illustrate potential effects of different situations.

Results and Discussion

The effect of animal weight on N excretion was small, and the results (Table 1) were therefore averaged for the two live weights of 600 and 650 kg. As expected, there was a close correlation between N excreted in manure and feed crude protein content. The mean N excretions across all diets represent a diet containing 14% crude protein in the roughage and 18.5% crude protein in the concentrate. This is slightly lower than the average dairy cow ration in Norway and, accordingly, our estimates of 98, 111, 128 and 145 kg/year for cows yielding 5000, 7000, 9000 and 11000 kg ECM/year, respectively are probably slightly underestimated. However, these

results are in agreement with values used in Sweden; 117 and 139 kg/year for cows yielding 8000 and 10000 kg of milk per year, respectively (Jordbruksverket, 2010) and Denmark; 130 kg N/year for cows yielding 9239 kg of milk per year (Poulsen, 2009). Expressed as g N excreted/kg ECM, the mean estimated numbers were 18.5, 15.5, 14.1 and 13.2 for cows yielding 5000, 7000, 9000 and 11000 kg milk/year respectively.

Table 1 Effect of milk yield and feed protein content on annual nitrogen excretion (kg) from dairy cows

	Potential milk yield (kg ECM/year)[a]	NE[b] (14% PC[c])	NE[b] (17% PC[c])	NE[b] (20% PC[c])	NE[b] (23% PC[c])
10% PR[d]	5000	65.1	69.6	74.1	78.7
	7000	74.1	82.6	91.4	100.0
	9000	84.8	97.8	111.1	124.0
	11000	96.1	113.6	131.4	148.6
14% PR[d]	5000	90.6	95.2	99.3	103.7
	7000	98.4	106.7	115.0	123.0
	9000	108.2	121.0	133.9	146.2
	11000	118.9	136.4	153.8	170.5
18% PR[d]	5000	116.9	121.6	125.8	129.9
	7000	123.8	132.0	140.0	147.7
	9000	132.6	145.3	157.7	169.7
	11000	142.8	160.2	177.2	193.6

[a]ECM= energy corrected milk. [b]NE=nitrogen excretion. [c]PC=Crude protein content in the concentrate. [d]PR=Crude protein content in the roughage.

The excretion of P decreased slightly as the concentration of protein in the feed increased. Excretion of P was approximately 10% lower when the highest protein ration was used compared to the lowest protein ration, and the milk yield was set to 11000 kg ECM/year. The results (Table 2) represent the mean P excretion of all feed protein concentrations and animal weights. By increasing the milk yield from 5000 to 11000 kg ECM/year, the P excretion increases from 11.8 to 19.4 kg/year per animal. However, under the same conditions, the excretion is reduced from 2.24 to 1.77 g/kg ECM.

The numbers used in Sweden are currently 14.9, 15.9, 17.4 and 19.1 kg P/year for cows yielding 6000, 8000, 10000 and 12000 kg milk/year respectively (Jordbruksverket, 2010). Accordingly, the Swedish numbers are slightly higher for the lower milk yields and slightly lower for the higher milk yields. The numbers used in Denmark are considerably higher than the numbers calculated using the NorFor system; 20.9 kg P/year for cows yielding 9239 kg milk/year. This might partly be due to a higher concentration of P in the diet (4.43 g/feed unit) in Denmark compared to a value of 3.9 g/feed unit in Norway.

Table 2 Effect of milk yield on phosphorus excretion from dairy cows

	5000 kg ECM[a]	7000 kg ECM[a]	9000 kg ECM[a]	11000 kg ECM[a]
P-excretion (kg/year)	11.8	13.9	16.6	19.4
P-excretion (g/kg ECM[a])	2.24	1.94	1.83	1.77

[a]ECM=energy corrected milk

Conclusions

The NorFor system seems to produce reliable estimates on excretion of P and N from dairy cows. Concentration of protein in the feed is of great importance for the excretion of N. Annual excretion as well as excretion per kg ECM of both N and P is highly affected by milk yield

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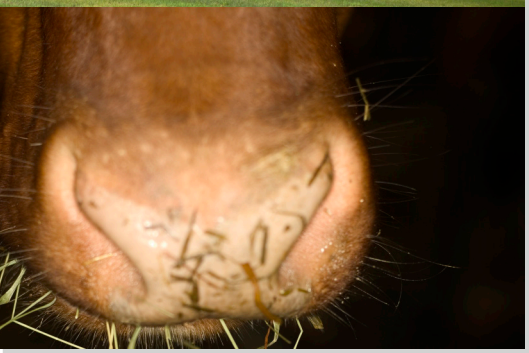

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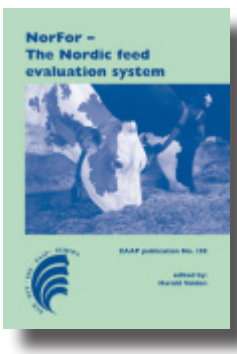

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