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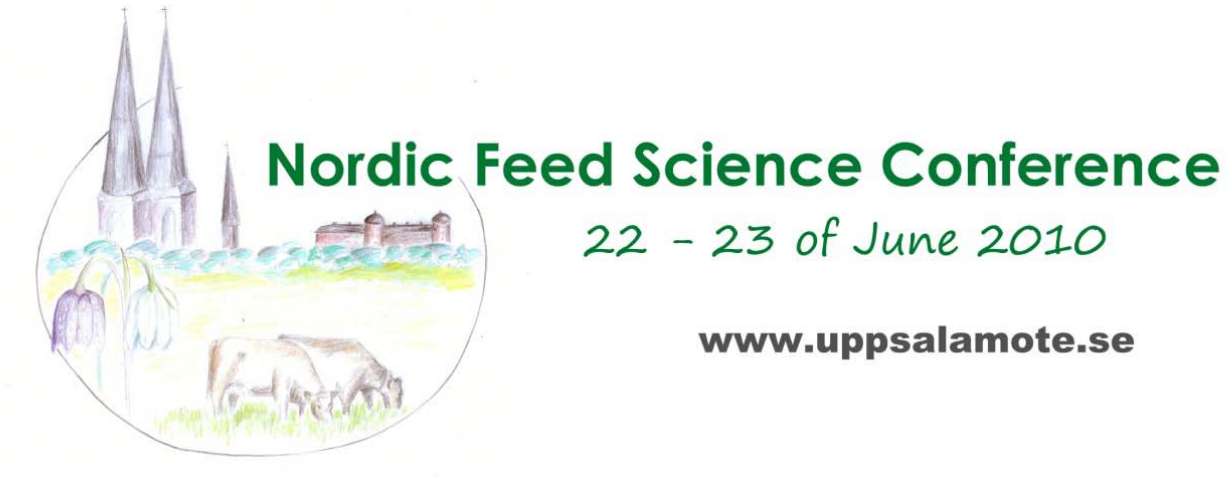

Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management

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Organising Committee:
Peter Udén
Rolf Spörndly
Cecilia E. Müller
Torsten Eriksson
Marie Liljeholm
Kerstin Burstedt

Edited by:
P. Udén
T. Eriksson
C.E. Müller
R. Spörndly
M. Liljeholm

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Foreword

The Nordic countries are small and few scientists work in the area of Animal Science. Still fewer are working with the feeding of our domesticated grass eating species, such as cows and horses. Therefore, we need to pool our resources within the Nordic countries and work together, rather than competing with each other or duplicate research, in order to progress. These ideas are, in fact, demanded by some research foundations in Denmark, Norway and Sweden.

Feed Science is a discipline which includes both the feeds and the animals and it borders to sciences such as Agronomy, Engineering, Chemistry and Physics, as well as Statistics, Environmental Sciences and a number of others. Our area is not a rapidly advancing one such as nanotechnology, molecular biology, biotechnology, medicine, etc; therefore, we need to keep our eyes open to what's going on there, as many discoveries may prove useful to our own research. This is particularly true for analytical procedures.

For many years, Sweden has lacked a 'backyard' Feed Science conference which brings together both ruminant and equine scientists. We have previously had an Agriculture Conference in Sweden, but it was diverse in nature and mostly focused on useful findings for advisors. Its popularity dwindled in the 1980's and was discontinued for a while, reappeared in a different form, but died again in the end of the 1990's. Norway has continued until today with an annual Animal Science Conference and the dairy associations in Denmark and Sweden organize conferences for advisors and farmers, which attract scientists also from neighboring countries.

Attending the Cornell Nutrition Conference a number of times has made it clear to some of us that we are sadly lacking something similar in the Nordic countries. After discussions with a number of Nordic colleagues, it became clear that our interests in a Nordic conference, similar to the Cornell Nutrition Conference, were shared by many. So, after some years of procrastination, you are now welcome to the 1st Nordic Feed Science Conference. We hope that you will enjoy your stay and that you will both support this conference and advice us how to continue with an improved 2nd conference in 2012.

Peter Udén

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Proportion of red clover in forage can be estimated based on calcium concentration

M. Rinne[1], A. Nykänen[1], J. Kemppainen[1], L. Nyholm[2] and J. Nousiainen[2]
[1]*MTT Agrifood Research Finland*
[2]*Valio Ltd, Finland*

Introduction

Adding red clover (*Trifolium pratense*) into the leys for ruminant feed production has several advantages. Red clover as a legume has the ability of biological nitrogen (N) fixation, which provides a N source into organic farming systems and savings in fertilization costs in conventional farming. The decline in digestibility during primary growth is slower in red clover compared to the common grasses such as timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) grown in Nordic countries (Rinne and Nykänen 2000), which provides flexibility into the timing of harvest of first cut forage. The intake potential of forages including red clover is greater than forages containing only grass species (Huhtanen et al. 2007), which improves animal production. The cation-anion balance [(Ca+Mg)/K] of red clover is more favourable than that of grasses regarding the risk of milk fever in dairy cows. Including red clover into the diet of ruminants is also likely to improve the fatty acid composition of milk in terms of human health (Vanhatalo et al. 2007).

There are however some challenges related to the cultivation of red clover, and the proportion of clover in the forage is highly variable. The clover concentration is typically lower in primary growth than in regrowth, and it declines as the ley gets older (Nykänen et al. 2000, Rinne & Nykänen 2000). The clover content is also sensitive to the level of N fertilization used. High N fertilization improves the competitiveness of grasses and proportion of clover decreases (Mela 2003).

It would be useful to be able to estimate the proportion of red clover in a particular ley or batch of forage. It can be used to estimate the amount of biological N fixation, and subsequently the nutrient balances in the field (Nykänen 2008). The information of the clover concentration can be used in adjusting the harvest time and N fertilization, and it can be used to refine the silage dry matter (DM) intake index (Huhtanen et al. 2007). Knowledge of clover proportion in different fields assists in deciding about renewing or re-seeding the ley, and gives in the long run feedback about how clover is growing under the conditions of the various fields.

There are several options to measure the clover content in a ley or in the feed. The standard reference method is to conduct a botanical analysis by manually separating a herbage sample into the different species. This method is however too laborious to be used routinely. Subjective visual assessments give some idea of the proportion of clover. If combined with e.g. model photographs (Figure 1), the accuracy of the method can be improved somewhat. Methods such as calculating the number of clover plants in the spring or the so called Rod Point method did not give very promising results under Finnish conditions (Nykänen 2007). Direct NIRS (near infrared reflectance spectroscopy) calibrations have also been developed with reasonable results (R^2=0.79; Nykänen 2005). This approach does however require the calibration of the NIR with samples of known clover content.

In the current work, we utilized the difference in calcium (Ca) concentration between the species in estimating the proportion of clover in grass material. According to the Finnish feed tables (MTT 2010), the Ca concentrations in red clover and grass are 14 and 4 g/kg DM,

respectively. In Finnish material, the Ca concentrations in pure red clover, lucerne, birdsfoot trefoil and goat's rue were 14.7, 15.5, 11.8 and 9.0 g/kg DM, respectively (Tuori et al. 2006).

The objective of the current work was to find out if the Ca concentration of a mixed red clover-grass forage could be used to estimate the proportion of red clover in total forage DM.

Figure 1 Mixed grass clover leys with 33 % (left) and 75 % (right) clover in DM. Photos: Arja Nykänen.

Materials and Methods

Mixed red clover grass swards were sampled at experimental sites of MTT Agrifood Research Finland in Juva and Sotkamo during years 2004 to 2006. Soil Ca concentration was determined in the beginning of the study. The clover varieties used were Betty and Bjursele, and they were grown in mixtures with timothy and tall fescue (*Festuca arundinacea*). The fields were under organic cultivation.

A total of 40 samples were collected, divided into two sub-samples, where botanical analysis was conducted on one sub-sample by manually separating red clover and grasses. After separation, botanical fractions were dried at 60 °C to calculate the results on DM basis. The other sub-sample was dried at 60 °C and ground through a 1 mm sieve and the Ca, P (phosphorus) and K (potassium) concentrations were determined at the laboratory of Valio Ltd using X-ray fluorescence (MiniPal2, PANalytical, The Netherlands). The instrument was calibrated with forage samples analyzed with an ICP method (inductively coupled plasma mass spectrometry). The relationships between the Ca concentration and proportion of red clover in the sample DM was studied using regression analysis.

Results and Discussion

The proportion of red clover in total herbage DM was lower in primary growth compared to regrowth (Table 1). The variation in the proportion of red clover was large and great variation was also observed in sample Ca concentration. Rather large variation was observed in sample P and K concentrations, but the differences between the cuts were not as clear as for Ca concentration.

Table 1 Composition of clover-grass samples used for estimating clover proportion from calcium concentration. The material was collected from organically grown clover-grass leys at MTT experimental fields in Juva (n=21) and Sotkamo (n=19) during 2004−2006

	Primary growth (n=22)				Regrowth (n=18)			
	Mean	S.D.	Min	Max	Mean	S.D.	Min	Max
Clover (% in DM)	36.2	23.86	1.5	74.5	55.2	18.95	17.0	80.4
Soil Ca (mg/l)	923	458.1	378	1893	1007	540.9	378	1893
Concentration in clover-grass samples (g/kg DM)								
Ca	8.7	4.14	3.1	16.8	14.6	3.99	8.4	20.5
P	2.0	0.30	1.5	2.6	2.3	0.42	1.6	3.0
K	18.1	4.19	9.4	26.7	17.4	6.62	7.6	26.8

The level of P and K concentrations in the samples was rather low compared to the feed table values (MTT 2010) or average values analysed from Finnish farm samples (Artturi 2010), which probably reflects the low concentrations of these nutrients in the soil due to organic farming practices used.

The results of sample Ca concentrations and clover proportions (%) are plotted in Figure 2. The relationship was rather good with a R^2 of 0.800 and RMSE of 10.7 (Table 2). Taking the cut (primary growth vs. regrowth) or soil Ca concentration into account slightly improved the accuracy of estimation of clover proportion. Based on these results, this approach seems useful in obtaining a rough estimate of clover concentration in plant material. The data available was however rather small and derived from two organically grown fields. Enlarging and widening data material would probably yield more accurate and robust estimates of clover content based on sample Ca concentration.

Other information of clover and grass digestibility (stage of development) and chemical composition might also be useful, but currently data was not available. In the data set of Tuori et al. (2006), the Ca concentration of red clover declined 0.11 g/kg DM per day in progressing primary growth.

A calculator was produced so that by entering the Ca concentration and possible additional information of the cut and soil Ca concentration, an estimate of red clover concentration can conveniently be achieved. The calculator is available in the internet in Finnish, Swedish and English languages (Artturi 2010).

In this study, we used fresh samples, but since changes in Ca concentration are not likely during the ensiling process, the same approach can also be used for silage samples. Mineral concentrations are commonly determined to be used in ration formulation for the livestock, so that the information of Ca concentration of silages is often readily available. Annually over 20 000 silage samples are analysed by Valio Ltd. and typically the mineral analyses are conducted on one third of the samples (Artturi 2010). Estimation of feed clover content can thus be obtained without the need of additional measures.

Table 2 Estimation of clover proportion (%) from sward samples using regression equations. Statistically significant (P<0.05) regression coefficients are marked in bold

X1	X2	X3	Intercept	X1	X2	X3	RMSE[1)]	R^2
Ca			-3.1	**4.22**			10.7	0.800
Ca (1. cut)			-10.1	**5.31**			9.4	0.851
Ca (2. cut)			-3.5	**4.00**			10.5	0.710
Ca	Cut		3.7	**4.75**	**-8.91**		10.2	0.824
Ca	Ca^2		-18.4	**7.41**	-0.137		10.3	0.819
Ca	Ca^2	Cut	-11.9	**8.10**	**-0.143**	**-9.21**	9.7	0.844
Ca	Location		-3.2	**4.20**	0.236		10.8	0.800
Ca	SoilCa		-0.2	**4.66**	**-0.008**		10.2	0.822
Ca	Cut	SoilCa	11.8	**5.71**	**-13.4**	**-0.0129**	8.9	0.869

[1)]Root mean squared error; Ca = Herbage calcium concentration (g/kg DM); Ca^2 = Ca × Ca (Quadratic effect of Ca-concentration); Cut = Primary growth vs. regrowth; Location = Place where the grass was grown (Juva vs. Sotkamo); SoilCa = Soil Ca concentration (mg/l).

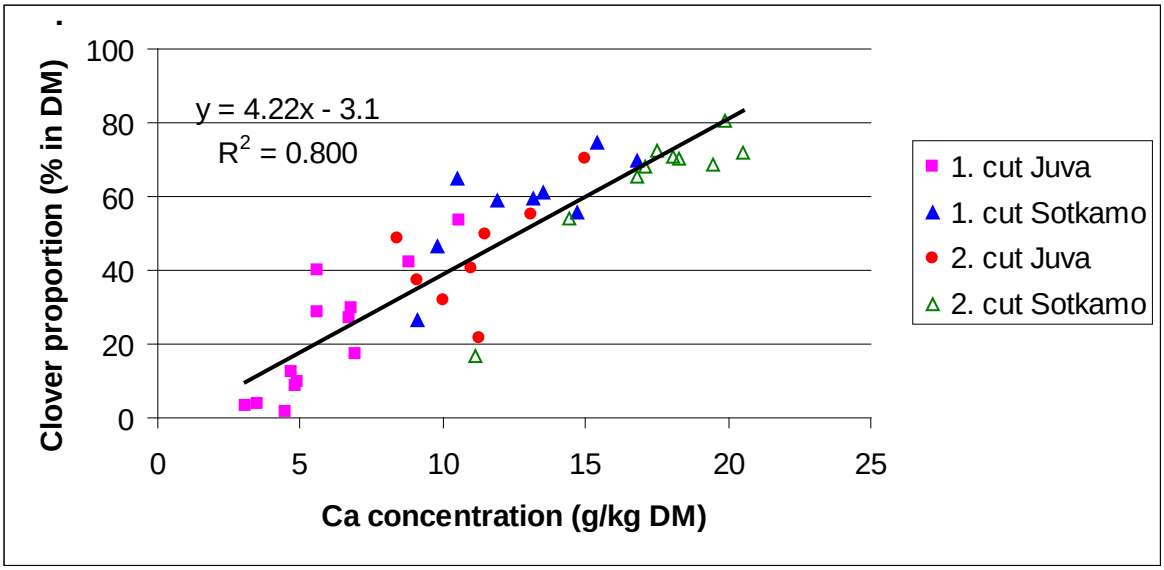

Figure 2 The relationship between herbage Ca concentration and proportion of clover was clear (n=40).

Conclusions

Based on calcium concentration of a herbage or silage sample, a rough but useful estimate of the clover content in the plant material can be achieved. Additional information such as cut (primary growth or regrowth) and soil Ca concentration can be used to improve the accuracy of the estimation. In Artturi web service, calculators in Finnish, Swedish and English languages are available to conveniently calculate the clover proportion if the sample Ca concentration is known. The data available for calculating the current results was rather small so that expanding the material would probably yield more accurate and robust estimates of clover content based on sample Ca concentration.

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A practical tool for the prediction of aerobic stability in grass silages

M. Bruinenberg and J. Bakker
BLGG AgroXpertus, P.O. Box 115, 6860AC Oosterbeek, The Netherlands.

Introduction

In The Netherlands, forages are ensiled at relatively high dry matter concentrations: dry matter concentrations of 50% or higher are very regular. With this type of silage, the pH remains high (e.g. pH > 5.0) and concentrations of lactic and acetic acid remain low (e.g. lactic acid < 20 g/kg DM, acetic acid < 5 g/kg DM). Silages with these characteristics may easily warm up after opening, which has negative consequences for the feeding value, palatability, intake, microbial composition, etc.

In 2005 BLGG AgroXpertus introduced a preservation index for grass silages in the Dutch market. This index combined the quality of the silage with an indication of aerobic deterioration. Silages with high dry matter concentrations (>550 g/kg) usually had a low preservation index, because of the sensibility for deterioration after opening. However, when such silages are fed at high speed, or when the silage consists of big bales instead of a clamp, the problems of warming up may not occur. In those cases, a low preservation index has no practical value, as the fermentation itself may have been very good and efficient. Therefore, we decided to investigate the perspective of splitting the preservation index in an indicator for aerobic stability and an indicator for preservation. In 2008/2009 the research was carried out and in 2009 BLGG AgroXpertus introduced an indicator for the risk on aerobic deterioration. In 2010 we will also introduce a new preservation index which does not have the risk on aerobic deterioration included anymore. This paper gives the results of the research on the perspectives of the indicator for aerobic deterioration stability.

Background and Model Development

Aerobic deterioration of grass silages usually starts with the growth of yeasts (Courtin and Spoelstra, 1990). Acetic acid and propionic acid are known to have an inhibiting effect on the growth of yeasts. Undissociated acids can diffuse into the cell membrane and lower the intracellular pH by releasing H^+ ions. This process will rapidly kill the yeast cell unless it is counteracted by an active, energy-requiring mechanism for removal of H^+ ions (Warth, 1988).

Acetic acid (HAc) usually has higher concentrations in grass silages than propionic acid because propionic acid is hardly formed spontaneously. Therefore we focus on HAc and the inhibition of the growth of yeasts. The inhibition depends on the pH. The pKa (log of dissociation constant; Moon, 1983) of acetic acid is 4.76. At pH 4.76, the solution is in an equilibrium: 50% of the acetic acid is available in the dissociated and 50% in the undissociated form.

$HAc \leftrightarrow H^+ + Ac^-$

At a pH < 4.76, more acetic acid is available in the undissociated form.

Courtin and Spoelstra (1990), but also Muck et al (1991) have designed a model which predicts the aerobic deterioration of silages. Both of them have a calculation rule about the inhibiting effect of acetic acid and lactic acid (to a smaller extent) on yeasts included in their model. We have distilled both calculation rules, and tested it on a dataset of 700 silages from

the year 2008. Of those silages, the chemical composition, including pH and lactic and acetic acid, was available.

We measured acetic acid (with Near Infrared Reflectance Spectroscopy) in fresh material and then calculated the ratio of undissociated acetic acid/acetic acid, based on pH and HAc concentration (Courtin and Spoelstra, 1990):

F1. $C_{au} = 1 / (10 (^{pH - pKa}) +1) * C_a$.

In which: C_{au} = concentration of undissociated acetic acid, C_a = concentration of acetic acid, pKa = log of dissociation constant of HAc.

Then we used the formula (Courtin and Spoelstra, 1990):

F2. $f_{aY} = e^{-76,09 * Cau - 0,00819 * (0,05-Clu)/0,05)}$

in which f_{aY} = inhibiting factor, C_{lu} = undissociated lactic acid. Undissociated lactic acid is calculated in a similar way as undissociated acetic acid. The pKa of lactic acid is 3.8.

The calculated f_{aY} is put on a scale of 1-60. This value is further referred to as the overheating sensibility (OS constant).

The calculation rules of Muck et al. (1991) gave approximately the same results. We chose to use the approach of Courtin and Spoelstra (1990).

Results and Discussion

The OS constant was calculated for all 700 grass silages in the database. In Table 1 a summary is given. Seven groups were made, depending on dry matter concentrations. In the low dry matter group, only 19 silages were available. Most silages had DM concentrations between 370 and 580 g/kg. Average HAc was ten fold higher in the wet silages (25.4 g/kg DM) than in the

Table 1. The silages in which the overheating sensibility constant is calculated

DM content (g/kg)	# of samples	HAc min (g/kg DM)	HAc max (g/kg DM)	HAc mean (g/kg DM)	pH mean	OS constant
<300	19	4	41	25.4	4.3	8.9
300-370	55	4	36	18.7	4.4	13.5
370-440	128	4	21	14.2	4.7	23.2
440-510	195	2	15	9.3	5.1	39.3
510-580	173	2	10	5.5	5.4	50.2
580-650	91	2	5	3.1	5.6	56.1
>650	46	1	5	2.5	5.7	57.3

HAc min/max = minimal/maximal HAc concentration per DM group, HAc mean = average HAc concentration per DM group. OS constant = average overheating sensibility constant per DM group. Concentrations in g /kg DM. Calculations are carried out in the fresh material.

dry silage (2.5 g/kg DM). Forty-six silages had a dry matter concentration of 650 g/kg and higher. Probably a large amount of those were big bales, in which aerobic deterioration hardly matters, because the whole bale is fed directly after opening.

Silages with a dry matter concentration over 45% often have a pH higher than 4.8, because the fermentation of the silage stopped earlier. The concentration of lactic and acetic acid will remain low, and the pH remains high. So, acetic acid will be available in low amounts, and

because the pH is also high, the available acetic acid will mainly be in the dissociated form. Growth of yeasts will then not be inhibited. Therefore, the OS constant is high with dry matter concentrations over 450 g/kg, meaning that the chance on warming up will be high. Silages with low dry matter concentrations (<450 g/kg) have a low OS constant, meaning that the chance of warming up after opening is smaller. However, even if the OS constant is below ten, after several days, warming up of the silage will occur.

In Table 2 some examples are given of the OS constant with varying DM concentrations. It is clear that pH is crucial for the value of the OS constant. E.g. silage no. 4 and 5 have similar DM and HAc concentrations, but because pH in silage 4 is much higher than in silage 5, the OS
constant is also much higher. i.e. the chance that silage 4 will warm up within 48 hours after opening is much higher than the chance that silage 5 will warm up within the same period. Also silage 2 and 3, and 6 and 7, have approximately similar DM and HAc concentrations, but because of the difference in pH, the OS constant also differs. Because of the difference in pH, silage 7 will probably warm up quicker than silage 6, which is shown by the higher OS constant in silage 7.

Table 2. Examples of grass silages and the OS constant

	DM	pH	HAc	OS constant
1: Wet silage	315	4.5	25	6
2: Low DM	374	4.3	15	13
3: Low DM	377	4.8	16	25
4: Average DM	449	5.4	11	47
5: Average DM	449	4.4	11	19
6: High DM	502	4.7	10	27
7: High DM	502	5.2	9	43
8: Dry silage	572	5.4	5	52

The effect on pH on the dissociation of HAC is shown in Figure 1.

To validate whether the OS constant really indicates which silages have a higher change on aerobic deterioration, 40 silages on dairy farms were selected based on OS constant, NDF and DM. The silages were sampled and the temperature of the silage and environment were registrated. Silage with a high OS constant had higher differences between temperature of the silage clamp and the environment than silages with a low OS constant. Silages with an OS constant < 15 (13 silages) had on average a difference in temperature of 7.8°C, and silages with an OS constant > 45 (10 silages) had on average a difference in temperature of 17.1°C. The experiment was carried out in the winter. Probably differences would have been more prominent if the experiment had been carried out in spring or summer.

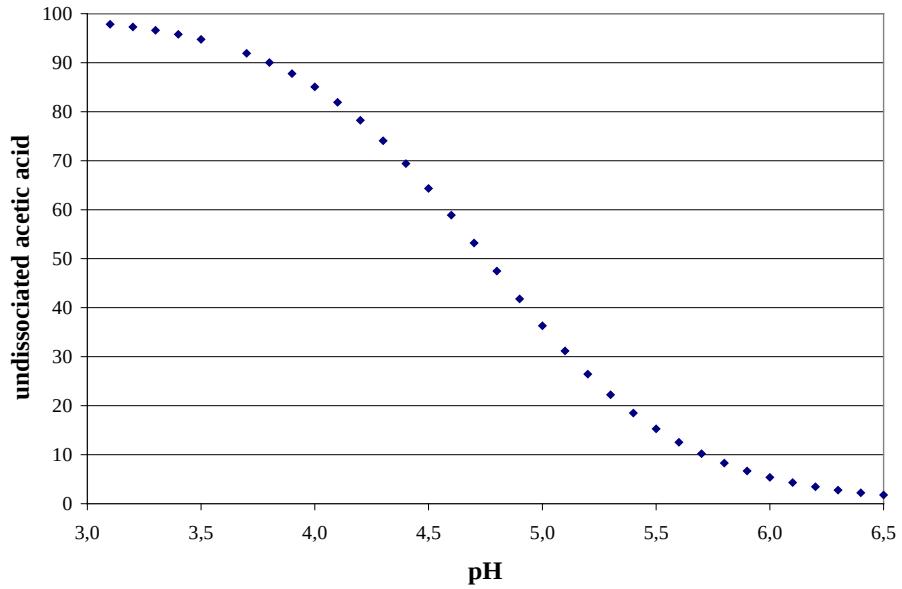

Figure 1. The dissociation of HAc with differing pH.

Conclusions

The OS constant provides a useful tool to estimate whether farmers will have to be keen on warming up of silages after opening or not.

In our grass silage reports we give the overheating sensibility and the preservation index (Table 3). Furthermore, we report the pH (not shown), lactic acetic and acetic acid. In the example in Table 3 the overheating sensibility has an OS constant higher than the target value. The value therefore got a color to warn the farmer. Based on the results, the farmers are given advice about 1. how and when to feed the silage and 2. measurements to prevent silages with a high overheating sensibility in the future.

Table 3. Results on grass silage reports considering preservation characteristics

Preservation indices	Result	Target value	Average sandy soils
		Harvest before June 15th	
Butyric acid (g/kg DM)	2	< 3.0	1.6
Acetic acid + propionic acid* (g/kg DM)	9	10 - 21	7
Lactic acid* (g/kg DM)	33	15 - 40	16
Preservation index	83	80 - 100	79
Overheating sensibility	32	1 - 20	41

*Target values of acetic acid, propionic acid and lactic acid depend on the dry matter concentration. In the section considering preservation characteristics, acetic acid and propionic acid are added up and reported as a combined value. In another section of the report, acetic acid is also reported separately.

The current indexes are developed for grass silages. Both the preservation index and the overheating sensibility will also be used for maize silage, starting in 2010. An extra measurement for maize silage will be the degradability of starch and the stability of the silage during ensiling.

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Estimation of indigestible NDF in feedstuffs for ruminants

M. Krämer, M. Riis Weisbjerg and P. Lund
Aarhus University, Faculty of Agricultural Sciences, Department of Animal Health and Bioscience, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark

Introduction

Intrinsic properties of plant cell walls determine the digestibility of ruminant diets, as they establish the maximum degree of the rate and extent of cell wall digestion in ruminants. The determination of INDF is important for the estimation of potentially digestible NDF (DNDF), and has been shown to be a suitable parameter in prediction models of energy and protein values in feedstuffs for ruminants, as the NorFor system. Therefore, there is a need to develop laboratory methods, applicable in practice, that determine the INDF content in feedstuffs. The present paper aims at presenting correlations and ratios between INDF and related measurements as lignin (ADL) and organic matter digestibility (OMD), for evaluating the ability of these feed variables to predict the INDF concentration.

Material and Methods

Data
The data presented is from our own laboratory. Only data for feedstuff samples with both the ADL concentration and INDF concentration determined in situ at an incubation period of 504 h were included in the dataset. The dataset included a total of 182 measurements of different legume and grass species as well as concentrate feeds and by-products. NDF and ADL concentrations were analysed as ash-free and the NDF analyses were including amylase treatment. The mean values of the cell wall fractions analysed in different feedstuffs, the *in vivo* organic matter digestibility (IVOMD) and the ratio between INDF and ADL are given in Table 1. The lower and the upper bound of the parameters within each feedstuff group are shown in bold. In general, the cell wall components in the heterogeneous group of concentrates and by-products showed greater variation than legumes and monocotolydoneae.

Calculations
In vivo organic matter digestibility was measured in sheep fed at maintenance level. If data were missing, *in vivo* digestibility was estimated from *in vitro* digestibilities using rumen fluid or enzymes with the equations developed by Møller et al. (1989), Søegaard et al. (2001), Hvelplund et al. (2000) and Weisbjerg and Hvelplund (1993) (cf. Møller et al. (2005)) according to the feedstuffs groups. Pearson correlations between the INDF content and corresponding measurements as ADL content and IVOMD were calculated.

Results and Discussion

Different methods are applicable in order to measure the INDF content in feedstuffs such as in situ (nylon bag) and in vitro disappearance. The in situ method is the predominant method used. Due to many factors that may cause erroneous prediction of the INDF fraction using the in vitro method and because the measurements in vitro are not as close to the animal as those obtained by the in situ method, only INDF determinations in situ were included in the present dataset. Overall, the data displayed a high variation in all parameters shown.

Table 1 Cell wall fractions and organic matter digestibility in different feedstuffs, ratios and correlations between these parameters. Given values are means of each parameter, figures in parentheses are standard deviations

	n	INDF (%DM)	NDF (%DM)	ADL (%DM)	IVOMD[5] (%OM)	INDF/ ADL
Monocotolydoneae						
Grass[1]	51	9.0 (4.12)	51.9 (9.01)	3.7 (2.07)	68.6 (9.86)	**1.8** (0.56)
maize silage	9	8.4 (2.00)	42.4 (5.87)	7.0(12.05)	72.0 (4.17)	2.5 (0.97)
Grass/clover[2]	41	**5.1** (2.68)	**39.8** (9.60)	**3.0** (1.60)	**72.9** (14.04)	**1.8** (0.56)
Barley whole crop	14	9.1 (3.34)	44.2 (5.67)	3.7 (0.71)	69.3 (2.96)	2.5 (0.49)
Wheat whole crop	12	8.5 (1.22)	**39.8** (1.63)	4.3 (1.08)	70.5 (1.51)	2.1 (0.43)
Barley straw	1	**26.4**	**77.5**	**9.8**	**45.4**	**2.7**
Legumes						
Red clover	2	13.8 (6.54)	35.3 (7.10)	**3.8** (1.73)	75.2 (5.13)	**3.9** (0.07)
White clover	2	**8.2** (3.49)	28.4 (7.83)	4.5 (2.51)	**76.0** (6.56)	**2.2** (0.05)
Alfalfa[3]	4	**17.6** (5.44)	**40.7** (7.47)	**6.9** (2.29)	**65.9** (8.06)	2.5 (0.96)
Pea whole crop	5	10.3 (8.40)	**25.7** (25.56)	4.1 (1.11)	74.1 (5.00)	3.1 (0.73)
Lupine whole crop	2	14.2 (4.14)	40.1 (7.02)	4.8 (1.20)	72.4 (3.57)	3.0 (0.13)
Concentrates and by-products						
Wheat	5	**1.0** (1.07)	**8.2** (9.11)	**0.4** (0.32)		2.5 (0.95)
Barley	2	4.6 (1.02)	20.7 (3.17)	1.2 (0.37)	**86.4**	**4.7** (1.51)
Rye	1	4.8	15.4	1.6		3.1
Oat	1	9.3	28.6	3.4		2.8
Maize	3	2.5 (0.89)	17.9 (7.01)	1.5 (0.45)		1.7 (0.22)
Pea	1	3.0	17.9	1.7		1.8
Fodder beets	1	3.5	14.2	2.9		1.2
Soypass[4]	1	2.62	30.45	5.72		0.5
Soybean hulls	2	1.1 (0.65)	**67.0** (4.65)	3.3 (0.39)		**0.3**
Sugar beet pulp	2	4.7 (0.02)	43.3 (3.25)	7.3 (1.80)		0.7 (0.17)
Sunflower cake	2	**15.2** (7.20)	28.7 (0.64)	8.2 (0.99)		1.9 (0.23)
Sorghum distillers grain	2	8.8 (3.74)	36.0 (0.64)	**16.5**(0.99)		0.5 (0.23)
Coconut cake	1	9.8	42.6	8.6		1.2
Maize distillers grain	1	5.9	35.3	11.6		0.5
Cotton seed cake	2	8.4 (4.17)	25.7 (14.42)	10.2(7.64)		0.9 (0.30)
Palm kernel cake	1	12.9	56.3	10.6		1.2
Guar meal	1	1.3	25.6	1.3		1.0
Rapeseed cake	2	12.0 (2.38)	23.6 (2.79)	14.1(3.36)	79.0	1.0 (0.15)
Rapeseed meal	2	11.4 (2.20)	24.8 (4.25)	12.1		1.1
Soybean meal	7	**1.0** (0.62)	13.0 (2.96)	2.2 (1.68)		0.5 (0.33)

n = number; INDF = indigestible NDF; NDF = ash-free NDF; ADL = ash-free ADL; IVOMD = *in vivo* OMD determined in sheep fed at maintenance level, or predicted IVOMD from *in vitro* analysis; [1]16 grass silages, 6 hays, 27 fresh grasses, 2 grass pellets; [2]21 silages, 1 hay, 19 fresh grass/clovers; [3]2 hays, 2 fresh; [4]xylose and heat treated soybean meal; [5]calculated from a sub dataset; upper and lower bound of parameters within each group are printed in bold.

Monocotolydoneae

The group of monocotolydoneae includes also grass/clover mixtures. Within this group, barley straw deviated considerably from the other feedstuffs and correlations between the parameters shown varied severely between the feedstuffs. High correlations were found between the INDF (%DM) and ADL concentration and between INDF (%DM) and IVOMD

for barley whole crop (r = 0.90 and r = -0.82, respectively). Figure 1 shows the relationship between INDF content and ADL content in the feedstuffs of the monocotolydoneae category. For all feedstuffs in the group of monocotolydoneae, the correlation between INDF and ADL was positive. IVOMD and ADL could be potential predictors of the INDF content in barley whole crop and the IVOMD might also be a suitable predictor for the INDF content in grass, whereas they were not suitable for maize silage, wheat whole crop and grass/clover. If these parameters are applicable for the prediction of INDF in barley straw remains questionable, due to the few data.
Large variation was found in the INDF/ADL ratio within feedstuffs. It is well known, that the organic matter digestibility decreases as the INDF content increases, as can be shown by the negative correlation between these two parameters over all feedstuffs (data not shown).
The ratio between INDF and ADL was lowest for grass/clover and grass with 1.8 and highest in barley straw with 2.7. The INDF/ADL ratio for maize silage and barley whole crop was close to the 2.4 used in the Cornell Net Energy and Protein evaluation system (CNCPS).
The INDF/IVOMD ratio ranged between 0.1 (grass/clover) and 0.6 (barley straw) (data not shown).

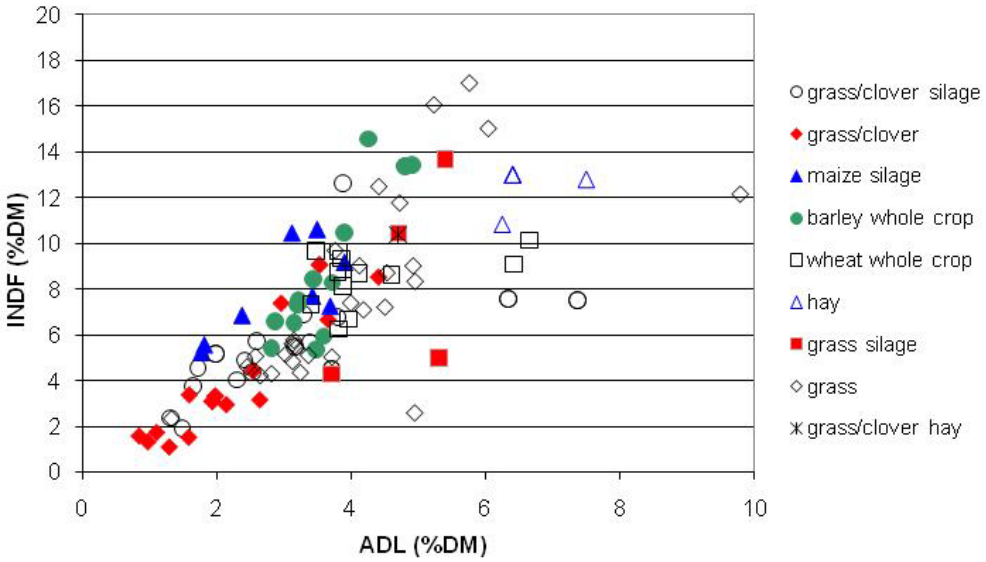

Figure 1 INDF concentration plotted against ADL concentration in monocotolydoneae (barley straw not included).

Legumes
The plot of INDF vs. ADL in Figure 2 reveals the greater slope for legumes than seen in Figure 1 for monocotolydoneae. Alfalfa established an outlier compared to the other legume species with higher cell wall contents and consequently a lower IVOMD. The INDF content in legumes is higher than in grasses although the ADL contents in the two feedstuff groups are similar. Differences between legumes and grass species in the chemical composition and degradability of cell walls are described several times in the literature as legumes generally contain more lignin, less NDF but more INDF and consequently less DNDF compared to grasses. The morphological structure of the cell wall fractions in legumes and grasses differs, so that the NDF digestion in legumes is often faster (higher rate of DNDF digestion) compared to grasses.
The INDF/ADL ratio ranged between 2.2 (white clover) and 3.9 (red clover) and deviated considerably from the 2.4 factor (CNCPS), except for alfalfa with the value 2.5. The ratio between INDF and IVOMD was lowest for white clover (0.1) and highest for alfalfa (0.3).

Except for alfalfa, the correlation between INDF and ADL was positive with high correlations for pea whole crop (r (INDF (%DM) vs. ADL) = 0.73)). Pea whole crop was the only legume which showed a highly negative correlation between the IVOMD and INDF content (%DM) (r = -0.87). As a result, IVOMD and ADL could potentially be predictors of the INDF content in pea whole crop.

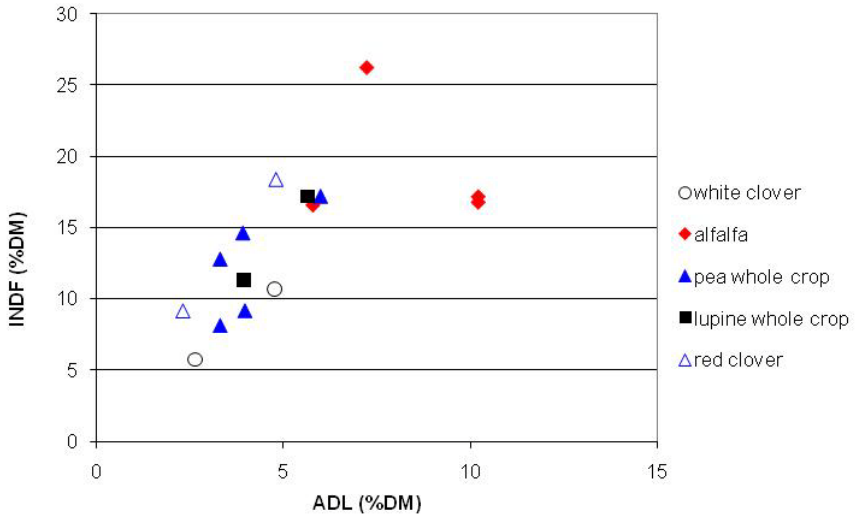

Figure 2 INDF (%DM) plotted against ADL (%DM) for legumes

Concentrates, by-products, etc.
For most of the feedstuffs in this category, no data was available for IVOMD. Both positive and negative correlations between INDF and ADL content were found. ADL content in maize and wheat could be a potential predictor of the INDF concentration, as high correlations with r = 0.93 and r = -0.89 were observed. Within the group of concentrates and by-products, sunflower and rapeseed cake were the feedstuffs with the highest INDF content (Table 1) expressed as proportion of DM or NDF. More than 50% of the NDF fraction is INDF in these two feedstuffs. Thus, a low IVOMD would be expected. On the other hand, pea showed with 0.2 INDF (%NDF) a very low content although the total NDF content did not differ widely from that of the other feedstuffs. This shows that feedstuffs with a high NDF content do not necessarily show a high INDF content, but the distribution of NDF between DNDF and INDF can vary highly between feedstuffs, therefore INDF determination should be included in all basic feedstuff analyses. The INDF/ADL ratio showed the largest deviation from the 2.4 factor used in the CNCPS and ranged between 0.3 (soybean hulls) and 4.7 (barley). Only wheat approximately fits the 2.4 factor.
In general, the INDF and ADL concentration in this feedstuff group varied widely as shown in Figure 3. Because of the limited amount of data for concentrate and by-products the mean values should be interpreted with care.

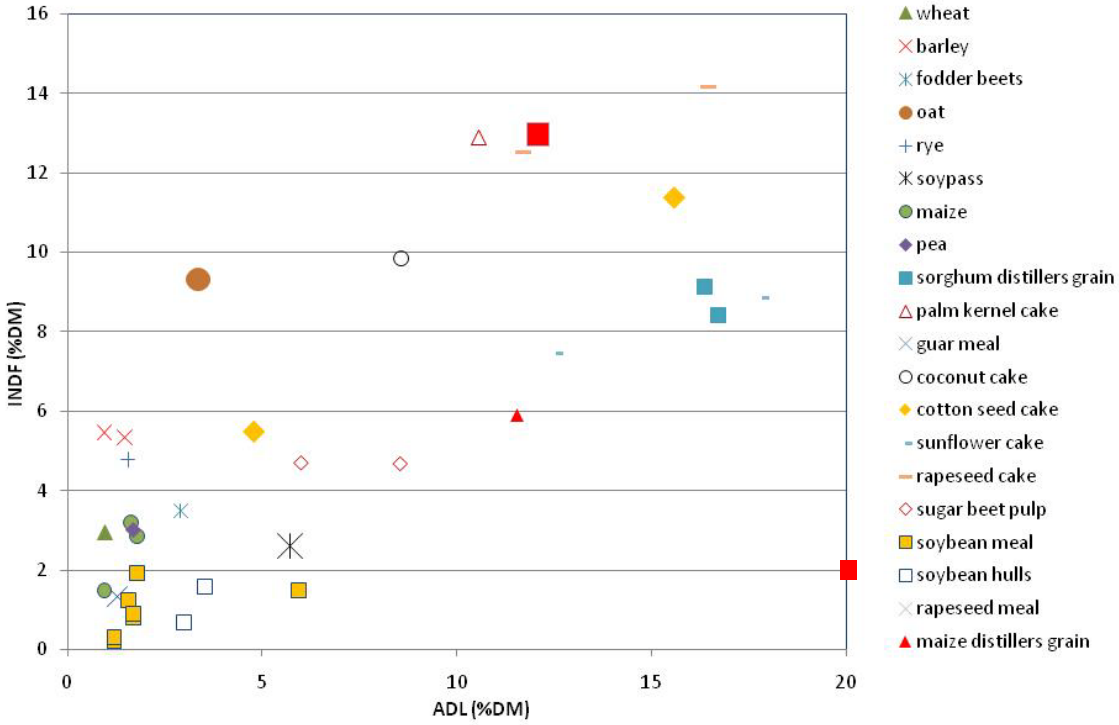

Figure 3 INDF (%DM) plotted against ADL (%DM) for concentrates and by-products

Conclusions

The ratios and correlations of the parameters tested (INDF, NDF, ADL, IVOMD) showed large variations between feedstuff groups, within the groups and also within one feedstuff type. The INDF/ADL ratio deviated largely from the 2.4 factor used in CNCPS except for maize silage, barley whole crop, alfalfa and wheat. Correlations shown indicate that ADL content and/or IVOMD could be acceptable predictors of INDF within feedstuff group, but not across groups.

Acknowledgements

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Micro- and nano-technologies - possibilities and challenges

A. Krozer
Imego AB, Arvid Hedvallsbacke 4, SE-411 33 Göteborg, Sweden
E-mail: anatol.krozer@imego.com

Introduction

The developments in micro and nanotechnologies paired with the progress in biological receptor-ligand interactions and surface chemistry has paved the way for commercial applications of sensors in human-related Life Sciences (Velusamy et.al., 2010; Biosite Inc., 2010), but only to a limited extent (Velusamy et.al., 2010) in veterinary applications and agriculture. One obvious reason for that is a much higher cost pressure in the latter fields as compared to the human-related Life Sci., another reason is the need for still further automatization of the sampling and measuring system (Bladh, 2010).

The commercial focus of Imego Institute - the design and realization of micro sensing for in-field use (see below), is very much in line with these needs. Therefore we have recently started collaboration with several departments at SLU.

I will try to illuminate several examples of activities within the institute that are of direct interest for the veterinary and agricultural community. It will not be an exhaustive overview of the whole field, nor will I cover all of the technologies under development at Imego. The intention is to give a flavor about the present possibilities of micro- and nano-technologies and large scale manufacturing and promote further collaborations.

Presentation focus

After a short overview of the institute I will turn to discuss mainly three fields:

- Measurements of limp or rut in cows using micro electromechanical components and systems (MEMS). The detection of changes in walking patterns of individual animals is made using accelerometers.
  Figure 1 below is taken from Fornara (2008) illustrates one of the possibilities of MEMS technologies.

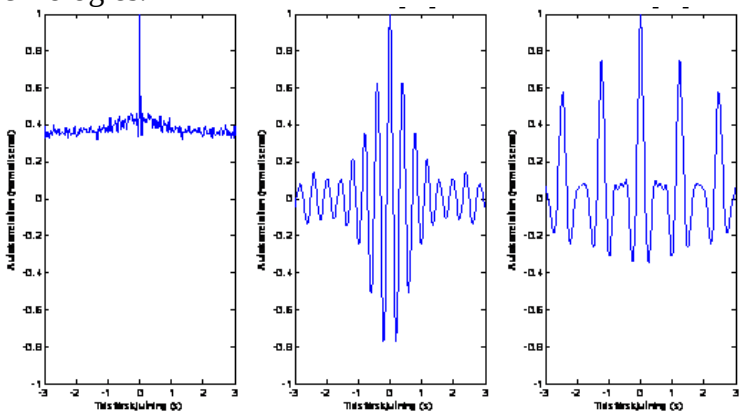

Figure 1. The characteristics of different patterns in a cow. LHS – standing “still”; middle “normal” walk; RHS – limping animal

- The in-vitro measurements of antibodies against *Brucella abortus* by monitoring changes of Brownian motion of magnetic nanoparticles.

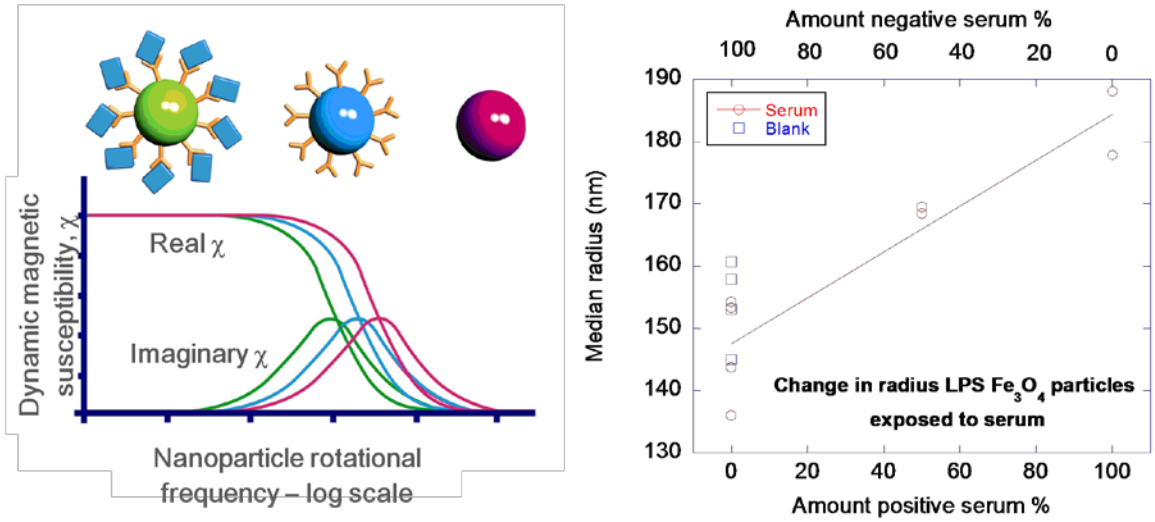

Figure 2. RHS illustrates the detection principle. When a protein (or in general, a biomolecule) binds to a nanoparticle its Brownian rotational motion slows down, - the frequency decreases. If the particle is magnetic and its magnetic moment is blocked to a certain direction with respect to nanoparticle it rotates, too. This rotation is detected by measuring the dynamic magnetic susceptibility.

LHS of Figure 2 illustrates one application of the method. The magnetic nanoparticles functionalized with the Brucella abortus antigen were mixed with milk having different proportion of brucella antibody. The figure illustrates the increase of nanoparticle diameter due to antibody adsorption. The lowest diameter is for the milk from the healthy cow. The largest increase of size is for the milk from sick cow while the in-between value is after a mixture 50%/50% of both milks.

Finally we will discuss the possibility to measure the breath from the cow using a novel sensor developed for Alcohol lock for vehicles.

If time will permit we will discuss also other possibilities, e.g., wireless in-vivo measurement of pH in animals.

Shortly about the Imego Institute

Imego AB is a research institute started 1999 has become a part of RISE (Research Institutes in Sweden) last year and sorts under the Swedish ICT branch. Imego has a strong commercial focus. The business idea is to develop micro sensor systems. The field of activities is broad since the ambition of Imego is to act as one-stop-shop for a potential customer. The institute is divided into a R&D department and the System Design department. Imego develops micro sensor systems within four main business areas: MEMS-based motion sensors and inertial navigation systems, electromagnetic sensors and biological & chemical sensors. The system Design dept acts as a support for R&D. It deals mainly with signal processing, close-to-sensor electronics and software development, sensor packaging and contains also an industrial designer.
The present project portfolio is split app. equally between the long and medium term projects funded by the industry as well as the development of the proprietary techniques. The institute

employs app. 37 persons and has turnover of over 50 MSEK. Approximately 50% of the employees are PhDs and 25% have more than 10 years of relevant industrial experience.

We work actively with intellectual properties right and have more than 30 patents/pending patents. Together with the School of Entrepreneurship at the Chalmers Imego has spun – out two successful companies based on our IP. *VasaSensor* (focused on wireless sensing of velocity, friction and wear of moving objects) has received several awards and attracted substantial venture capital.

Imego is ISO 9001/2000 certified.

Apart from industrial collaboration the Institute takes active part in national (Vinnova, VR, Formas, etc.) as well as European (FP7) programs. At present we participate in five FP7 projects and receive support national support, e.g., from Vinnova. We collaborate closely with several academic partners both in Sweden and abroad.

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HPLC – applications for agricultural and animal science

B. Ericson and J. André
Department of Animal Nutrition & Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences, S-753 23 Uppsala, Sweden

Introduction

High-performance liquid chromatography (HPLC)
HPLC has been optimized to provide rapid high resolution separations. It evolved over nearly a century ago from the early work of Tswett in the 1900s to the high sophisticated reliable and fast liquid chromatography techniques in common use today. HPLC is today one of the most widely used of all analytical separation techniques in laboratories. The popularity of HPLC analysis can be attributed to its powerful combination of separation and quantification capabilities, its sensitivity, and its suitability for separating species that are thermally fragile. HPLC instrumentation has reached a state of maturity.

HPLC is a form of liquid chromatography to separate compounds that are dissolved in a solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector (Fig. 1). Compounds are separated by injecting a plug of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in the partitioning behaviour between the mobile liquid phase and the stationary phase.

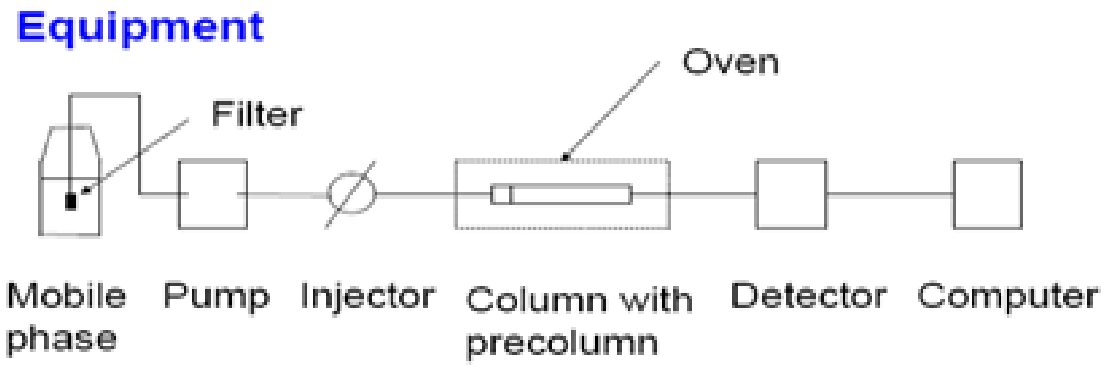


Figure 1 Example of the chromatography technique

Type of samples analysed using HPLC at Kungsängen Research Centre
The biological samples that are most frequently analyzed using the HPLC technique at Kungsängen Research Centre are silage and rumen fluid. The quality of silages is evaluated according to the content of organic acids and alcohols such as lactic, acetic, butyric, propionic, succinic acids and ethanol and 2,3-butanediol In rumen fluid, we analyse for the n- and iso-forms of the volatile fatty acids which are of interest in nutritional and metabolic studies.

Materials and methods

Reagents
All reagents and standards used in the analysis are of analytical grade p.a. Laboratory reagent water is Milli-Q water.

Apparatus and operating conditions
The HPLC-system at use consists of:
Alliance 2795 Separations Module with a Temperature control Module II range 40-70°C and 2414 RI Detector (Waters Assoc. USA)

Separation column; Column packet ReproGel H 9µm 300 x 8 mm (Dr.A.Maisch, Ammerbuch, Germany).

Pre-column: ReproGel H, 9µm 30 x 8 mm (Dr.A.Maisch, Ammerbuch, Germany).

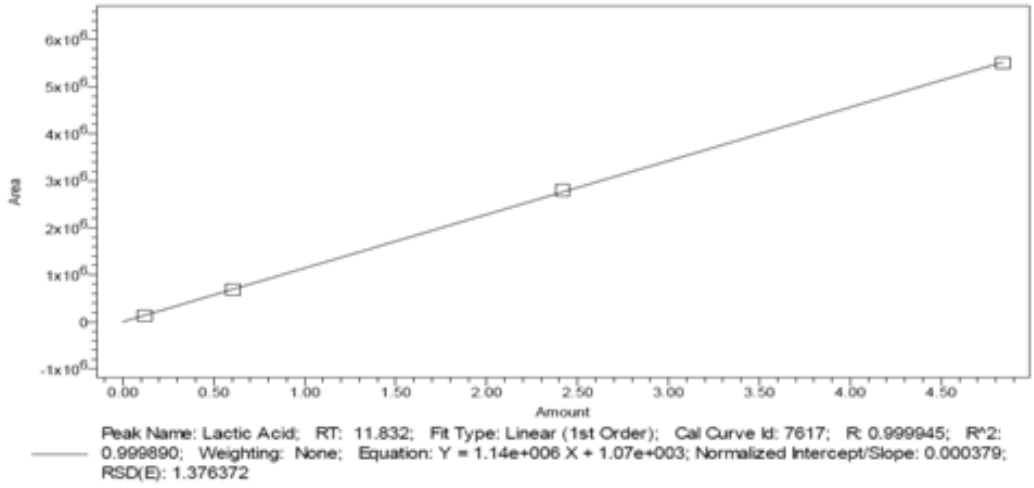
The conditions for the HPLC analysis are:

Mobile phase: 0.005M sulphuric acid; flow rate: 0.8 ml/min

Column temperature: 60°C.

Injection volume: 20 µl

Sample preparation
The biological samples are centrifuged for 5 minutes at 13000 *x g*. A volume of 200 µl of the clear solution is mixed with 800 µl of the internal standard solution (0.1% of Pivalic acid in 5mM sulphuric acid). The solution obtained is analysed directly by the HPLC method described below.



Peak: Lactic Acid

	Sample Name	Result Id	Peak Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore
1	Std1	7630	Lactic Acid	1	0.121	127834.035	0.111	-8.14	No	No
2	Std2	7631	Lactic Acid	2	0.605	681822.087	0.597	-1.34	No	No
3	Std3	7632	Lactic Acid	3	2.420	2798956.855	2.453	1.38	No	No
4	Std4	7633	Lactic Acid	4	4.840	5503210.738	4.825	-0.32	No	No

Figure 2 The calibration curve for lactic acid

Calibration and quantification
The instrument is linear multilevel calibrated by injecting solutions containing known amounts of organic acids, ethanol and 2,3-butanediol. Prior to injection, the calibration

solutions are mixed with the internal standard solution used for sample preparation. The calibration is performed using peak-area estimates and the internal standard method (Fig. 2).

Results calculations
The determination of organic acids, ethanol and 2,3-butanediol (OAcs; EOTH; 2,3butandiol) concentration present in an unknown sample is measured by the calibration curve results:

$x = \frac{y - b}{m}$ where,

x = sample analysis result (concentration of organic acids, ethanol and 2,3-butandiol measured)

y = raw data (mV- sec.)

b = y intercept (mV- sec.)

m = slope or calibration factor (mV-sec./mM).

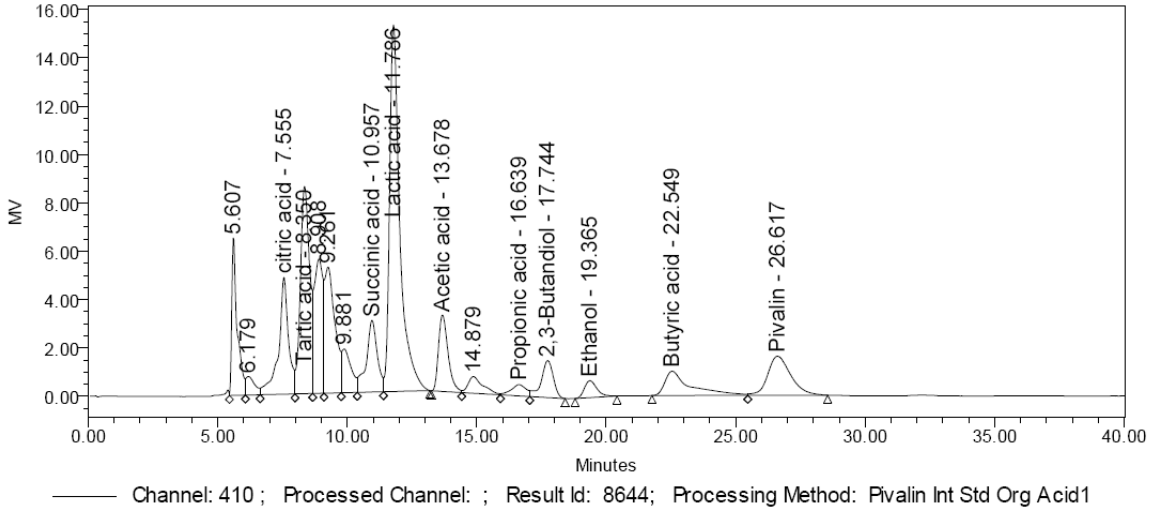


Figure 3. Chromatogram of a typical silage sample

Method detection limit (MDL)
The detection limit is a estimated as the lowest possible concentration of each organic acid and alcohol that can be measured by the HPLC instrument. In other words, the MDL is the analyte concentration that is required to produce a signal greater than three times of the noise level.

$$MDL = \frac{3 \times Noise(mV)}{Tophigh(mV)} \times [analyte(\%)]$$

Limit of quantification (LOQ) and practical quantification limit (PQL)
The LOQ is the limit at where we can reasonably well tell the difference between two different values. The LOQ is drastically different between laboratories so another detection limit is commonly used that is referred to as the PQL. The PQL is defined simply as about 5 times the MDL.

$$PQL = 5 \times MDL = 5 \times \left(\frac{3 \times Noise(mV)}{Tophigh(mV)} \times [analyte(\%)] \right)$$

Conclusions

The number of samples we are analyzing at Kungsängen are about 20 samples a day. Most of the samples are for research purposes, from method development to established methods.

The HPLC method is giving very good results with replicates RSD<0.5 % and quantification limits of 0.005 g/100 ml for most substances analyzed (organic acids, ethanol and 2, 3-butandiol).

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A macro-in vitro system for short-time fermentation studies

H. Wallin and P. Udén
Department of Animal Nutrition & Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences, S-753 23 Uppsala, Sweden

Introduction

In vitro techniques offer advantages over in vivo techniques. End-products are not absorbed and can be measured accurately and single substrates can be studied more easily. However, long-term fermentation in batch or continuous cultures will inevitably change the flora and there is a great risk that fermentation end-products will be altered. The appealing idea of the short term incubation of whole rumen content of Carroll and Hungate (1954) is the greater chance of maintaining the original flora and, hence, degrade substrates and produce similar end-products as in vivo. One drawback, however, is the greater dependence on accurate and precise data due to the smaller absolute changes during the short incubation period.

The objectives were to develop a short-term in vitro system with a capacity of up to 10 kg rumen contents to simulate fermentation in vivo.

Materials and Methods

A novel macro in vitro system for whole rumen content was developed. The latest version consists of a thermally insulated box made from 5 cm thick polystyrene panels and has the following outer dimensions (cm): 110 (l) x 55 (w) x 60 (h). The box is held at a constant temperature of 39ºC by a 400 W external heater and the air is circulated in the box by a fan. Eight 18.8 (o.d) x 76 cm drainage pipes made of 6-mm polyethylene with water tight bottom stoppers are placed in the box extending 10 cm above the box through holes in the lid. The pipes are fitted with 6-cm thick closed cell foam rubber lids with four holes for the stirring mechanism, infusions, continuous gassing with CO_2 during incubations and for sampling. The mixer consists of a grain auger (cm) 50 (l) and 10 (Ø) with 8 turns/m and a 45-cm shaft extending through the centre hole of the lid. The shaft is attached to a 750 W electric drill and the bottom part is centered by a protruding short bolt which fitted in the hollow shaft. The whole assembly including heater is mounted on a pallet for easy transport between in vitro room and stable. Sampling is normally done after 30 sec of vigorous mixing by pressing down a perforated rigid plastic tube (80 x 1.6 cm) with a bottom stopper through one of the holes. The liquid entering the tube is collected into a 50-mL centrifuge tube; using a 1.2 m plastic tube and suction (see Fig 1).

Incubations are normally done as follows:

1. 1Day before the experiment: prepare sufficient quantities of buffer. Fill glass jars with 2-L of the buffer (blank) or the test substances in question dissolved in the same amount of buffer. Record tare weights of in vitro tubes and assemble the in vitro unit. Add 2 L of buffer to all tubes, gas with CO2, seal the lids, and heat over night with the thermostat set at 39°C. Bubble CO2 in all solution over night.
2. Experimental day: add more CO2 and move in vitro assembly to the stable and plug in heater. Record rumen pH and transfer whole RC (6 to 10 kg) from rumen fistulated cow(s) to each in vitro tube with minimum exposure to air.
3. Move assembly back to the in vitro room and plug in heater again. Add more CO2 and, if substrate degradation is investigated, pour the first substrate solution (1 L) into in vitro tube no 1.

4. Start timer, mix the in vitro content vigorously for 30 sec and take a 50-mL sample of the liquid fraction by aspiration (see Udén, 2010). Record pH and cool sample rapidly in ice bath. Continue with the tube no 2 and solution no 2, etc. Space samplings 2.5 min apart, which will allow for a minimum time interval of 20 min for each tube when using a total of 8 tubes.
5. Sample again according to schedule and continue sampling for a maximum of 3 h.
6. Centrifuge samples at 1500 x g for 5 min and split the supernatant into sub-samples. Transfer supernatants to refrigerator (4°C) and analyze samples the following morning.
7. Record weights of the in vitro tubes and determine DM concentrations of remaining ingesta.

Results and Discussion

The macro-in vitro assembly is shown in Figure 1.

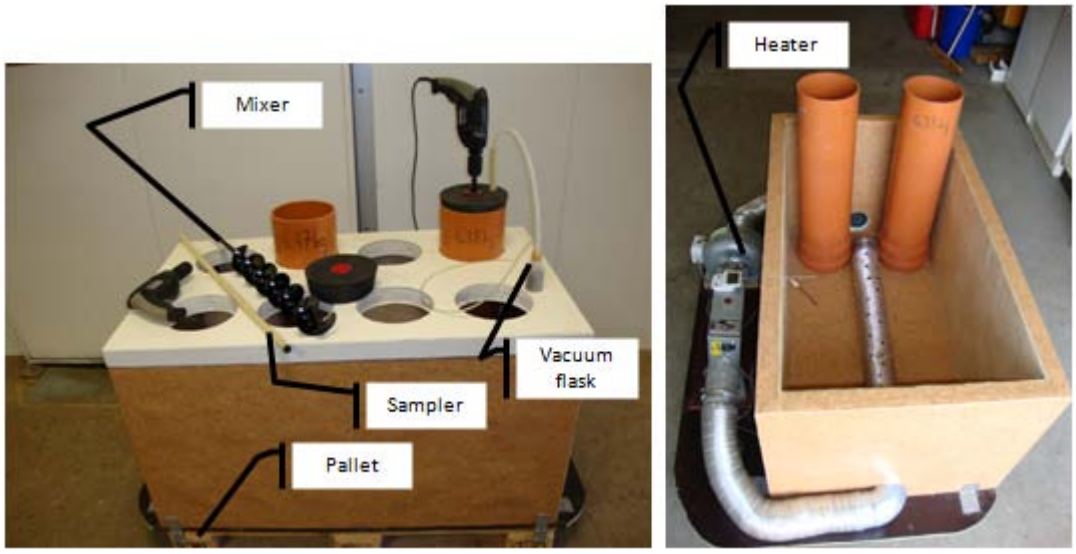

Fig 1 Macro-in vitro assembly for rumen fermentation studies

We removed large amounts (6 to 10 kg), representative of the entire rumen content. Using large amounts of digesta we also believed to ensure better anaerobic conditions and less chance for temperature drops. The large volume of rumen fluid made it possible to take at least 50-mL samples which were regarded advantageous for further fractionations and multiple analyses. The addition of 5 to 8 L of a bicarbonate buffer was done in order to prevent early pH drops and increases in osmotic pressure.

It required two persons attending it during frequent sampling with elapsed time sheets to monitor the staggered samplings. The system was found to be relatively easily managed and studies of volatile fatty acid (VFA) production (Udén, 2010) and degradation of glycerol (Holtenius, unpubl.) and soluble proteins (Udén, unpubl.) have been performed (Fig 2 to 4). The only problem experienced with the system was a slight reduction of VFA production, mainly from rumen ingesta, during the first hour of fermentation. This caused some problems in the study of Udén (2010) due to the particular design of that study with the simultaneous infusion of VFAs. It is not believed to have any major effects in studies on the degradation of soluble substrates. Studies of slowly degradable or insoluble substrates may not be feasible with this system due to lack of data on curve asymptotes and problems of differentiating between remaining substrate and ingesta.

A modification to allow also the measurement of gas production may be possible but liquid samples must then be taken through outlets in the tube, rather than from the lid.

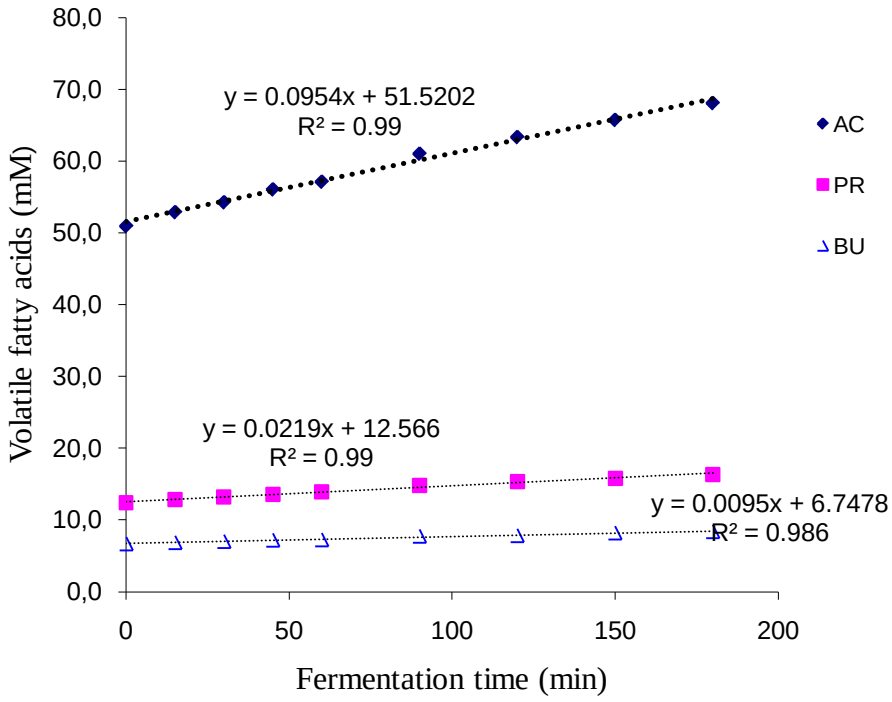

Fig 2 Volatile fatty acid concentrations during 3-h incubations.

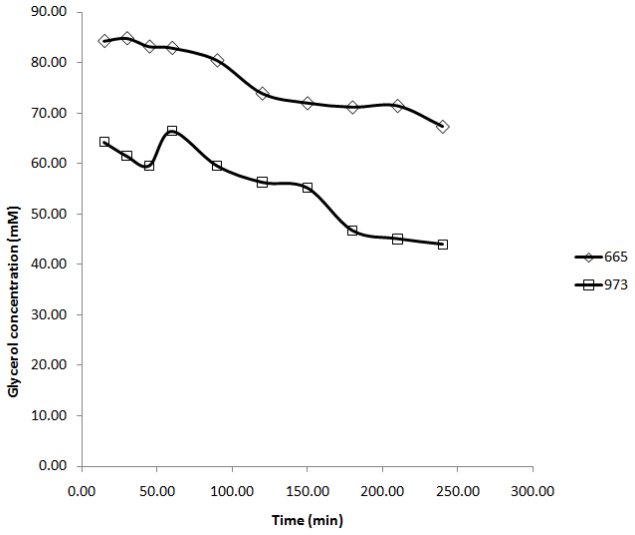

Fig 3 Glycerol concentrations during 3-h incubations using rumen contents from two cows (courtesy of K. Holtenius).

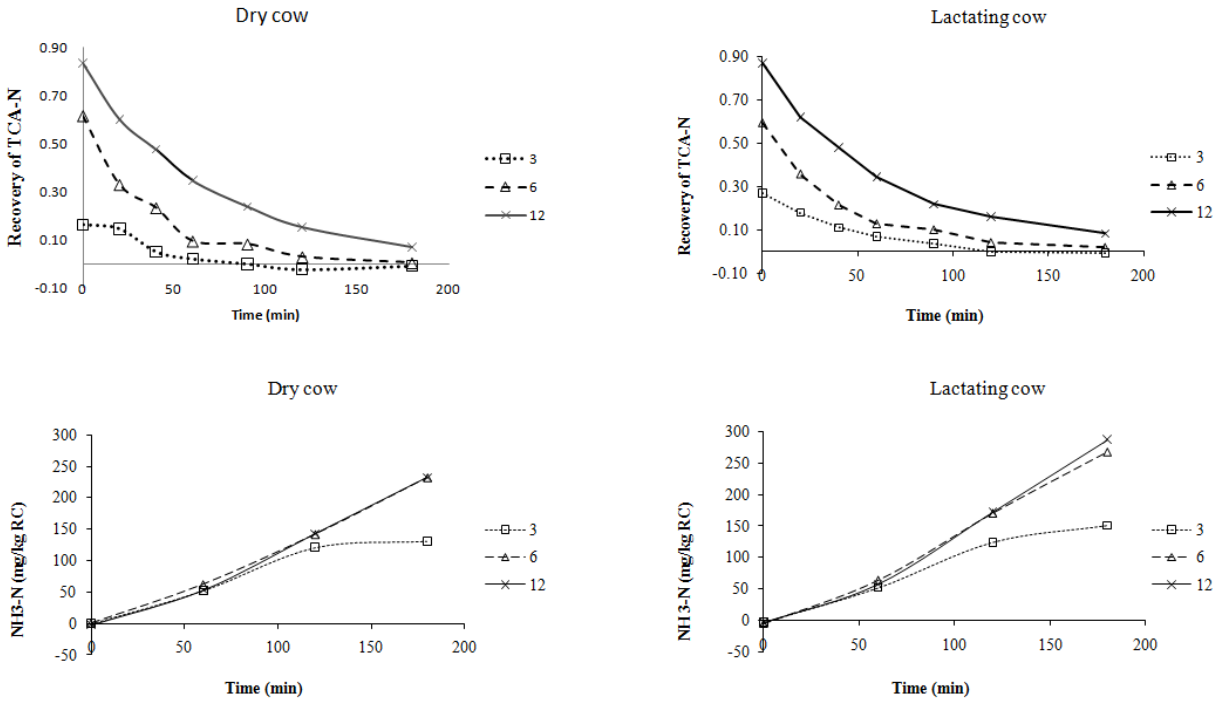

Fig 4 Recovery of casein (TCA-N) after three different dosages (3, 6 and 12 g TCA-precipitable N/tube) and ammonia-N (mg/kg rumen content) evolution in rumen contents from a dry and a lactating cow.

Conclusions

The system appears useful for studying formation of fermentation end-products and the degradation of soluble substrates. Removing large amounts of rumen contents seemed to produce in vivo-like conditions in vitro with only minor fermentation lag phases. Repeated sampling of relatively large amounts of liquid is an added advantage of the system.

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Comparisons of estimated rumen protein degradation using a new *in vitro* gas production technique and the *in sacco* technique

L. Karlsson[1], M. Hetta[1], P. Udén[2] and K. Martinsson[1]
[1]Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden. [2]Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Kungsängen Research Centre, SE-753 23 Uppsala, Sweden.

Introduction

Rumen availability of feed protein is often expressed as effective protein degradation (EPD). The concept is an important feed characteristic in nutritional models and in most cases estimated by using the *in sacco* technique (Ørskov and McDonald, 1979). The *in sacco* technique records the disappearance of crude protein (CP) from feed samples incubated in nylon mesh bags in the rumen. However, the *in sacco* method is laborious, expensive and has inherent problems with losses of undegraded protein from the bags. The losses may result in overestimation of the rumen availability of feed proteins (López, 2005). An alternative technique is to record the release of ammonia during rumen fermentation of the protein. However, estimating protein degradation in the rumen through ammonia release is complex, since feed protein degradation and ammonia uptake from the *de novo* synthesis of microbial proteins occur simultaneously (López, 2005). To overcome this problem, Broderick (1987) developed an *in vitro* inhibitor method, in which hydrazine sulphate and chloramphenicol are used to limit microbial protein synthesis in the rumen. The inhibitor method is however limited by the fact that the chemical therapy will limit bacterial growth and thereby the efficacy of the microbes to degrade protein.

A different approach to overcome the problems with uptake of ammonia caused by microbial protein synthesis during fermentation is the gas production (GP) technique described by Raab *et al*. (1983). In this technique, *in vitro* degradable CP (IVDP) is determined via ammonia-N and GP measurements when feed and graded levels of carbohydrates are incubated in buffered rumen fluid. Extrapolation of the linear regression between ammonia-N and GP gives a y-intercept that is assumed to represent the amount of ammonia-N released when no fermentable carbohydrates are available and hence, no bacterial protein synthesis would occur. However, the technique has some shortcomings such as large impact of the quality of the inocula on the results and the requirements of several incubation bottles for each sampling time. To improve the practicality and reliability of the method, a number of methodological changes were made by Karlsson *et al.* (2009), referred to as the new GP technique. The aim of the presented study was to compare estimates of rumen protein degradation using the new GP technique and the *in sacco* technique.

Materials and Methods

The IVDP was determined for five protein feeds (cold-pressed hempseed cake, cold-pressed rapeseed cake, rapeseed expeller, heat-treated rapeseed meal and soybean meal), using the new GP technique presented by Karlsson *et al.* (2009). The feeds were incubated in 90 ml of buffered rumen fluid with addition of a mix of carbohydrates at four concentrations. Blank samples, containing buffered rumen fluid only, were included in duplicates. Amounts of ammonia-N and gas produced were recorded at 4, 8, 12, 16, 24, and 30 h. The new GP technique included a number of methodological changes compared to the original method described by Raab *et al*. (1983). The recordings of ammonia-N and GP were collected within

the same incubation bottle at all incubation times instead of using one bottle for each recording. The GP was recorded in an automated system, while 2 ml of the liquid phase was repeatedly sampled for ammonia-N analysis by attaching a syringe to a valve in the modified fermentation unit.The rumen fluid was pre-incubated for 3 h with a mix of carbohydrates to improve the microbial activity and reduce the level of background ammonia-N in order to improve the sensitivity of the method. The IVDP value was estimated for each feed at each incubation time via linear regression of ammonia-N *vs.* GP, as described by Raab *et al.* (1983). The difference between the intercept and the blank ammonia-N content was assumed to represent the amount of ammonia liberated from degradation of the protein in the incubated feed.

The *in sacco* CP disappearance was determined according to NorFor standard procedure (Eriksson *et al.*, 2007) by incubating the feeds in the rumen for 2, 4, 8, 16, 24 or 48 h, using three rumen cannulated non-lactating dairy cows. The EPD of the feeds was calculated after fitting the IVDP and *in sacco* CP disappearance data to a non-linear equation (Ørskov and McDonald, 1979) estimating the first order rate of degradation, in combination with an assumed fractional rate of passage of 0.08/h. The results yielded by the *in vitro* and *in sacco* techniques were compared by subjecting the EPD values to analysis of variance. The linear relationships between IVDP and *in sacco* CP disappearance at 4, 8, 12 and 24 h of incubation were determined by linear regression.

Results and Discussion

The new GP technique provided useful estimates of the IVDP that enabled calculations of EPD values of the protein feeds. The estimated EPD values were affected by feed and method, as well as feed × method interactions (Table 1). Pairwise comparisons within feed showed that the estimated EDP values using the two techniques differed for the cold pressed feed cakes, while there were no between-method differences for the other feeds (Table 1).

Table 1 Least square means of effective protein degradation proportion (EPD) calculated at a passage rate of 0.08/h from results obtained with the new *in vitro* gas production (GP) technique and the *in sacco* technique.

	GP technique			*In sacco* technique			Significance (*P*)			
	n	EPD	SD	*n*	EPD	SD	M	F	M × F	M within F
Hempseed cake	3	0.33	0.03	3	0.84	0.00	<0.001	<0.001	<0.001	<0.001
Rapeseed cake	4	0.59	0.05	3	0.89	0.01				<0.001
Rapeseed expeller	4	0.46	0.06	3	0.40	0.04				0.696
Rapeseed meal	4	0.36	0.06	3	0.37	0.03				1.000
Soybean meal	4	0.67	0.04	3	0.65	0.04				1.000

n =number of runs behind each mean; SD=standard deviation; F=feed; M=method.

There were significant linear relationships between IVDP and *in sacco* CP disappearance with R^2 values of 0.87 and 0.93, respectively, when the data for the cold-pressed cakes were compared separately from the data for the expeller and meals (Fig.1). Both the comparisons of the EPD values estimated using the two techniques and the relationship between the IVDP and *in sacco* CP disappearance show that the differences were feed-dependent. The feed cakes had a considerable higher fat content than the other feeds. It is possible that high amounts of lipids affected the microbial fermentation more in the bath culture *in vitro* system than when the bags were incubated in the rumen, resulting in lower IVDP values compared to *in sacco* CP disappearance.

The largest between-method differences were obtained for samples taken at early points in the

incubations, for which the estimates of protein degradation yielded by the GP technique were considerably lower than those provided by the *in sacco* technique (Fig. 2). One possible explanation for this is loss of undegraded and soluble protein from the nylon bags used in the *in sacco* technique. All protein that disappears from the bags is assumed to be degraded. However, some proteins may pass through the bag pores without being degraded, resulting in overestimated degradability (Dewhurst et al., 1995; López, 2005). The technique also assumes that solubility is synonymous with degradation, but soluble proteins have been shown to vary in degradation rates and cannot be assumed to be completely degraded in the rumen (Hedqvist and Udén, 2006).

Improvements to the sampling of the liquid phase during incubations increased the analytical capacity of the new GP method (Karlsson et al. 2009) and made it more practical and consistent compared to the original method described by Raab et al (1983). The pre-incubation of the rumen fluid, which is included in the new GP technique, has two main purposes. One is to enhance the microbial activity to avoid a fermentation lag time at the start of the incubation. The second is to lower the background ammonia-N in the rumen fluid and hence, in the blanks. This increases the sensitiveness of the method to record differences in ammonia-N concentration that are caused by the graded levels of added carbohydrates.

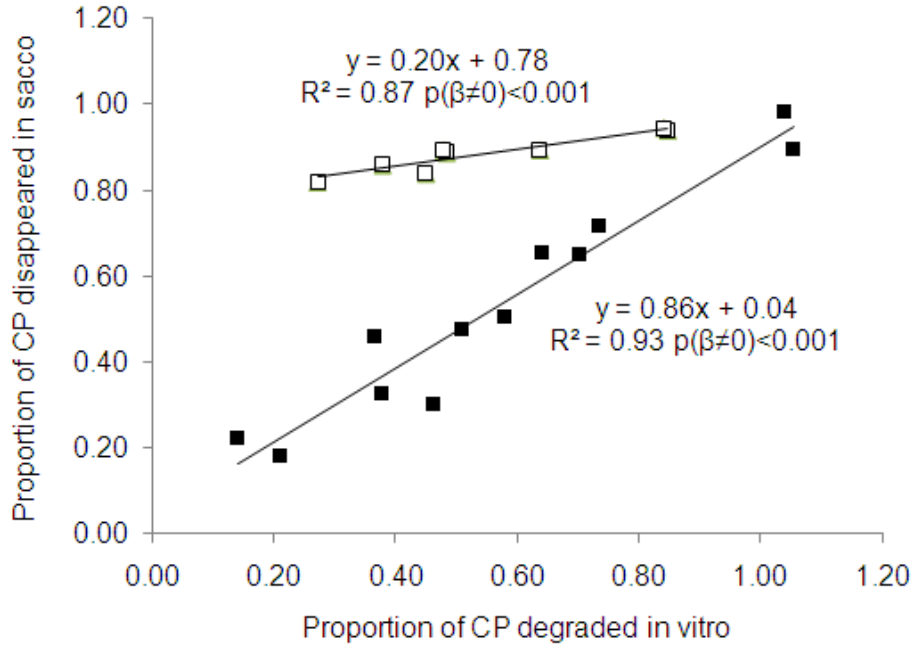

Figure 1 Relationships between *in vitro* degradable crude protein (CP) and *in sacco* CP disappearance estimated for hempseed cake and rapeseed cake (□), and rapeseed expeller, rapeseed meal, and soybean meal (■) after 4, 8, 12, and 24 h incubation.

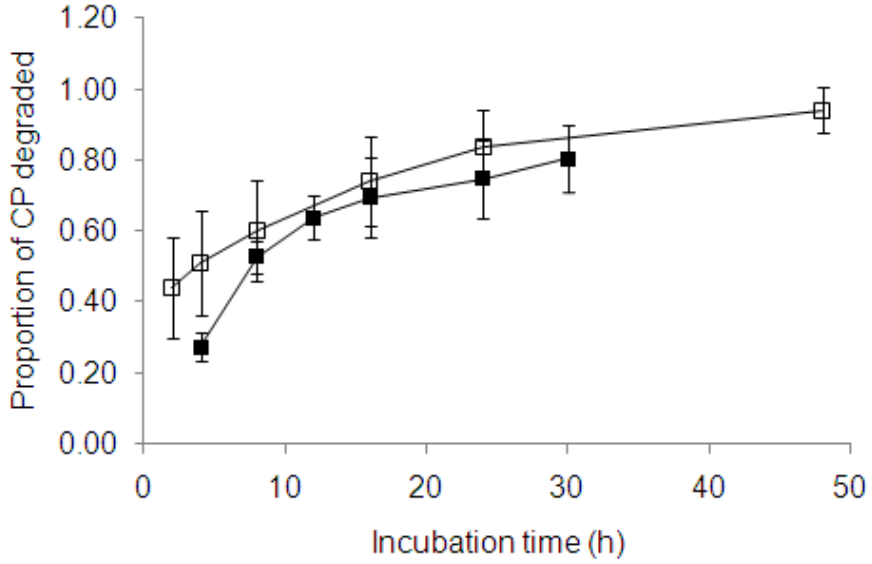

Figure 2 Comparison of mean *in vitro* degradable crude protein (CP) (■) and *in sacco* CP disappearance (□) measurements obtained in experiments with five protein feeds. Bars indicate standard errors.

Conclusions

Differences between estimated rumen protein degradation, using the new *in vitro* GP technique and the *in sacco* technique, were feed-dependent. The EPD values obtained by using the GP technique were lower for the cold-pressed feed cakes, but no differences were found for the feed expeller or meals. This may be related to the higher fat content of the feed cakes and emphasizes the importance of including different types of feed sources when evaluating analytical techniques. The largest differences between the two methods were observed during early incubation, where the GP technique provided lower estimates of protein degradability. A possible explanation is losses of soluble and undegraded feed protein particles from the bags used in the *in sacco* method, resulting in an overestimation of rumen availability of protein. The new GP technique has potential to provide accurate and biologically relevant estimates of protein degradation in the rumen. However, the technique needs to be further validated using protein feeds with known *in vivo* responses. The method has potential to be automated and could then become more interesting for commercial analysis of protein degradation in feeds for ruminants.

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The influence of sample preparation on the level of soluble and non-structural carbohydrates in forage crops and silages

P. Udén
Department of Animal Nutrition & Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences, S-753 23 Uppsala, Sweden

Introduction

Oven drying at around 60°C is by far the most common method for preparing feed samples for analysis (e.g. Undersander et al., 1993) and is recommended by the European Union (71/393/EEC) for high-moisture solid feeds and for neutral detergent fibre analysis (ISO 16472:2006 IDT). Sugar losses during sample preparations are often ignored for reasons of not being volatile. Substantial losses were, however, seen after drying by Jones (1962), Lancaster et al. (1977), Deinum and Maassen (1994), Nielsen et al. (2007) and Pelletier et al. (2010). Deinum and Maassen (1994) investigated the effects of different oven drying temperatures on sugar recovery and attributed an increasing loss of sugars with decreasing oven temperature to respiratory losses. Similar results were reported by Pelletier et al. (2010) and the authors also reported that a 1-min microwave oven treatment before drying at 55°C gave similar values as for freeze drying by denaturing proteins that cause enzymatic conversions and respiratory losses. Metabolism of sugars during oven drying of silage is also likely to have been the reason for the 45% loss of glucose in maize silage (Nielsen et al., 2007).

Drying samples is not an option in the analysis of volatile silage components. Therefore, ammonia, organic acids, alcohols and other volatiles are extracted directly from the fresh forage or silage sample either after squeezing out the juice with a hydraulic press or after macerating the sample in a known amount of water. Little information is available on the possibility of using fresh crop or silage extracts for analysis of sugars, something which should reduce labor costs, particularly, in the case of subsequent analyses of fermentation products with the same type of method – chromatographic or spectroscopic. Normally, forage samples are extracted in water before the determination of soluble carbohydrates. However, many whole-crops contain starch requiring an acetate buffer for an enzymatic analysis of starch. In a sequential analysis of soluble sugars and starch it would be useful if acetate could replace water in the extraction of soluble carbohydrates.

In this study we compared: i) the effect of four extraction-preparation methods on the analyzed levels of water extractable soluble carbohydrates in grasses and legumes and three preparation methods for acetate extractable whole-crop cereals and ii) the ability of hot water and acetate to extract sugars in grasses and legumes.

Materials and methods

Samples

Samples were collected in Norway and Sweden and consisted of 24 direct-cut and wilted grasses (16), clovers (4), grass-clover mixtures (3), birdsfoot trefoil (1) and of 12 whole-crops of wheat (2), barley (2), oats (2), maize (4) and peas (2). Half of the samples were silages and half were crops. Approximately 2 kg of all samples were frozen at -25°C and ground in a meat grinder to pass a 13-mm sieve. The frozen and ground samples were thoroughly mixed and then immediately divided into three subsamples of equal size and prepared for analysis. One subsample was freeze dried (FDR), one was dried in a forced draught oven (ODR) and the remaining subsample was further divided into two sub-

subsamples and frozen fresh (FF) in two different ways. One sub-subsample was used to fill four 50-mL plastic test tubes with 10.000 g of the material, to be extracted later by hot water (grass-legume samples) or acetate buffer (whole-crops), and re-frozen. The second sub-subsample (grass-legume samples only) was put in duplicate in 'Ziplock' bags (100.00 g) and 100.0 mL water was added for the cold water extraction and re-frozen.

The samples for oven drying were placed on aluminum trays at a thickness of 1 cm and dried for 16 h at 60°C. Samples for freeze drying were first frozen to -80°C before starting the drying process. All dried samples were allowed to equilibrate at room temperature, ground in a knife mill (Brabender OHG, Duisburg, Germany) to pass a 1-mm sieve and bottled.

Extraction of soluble carbohydrates

Water (hot or cold) was used to extract all grass-legume preparations and acetate for all whole-crop samples and also, for comparative purposes, for the grass-legume ODR and FDR preparations. Specific sample amounts, extraction volumes, times and temperatures are specified in Table 1. Soluble carbohydrates [Su(s)] were assumed to consist of glucose, fructose, and sucrose and, as was discovered later, some "soluble" starch [St(s)]. Sub-samples taken were used for subsequent enzymatic analysis. Acetate extractions (0.0500 M, pH 5 with 280 mg CaCl2/L) were done on whole-crop samples to enable also the sequential analysis of starch and, for comparative purposes, on the grass-legume ODR and FDR preparations.

Table 1 Details on sample drying and extraction procedures for the samples which all had been stored frozen

Sample type	Treatment abbreviations[a]	Drying method	Solvent	Temp.[b]	Extraction time
Grasses and legumes	ODR, FDR	Oven or freeze drying	Water	100°C	3 min
	FF	None	Water	100°C	3 min
	FF	None	Water	-25°C/20°C	>24 h/0.5 h
	ODR, FDR	Oven or freeze drying	Acetate	60°C	40 min
Whole crops	ODR, FDR	Oven or freeze drying	Acetate	60°C	40 min
	FF	None	Acetate	60°C	40 min

[a]ODR = oven dried; FDR = freeze dried; FF = fresh-frozen; [b]-25°C/20°C means that the defrosted samples were re-frozen after adding water for >24 h, then defrosted again and kept in room temperature for 30 min before filtering.

Enzymatic analyses

The analysis of soluble sugars was based on an acid hydrolysis of sucrose and fructans, followed by enzymatic conversions of all fructose and glucose to glucose-6P. The amount of glucose equivalents was finally measured in a spectrophotometer from the absorbance change at 340 nm due to the conversion of NADP to NADPH. Whole-crop samples were analyzed for both soluble and insoluble starch [St(i)]. Soluble starch was defined as starch still in suspension after centrifugation for 5 min at 2000 x *g*. The starch analysis was similar to the soluble sugar analysis but included also two initial hydrolytic steps with amylase and amyloglucosidase.

Statistical analyses
All values were calculated per unit of fresh matter (FM) as these were considered unaffected by losses of volatiles and would therefore be better for evaluating any possible loss of sugars by metabolism during sample preparation.

Analysis of variance for the effect of sample preparation and extraction method was done using the GLM procedure of Minitab (v. 15, Minitab Inc., State College, PA, USA).
Grass-legume samples:
1. Effects of sample preparation on Su(s): 96 observations (duplicate means) from the water extracted grass-legume samples were used which were made up of 24 samples and 4 preparations (FDR, ODR and FF extracted hot or cold). The fixed factors of the model were sample and preparation.
2. Effect of solvent on Su(s): 96 observations made up of 24 samples, 2 preparations (FDR and ODR) and 2 extractions (water and acetate) with sample and extraction method as fixed factors. Whole-crop samples:
Effects of preparation method on Su(s), St(s), St(i) and total non-structural carbohydrates: 36 observations from 12 samples and 3 preparations (FDR, ODR, FF). Fixed factors in the model were sample and extraction method.

Results and Discussion

Grass-legume samples
Table 2 shows results for the grass-legume Su(s) analysis. No clear differences were seen among the FDR and the two FF variants. The ODR preparation resulted, however, in lower levels than all the other preparations with a 19% lower level compared to the FF hot water treatment ($P<0.05$; total average) and for silage, this value was 29% lower ($P<0.05$). Extraction with either water or acetate buffer resulted in very similar Su(s) levels in the grass-legume samples with 25.3 and 24.4 g/kg FM for acetate and water, respectively. A possible explanation for the lack of effect on the grass-legume crops may have been a rapid dehydration, as a result of using only a 1-cm layer of sample material on the trays as opposed to 6 to 10-cm layers used by Pelletier et al. (2010). In silage, a more rapid metabolism due to the presence of microorganisms may have caused an increased loss of sugars.

Table 2 Comparisons of sample preparation of 24 water extracted grass-legume crop and silage samples on recovery of soluble sugars [Su(s)] expressed in g/kg of fresh matter

	Preparation:	ODR	FDR	FF	FF		
Form	Extraction:	Hot	Hot	Hot	Cold	SEM	P=
Crop		25.9a	29.1	28.5	30.0b	0.85	0.012
Silage		19.2a	23.5ab	27.0b	23.9b	1.17	0.001
All		22.6a	26.3b	27.8b	27.0b	0.53	<0.001

ODR = oven drying; FDR = freeze drying; FF = fresh-frozen; Hot = hot water; Cold = cold water; a,b = treatment means followed by different letters differ significantly ($P<0.05$).

Whole-crop samples
Small differences were detected in Su(s) values with slightly (average 7%) lower values for the ODR preparation (Table 3), compared to FDR and FF ($P<0.05$). A surprisingly high proportion of the starch was soluble in the acetate buffer with mean of 0.34 for the FF preparation. It varied from very low (0.06) in pea silage to very high (0.85) in the early cut maize crop (not shown). More St(s) was solublized by the FF preparation than the FDR

preparation (P<0.05) but there was no effect of sample preparation on total starch (Table 3). This also resulted in a higher level of soluble carbohydrates (sugars and starch) for the FF preparation (P<0.05).

Table 3 Effect of sample preparation method on concentrations of soluble sugars and starch in 12 whole-crop crop and silage samples extracted in hot water (g/kg fresh matter)

	ODR	FDR	FF	SEM	P=
Soluble sugars	21.3a	22.7b	23.2b	0.41	0.008
Soluble starch	9.8a,b	6.4a	15.7b	1.84	0.006
Insoluble starch	51.2	54.7	46.9	2.34	0.086
Total	82.3a	83.8	85.9b	0.76	0.012

ODR = oven drying; FDR = freeze drying; FF = fresh-frozen; a,b = treatment means followed by different letters differ significantly (P<0.05).

Analytical considerations

For practical and economical reasons, it is advantageous to analyze soluble sugars and other soluble components, using a single preparation. This study showed that cold water gave similar results as for hot water extraction of the FF preparations. Cold water extraction of fresh-frozen samples can therefore be recommended for the analysis of soluble components. It is also likely that a procedure using a hydraulic press will give similar results. The best drying procedure seemed to be freeze drying, even though it did not always differ from ODR (Table 2). The risk of oven drying is a prolonged drying time when overloading the dryer with too thick sample layers and too many trays.

If both starch containing whole-crop samples and grass-legume samples are analyzed routinely, this study shows that the acetate buffer gives similar results as water extraction. The acetate buffer can therefore be recommended as a single extraction medium to avoid having separate protocols for the two sample categories. However, if silage fermentation products are also analyzed, water must be used as the acetate buffer would otherwise swamp the HPLC chromatogram. The most convenient method would then be to use the FF-cold water extraction procedure and add a higher strength acetate buffer after taking a sub-sample for fermentation end-products and before continuing with analysis of sugars and starch.

The presence of high levels of St(s) in the whole crop samples, particularly in the FF preparations, was surprising and has not been widely recognized in the feed science literature. Soluble starch, as a proportion of total starch, ranged from 0.06 in late cut maize silage to 0.85 in early cut maize crop with an average of 0.34 (data not shown). The forms of soluble starch (amylose, amylopectin or dextrins) or if it was part of any granular structure were not investigated. If this type of starch exists in the form of granules, these granules must be small enough to resist centrifugation at 2000 x g for 5 min. It is likely that St(s) has a more similar rate of degradation to Su(s) than to St(i) and that it should, therefore, be included in the water soluble carbohydrate fraction. If ignoring the presence of St(s), an underestimation of total starch will occur in a sequential analysis of Su(s) and St(tot), depending on the amount of extract that is removed for the Su(s) analysis.

Conclusions

Fresh, freeze dried or cold-water extracts are recommended for analysis of soluble sugars in forage crops and silages. Acetate buffer or water extract similar amounts of sugars and can

be recommended for both grass-legume and whole-crop samples. A large proportion of the starch may exist in a “soluble” form. This fraction should not be ignored in the analytical procedure and may also have other nutritional properties than the insoluble form.

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In vitro methods for indigestible Neutral Detergent Fiber (iNDF)

T. Eriksson
Department of Animal Nutrition & Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences, S-753 23 Uppsala, Sweden

Introduction

The most important determinant of the ruminant's forage utilization is to what extent the fiber fraction is degraded. The ruminal effective fiber degradability, i.e. the proportion of fiber degraded in the rumen under standard conditions, results from the competition between degradation and passage. Forage evaluation has traditionally been based on the proportion of organic matter (OM) degraded at maintenance. Routine evaluation of farm samples has been performed by in vitro methods calibrated against values from sheep fed at maintenance (Lindgren, 1979).

In feed evaluation models such as the Cornell Net Carbohydrate and Protein System (CNCPS) or NorFor Plan, the potential digestibility of neutral detergent fibre (NDF), rather than the effective digestibility of organic matter, is input to the model as well as the intrinsic NDF degradation rate. The potential digestibility of a feed fraction is understood as the proportion that would be degraded at zero ruminal outflow. Potentially degraded NDF (PDNDF) has been assayed by measuring its counterpart indigestible NDF (iNDF) in long term in sacco incubations of up to 28 days (Robinson and Kennelly, 1989). In the Nordic countries, several different incubation lengths from 96 to 504 h have been used for estimating iNDF for research purposes. Together with differing equipment and analytical routines, this has resulted in different iNDF estimations (Lund et al, 2004). Within the NorFor cooperation, analytical methods have been standardized and the reference method is now a 288-h incubation under relatively specified conditions. Alternative methods are required to substitute for the reference method in routine analysis. Near infrared (NIR) calibrations have been developed, although they do not perform satisfactorily on all sample types. Koukolova et al. (2004) could explain 0.79 of the variation in iNDF of typical Danish grass and grass/clover samples from in vitro OM digestibility and NDF analysis and 0.88 if also ADF and lignin measurements were included in the regression. Lignin was the single variable most correlated to iNDF but the iNDF estimation from lignin analysis in CNCPS has recently been shown to vary considerably for Scandinavian forages (Krämer et al., 2010).

The objectives of the experiments reported here were:

1. To investigate to what extent the routine Swedish in vitro OM assay VOS (Lindgren, 1979) could be used for predicting iNDF in forage samples

2. To develop alternative methods by increasing VOS incubation length and including ND treatment of sample residues

Materials and Methods

A total of 293 forage samples were included in the analysis. These samples originated from Swedish farms or from feeding trials and crop production experiments. Additional samples were provided from Norway (12) and Denmark (18). The sample types comprised grass (timothy, meadow fescue and perennial ryegrass), red clover, white clover, lucerne, maize and whole crop silages from barley, peas and lupins. The botanical composition was defined from manual sorting except for 55 mixed ley samples that were only broadly classified to

have a legume content over or less than 25% on a DM basis. All whole crop, including maize, and 55 of the ley samples were silages, while the rest were laboratory dried green material and in a few cases hay.

The in sacco 288-h iNDF analysis (NorFor) was performed at Kungsängen research center except for the Danish and Norwegian samples that were analyzed at the same research institutes that provided the samples. All in vitro fermentations were performed at the Kungsängen Research Center. Routine in vitro assays were according to Lindgren (1979). Briefly, 500-mg samples were ground in a hammer mill through a 1-mm screen and incubated in 49 ml buffer and 1 ml of coarsely strained ruminal fluid for 96 h in glass filter crucibles with Porosity 1 (100-160 μm). At incubation termination, liquid was filtered off and samples were rinsed three times with deionized water and three times with acetone before drying, weighing and ashing to determine OM residue.

Table 1 Forage samples analyzed for iNDF 288 h in sacco and 96 h in vitro organic matter residues (IVOMR) according to the VOS method (Lindgren, 1979). Maximum and minimum values within parentheses denotes the range after removing two outlier samples

	Mean	Min	Max
Grasses, legumes and mixed leys (249)			
NDF, g/kg OM	502	302	672
iNDF, g/kg OM	81 (80)	23 (23)	298 (187)
96 h IVOMR, g/kg OM	147 (145)	39 (39)	427 (299)
Maize silage (22)			
NDF, g/kg OM	424	310	568
iNDF, g/kg OM	87	50	125
96 h IVOMR, g/kg OM	157	89	247
Whole crop silage (22)			
NDF, g/kg OM	542	464	605
iNDF, g/kg OM	144	77	203
96 h IVOMR, g/kg OM	230	160	311

A sub-set of 9 grass-grass/clover samples were subjected to a series of treatments consisting of in vitro incubations of 96, 192 and 288 h, respectively, with or without subsequent treatment of incubation residues with ND solution. Incubation procedures were the same as for the routine assay (Lindgren, 1979) with the following exceptions: Glass filter crucibles with Porosity 2 (40-100 μm) were used, according to the standard for NDF analysis and samples subjected to ND treatment were at incubation termination only rinsed with deionized water before ND solution was added to the crucible and oven NDF determination was performed (Chai & Udén, 1997; Mertens, 2002).

iNDF results as well as in vitro OM residues (IVOMR) were expressed in g/kg OM to allow for direct comparison without confounding results by different ash contents. Regressions of iNDF against IVOMR were performed with procedures GLM and REG of SAS, using Cook's distance for identifying outliers.

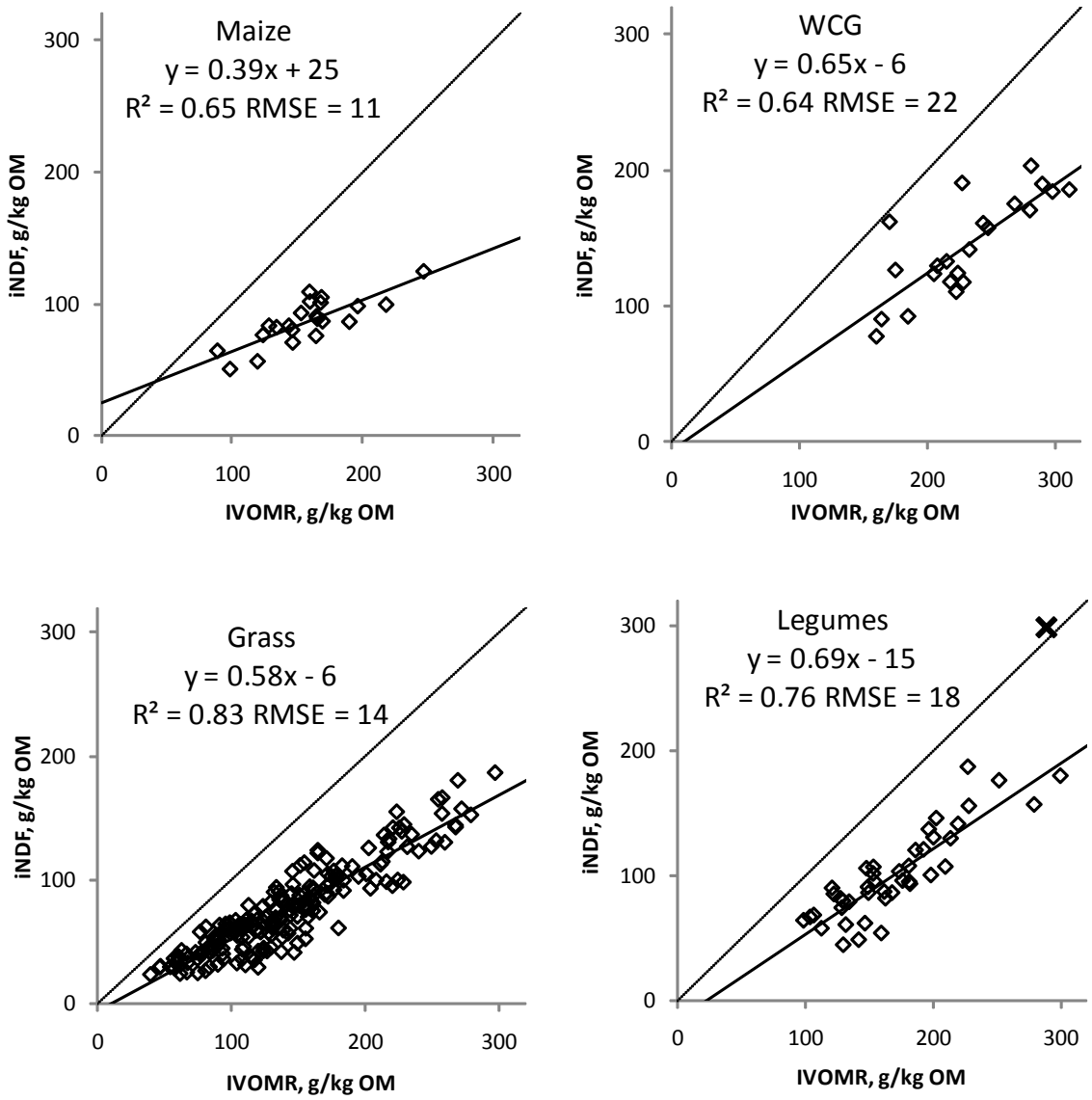

Figure 1 Plots of iNDF in sacco 288 h versus in vitro organic matter residue (IVOMR) from the standard Swedish 96 h in vitro procedure. WCG = Whole Crop Grain. Legumes = > 50% legumes on a DM basis. Regression line and the line y = x is displayed in all graphs. "X" in the legume graph denotes outlier lucerne sample not included in regression.

Results and Discussion

When iNDF values were regressed against IVOMR for the entire data, this resulted in different slopes for maize compared to leys and whole crop grain, but not in significant intercepts for any of the categories. This would partially be due to large variation among the different crops. However, the forage type would be known in a practical situation when equations are to be applied, so separate regressions were made for the three categories and then grasses and legumes were tested for different regressions, with the dividing line set to 50% legumes on a DM basis. Slopes and intercepts did not differ between grasses and legumes. This could be explained by the more narrow range of IVOMR values with >50% legumes, where results below 100 g/kg OM were absent. Results are displayed in Figure 1, with separate graphs for grasses and legumes, respectively. Among the legumes, a lucerne

sample was identified as an outlier and removed from the regression. There were only three lucerne samples included in the data, so it was not possible to test for a separate lucerne regression, even if it could be assumed that the combination of large iNDF content and relatively high DNDF degradation rate typical for lucerne should result in a different equation.

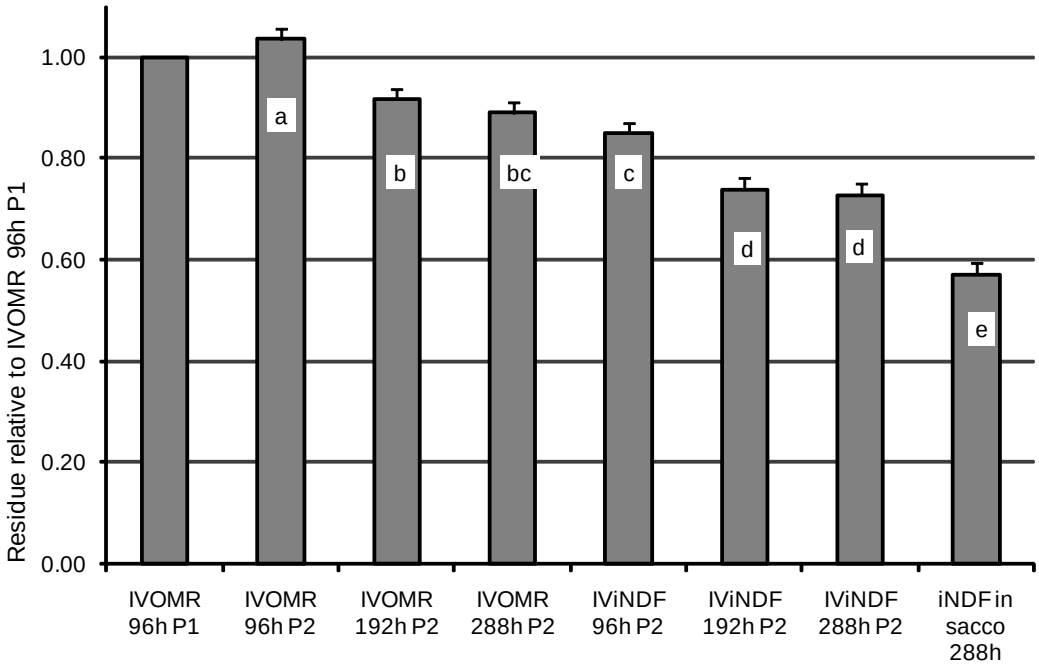

Figure 2 Relative ash-free incubation residue for 9 grass-grass/clover samples. IVOMR = In Vitro Organic Matter Residue; IViNDF = In Vitro NDF residue. P1 and P2 refers to porosity 1 and 2, respectively, of the glass filter crucibles used for in vitro incubation. P1 filters were only used for the control treatment (Lindgren, 1979). Bars indicate standard error of difference. Treatments without a common letter differ ($p<0.05$ for Bonferroni test).

The in sacco iNDF value was lower than all in vitro treatments (Figure 2). IViNDF was 81% of IVOMR, when compared by incubation time. Prolonging the incubation time from 96 to 192 h lowered amounts of IVOMR and IViNDF with 25 g/kg OM, whereas further prolonging to 288 h only changed in vitro results numerically with 4 g/kg OM. As a comparison, Koukolova et al. (2004) reported a marginal disappearence of 16 g iNDF/kg DM when prolonging in sacco incubation from 168 to 504 h. The higher IViNDF compared to in sacco iNDF is probably a result of the more harsh microbial conditions in vitro. It is also possible that losses in the in sacco procedures contribute to the differences.

Regressions of in sacco iNDF against the in vitro treatments were worse with ND treatment of the in vitro residues (IViNDF vs. IVOMR, Table 3). This may be due to the small residual amounts (35- 120 mg of the 500 mg feed sample) and the larger variation this resulted in with the analytical steps and weighing necessary for IViNDF determination.

Table 3 Regressions of iNDF in sacco (g/kg OM) vs in vitro organic matter residue (IVOMR) or in vitro iNDF (IViNDF). 9 grass-grass/clover samples

	IVOMR 96h P1	IVOMR 96h P2	IVOMR 192h P2	IVOMR 288h P2	IViNDF 96h P2	IViNDF 192h P2	IViNDF 288h P2
Intercept	-12	-15	-8	-3	-2	8	11
Slope	0.63	0.63	0.67	0.65	0.69	0.70	0.69
R^2	0.89	0.89	0.91	0.88	0.76	0.81	0.79
RMSE	18	18	16	19	27	23	25

Conclusions

The routine 96 h in vitro incubation of Lindgren gives results that are well correlated to iNDF in sacco within forage categories but there is not a universal relationship across all forages. Prolonging in vitro incubation to 192 h has a potential to improve correlation between in sacco and in vitro methods. The crude in vitro organic matter residue gave better estimates of iNDF in sacco than did the in vitro iNDF residue.

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Eurofins is an international group of laboratories providing a range of testing and support services to food and agricultural industries.

Sweden
Charlotte Åkerlind
Key Account Manager
Eurofins Food & Agro Sweden AB
Box 887
531 18 Lidköping
Sweden
Phone: +46 10 490 84 12
Mobile: +46 70 660 94 34
e-mail:CharlotteAkerlind@eurofins.se
Website: *www.eurofins.se*

Denmark
Martin Frandsen
Key Account Manager
Landbrug
Eurofins Steins Laboratorium
Petersmindevej 1
8362 Hørning
Denmark
Phone:+45 7660 4242
Mobile:+45 2177 3744
e-mail: mrf@eurofins.dk
Website: *www.eurofins.dk*

Norway
Cathrine Eriksen
ASM Forage
Eurofins Norsk Matanalyse AS
Møllebakken 50
NO-1506 Moss
Phone: +47 94 50 42 78
Mobile: +47 94 50 42 78
e-mail:Cathrine.Eriksen@eurofins.no
Website: *www.eurofins.no*

BLGG AGROXPERTUS

BLGG AGROXPERTUS

Jan Bakker
M +31 (0)6 52 00 21 02
E jan.bakker@blgg.agroxpertus.com

PO Box 115, NL- 6860 AC Oosterbeek
Nederländerna

Energy and protein requirements for horses in the Nordic countries

D. Austbø
Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, N-1432 Ås, Norway

Introduction

In 1992, a NJF working-group "Feed evaluation and nutrient recommendations for horses" was established. The mandate was to find a common basis for feed evaluation and nutrient recommendations in the Nordic countries. The members of the working-group were researchers representing Finland, Sweden, Denmark, Norway and Iceland. Until this time, systems for energy and protein evaluation of feedstuffs were based on existing systems for ruminants (and sometimes swine). The calculations of energy and protein requirements differed between the Nordic countries and were based on foreign and national recommendations and research findings. In Sweden, Axelsson published data from digestibility experiments with horses in 1940 and 1941 (Axelsson, 1940 and 1941). Some years later Olsson and co-workers published more data from digestibility experiments with horses (Olsson et al., 1949). Occasionally, experiments on horse nutrition were also carried out in the other Nordic countries providing information about the use of local feedstuffs and roughage qualities (Christensen, 1967).

Several systems were studied including the German system by H. Meyer (DLG, 1992), the American system (NRC, 1989) and the French system (UFC/MADC) (INRA, 1984). In the Netherlands, they had been using the French system for several years. During this period they had tested the system and modified it using results from Dutch experiments with horses, in addition to German and American data. The Dutch feed evaluation and nutrient recommendations for horses is therefore mainly based on the French system (CVB, 1996). The Dutch (and French) requirements for minerals and vitamins were in accordance with NRC (1989).

The Nordic working-group delivered its report in 1996 recommending that the Nordic countries should use the French energy evaluation system for horses (UFC-system) (INRA, 1984) with the modifications made by the Dutch (CVB, 1996). This system is based on experiments with horses and is built up in a traditional manner from gross energy (GE), digestible energy (DE), metabolisable energy (ME) to net energy (NE). The NE is expressed as Feed Units for Horses (UFC) and is defined as the NE content of 1 kg standard barley fed to horses on maintenance, which is equal to 9,414 MJ.

Given this system, Sweden could go on defining energy as MJ ME, Finland as ME based Feed Units and Denmark, Norway and Iceland as Feed Units of net energy (NE). Later on, Finland has changed the energy unit to MJ ME.

In the French system is also defined a new protein evaluation system (MADC). The working group did not accept this system and decided to follow the Dutch recommendation and go on using Digestible Crude Protein (DCP), based on digestibility figures obtained from experiments with horses or sheep.

To calculate energy in the different energy units, the following factors can be used:

1 UFC (NE) = 13.45 MJ (ME) Denmark, Norway, Iceland → Sweden, Finland

1 MJ (ME) = 0.0743 UFC Sweden, Finland → Denmark, Norway, Iceland

Feed evaluation

The French feed evaluation system is based on analysis of crude fibre (CF) and crude protein (CP) in addition to digestibility figures obtained with horses. Alternatively, equations including water soluble carbohydrates can be used. If horse digestibility figures are not available, ruminant values can be used after recalculation.

Maintenance energy requirement

The maintenance requirement is calculated according to metabolic body weight ($BW^{0.75}$). The calculations give requirements for cold-blooded mares and geldings (Table 1 and 2).

Table 1 Calculation of the maintenance requirement for adult horses (cold-blooded).

Form	Conversion	Requirement	Unit	Country
NE		0.351	MJ/kg $BW^{0.75}$	
NE	NE/9.414	0.0373	UFC/kg $BW^{0.75}$	Denmark, Norway & Iceland
ME	NE/0.7	0.50	MJ/kg $BW^{0.75}$	Sweden & Finland

Corrections are being calculated for different types of horses.

Cold-blooded ponies and draught horses	No correction
Warm-blooded horses and cold-blooded trotters	5% addition
Thoroughbred	10 % addition

For stallions an extra 10 % is calculated compared to mares and geldings.

Table 2 Calculation of the maintenance energy requirement for adult horses, BW 500 kg

Country	Type of horse	Mare/Gelding	Stallion
DK, NO IS (UFC)	Cold-blooded, ponies and draught horses	3.9	4.3
	Warm-blooded horses and cold-blooded trotters	4.1	4.5
	Thoroughbred	4.3	4.7
SE, FI* (MJ ME)	Cold-blooded, ponies and draught horses	52.9	58.1
	Warm-blooded horses and cold-blooded trotters	55.6	61.1
	Thoroughbred	58.1	63.9

*****Finland has made some modifications, but they are only published in Finnish.

Maintenance protein requirement

The protein requirement of adult horses is calculated as 3 g digestible crude protein (DCP) per kg $BW^{0.75}$ per day (Table 3).

This is in accordance with recommendations from Germany (3,0 g per kg $BW^{0.75}$ per day), France (2,8 g per kg $BW^{0.75}$ per day) and USA (calculated mean value 2,8 g per kg $BW^{0.75}$ per day).

Table 3 Maintenance requirements of DCP per energy unit in the different Nordic countries:

Sweden, Finland	6 grams DCP/MJ
Denmark, Norway, Iceland	80 grams DCP/UFC

Energy requirement for work

The energy requirement for work is difficult to describe in detail for different types of work. The Nordic countries use a similar system as described by NRC (1989).

Energy requirement for work/training (percentage of maintenance requirement):

Light work : + 25%

Medium work: + 50%

Hard work : + 75%

Intense work : + 100%

Protein requirement for work

The protein requirement for work is defined as DCP per energy unit (Table 4). In Germany, France, USA and The Netherlands, the DCP-content per unit energy for work is the same as for maintenance.

Table 4 Protein requirement for work per energy unit in the different Nordic countries:

Sweden, Finland	6 grams DCP/MJ
Denmark, Norway, Iceland	80 grams DCP/FEh

Requirements for pregnancy, lactation and growth are also defined.

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The history of feed evaluation for ruminants, with special emphasis on the Nordic countries

M. Riis Weisbjerg, M. Rinne[1], R. Spörndly[2], A. Ekern[3] and O. M. Harstad[3]
Danish Institute of Agricultural Sciences, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark.
[1]MTT Agrifood Research Finland, Jokioinen, Finland.
[2]Kungsängen Research Centre, Swedish University of Agricultural Sciences, Uppsala, Sweden.
[3]Norwegian University of Life Sciences, Ås, Norway.

The history of feed evaluation

Energy evaluation.
It has been known for centuries that different feedstuffs can have different feeding values for animals. Thaer (1754-1828) was probably one of the first to systematize this knowledge into a feed value (hay value). Chemical fractionation became possible in the 19th century, and was used in combination with calorimetric measurements to determine the energetic contribution of different feed components (Weende analysis). The Weende analysis, also called the Proximate analysis, separates feed dry matter (DM) into crude ash, crude protein, crude fat, crude fibre, and nitrogen free extract (rest fraction). For nearly 150 years, the Weende analysis has been the basis for all feed evaluation systems for dairy cows. Digestibility was used as basis for biological availability of feeds from the mid of the 19th century (Henneberg & Stoman, 1860; Flatt, 1988).

In the late 19th and the early 20th century, the principles used in all 'classic' feed evaluation systems were established by the work of Wolf, Armsby, Kellner and others. In all classic systems (DE, ME or NE) energy was the primary unit determining the value of the digested nutrients. Gross energy (GE) is the heat of combustion of the feed. GE minus energy in faeces is defined as the digestible energy (DE). DE subtracted by the energy in excreted urine and methane is defined as metabolisable energy (ME). In net energy systems, ME is separated into: a) thermic energy, b) energy for maintenance, and c) energy for production (milk, weight gain, foetus, or work).

Total Digestible Nutrients (TDN) became the basis of the American system, calculated as the sum of all digestible Weende components with energetic correction of 2.25 for fat. In Europe feed evaluation was based on Kellner's starch unit, which in theory is a net energy (NE) system predicting fat accretion in steers. The fattening feed units (FU) were essentially Kellner's starch units. With a modification of the energetic value for digestible protein, the starch unit also became the basis for the Scandinavian FU. In the middle of the 20th century, systems based on metabolisable energy (ME) were proposed in the UK, and in Holland a net energy system based on ME was introduced. The Dutch system has gained popularity in many countries. However, these systems are all based on digestible Weende fractions.

Correction factors.
In Kellners starch unit system for fattening, the digestible nutrients were multiplied with energy factors on a carbohydrate basis. The factors were: digestible NFE and crude fibre, 1; digestible protein, 0.94; and digestible ether extract, 2.41 (oilcakes), 2.12 (cereals), and 1.91 (forages). Kellner parameterised his system using ground and purified feed fractions but noted that natural feedstuffs did not result in the expected fat deposition. Kellner therefore introduced a reduction factor (value number) based on the crude fibre concentration in a feed. In several of the systems based on the starch unit, e.g. the Scandinavian FU (SFU), the value

number should, in principle, be determined in production trials and tabulated. However, for practical feed evaluation, especially on forages, it was not satisfactory to rely on single table values, due to the great variation between forage batches. The Scandinavian feed unit system, as used in Denmark, was therefore changed to a calculation based on multiple regression on DE and crude fibre concentration, so back to Kellners crude fibre correction! (Weisbjerg and Hvelplund, 1993). Corresponding corrections in the Dutch system are currently based on the energy density q (100*ME/GE) (Van Es, 1978) and a similar principle was introduced in the NRC (2001) system.

The basic values for digestible Weende nutrients were determined in digestibility trials using animals fed at maintenance level. Up to the middle of the 20th century, the experimental animals were usually cattle (steers or cows) but, in the last 40 years, the standard animal has been the sheep, as experiments with sheep are less resource demanding. This change had a minor effect on the systems, as digestibility in cattle and sheep are comparable for organic matter, although protein digestibility is slightly higher and fibre digestibility slightly lower in sheep compared with cattle (Südekum et al., 1995). Around year 1900, the feed intake of milking cows was much closer to cows fed at maintenance as compared with today. Digestibilities obtained at maintenance, therefore, gave reliable values when compared with milking cows. However, as production and feed intake increased, especially during the last half of the 20th century, it became clear that there was a diminishing production return with increasing feeding level. In the Dutch system, this was managed by a reduction in the predicted NE with 1.8 %-unit per multiple of maintenance. In Denmark a correction called 'Feed Efficiency' was introduced, where feed efficiency was estimated in production experiments as the NE (FU) obtained in maintenance and production in proportion of FU input in feed, and feed efficiency was related to feeding level (Danfær, 1983). This approach has been further developed recently by Kristensen et al. (2003). They predicted feed efficiency from feeding level, feed intake capacity of the cow, and digestibility of the forage in the ration. Similar analyses have also been performed in Finland (Nousianen et al., 2009; Huhtanen et al., 2009).

The classic energy evaluation systems, which are still used worldwide, are all based on work done more than 100 years ago, and only slight modifications have been introduced during the last half of the 20th century. The underlying reason for choosing DE, ME or NE systems, and different subsystems, are probably more historical in nature than founded on science. In theory, NE systems should better describe feed induced variation than ME systems, which again should do better than DE systems. However, variation in digestibility is by far the greatest contributor to variation among feedstuffs. The prediction of energy in methane and urine is required for the estimation of ME and for the NE systems, also heat increment must be estimated. The variables contribute less to the final energy value and are also more unpredictable. Therefore, despite of the theoretical benefits of having a more detailed system, the increasing need for estimations, assumptions and use of tabulated values often counteract these advantages.

Protein evaluation.

It has been known for many years that absorbed protein should be quantified in the form of amino acids. The first protein evaluation systems for ruminants were therefore based on digestible true protein. However, in the mid 20th century, it was recognised that rumen microbes are able to utilize ammonia for microbial protein synthesis as demonstrated by Virtanen (1966) who maintained milk production on a ration free of true protein. In 1961, the Nordic countries decided to change to digestible crude protein (Eriksson, 1961). Further research in the microbial degradation and synthesis of protein in the rumen, and the major

impact that rumen protein metabolism has on the supply of amino acids to the host, resulted in development of a number of 'modern' protein evaluation systems in the period from 1970 to 2000 (e.g. INRA, 1978; ARC, 1984; NKJ, 1985; Tamminga et al., 1994). These protein evaluation systems were the first feed evaluation systems to introduce a more mechanistic approach of describing nutrient metabolism in the digestive tract.

Like the classic energy evaluation systems, all 'modern' protein evaluation systems are based on similar principles, where the main variables are feed protein degradability in the rumen (measured) and estimated microbial protein synthesis.

Additivity.
Both the classic energy evaluation systems and the modern protein evaluation systems assume additivity except for some corrections for feeding level and different nutrient limitations (starch, sugar, fibre etc.), which have been included in some of the ration formulation systems. It is therefore not possible to describe and predict all the interactions (associative effects) especially related to ruminal fermentation.

Requirements.
Except for true NE systems, energy and protein evaluation systems include both feed values and nutrient requirements for maintenance and production. Comparisons of different systems for their ability to predict production have shown that the different systems differ little in their ability to predict production when energy values and requirements of each specific system are used for the comparisons (Kaustell et al., 1997), probably because requirements absorb some of the inaccuracies in energy values.

Ration formulation.
Will the different systems, as used in different countries, result in differences in ration composition? It is known that the different energy evaluation systems rank feedstuffs differently. If barley is set to 100 in all systems, then for example SFU (Denmark), VEM (Holland) and TDN (USA) estimate barley straw to 24, 45 and 54; grass silage to 67, 75 and 82; and soy bean meal to 119, 102 and 101, respectively (Van der Honing and Alderman, 1988). However, as the requirements are linked to the energy values, and the intake capacity of the animals, systems will not vary much in concentrate:forage ratio and, therefore, rations obtained, using different energy evaluation systems, will not vary very much. Therefore, different energy evaluation systems, will probably not result in large differences in ration composition, if other conditions are similar. Of much larger importance for ration composition, is the availability and price of feeds, technology for feeding, strategies for feeding and restrictions other than energy and protein values used in the respective ration formulation systems.

Forage availability will typically be determined by both climate and tradition. An example of tradition is that, until recently, fodder beets were used in large amounts in Denmark, but not in neighbouring countries even in areas with similar potential for beet production. Strategies chosen for feeding can heavily affect the ration fed to cows, especially at peak lactation. In Denmark there has been a tradition for flat rate feeding. This means that all cows are fed the same amount of concentrate in the first part of lactation, independent of individual milk yield. The cows can, therefore, only regulate energy intake by increasing forage intake, a system which requires ad libitum feeding of high-quality forages. In contrast to this is the feeding according to requirements also in early lactation, which has been a tradition in many countries, and which can result in large quantities of concentrate to high yielding cows. Total mixed rations (TMR), where all cows or cows in a certain lactation stage are fed the same mixture of feeds, have become very popular recently.

Feeding principle and individual cow yield will therefore result in heavy interactions in energy concentration in rations fed to individual cows. TMR feeding will result in the same energy density to all cows fed the same mixture, feeding according to requirements will result in increased, and flat rate feeding will result in decreased ration energy density with increased milk yield as increased milk production normally is followed by increased feed intake.

Two major sources of variation in actual ration formulation between countries are ration restrictions (e.g. limits on starch, structure etc.) and ad libitum feed intake predictions used. These parts of the ration formulation systems vary greatly among countries and in some countries, they are not 'officially' part of the ration formulation system, but are based on knowledge gained by farmers and extension specialists of well-functioning rations. The intention with most restrictions is to ensure that the rations do not induce production disorders as e.g. acidosis, laminitis and abomasal displacements. In Denmark, maximum limits for sugar and starch, and minimum limits for chewing time and digestible cell wall carbohydrates have been used to ensure formulation of healthy rations for dairy cows. In some countries, minimum limits for total NDF or forage NDF are used with the same purpose. In USA, recommendations are given for a combination of minimum forage NDF, minimum total ration NDF, maximum dietary non-fibre carbohydrates and minimum dietary ADF with the same purpose to ensure a healthy ration for the cows (NRC, 2001). The ration restrictions, feeding strategies, feed intake predictions and forage availabilities can vary considerably among countries and they can result in large variations in the formulated rations, especially at peak lactation, and are much more important for country differences than the energy evaluation systems.

The Nordic feed evaluation history in brief

The Nordic countries have to a large degree had a common early feed evaluation history. Based on Niels Johannes Fjords (1825-1891) and others (Winkel, 1880; Svendsen, 1886, cf. Breirem and Homb, 1970) work with production experiments with dairy cows, feed values were estimated for a number of feedstuffs and simple feed units were introduced together with the start of milk recording in 1885 in Denmark and 1898 in Sweden (Hansson, 1913).

Table 1 Pound[1] of feed in the 1885 Danish feed unit, and kg of feed in the 1898 Swedish feed unit to supply one feed unit (Hansson, 1913).

1	Concentrate
2.5	Hay
4	Straw
10	Green forages, fodder beets, swedes
12.5	Turnips
	1/6 day grazing

[1] Pound and kg as fed, thereby a 1898 Swedish feed unit was twice a 1885 Danish feed unit

The concentrate in Table 1 was an unspecified concentrate mix. The evaluation of concentrates was refined by Hansson (1913) based on *53de Beretning* (Anom, 1902) and *55de Beretning* (Anom, 1904) from *Laboratorium for landøkonomiske Forsøg*. Kellner published his starch units in 1905, and based on this work and Nordic experiments, Hanson (1913) proposed the factor 1.43 instead of 0.94 (protein/carbohydrate factor) for the Scandinavian feed unit (SFU). The factor 0.94 in the starch unit was based on the value of protein for fat accretion, however as dairy cows produce milk protein, Hanson argued that digested protein should be valued with the full combustion heat.

After publication of Hansson's Handbook in 1913, a meeting was held in Copenhagen in the autumn of 1915 where researchers and representatives for milk recording in Denmark, Norway and Sweden agreed on the unit for feed as 1 kg of 'normal' grain, and after some years, full agreement on the SFU was established (Hansson, 1939). The SFU was then calculated using relative values for the digested nutrients given in Table 2 and the value obtained, using these relative values, were further corrected using value numbers obtained in experiments. In Kellner's starch unit system, a crude fibre correction was used and this was also discussed in the Nordic countries but Hansson (1939) conclude: *"Vi göra därför säkerligen klokt i at fasthälla, att värdetalet i princip bör vara lika med fodermedlets genom försök uppmätta effekt i procent av den på grund av näringsämnenas smältbarhet teoretisk beräknade och att de på desammas halt av växttråd fotade avdragen endast äro att betrakta som genvägar, värda att använda som hjälpmedel, då inga direkta försöksresultat föreligga*" (We will do best holding on to the concept that the feeding values, in principle, should be equal to what's been achieved in animal experiments, expressed in percent of theoretically calculated and that using crude fibre corrections can only be regarded as a solution when no animal experiments are available). The use of experimentally determined value numbers (Breirem, 1969) were probably one of the strengths of the SFU as it guaranteed a production response according to SFU concentration (if the feeds had been tested!). But, it was also one of the main problems as value numbers always were historical. Estimation of value numbers for feeds at hand could, therefore, only be 'guestimates'. Requirements for milk production were settled to equal 0.4 SFU per kg of fat corrected (4% fat) milk by Frederiksen (Frederiksen and Østergaard, 1931). The SFU continued as common system for many years until Finland introduced the fattening FU (FFU) in 1958-59, Sweden introduced ME in 1967 and Norway FFU in 1969, whereas Denmark kept the SFU (NJF, 1969).

Table 2 Reduction factors for digestible nutrients in Kellner's starch unit and in the Scandinavian feed unit (SFU) (Hansson, 1913).

Digestible nutrient	Starch unit	SFU
Nitrogen free extract (NFE)	1	1
Crude fibre	1	1
Protein	0.94	1.43
Fat oilcakes	2.41	2.41
Fat cereals	2.12	2.12
Fat forages	1.91	1.91

The above account indicates that there existed a consensus in the Nordic countries during the first half of the 20th century for a common energy system many years. However, behind the scenes considerable disagreements were aired during a number of dramatic discussions. A very heavy critique of the FU had started in Demark around 1901 by Henriques and continued in 1904 by Hindhede. Ærsøe (1943) defined this period as *Kampens år* (The Fighting Years), where the main argument was that evaluation of feeds based on their names instead of chemical composition, especially protein content, would result in erroneous evaluations. In Sweden, Axelsson (1941, 1949) argued for a ME system and Breirem and Homb (1970) in Norweay discussed the pros and cons of the different systems in details. The root of the problem was that the SFU had from the start been developed as a practical tool more than a scientifically based instrument.

Since the founding of NJF (Nordic Association of Agricultural Scientists) in 1918, and the organisation of an Animal Science section in the 1920's (Eriksson, 1998), NJF has had a major role in discussions on a common Nordic feed evaluation system. A return to a common energy evaluation system has been discussed several times, also together with the formulation of a Nordic feed table (NJF, 1969). Later a NJF working group (1987-1991) funded by

Nordisk Ministerråd with the mandate: "To assess the present systems in various countries, and to prepare a proposal and a project application for research needed to work out a common Nordic system". However, this group, lead by Frik Sundstøl, could not recommend changing to a common classical system, but recommended Nordic cooperation in development of a 'substrate based' feed evaluation system (Kristensen et al., 1991). Based on this, a Nordic project, lead by Torben Hvelplund, worked in two periods from 1994-1998 and from 2000-2004 (Karoline, 1998; Karoline Seminars, 2004), mainly on developing the 'Karoline' model based on previous work by Danfær (1990). However, this work did not result in a system used in practice.

The dairy farmers unions in Sweden, Norway, Iceland and Denmark decided that they wanted a common ration formulation system, and decided after some deliberation to choose the model developed by Harald Volden in Norway. After further developments, lead by the extension service in respective countries, the system was introduced in practice in 2007 (Rygh et al., 2006). However, Finland is not a partner in this cooperation.

Protein evaluation

Originally, protein was evaluated as digestible true protein, however, both problems in determining and defining true protein, and the degradation and synthesis of protein in the digestive tract were reasons for a proposal to substitute digestive true protein for digestive crude protein (Axelsson, 1942). This took time and not until 1961, the change was officially decided in the Nordic countries (Eriksson, 1961). Digestible crude protein was used until the introduction of the AAT/PBV system (Madsen, 1985). Later, some updates of the AAT/PBV system followed as described by Madsen et al. (1995).

Country specific feed evaluation history

The country specific developments in the Nordic countries are described below, and in Table 3, the major changes taking place in Denmark, Finland, Norway and Sweden are shown.

Table 3 Development in feed evaluation in the Nordic countries, year for introduction of systems

Country	Energy					Protein			
	SFU	FFU	ME	FUm	NorFor	Digestible true protein	Digestible cru. protein	AAT/PBV	NorFor
Denmark	1915				2007	Until 1961	1961[2]	1985	2007
Finland	1915	1959	1995[1]			Until 1961	1961[2]	1995	
Norway	1915	1969		1993	2007	Until 1969	1969	1993	2007
Sweden	1915		1967		2007		1961[2]		2007

[1]Maff, (1975) [2]Year for Nordic decision

Denmark

Energy. The Scandinavian feed unit (SFU) was introduced in early 20th century, and is still in use. Feed efficiency was introduced in mid 1980's and the last version was published by Kristensen et al. (2003). Calculation of FU changed from using value numbers, requiring feed table values, to a multiple regression on digestible energy and crude fibre requiring analytical values for chemical composition and digestibility, determined by in vitro rumen fluid or enzymatic methods (Weisbjerg and Hvelplund, 1993). In between, there have been attempts to exchange value number with digestible crude fibre (Møller et al., 1983) but, due to unavailability of crude fibre digestibility values, this system was newer introduced in

practice. A practical forage evaluation system, using calculated digestible cell wall carbohydrates and based on ideas from Verner Friis Kristensen was in use for a few years (Frank, 1988). The SFU system is presently slowly replaced by the NorFor system which was introduced recently and is expected to be the dominant system in Denmark. NorFor is today (spring 2010) still only used in a minority of feed plans made in practice.

Protein. Estimation of digestibility of crude protein changed around 1980 to use of the Lucas principle, where apparent CP digestibility is a function of the CP concentration in DM (Thomsen, 1979). The AAT/PBV system was introduced in 1985 (Madsen, 1985) and was, after few years, used for practically all dairy cow ration formulations, however digestible crude protein is still used for young stock. Norfor is now introduced also to cover protein evaluation, whereby energy and protein evaluation will be integrated.

Feeding practices. Dairy cows in Denmark were traditionally fed forage and concentrate separately and concentrate was fed according to yield. In the 1980's, flat rate feeding took over based on the work of Østergaard (1979). Cows were fed a constant concentrate level in early lactation, independent of milk yield, and allowing a forage or mixed basal ration ad libitum. In the 1990's, TMR, especially TMR1, with only one mix for all lactating cows, replaced flat rate feeding. In the last decade, robot milking has forced a change from true to partial TMR in many herds (approx. 20% of Danish cows are automatically milked) as concentrate feeding in the robot is necessary to obtain a satisfactory milking frequency.

Finland

Energy. Active work on feed evaluation and ration formulation took place in Finland in the early 1900's. The energy value, based on Nils Hansson (1913) from Sweden, was used until 1959, when the official calculated energy values, based on the coefficients presented by Oskar Kellner (1910), were taken into use. The energy values were presented as Finnish feed units relative to the energy value of barley.

Feed tables and feeding recommendations were originally published as appendices in animal husbandry handbooks and calendars. In 1982, "Feed Tables and Feeding Recommendations" were published for the first time as an independent publication, based on work led by docent Maija-Liisa Salo from the University of Helsinki (Salo et al., 1982). The publication included the principles of calculating feed energy and protein values, feed tables and feeding recommendations for the following animal species: ruminants, pigs, poultry and fur animals.

In 1995, new "Feed Tables" were published and values for horses were included in the publication (Tuori et al., 1995). Major changes took place in the formulation of principles for feed evaluation. An energy value system, based on metabolizable energy (MAFF 1975), was taken into use. Finnish feed units were still used to express the values, but now they were calculated as metabolizable energy (MJ/kg DM) / 11.7.

Protein. Protein value was expressed as digestible crude protein until 1995, when a metabolizable protein system AAT/PBV, developed in Nordic cooperation, was adopted. Some national modifications were implemented, and a national development continued in the following years in terms of e.g. adjusting values of effective protein degradability values of some feeds. The effective degradability values were originally based on *in situ* (nylon bag) incubations, but as the limitations of that method became more evident, the values were adjusted, based on information from in vivo experiments (both physiological and production experiments) and the Cornell protein fractionation system.

Feeding practice. Similar trends in the practical feeding solutions have been observed in Finland as reported above for Denmark. Due to smaller herd size, the uptake of new systems, such as TMR and robotic milking, has been somewhat slower.

Further developments. Since 1990's, the changes in feed evaluation systems have been systematically tested using large data sets from production trials to see if theoretical concepts can be verified in practise. The latest updates of ruminant feed values in conjunction with several updates involving pig, poultry, horse and fur animal feeds and feeding recommendations were done in the spring of 2010 and will be implemented from 1 September 2010 and onwards. The current version of the "Finnish Feed Tables and Feeding Recommendations" is published on the Internet (MTT 2010). Also Swedish and English language versions of the website are available. A publication will be published in Finnish by MTT Agrifood Research in autumn 2010.

The ongoing modifications include presentation of feed energy values as MJ of metabolizable energy which means that the Finnish feed units will no longer be used. This improves the transparency of the system and aids in international comparisons of energy values. The energy value continues to be based on ME (MAFF, 1975).

Further modifications have also been made to the AAT/PBV system (MTT, 2010). The substrate for microbial protein was changed to digestible organic matter minus undegradable protein (previously digestible crude carbohydrates plus degradable protein). Also, the proportion of amino acid N in microbial protein was increased from 0.70 to 0.75, and for undegradable feed protein from 0.85 (concentrates) or 0.65 (forages) to 1.

Ration formulation for dairy cows in Finland will continue to be based on static feed values, but new elements will be taken into use in practical ration formulation. This is done through the programme CowCompass, which is under development in a project led by Finnish rural advisory organization ProAgria. The ration optimization of CowCompass is based on the Lypsikki model, which was developed originally at MTT in a farm nutrient balance project.

The system is based on relatively simple and well-established feed analyses. Using empirical relationships, based on a large data set, allows realistic estimates from feed intake, associative effects in digestion and milk production responses to be made. Rations can be optimized, based on least-cost ration or maximum milk income minus feed cost.

The relative feed intake indices (Huhtanen et al. 2007, 2008) and effects of animal factors on feed intake (Huhtanen et al. 2010) will be taken into account when estimating the feed intake of the cows. The level of DM intake and ration composition affects the digestibility (Huhtanen et al. 2009) and thus, the amount of energy available for the cow. Finally, milk production responses to energy and nutrient supply will be calculated based on empirical relationships based on the large data set of milk production experiments used in the analyses mentioned previously.

Norway

Energy. The SFU was introduced in 1915-16 and was in use until 1969, when it was replaced by the FFU system. Based on extensive experimental work, the FU milk (FUm) was introduced in 1993 (Ekern et al., 1991), where 1 FUm was defined as 6900 KJ NE for lactation according to van Es (1975).

NorFor, the Nordic Feed Evaluation System for cattle, was introduced by the Dairy Association (TINE) in 2007 and is currently used by a majority of the dairy farmers. In order to improve and modernize the feed evaluation and ration formulation, NorFor takes into account interactions between feed and animal characteristics in a non-linear model. Thus, the feeds energy (NE) and protein (AAT and PBV) values will vary with diet composition and dry matter intake. The system is based on knowledge about; 1) chemical composition of the feeds; 2) dry matter intake of the cow; 3) degradation and absorption in the gastrointestinal

tract; 4) microbial synthesis of organic compounds in the rumen and large intestine; and 5) the efficiency of the intermediary metabolism.

Protein. Digestible true protein was officially used until 1969, when it was replaced by digestible crude protein. In praxis, digestible crude protein had been used from 1941-42 but 50 years later, the AAT/PBV system took over in 1993. From 2007, protein evaluation has been integrated in Norfor

Feeding practices. Also in Norway, restrictive feeding with both forage and concentrate according to yield, used to be the most common feeding system. During the last decades, it has been more and more common to practise ad libitum feeding of roughage, and supply concentrate individually, calculated as the difference between requirement and estimated intake of roughage. The use of mixed rations has increased during the last years, but still constitutes only a small proportion. Partial Mixed Ration System (part of the concentrate given separately) is more commonly used.

Sweden

Energy. As mentioned earlier, the SFU system was replaced by the ME system in Sweden 1967. The major concern was the problem of a reliable estimation of the feeding value of roughages. Work was intensified in refining the method to evaluate the feeding value of forages at different stages of development. This resulted in the introduction of the VOS-method for estimating the ME-value of roughages (Lindgren 1979, 1981). The method was based on an *in vitro* incubation of the feed sample in rumen liquor. For estimation of the ME in concentrates, national feed tables containing feed digestibility values and the ME coefficients of Axelsson (1941) were used. The ME system was used with a constant allocation for maintenance, growth, gestation and milk production until 1994. In 1995, a correction was introduced which increased the allocation of ME at higher intake (Andresen, 1994). This "classical ME-system" was without exception used in ration calculations for all dairy and beef cattle as well as sheep and goats until the year 2000. The system is still in use, as described in the national feed tables (Spörndly, 2003), but in the new millennium two new alternative systems have been introduced in Sweden.

In 2001, the main commodity and feed supplier in Sweden, *Lantmännen*, introduced an alternative system, the LFU-system (Lantmännen, 2003) for dairy cows. The system, although indirectly based on energy, is a substrate-based system. It does not include any energy concept. Instead, it is based on the estimated DM-intake depending on the milk production. For each production level, the DM is partitioned into defined percentages of crude protein, ether extract, starch, NDF and a rest named easily soluble carbohydrates. Crude protein and NDF are further divided into a rumen degradable and a rumen undegradable fraction. These variables were all well known from existing systems, except for the degradable NDF fraction. A new NDF variable was estimated in the same way as for effective protein degradability (EPD) and consequently named EFD, effective fibre degradability.

Between 2006 and 2009, the non-additive NorFor system (described elsewhere) is slowly being introduced in Sweden by the Swedish Dairy Association, resulting in three feeding systems for dairy cattle presently being used in parallel in Sweden.

Protein. Digestible crude protein, using national tabulated digestibility values for the different farm animals, was used for ruminants in Sweden until 1990. In 1991 the AAT/PBV-system was introduced with the intention to be a common Nordic system (Madsen, 1985 and Madsen et al., 1995). However, the system tended to be slightly different in the different Nordic countries. The way the AAT/PBV-system is applied in Sweden to estimate protein

supply to cattle, sheep and goats is described in "*Fodertabeller för idisslare*", (Feeding Tables for Ruminants) by Spörndly (2003). The NorFor system in use in Sweden also uses the concept of amino acids absorbed in the duodenum, but unfortunately the AAT in NorFor is calculated in a slightly different manner, possibly leading to misunderstanding as long as the two systems are used in parallel. The LFU system, also in use in Sweden, does not operate with AAT. The LFU system only calculates a recommended level of degradable and un-degradable crude protein.

Feeding practices. Semi-free access to forage and individual allotment of concentrates to neck tied cows was typical for Swedish dairy cow feeding during the second part of the 20th century. The forage was given *ad libitum* in the first half of the lactation but normally restricted thereafter. The level of concentrate feeding was higher than in most countries due to the relationship between feed cost, milk price and housing cost, resulting in concentrate levels of up to about 18 kg/cow/ day at the end of the 20th century. The high concentrate allotments naturally restricted early lactation forage intake when milk yield was high. Decreasing milk to concentrate prices during the first decade of the 21st century has lowered the concentrate allotments. The relatively fast shift from neck tied cows in smaller herds to loose housed bigger herds of about 100 cows has also changed the feeding strategy. Cows receiving less concentrates when the milk yield is decreasing have increased their forage consumption in loose housing systems. Therefore, it has become increasingly difficult to regulate complete rations according to the yield as compared to tie-stall systems. Thus, the traditional Swedish rations with large differences between concentrate level over the lactation has changed towards flat rate feeding where the cows have to regulate their intake to a greater extent by eating more or less forage. The TMR and PMR (partly mixed ration) have also gained in popularity. Predicting the voluntary feed intake has therefore become more important as a result of changing to loose housing systems and feeding of mixed rations. Estimation of the feed intake was absent in all of the classical feed evaluation systems but is now an important component of the newly introduced NorFor system.

The future

Increased understanding of the ruminal ecosystem and nutrient metabolism in dairy cows highlight the shortcomings of the classic additive feed evaluation systems. The aims of future feed evaluation tools are to enable a more detailed simulation/prediction/monitoring of nutrients available to the cow from each ration. Only by modelling how cows under specified physiological conditions metabolize specific nutrients, will enable us to make more accurate dietary adjustments to target specific production goals.

The modern protein evaluation systems were the first to take a more mechanistic approach to nutrient metabolism in the digestive tract and have now been followed by a number of attempts to also base energy evaluation systems on modelling nutrient metabolism in the digestive tract or in the whole animal. Some of these systems/models, which are applied in practice, are the CNCPS (Cornell Net Carbohydrate and Protein System; Russell et al., 1992) and the NorFor system which has been introduced in Norway, Sweden, Iceland and Denmark (Gustafson and Aaes, 2004).

Generally, new feed evaluation models/systems describe the complete ration, because they use interactions between feedstuffs to describe effects beyond systems assuming additivity. However, feed evaluation will still be based on individual feeds, and a great challenge in coming years is to provide data for all the new variables used in the new system (potential digestibility, rate of digestion etc.). There will be a need for tabulated values to be used as

default values, but the greatest challenge is to develop analytical tools for estimation of these feed characteristics on samples from practical agriculture.

Conclusion

The classic feed evaluation systems differed, but they all used the same basic principles (Weende analysis, digestibility, calorimetry data etc.).

Despite the differences among systems, the advice given to farmers on ration formulation has not been that different, and differences in the energy evaluation systems have only been responsible for a small part of the differences. With the coming generations of feed evaluation systems, we can foresee systems which will be really different from each other.

The Nordic countries have a large part of the feed evaluation history in common, and with the NorFor system also the future seems to be common for the countries with the exception of Finland.

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The potential of using the NorFor model to evaluate dietary strategies to reduce methane production and nitrogen excretion in cattle

H. Volden
TINE Norwegian Dairy Association, P.O. Box 58, 1430 Ås, Norway and Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway

Introduction

During the last few years an increased interest in reducing methane (CH4) emission from ruminants has been observed, and increased N-efficiency by the animal is a key to minimizing nitrous oxide (N_2O) emissions. The NorFor system is a semi-mechanistic feed evaluation system that optimises cattle nutrient supply based on feed composition, animal requirements and environmental conditions at different production situations. The system can effectively be used on dairy farms to evaluate the environmental impact of different feeding strategies on enteric CH4 emission and N from animal excreta (faeces and urine). Therefore, NorFor has a potential as a tool for improvement of the efficiency in future cattle production. The objectives of this paper are to: 1) briefly describe a CH4 sub model in Norfor, which can be used to evaluate different nutritional strategies to reduce CH4 production and 2) demonstrate how the Norfor system can be used to evaluate and improve the nitrogen efficiency in dairy cattle production.

Materials and methods

The NorFor model was used to simulate the effect of different nutritional strategies on methane production. Excess hydrogen produced during the fermentation of nutrients in the rumen and the large intestine to lipogenic volatile fatty acids (VFA), i.e., acetate (Ac) and butyrate (Bu), is used for microbial growth, biohydrogenation of unsaturated fatty acids and the production of the glucogenic VFA's propionate (Pr) and valerate. Based on an assumption of hydrogen balance, the remainder of hydrogen produced is used for the production of CH4.

Nutrient digestion in the rumen and large intestine

The ruminal digestion and fermentation of nutrients are predicted from the fractional rates of degradation and passage. Ruminal degradation of crude protein (CP), starch (ST) and neutral detergent fibre (NDF) in concentrate feeds are assumed to follow first-order single-compartment kinetics, while degradation of NDF in roughage is modelled as a two-compartment system, with a non-escapable and an escapable pool. In the rumen the nutrients available for microbial growth originates from degraded NDF, ST, residual carbohydrates (RestCHO), glycerol, CP and lactic acid (LAF). The efficiency of microbial synthesis depends on the level of feed intake (FL) and diet composition. The NDF, ST and RestCHO passing into the large intestine is subjected to microbial fermentation using fixed digestibility coefficients.

Stoichiometry of rumen fermentation

The main products of nutrient fermentation are the VFA's, CO2 and CH4. Calculated on glucose basis the following equations can be used to predict the fermentation products:

$$0.5C_6H_{12}O_6 + 2(H) \rightarrow Pr + H_2O \quad \text{(Eq. 1)}$$

$$0.5C_6H_{12}O_6 + H_2O \rightarrow Ac + CO_2 + 4(H) \quad \text{(Eq. 2)}$$

$$C_6H_{12}O_6 \rightarrow Bu + 2CO_2 + 4(H) \quad \text{(Eq. 3)}$$

, where glucose, acetate (Ac), propionate (Pr), butyrate (Bu), hydrogen (H) are calculated on a molar basis.

If the equations 1, 2 and 3 are multiplied by their respective molar proportions of Ac (a), Pr (b) and Bu (c) and assuming that a + b + c = 100, it is possible to derive the following equation (Czerkawski, 1986):

$$C_6H_{12}O_6 + \left[\frac{2(3a+b+2c-200)}{(200-a-b)}\right]\cdot H_2O \rightarrow \left(\frac{2a}{(200-a-b)}\right)\cdot Ac + \left(\frac{2b}{(200-a-6)}\right)\cdot Pr + \left(\frac{2c}{(200-a-b)}\right)\cdot Bu + \left[\frac{2(100+c-b)}{(200-a-b)}\right]\cdot CO_2 + \left[\frac{4(3a+3c-100)}{(200-a-b)}\right]\cdot (H) \quad \text{(Eq. 4)}$$

, where $C_6H_{12}O_6$ is glucose, mol; Ac, Pr and Bu are acetate, propionate and butyrate, respectively, mol; a, b and c are acetate, propionate and butyrate, respectively, molar proportions; CO_2 is carbon dioxide, mol; H_2O is water, mol; H is hydrogen, mol.

The fermentation of glucose results in the production of excess hydrogen, which is converted to methane according to the equation:

$$CO_2 + 8(H) \rightarrow CH_4 + 2H_2O \quad \text{(Eq. 5)}$$

,where CO_2, H , CH_4 and H_2O are moles of carbon dioxide, hydrogen, methane and water, respectively.

The ruminal VFA profiles used in equation 4 were calculated from the stoichiometrical model of Sveinbjörnsson et al. (2006). The stoichiometrical coefficient values were predicted from the conversion of six classes of digested nutrients; forage NDF (fNDF), concentrate NDF (cNDF), ST, CP, LAF and RestCHO. The glycerol fraction of crude fat was added to the RestCHO fraction. In addition to digested nutrients the model takes into account the effect of FL and concentrate crude fat (cCFat) on the ruminal VFA profile. In the stoichiometrical model it was assumed that per mol of hexose, amino acid equivalent (AAeq) and LAF, 2.0, 1.1 and 1.0 mole pyruvate was formed, respectively; and that per mol of pyruvate 1.0, 1.0 and 0.5 mol of Ac, Pr and Bu was produced. The same assumption was used when converting AAeq and LAF to glucose units for further calculation in equation 4. When predicting the CH4 production from fermentation in the large intestine it was assumed that the stoichiometrical VFA profile was similar to that of the rumen.

Synthesis of microbial organic matter (mOM) and biohydogenation of unsaturated fatty acids
The dietary components are in the fermentation process broken down and either used as an energy source or as a substrate for synthesis of mOM. In NorFor the efficiency in the mOM synthesis is dependent on the FL and the concentration of RestCHO + ST in the diet. The chemical composition of the mOM is assumed to be constant: 512, 167, 51 and 270 g/kg mOM for protein, cFat, ST and residual fraction (cell wall), respectively. Based on the chemical composition of Norfor and that ammonia (NH3) is the solely nitrogen source, it is possible to derive the balanced equation:

$$C_6H_{12}O_6 + 0.96NH_3 + 0.0016H_2S + 2.5(H) \rightarrow C_{6.0}H_{9.8}O_{2.16}N_{0.96}S_{0.016} + 3.80H_2O \quad \text{(Eq. 6)}$$

The first term on the right represents the mOM and shows that 1 mol of glucose will be converted to 130.3 g mOM. When then mOM synthesis in the large intestine is calculated, it is assumed that microbial chemical composition is similar to that of the rumen.

In the rumen dietary unsaturated fatty acids are reduced to form saturated fatty acids. In the model the following equation is used:

$$C_{18}H_{32}O_2 + 2H \rightarrow C_{18}H_{34}O_2 \qquad \text{(Eq. 7)}$$

,where, Linoleic (left term) and oleic fatty acids (right term) are used as reference acids in the calculation.

Model simulations

In the model simulations, the CH4 production was expresses either as MJ/d, in percent of gross energy (GE) intake or in MJ kg/energy corrected milk (ECM). Gross energy was calculated according to NorFor. When predicting the N efficiency it is assumed that N ingested by the animal is either used for maintenance, growth, gestation or milk production, and N not utilized for production is excreted in faeces and urine. To simulate the effect of different nutritional strategies on digestion kinetics and ruminal fermentation, different theoretical diets were formulated. The chemical composition and characteristics of feedstuffs making up the diets are presented in Table 1.

Table 1 Characteristics of feedstuffs used to simulate different dietary strategies on methane production

	Chemical composition, g/kg DM			Degradation rates, %/h	
Feed	CP	NDF	Starch	kdCP	kdNDF
Grass silage, medium dig.	159	560		9.6	4.3
Grass silage, high dig.	173	501		12.1	5.0
Maize silage	78	342	338	4.6	3.4
Concentrate mix. High CP	211	217	358	6.4	3.2
Concentrate mix. medium CP	188	217	390	6.1	3.2

[1]For abbreviations see text

Simulations were conducted for a 600-kg body weight cow. Unless indicated, all simulations were performed at intake of 20.0 kg of DM/day (DMI).

Results and discussion

Strategy 1. Increasing dry matter intake

Simulations to evaluate the effect of feed intake level on methane production were performed for diets based on high digestibility grass silage (50% of forage), maize silage (50% of forage) and a concentrate mixture high in CP. Simulations (Table 2) demonstrated that changing the DMI altered rumen fermentation and digestion processes. The DMI showed small effects on the VFA proportions, and was less sensitive than observed in experimental data (Volden, 1999). The production of CH4 (MJ/d) increased with increased feeding level, but when expressed in percentage of GE or per kg ECM, it decreased, which is in agreement with experimental data (Yan et al. 2000). The proportion of dietary N converted to milk or excreted in faeces increased with increased DMI, while the percentage of N excreted in the urine decreased, which is in accordance with the changes in protein balance in the rumen (PBV) (Volden, 1999).

Table 2 Effect of dry matter intake on digestion kinetics, methane production and nitrogen utilization

	Dry matter intake, kg/day		
Item	15	20	25
Intake, g/day			
OM	14085	18780	23475
CP	2450	3266	4083
NDF	5096	6794	8492
ST	3669	4892	6115
Passage rates, %/h			
Liquid	10.7	13.0	15.2
Concentrate CP	5.1	6.3	7.4
NDF roughage	1.39	1.67	1.82
Ruminal efficiency, g CP kg/RDOM	161	177	189
Total tract digestibility, %			
OM	80.5	78.7	77.6
CP	69.2	67.2	65.8
NDF	66.6	63.9	62.4
VFA, molar %			
Acetate	67.0	66.5	66.4
Propionate	18.8	19.2	19.3
Butyrate	14.2	14.3	14.3
Methane production			
MJ/d	20.0	24.1	27.9
% GE intake	6.0	5.4	5.0
g/kg ECM	17.1	14.0	12.3
N utilization, % of N intake			
Milk	27.4	30.4	32.4
Faeces	30.8	32.8	34.2
Urine	41.8	36.8	33.5
PBV, g/kg DM	28.0	17.0	8.1
CP, g/kg DM	163	163	163

[1]For abbreviations see text

Strategy 2. Increasing proportion of concentrates in the diet

The effect of increasing the amount of concentrate in the diet on digestion kinetics and CH4 production was investigated using different forage/concentrate ratios: 75:25, 50:50 and 25:75. The simulations were performed using the medium quality forage and the concentrate mixture with medium CP content. Increasing the proportion of concentrate in the diet reduced ruminal passage rates (Table 3). The total tract digestibility of NDF decreased duo to a negative effect of rapidly degradable carbohydrates on the fiberolytic activity. The rumen fermentation pattern was also altered: increasing the proportion of concentrate decreased the molar % of Ac and increased the molar % of Pr and Bu. The CH4 production changed in a curve-linear way. In general, the effect of concentrate proportion on the CH4 production was small. Several reports have demonstrated a reduced CH4 production (Yan et al., 2000), whereas others have observed only a small reduction (Ferris et al. 1999; Garmo et al. 2009) by changing the concentrate proportion.

Table 3 Effect of forage to concentrate ratio on digestion kinetics, methane production and nitrogen utilization. 20 kg dry matter intake per day

	Forage/concentrate ratio		
Item	75:25	50.50	25:75
Intake, g/day			
OM	18560	18600	18660
CP	3325	3470	3615
NDF	9485	7770	6055
ST	1950	3900	5850
Passage rates, %/h			
Liquid	13.7	12.5	11.7
Concentrate CP	6.6	6.1	5.6
NDF roughage	1.86	1.77	1.57
Ruminal efficiency, g CP kg/RDOM	186	187	168
Total tract digestibility, %			
OM	75.5	76.9	77.3
CP	67.3	68.0	70.2
NDF	66.4	62.6	52.4
VFA, molar %			
Acetate	68.7	67.1	65.2
Propionate	17.8	19.0	20.2
Butyrate	13.5	13.9	14.5
Methane production			
MJ/d	23.4	22.5	23.3
% GE intake	5.3	5.0	5.1
g/kg ECM	14.8	13.7	13.8
N utilization, % of N intake			
Milk	26.9	27.8	27.2
Faeces	32.7	32.0	29,8
Urine	40.3	40.1	43.0
PBV, g/kg DM	30.0	26.0	36.0
CP, g/kg DM	166	174	181

[1]For abbreviations see text.

The present simulations indicate that there is an interaction between ruminal fermentation pattern, efficiency in the microbial synthesis and the amount of nutrients digested in the rumen. The proportion of dietary N excreted in the faeces decreased linearly with increased concentrate proportion due to a reduced content of indigestible CP in the diet. The excretion of N in urine was highest for the diet highest in PBV and CP.

Conclusions

This work demonstrated the usefulness of a semi-mechanistic model of gastro intestinal digestion to understand and to assess the effectiveness of different strategies aiming at reducing CH4 emission and to increase the N utilization in dairy cows.

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Life cycle assessment of locally produced feed for dairy cows

I. Strid[1] and J. Bertilsson[2]
[1]*Department of Energy & Technology, Post Box 7032, Swedish University of Agricultural Sciences (SLU), S-750 07 Uppsala, Sweden*
[2]*Department of Animal Nutrition & Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences (SLU), S-753 23 Uppsala, Sweden*

Introduction

This study aims to contribute to the knowledge on environmental consequences of feed choice in dairy production, based on both feed production and animal feed utilisation. Life cycle assessment methodology was used to estimate energy use and land use as well as potential contribution to climate impact, acidification and eutrophication for a scenario of a 100-cow dairy farm in western Sweden (Västra Götaland county). Five different feeding alternatives were compared in order to test the hypothis that more home-grown feeds leads to a more 'environmently friendly' dairy production. Full reports are available in Swedish (Liljeholm et al., 2009 and Strid et al. 2010)

Materials and methods

Five feed rations, all calculated for 9000 kg ECM milk annually, have been compared: one 'normal' representing standard feeding in Sweden, three locally produced and one maize silage-based. Feeds used in the different feed rations are presented in Table 1. The feed rations have been balanced using the 'old' system, MJ ME and AAT/PBV. The functional units that have been compared are feed rations that support a dairy cow with feeds to produce 9,000 kg ECM per year. Only silages are supposed to be produced at the farm –other feeds have been transported to the farm.

Result and discussion

The results gave that no feed ration was consistently better for all environmental categories (Table 2). Feed production (incl. farm yard manure spreading) was more important than animal emissions for all environmental effects except climate impact. Feed production was also the part of the production chain with the largest variation between the alternatives. The small difference in contribution to climate impact was partly due to the choice of method for calculation of enteric methane emissions, where the used method did not chemical composition of feeds (Lindgren, 1980)

Energy use was the environmental category that was most effected by feed choice, where the feed ration with rapeseed products, peas and clover silage (ration 5) had the lowest primary energy use followed by the more and better silage (ration 3). Feed ration 5 had, however, a higher contribution to eutrophication and used more land and ration 3 had a higher contribution to acidification. The latter was though partly an effect of methodology, since the feed received had a higher share of the manure than the other feeds. The feed that managed to lower its contribution to climate impact most was ration 5 containing rapeseed products, peas and clover silage.

Table 1 Feeding in five different rations balanced for 9000 kg ECM annual production (kg feeds if not else stated)

Feed ingredient	Ration 1 'Normal '	Ration 2 DDGS	Ration 3 More and better silage	Ration 4 Beet pulp and maize	Ration 5 Rapeseed, peas, clover
Grass silage, 2 harvests, kg DM	3367	3346		1601	-
Grass silage, 3 harvests, kg DM	-	-	4499	-	-
Clover-grass silage, kg DM	-	-			2989
Maize silage, kg DM	-	-		549	-
Pressed sugar beet pulp, kg DM	-	-		427	488
Cereal	1620	1373	1007	1818	1278
Distillers dried grains with solubles, DDGS	-	549		-	-
Dried sugar beet pulp pellets	275	275		-	-
Rapeseed meal	-	-		-	204
Rapeseed cake	-	-		-	400
Peas	-	-		-	881
Soybean meal	-	-		85	-
Commercial protein (Unik 52)	1196	924	726	1473	-
Kg DM	6090	6095	6020	5542	5736
Roughage, %	57	57	75	47	61
Soya, kg	239	185	145	380	0
Locally produced, %	94	96	97	91	100

Table 2 Relative environmental impact (%) compared to the 'Normal feed ration' (feeding is explained in Table 1)

	Ration 2	Ration 3	Ration 4	Ration 5
Energy use	97	83	101	74
Land use	96	103	93	111
Climate impact	100	99	98	89
Acidification	101	114	88	94
Eutrophication	98	106	92	114

Transport energy use was assessed. For the 'normal' diet (ration 1) and the diet containing DDGS feeds (ration 2), transport energy use represented 15 % of the total energy use, while for ration 3, it was 10 % and for rations 4 and 5 it was 28 %. The last two feeds got high

values due to their content of pressed sugar beet pulp, which is a moist product that was assumed to be transported relatively far. The concept of locally produced feed in the sense Swedish feeds did not unambiguously give lower transport energy use, except in a sensitivity analysis where the farm was assumed to be situated within 100 km from the sugar mill producing the beet pulp. On the contrary, the feed ration with a high share of on-farm produced feed (Ration 3) had a noticeably lower transport energy use.

The transport energy varied largely between the feed rations, but had only a moderate impact on total energy use. The low total energy use of the Rapeseed, peas and clover feed, despite its high transport energy use, was explained by the high share of nitrogen fixing crops (peas and clover) that enables low use of mineral fertilizers in the feed production. *Locally produced* feed thus seam to have lower relevance than *mineral fertilizer saving* feed, when aiming for low total energy use by the choice of feed.

The feed ration with only locally produced protein feed and silage from nitrogen fixing clover-mixed leys (ration5) had environmental advantages in the form of lower energy use and lower contribution to climate impact than the normal feed of this study. However, this feed ration was also estimated to give a higher contribution to eutrophication than the other alternatives. With measures to lower the risk of eutrophying emissions, such as nitrogen leaching, this feed ration was concluded to be the best of the studied alternatives.

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Choice of statistical model and computer procedure or vice versa?

L. Norell
Unit of Applied Statistics and Mathematics, Department of Economics, P.O. Box 7013, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden

Introduction

The issue whether a factor in an experiment shall be considered as fixed or random in a statistical model leads to consequences for the interpretation of the results. In some cases, the effect is not clearly visible. Two examples, one with milk yields of ewes and one with protein measurements from cows, will be examined.

Materials, Methods, and Results

Example 1

Data (Table 1) are from a sheep experiment (Peart, 1968) with nine ewes randomly divided into groups of three for three feeding strategies. Response variable was residual milk (milk after lamb had suckled the ewes):

1. Ewes and lambs fed *ad lib*.
2. Ewes fed *ad lib*, lambs' feed restricted.
3. Ewes' feed restricted, lambs fed *ad lib*.

Are there any differences among the feeding strategies?

Table 1 Extract of data from Peart (1968). Residual milk in grams/d for lactation week 5 to 9 in ewes subjected to three different feeding strategies

		Lactation week				
Feeding strategy	Ewe	5	6	7	8	9
1	1:1	240	270	280	280	280
	1:2	370	390	400	390	360
	1:3	200	220	230	260	290
2	2:1	580	570	560	570	490
	2:2	200	200	170	240	290
	2:3	110	100	100	80	110
3	3:1	110	90	110	100	100
	3:2	130	130	130	130	100
	3:3	210	200	130	140	120

Questions:

What are the factors? Are they fixed or random?

Factors: Feeding strategies (FS), Ewes within FS, Weeks
FS: inference intended for those three used $\Rightarrow$ fixed factor
Ewes: inference intended for a population of similar ewes $\Rightarrow$ random factor
Weeks: inference intended for the weeks used $\Rightarrow$ fixed factor.

Choice of model: Statistical model or computer program first?

Statistical model for residual milk from FS (i), Ewe (j) within FS, and Week (k):

$$y_{ijk} = \mu + \alpha_i + s_{ij} + \omega_k + (\alpha\omega)_{ik} + e_{ijk}$$

where

μ = overall expectation

α_i = fixed effect of FS, i=1,2,3

s_{ij} = random effect of Ewe j within FS, j=1,2,3

ω_k = fixed effect of Week, k =5,6,7,8,9

$(\alpha\omega)_{ik}$ = interaction effect of FS and Week

e_{ijk} = random residual effect

Correlations among residual errors within ewes might be considered by various models for repeated measurements. Primary interest in comparison of feedings. Effect of considering ewes as randomly chosen. Pitfalls in computer programs?

In SAS, Proc GLM and Proc Mixed can be used. Consequences?

```
Proc GLM;
  class FS Ewe Week;
  model Yield=FS Ewe(FS) Week FS*Week;
  random Ewe(FS)/test;
  lsmeans FS/tdiff   e=Ewe(FS);
```

Table 2 The standard ANOVA output table for residual milk yield from Proc GLM in SAS for the study of Peart (1968), where Feeding strategy (FS) is tested against Error (Ewes considered fixed). Below that the requested F-test of FS against Ewe(FS), which implies that Ewes are considered random and are representing a population

ANOVA and F-test with Ewe considered fixed				
Source	df	MS	F	Pr>F
FS	2	137362	148.8	<.0001
Ewe(FS)	6	103007	111.6	<.0001
Week	4	102.2	0.11	0.9776
FS*Week	8	903.9	0.98	0.4757
Error	24	923.3		
F-test with Ewe considered random				
FS	2	137362	1.33	0.3318

Table 3 Lsmeans for residual milk (g/d) in the study of Peart (1968). Estimates do not differ if ewes are considered as fixed or random factors

FS	1	2	3
Grams milk/d	297.3	291.3	128.7

Table 4 Tests for treatment differences in the study of Peart (1968) with ewes considered either fixed or random. FS = feeding strategy

		FS 1-FS 2	FS 1-FS 3	FS 2-FS 3
Ewe fixed	*T*	0.541	15.20	14.66
	Pr>|*t*|	0.5937	<.0001	<.0001
Ewe random	*T*	0.051	1.439	1.388
	Pr>|*t*|	0.9608	0.2001	0.2145

The other procedure is implemented according to:

```
Proc Mixed;
    class FS Ewe Week;
    model Yield=FS  Week FS*Week;
    random Ewe(FS);
    lsmeans FS/tdiff ;
```

This procedure yields correct results for tests and comparisons of fixed effects considering the effect of the random factor. This is a consequence of the built-in property of models containing both fixed and random factors. The very first versions of Proc GLM did not contain possibilities for random factors and today's supplements need some care. However, the test of the variation among ewes is not appropriate in Proc Mixed since it is based on normal approximation of the *F*-test and is not valid for this few ewes.

Example 2
Data (Table 5) from an experiment at Kungsängen, SLU. Comparison of two diets (A and B). There are 6 cows per diet and 2 observations (periods) per cow. Response variable in the example is PBV (protein balance value in the rumen)

Table 5 Protein balance in the rumen (PBV), g/d in the Kungsängen experiment. Data extract

Diet	Period	Cow					
		1	2	3	4	5	6
A	1	125	177	219	164	170	197
	2	289	351	377	339	246	205
B	1	104	105	106	134	88	90
	2	141	172	117	164	131	147

Statistical model as earlier:

$$y_{ijk} = \mu + \alpha_i + c_{ij} + \pi_k + (\alpha\pi)_{ik} + e_{ijk}$$

where

μ = overall expectation

α_i = fixed effect of Diet, i=1,2

c_{ij} = random effect of Cow within Diet, j=1,2,3,4,5,6

π_k = fixed effect of Period, k = 1,2

$(\alpha\pi)_{ik}$ = interaction effect of Diet and Period

e_{ijk} = random residual effect

```
Proc GLM;
   class Diet Cow Period;
   model PBV=Diet  Cow(Diet)  Period  Diet*Period;
   random Cow(Diet)/test;
   lsmeans Diet/tdiff   e=Cow(Diet);
```

Table 6 The standard ANOVA output table from Proc GLM in SAS for the data extract from the Kungsängen study, where Diet is tested against Error (Cows considered fixed). Below that the requested F-test of Diet against Cow(Diet), which implies that Cows are considered random and are representing a population

ANOVA and F-test with Cow considered fixed				
Source	df	MS	F	Pr>F
Diet	1	77066.7	60.28	<.0001
Cow(Diet)	10	1783.4	1.39	0.3043
Period	1	41666.7	32.59	0.0002
Diet*Period	1	10866.7	8.48	0.0155
Error	10	1278.5		
F-test with Cow considered random				
Diet	1	77066.7	43.21	<.0001

Table 7 Lsmeans for PBV (g/d) for the data extract from the Kungsängen study. Estimates do not differ if cows are considered as fixed or random factors

Diet	A	B
PBV, g/d	238.3	124.9

Table 8 Tests for treatment differences for the data extract from the Kungsängen study with cows considered either fixed or random

		Diet A - Diet B
Cow fixed	*t*	7.76
	Pr>|*t*|	<.0001
Cow random	*t*	6.57
	Pr>|*t*|	<.0001

Proc Mixed (detailed results not shown) gives the test for the diets assuming cow as a random factor. The test of the cow variation is not proper (although the *P* values are rather close in this case).

Conclusions

The choice of model is important. There are great risks of too drastic conclusions if animals are used for repeated measurements and are not considered as a random factor. Some computer procedures take care of the model properly. In some others, extra options are needed as in Proc GLM above.

A recommendation is to first consider the choice of the statistical model and then to choose an appropriate computer program. Often, several computer procedures are needed for answering different questions.

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Uppsala Livestock Research Centre

M. Emanuelson[1], M. Pehrsson[2] and G. Pettersson[1]
[1]*Department of Animal Nutrition and Management,* [2] *The faculty of veterinary medicine and animal sciences, Kungsängen research centre, SE-753 23 Uppsala, Sweden.*

Introduction

The Faculty of Veterinary Medicine and Animal Science (VH) is building new facilities for housing farm animals at Lövsta, seven km south-east of Uppsala. This area is of considerable interest in terms of natural history and cultural heritage. Here, the open agricultural landscape combines with pastures of varied topography, rich in deciduous trees, ideal for various forms of livestock grazing. Bordering the Linnaeus Hammarby estate, with its waymarked Linnaeus paths, this location was a meeting place for one of Sweden's most prominent scientists and his students and research colleagues in the 18th century. Adjacent to the new animal facilities lies the Lövsta estate, dating back to the 1700s. It takes about 20 minutes to travel between the Ultuna campus and Lövsta, about 30 minutes by car from Stockholm Arlanda Airport to Lövsta, and it is less than one hour by car to Stockholm city. Ultimately Lövsta will be provided with well-appointed facilities capable of accommodating up to 100 visitors.

In 2003 the Vice-Chancellor of the Swedish University of Agricultural Sciences (SLU) instructed the Dean of the VH faculty to examine the possibility of building new animal facilities at SLU in Uppsala to replace those that were more than 30 years old, worn out, and no longer fit for their purpose. However when it became clear that modernising them would not be economically viable the Vice-Chancellor and the Dean decided that Lövsta would be an ideal location for new, modern facilities for teaching and research on livestock: cattle, pigs and poultry. Program development began in 2006, and in October 2008 the University Board decided that the Vice-Chancellor should assume responsibility for implementing the Lövsta construction project. The first turf was cut in August 2009 and animals will start to move in to new accommodation in September 2010, and the facility will be completed and fully operational by September 2011.

Uppsala Livestock Research Centre

The centre will be a modern facility for teaching and research on dairy cattle, pigs and poultry and all undergraduate and postgraduate students of the VH faculty will spend some time here during their studies. The VH faculty has an annual intake of over 200 students studying veterinary medicine and animal science as well as providing master's courses for international applicants. In addition the new research centre will offer fellow scientists from around the world, and including those in industry, a unique environment where it will be possible to undertake research of high quality with emphasis on animal welfare and animal health, climate-friendly animal management and sustainable food production. Animal production, feeding, behaviour, reproduction and health will be continuously monitored and recorded, and the data will be available to students and researchers. The faculty is also working with the management at the Lövsta facility on plans for a biogas unit and a slaughterhouse.

The dairy cattle barn

The cattle barn is built as a loose housing system and will accommodate a closed herd of 300 dairy cows plus replacement animals for teaching and research. The herd will consist of animals of the Swedish Red Breed and the Swedish Holstein breed in equal proportions.

The lactating cows will be hold in 4 groups of 60-65 cows respectively. One group will be in an automatic milking system (AMS) and three groups will be milked in an automated rotary system. In the AMS-group and in one of the rotary groups there will be concentrate stations as well as roughage stations which make it possible for automatic registration of individual total feed consumption. In the other two rotary groups the concentrate will be distributed in stations and roughage on an ordinary feeding table. There will also be a separate area where heifers or lactating cows can be restrained for short periods to enable both intensive studies and teaching. In this area the feed will be distributed by registering feed wagons. I all three housing systems the milk yield and some milk quality parameters will be registered automatically at every milking.

Calving, planned to occur evenly throughout the year, will take place in a dedicated section. The calves will be housed in separate sections on litter beds in groups of 10 calves and fed by an automatic milk feeder up to weaning. During the period from 4 months to 6 months of age the heifers will be hold in groups of 20 calves on litter beds and the bull calves will be sold to farmers in the region. From 7 months of age up to calving the heifers will be housed in age groups in the section for replacement heifers and dried off cows. Feed consumption will automatically be registered individually up to weaning (milk and concentrate), from weaning to 6 months of age and from insemination to calving (concentrate).

The main part of the roughage fed to the herd will be silage stored in bunker silos. From the feed central in the barn the silage can be mixed with concentrate as PMR or distributed directly to the cows and heifers by a belt feeder and/or feed wagons. Bedding material, mostly as cut straw, will be distributed automatically to the lactating cows and the replacement heifers. Adjacent to the cattle housing there will be pastures extending over 50 ha, including 20 ha of separate pens for grazing studies.

On the second floor of the barn there will be visitors areas from where you can look out over the lactating cows in the AMS-group and the rotary groups. In the other direction you can see the calves up to 6 months of age and the cows in the calving section of the barn. There will also be a hall where up to 100 persons can sit down and listen to presentations and lectures.

The cattle facilities will open in September 2011.

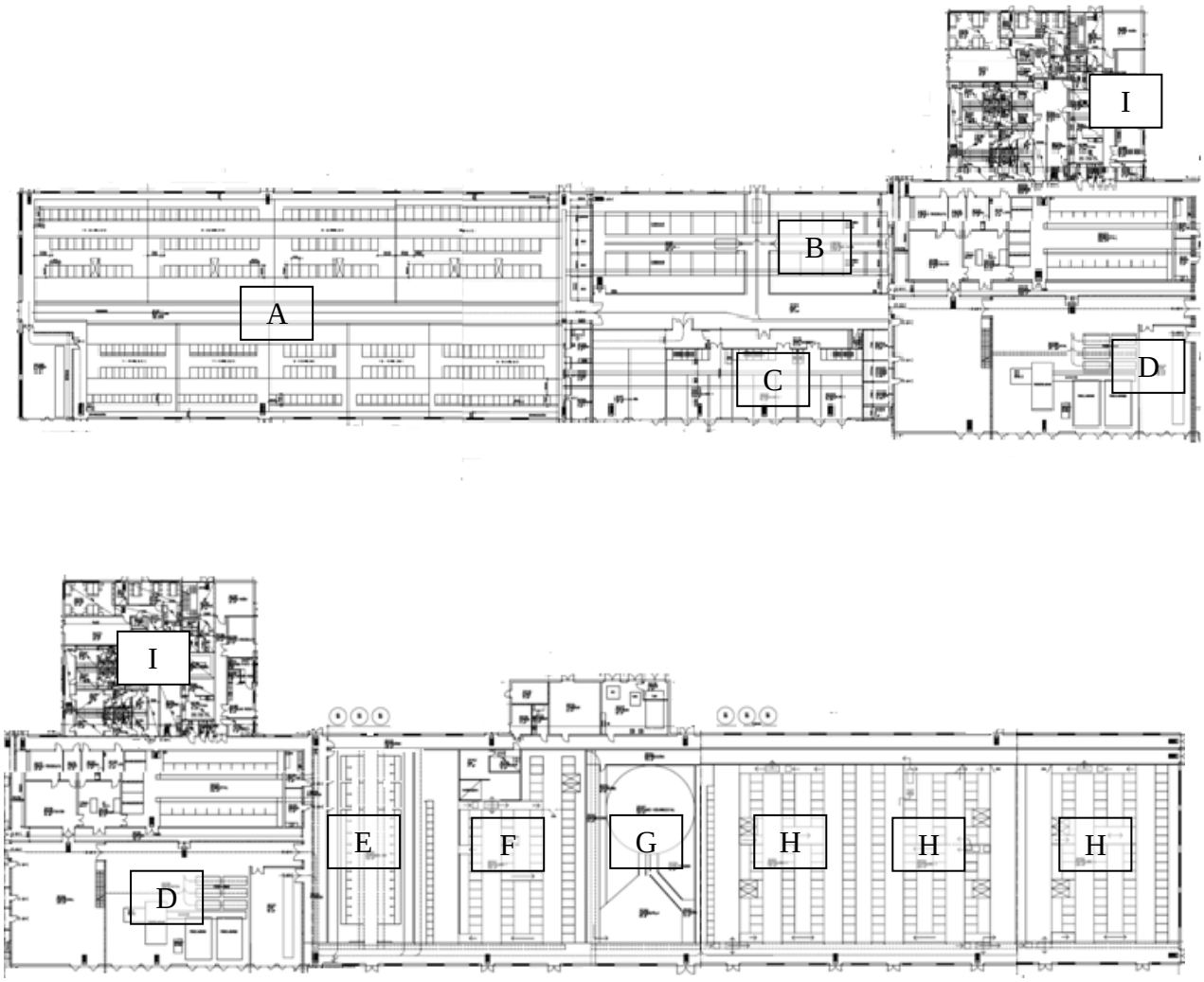

Figure 1 The layout of the cattle barn:
Replacement heifers (A), calving (B) and calf (C) sections and feed central (D), tied-up cows (E), AMS (F), Rotary (G), rotary group (H), staff (I)

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Molecular methods for detection of fungi in haylage

J. Schenck[1,2], R. Spörndly[1], C. Müller[1], A. Djurle[2] & D. Funck Jensen[2]
[1]Department of Animal Nutrition and Management, [2]Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, [1]S-753 23 Uppsala, Sweden

Introduction

The use of wrapped forage with dry matter (DM) contents between 500 and 800 g/kg, also known as haylage, has increased in recent years, especially in feeding of equines (Holmquist and Müller, 2002). The lactic acid bacteria (LAB) responsible for the ensiling process in silage are restricted in forage with higher DM contents (*e.g*. Finner, 1966). Haylage have comparatively higher content of residual sugars, which together with the low concentration of fermentation products may favour fungal (mould) growth. Since moulds can act as allergens and produce mycotoxins, more knowledge is needed about the fungal flora in haylage (Clarke, 1988).

Methods for analyses of the hygienic quality of haylage are limited. Most commonly classic microbiological cultivation methods are used, but they are expensive, time consuming and not very specific. Identification of mould species are commonly done on the basis of macro- and micro-structures of mould growth, and the risk of misidentification can be high (*e.g*. Boysen *et al.*, 1999). In addition, some fungi may not be culturable on artificial substrates and will remain undetected by conventional methods (Skouboe *et al.*, 1996). The application of a large-scale parallel pyrosequencing system, 454-sequencing with the ability to sequence hundreds of thousands fungi (mould) sequences in one sample, may therefore be of interest (Ellegren, 2008). Usually primer pair ITS1F and ITS4 is used for study fungi communities in 454-sequencing (O´Brien *et al.*, 2005). However, a new primer ITS9 has been developed with the advantage that together with primer ITS4, exclude the ITS1 region that has a large variation in sequence length between fungi species. Long sequence length could be a disadvantage during the 454 sequencing. Before the new primer pair is used for 454 sequencing it should be compared to a previously described primer pair, ITS1F and ITS4 (Lindahl B. *et al*., unpublished data and O´Brien *et al*., 2005).

The general aim of this project was to test two different primer combinations on samples previously confirmed to contain fungi using conventional microbiological cultivation. The wilted grass sample containing *Cladosporium spp.* and *Mucor spp*. and the haylage sample containing *Mucor spp.* (Müller C. and Schenck J., unpublished data, species identification verified by DNA-sequencing of isolates).

Materials and methods

Sampling and DNA extraction
The samples originated from a grass-dominated sward (composed of approximately 0.45 timothy (*Phleum pratense*), 0.45 meadow fescue (*Festuca pratensis*) and 0.10 red clover (*Trifolium pratense)*), cultivated outside of Uppsala, Sweden in 2009. Two samples; one grass sample (wilted to 490 g DM/kg) prior to baling and one haylage sample were chosen for further analysis (Müller and Schenck, unpublished data). Fifty grams of each sample were freeze-dried in ScanVac CoolSafe™ freeze-dryer and ground with a Mini MP 160V.V blender (Robot Coupe, France). Two grams of each sample were used for DNA extraction according to Stewart and Via (1993) with some modifications; DNA was extracted in 40 ml 3% CTAB buffer (cetyltrimetylammoniumbromid, 2 mM EDTA, 150 mM Tris-HCl and 2.6

M NaCl, pH 8) containing 2% PVP (polyvinylpyrrolidone) at 65 °C for 2 hours and purified twice with one volume of chloroform. The samples where precipitated from the supernatant with 1.5 volume of 2-propanol for 30 minutes in room temperature. The pellets where washed twice by adding 70% of cold ethanol and resuspended in 200 µl MilliQ water.

PCR, cloning, sequencing and sequence analysis
Wilted grass and haylage DNA extracts were used for polymerase chain reaction (PCR) with the two different primer combinations ITS1F (Gardens & Bruns, 1993) and ITS4 (White *et al*., 1990), and ITS9 (Lindahl B. *et al.*, unpublished data) and ITS4. Ten PCR-runs were performed for each sample and primer combination. PCR-reactions showing bands in gel electrophoresis were pooled within clone libraries (Table 1).

Table 1 Four clone libraries; C1, C2, C3 and C4.). A total of 96 clones were sequenced from each sample and resulted in 384 sequenced clones

Clone Library	Primer Combination	Description	Sequences	PCR reactions
C1	ITS1F + ITS4	Wilted grass	96	10 (pooled)
C2	ITS1F + ITS4	Haylage	96	10 (pooled)
C3	ITS9 + ITS4	Wilted grass	96	10 (pooled)
C4	ITS9 + ITS4	Haylage	96	10 (pooled)

PCR products were cloned using the TOPO TA Cloning Kit with the pCR®2.1-TOPO vector and One Shot TOP10 competent *Escherichia coli* (Invitrogen, USA). Bacteria was diluted in Milli Q water and directly used for PCR with primer pair M13F and M13R. The PCR products were purified with Agencourt® AMPure® XP PCR purification kit (Beckman Coulter, USA) and sequenced by Macrogen (Seoul, South Korea) in one direction (3`) using primer M13R. Sequences were aligned using the ClustalW algorithm of MEGALIGN (DNAStar Inc. USA) assembling with 95 % minimum match. Sequences were compared with data base sequences from NCBI (http://www.ncbi.nlm.nih.gov/BLAST) using the BLASTN algorithm (Altschul *et al.*, 1997).

Results and Discussion

The aim of this project was to test two different primer combinations in two feed samples; one wilted grass sample and one haylage sample. Sequence data from the PCR showed that the wilted grass sample contained *Cladosporium spp*. and *Davidiella spp*. (teleomorph state of *Cladosporium spp.)* using primer combinations ITS1F and ITS4, and ITS9 and ITS4. Compared to microbiological cultivation, *Mucor spp.* was undetected in the wilted grass using either of the primer combinations. Sequence data confirmed that the haylage sample contained *Mucor spp*. using primer pair ITS1F and ITS4. Both primer pairs were able to detect other fungi species present in the wilted grass and the haylage. Sequence data showed that primer ITS9 was not able to cut sequences from *Mucor spp*. and primer ITS9 efficiency to cut *Fusarium spp*. is unestablished (Lindahl B. *et al.* unpublished data). Further modification of the primer ITS9 is probably not practicable since the risk of amplifying plant DNA will increase. Therefore, if primer ITS9 will be selected for the 454 sequencing, *Mucor spp*. should be handled separately using primer combination ITS1F and ITS4. This pilot study shows that molecular methods can be useful tools to detect fungi present in haylage, but further development of methods is needed.

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Silage quality when the crop is infected with *Arion lusitanicus*

R. Spörndly and C. Haaga
Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences. 750 07 Uppsala, Sweden.

Introduction

The terrestrial gastropod mollusc *Arion vulgaris*, or often referred to as *Arion lusitanicus* and with the common name Spanish slug, is a slug without external shell with an adult size of 7-15 cm (NOBANIS, 2010). It originates from the Iberian Peninsula and has during the last decades migrated towards north in Europe. It was first reported in Sweden in 1975 and between 1988 and 1994 it was reported in Norway, Denmark and Finland. The climate change resulting in elevated winter temperatures and the trade with perennial horticulture plants for gardens are pointed out as possible vectors for its migration (von Proschwitz, 1997). In the summer 2007, an invasion on grasslands in south-west of Sweden created a heavy reaction in the farming society with great concern for animal health. Slugs were present in the grass at harvest or were moving into the crop after cutting and during wilting. In both cases, the slugs entered the silo along with the crop in great numbers. In an investigation made by the local farmers union, 1/3 of the farmers reported "problems" with the slugs. Unspecified health disturbances were ascribed the slugs and so was "bad smell" of the silage and animals unwilling to consume both pasture and silage.

It is well known that contamination of the crop with soil in connection to ensiling create disturbances. *Enterobacteriace* and *Clostridium spp.* are common in soil and reports of impaired silage quality resulting in high DM losses, elevated pH, high butyric acid, high ammonia level and spores of *Clostridium tyrobutyricum* are frequent when silage is contaminated by soil and slurry (Rammer & Lingvall 1997). Contamination with slugs living on the soil could be expected to give similar effect. The presence of dead animals, such as rodents, in the silage is also known to give rise to growth of *Clostridium botulinum* (Myllykoski et al, 2010). *Clostridium botulinum* is an anaerobe and spore forming bacteria known to produce the toxin botuline and give rise to botulism in man and animals.

As no information was found in the literature about the effect on silage quality with crops contaminated with slugs, an experiment was set up with the aim to investigate the silage quality parameters with classical chemical and microbiological methods. The aim was also to develop a method to refine *Clostridium botulinum Type C* in slugs and silage in order to detect the toxin gene with PCR. Results of the classical silage hygienic parameters will be reported in this paper while results concerning *Cl. botulinum* will be reported elsewhere.

Materials and methods

Ensiling procedure

Slugs (*Arion lusitanicus*) were collected from a grass ley at a farm in the south west of Sweden (N 57° 20′ 57″; E 12° 26′ 29″). Approximately 700 slugs in a juvenile stage were collected and placed in a clean cage on a bed of grass and closed with a net on the top. The species of the slugs was determined by Dr. von Proschwitz at the Museum of Natural History, Gothenburg, Sweden. Ten randomly chosen slugs were weighed and measured. The cage was transported 500 km north to the Swedish University of Agricultural Sciences in Uppsala where a slug-free grass crop was cut. The crop consisted of 75% Timothy (*Phleum pratense*) and 25% Meadow Fescue (*Festuca pratensis*) and it was wilted to 35 (low DM) or 55% DM (high DM). Twenty-seven hours after the collection of slugs, 36 experiment silos of 1.5 L size were filled with un-infected crop (UI) and with low (LS), medium (MS) or high (HS) levels of slugs. The crop from both dry matter (DM) levels was chopped in a stationary cutter

to an average of 5 cm particle length after being wilted. The low DM crop was ensiled with or without the addition of silage additive (Promyr 630, Perstorp AB, Sweden) whiles the high DM crop was ensiled without additive only. The additive consisted of propionic acid, formic acid and sodium formiate and was added in a concentration of 6 L/t fresh crop. Each treatment was replicated in three silos.

To the treatment LS 8 slugs, to MS 17 slugs and to HS 35 slugs were added to the silos. The highest amount was equivalent to the concentration of slugs per kg fresh weight found on the crop infected with slugs. Slugs were added both alive and cut into pieces to imitate the harvesting process. Slurry was produced from the leftover from the transporting cage, consisting of slug faeces, dead slugs and grass, homogenized with 1 L sterile water. Ina addition to the whole and chopped slugs, 20 mL slurry was added to the LS, MS and HS treatments. The low DM silages were packed at a density of 180 kg DM/m^3 and the high DM silages were packed at a density or 225 kg DM/m^3. The silos were sealed and fitted with water-locks in order to let out gases and were then stored for 100 days. The silos were weighed at the time of filling, at day 30 and at day 90 to determine weight losses. Weight losses, assumed to originate from the silage DM in the form of CO_2, were expressed as % of the DM content in the silo at filling.

Chemical and microbiological analysis

The fresh forage was analyzed for DM, crude protein (CP), ash, neutral detergent fiber (NDF), water soluble carbohydrates (WSC), lactic acid bacteria (LAB), enterobacteria, spores of *Clostridium tyrobutyricum*, yeast and mould. The silages were analyzed for the same constituents and in addition ammonia, pH, acetic, propionic, succinic, lactic, butyric, iso-butyric, n-valeric and iso-valeric acids, 2,3-butanediol and ethanol. The slugs and the slurry were analyzed for LAB, enterobacteria, *Clostridium tyrobutyricum*, yeast and mould.

Chemical analyses were performed as described by Knický & Spörndly (2009). Serial dilutions of samples were cultured for microbiological analyzes. LAB was cultured anaerobically for 72 h at 30°C on Rogosa agar plates and Clostridia spores anaerobically for 7 days at 30°C on RCM agar after heat treatment according to Jonsson (1990) and Pahlow (1990), respectively. Yeast and mould counts were cultivated aerobically at 25°C on malt extract agar supplemented with penicillin G (30 mg/L) and streptomycin sulphate (30 mg/L). For cultivation of enterobacteria, a VRBG agar was used. The above mentioned microbiological analyzes were also performed on a mixture of slugs and the slurry of slug faeces. Analysis of variance was carried out using SAS 9.1 General Linear Models procedure.

Results and Discussion

In total, 687 slugs were collected and the verification of the species *Arion lusitanicus* showed that only 1.3% of were of diverting species and these were discarded. The average weight of the slugs was 0.64 g, and the length was 2.14 cm. The wilting of the crop resulted in 37.1 and 61.4% DM for the Low DM and High DM, containing 8.6% ash, 17.5% CP, 15.1% WSC and 41.5% NDF of the DM. The microbial characterizations of the green crop and a mixture of slugs and slug fecal slurry used to inoculate the silages are presented in Table 1.

Table 1 Microbial characterization of the green crop and a mixture of added slugs and slurry (slugs +feces)

	Silage	Slugs + Slurry
	At harvest	
	Log cfu/g	
LAB	2.8	5.4
Cl. tyrobutyricum	1.5	<1.5
Enterobacteriacae	4.0	6.0
Yeast	1.5	2.7
Mould	2.4	<1.5

The silage additive, applied to the low DM crop, produced silage with lower microbial activity. Mean additive effect on all slug treatments was a higher residual WSC, 11.3 vs 4.1% of DM ($p<0.001$), and lower lactic acid content, 0.6 vs 4.6% of DM ($p<0.001$). The additive contained propionic acid which resulted in elevated propionic acid content in the silage. The lower ammonia-N content, 0.03 *vs* 0.08% of fresh matter ($p<0.001$) and lower losses during ensiling, 0.26 vs. 1.19% of DM ($p<0.001$), also demonstrates that the additive did have the typical influence of restricting the fermentation process.

Typical patterns were also evident when comparing the low DM silage in Table 2 with the high DM silage in Table 3. As an average over all slug treatments, pH was higher in high DM silage, 5.47 vs. 4.96, lactic acid was lower, 0.20 vs. 4.64% of DM, acetic acid, 0.12 vs. 0.87% of DM and so was also the case for succinate and 2,3-butandiole. Consequently, the WSC was higher in the high DM silage, 10.3 vs. 4.11% of DM ($p<0.001$). The ammonia-N content was also lower, 0.04 vs. 0.08% of DM ($p<0.001$) in the high DM silage. The storage losses were 0.79% of DM in the high DM silage while it was 1.19% in the low DM silage.

The effects of adding slugs in the two low DM silages can be seen in Table 2. Indications of improved silage quality, determined as significantly lower pH and higher lactic acid concentration were seen when slugs are added to the low DM silage without additive. Spores of *Clostridium tyrobutyricum , enterobacteriacae* and moulds did not increase. Indications of elevated yeast content could be seen but the numbers were still moderate. Increased ammonia-N and a weak tendency of increased fermentation losses also occurred. In Table 3, the same picture with increased lactic acid concentration when more slugs were added could be seen in the high DM silage. In contrast to the low DM silage, a higher number of LAB were detected at the highest addition of slugs. In both high and low DM silages, no increase in *Clostridium tyrobutyricum* spores or *enterobacteriacae* were seen but a slight increase in yeast was observed at the highest slug level.

The effect of adding slugs to silage resembles to some extent the effect of adding silage inoculants. This reflects the fact that the slugs contained a high number of lactic acid bacteria (Table 1). The *enterobacteriacae* in the slugs did not seem to disturb the fermentations process. However, at the highest slug level the yeast content had a tendency to increase. The fear of slugs destroying the silage could therefore not be verified in this study, but it should be kept in mind that the slugs were collected as juveniles in the beginning of the summer. Results could be different with older and fully grown slugs in the late season.

Table 2 Effects of low slug (LS), medium slug (MS) or high slug (HS) addition with un-infected (UI) crop. Least squares means and significance levels for low DM silages with or without silage additive.

	Low DM silage without additive					Low DM silage with additive				
	UI	LS	MS	HS	P-value	UI	LS	MS	HS	P-value
pH	5.14^{a}	4.95^{b}	4.89^{c}	4.87^{c}	<0.001	4.93^{a}	4.92^{a}	4.84^{b}	4.82^{b}	<0.01
In DM										
Lactic acid,%	2.78^{a}	5.20^{b}	5.14^{b}	5.47^{b}	<0.001	0.15^{a}	0.43^{ab}	0.63^{b}	1.22^{c}	<0.001
Acetic acid,%	0.71^{a}	0.90^{b}	0.87^{b}	1.00^{c}	<0.001	0.11^{a}	0.13^{a}	0.15^{a}	0.22^{b}	<0.01
Propionic acid,%	0.05^{a}	<0.02^{a}	<0.02^{a}	<0.02^{a}	n.s.	0.31^{a}	0.32^{a}	0.35^{ab}	0.38^{b}	<0.05
Succinic acid,%	0.59^{a}	0.84^{b}	0.78^{b}	0.82^{b}	<0.001	0.38^{a}	0.39^{a}	0.39^{ab}	0.42^{b}	n.s.
2,3-butanediol,%	0.42^{a}	0.33^{bc}	0.28^{b}	0.36^{ac}	<0.005	<0.02^{a}	<0.02^{a}	<0.02^{a}	<0.02^{a}	n.s.
Ethanol,%	1.19^{a}	1.05^{a}	1.16^{a}	1.14^{a}	n.s.	0.41^{a}	0.28^{b}	0.28^{b}	0.29^{b}	<0.05
WSC,%	5.00^{a}	3.91^{b}	4.06^{b}	3.49^{b}	<0.01	11.64^{a}	11.35^{a}	11.18^{a}	11.16^{a}	n.s.
Ammonia-N,% in FM	0.05^{a}	0.09^{b}	0.08^{b}	0.09^{b}	<0.001	0.02^{a}	0.02^{ab}	0.03^{b}	0.03^{c}	
Log cfu/g FM										
LAB	6.49^{a}	6.14^{b}	6.49^{a}	6.11^{b}	<0.05	5.24^{a}	5.86^{a}	6.07^{a}	6.07^{a}	n.s.
Cl. tyrobutyricum	<2.0	<2.0	<2.0	<2.0		<2.0	<2.0	<2.0	<2.0	
Enterobacteriacae	1.0	<1.0	<1.0	<1.0		<1.0	<1.0	<1.0	<1.0	
Yeast	<2.0	2.5	<2.0	3.9		<2.0	<2.0	2.3	3.9	
Mould	<2.0	<2.0	<2.0	<2.0		<2.0	<2.0	<2.0	<2.0	
Storage loss,% of DM	1.1^{a}	1.18^{ab}	1.19^{ab}	1.29^{b}	<0.05	0.21^{a}	0.25^{a}	0.25^{a}	0.34^{a}	n.s.
DM,%	33.05^{a}	32.8^{a}	32.73^{a}	33.08^{a}	n.s.	34.71^{a}	34.66^{a}	34.55^{a}	33.80^{a}	n.s.

^{abcd} Means with at least one identical letter within silage type do not differ at sign. level p<0.05.

Table 3 Effects of low (LS), medium (MS) or high (HS) levels of slug infection compared with un-infected (UI) crop. Least square means and significance levels for high DM silage without silage additive

	Treatment at high DM silage				
	UI	LS	MS	HS	P-value
pH	5.48[a]	5.48[a]	5.47[b]	5.43[b]	<0.001
In DM					
Lactic acid,%	<0.02[a]	0.05[a]	0.30[b]	0.45[c]	<0.001
Acetic acid,%	0.10[a]	0.10[a]	0.13[b]	0.14[b]	<0.001
Propionic acid,%	<0.02[a]	0.06[b]	0.06[b]	0.06[b]	<0.001
Succinic acid,%	0.39a	0.40a	0.39a	0.36a	n.s.
2,3-butanediol,%	<0.02[a]	<0.02[a]	<0.02[a]	<0.02[a]	n.s.
Ethanol,%	1.13[ab]	1.84[a]	0.89[ab]	0.56[b]	n.s.
WSC,%	10.5[a]	9.7[a]	10.5[a]	10.5[a]	n.s.
Ammonia-N,% in FM	0.04[a]	0.04[ac]	0.05[bc]	0.05[b]	<0.01
Log cfu/g FM					
LAB	4.58[a]	4.69[a]	5.41[a]	5.38[a]	n.s.
Cl. tyrobutyricum	<2.0	<2.0	<2.0	<2.0	
Enterobacteriacae	<1.0	<1.0	<1.0	<1.0	
Yeast	<2.0	<2.0	<2.0	2.5	
Mould	<2.0	<2.0	<2.0	<2.0	
Storage loss,% of DM	0.75[a]	0.95[a]	0.79[a]	0.68[a]	n.s.
DM,%	55.5[a]	54.7[a]	53.4[a]	53.7[a]	n.s.

[abcd] Means at the same row with at least one identical letter do not differ at sign. level p<0.05

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The influence of primary growth harvest date on microbial composition in grass-dominated haylage

C. E. Müller
Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, SE 753 23 Uppsala, Sweden

Introduction

In comparison with ruminants in production, most (but not all) equines have low nutritive requirements (NRC, 2007). In order to avoid over-feeding and fatness in horses, but still fulfil the behavioural and digestive requirement of forage in the diet, the harvest date of forages for horses with low nutrient requirements can be postponed. By delaying the harvest date, overall digestibility of the forage will decrease and provide less nutrients per kg dry matter (*e.g.* Edouard *et al.*, 2008; Ragnarsson & Lindberg, 2008). However, delaying the harvest date may also affect the hygienic quality of the forage. The counts of epiphytic yeasts, moulds, enterobacteria and lactic acid bacteria (LAB) have been reported to increase with increasing plant maturity (Fehrmann & Müller, 1990; Pahlow, 1991; Adler *et al.*, 1997; Behrendt *et al.*, 1997), and may influence the conservation and resulting microbial quality of the forage. In addition, delayed harvest dates have been reported to result in a decreased lactic acid content and increased pH and butyric acid content in silage (Podkówka & Potkanski, 1991). If the same is true for haylage is not known. As the fermentation in haylage is highly restricted compared to in silage (Jackson and Forbes, 1970), it may be that haylage conservation is less, or more, dependent on the composition of the epiphytic microbial flora on the crop at harvest. In order to gain more knowledge of the effect of plant maturity at harvest on the microbial composition pre- and post-conservation of haylage, an experiment was performed were the primary growth of grass was harvested as haylage in laboratory silos at three different harvest dates.

Materials and methods

The primary growth of grass dominated swards was harvested as haylage (on average 500 g DM/kg) on 29th May, 25th June and 13th August in 2008 and conserved in laboratory silos (25 L). The forages were sampled pre- and post conservation and analysed for microbial composition including yeast, mould, enterobacteria, LAB and clostridia using dilution and cultivation methods described by Müller (2009). Analysis of variance was carried out using SAS 9.1 General Linear Models procedure. Linear correlations and Pearson correlation coefficients were calculated using SAS 9.1.

Results and discussion

Microbial counts pre- and post conservation are reported in Figure 1 and 2, respectively. Counts of yeast, moulds and enterobacteria increased from May to August pre-conservation (Figure 1), but only yeast counts followed the same pattern post-conservation (Figure 2). Yeast counts post-conservation were positively correlated to yeast counts pre-conservation, but R^2 was low (Figure 3). The Pearson correlation coefficient between yeast counts pre- and post- conservation was 0.54 ($P<0.0001$).

Counts of LAB were highest in August both pre- and post-conservation (Figure 1 and 2). Counts of LAB pre- and post- conservation were positively correlated but R^2 was low (Figure 4). The Pearson correlation coefficient between LAB counts pre- and post- conservation was 0.52 ($P<0.0001$).

Counts of enterobacteria increased with increasing plant maturity pre-conservation but not post-conservation (Figure 1 and 2). Counts of enterobacteria pre- and post- conservation were negatively correlated but R^2 was low (Figure 5). The Pearson correlation coefficient between enterobacterial counts pre- and post- conservation was -0.44 ($P<0.001$).

Clostridial spore counts were not affected by harvest date pre- or post-conservation (Figure 1 and 2). Mould counts increased with increasing plant maturity at harvest pre- but not post conservation (Figure 1 and 2). The full result of the study is reported by Müller (2009).

The results of the study suggests that delaying the harvest date of grass haylage also results in higher yeast and LAB counts in the haylage compared to earlier harvest dates. The risk of increased counts of enterobacteria, mould and clostridial spores in haylage however seems to be low, even if counts of enterobacteria are high pre-conservation. These results are in accordance with the results of Heron *et al.* (1993), who found that enterobacterial counts in silage were lower than in the fresh crop and that a reduction of enterobacteria took place during silage conservation.

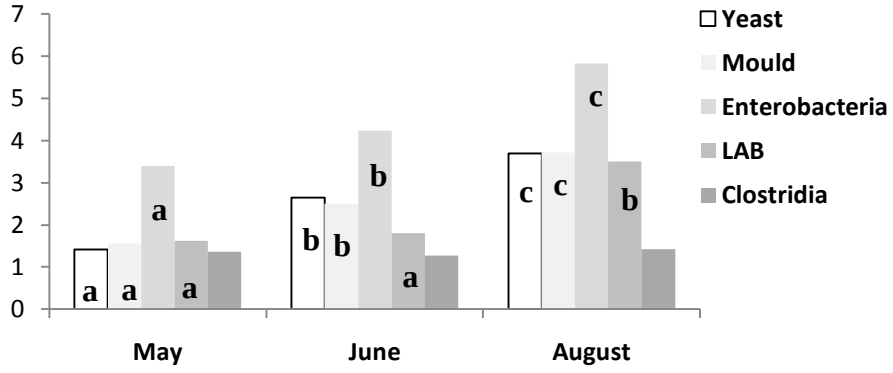

Figure 1 Microbial composition (log CFU/g) in primary growth grass pre-conservation. Different letters indicate differences between harvest dates at $P<0.0001$.

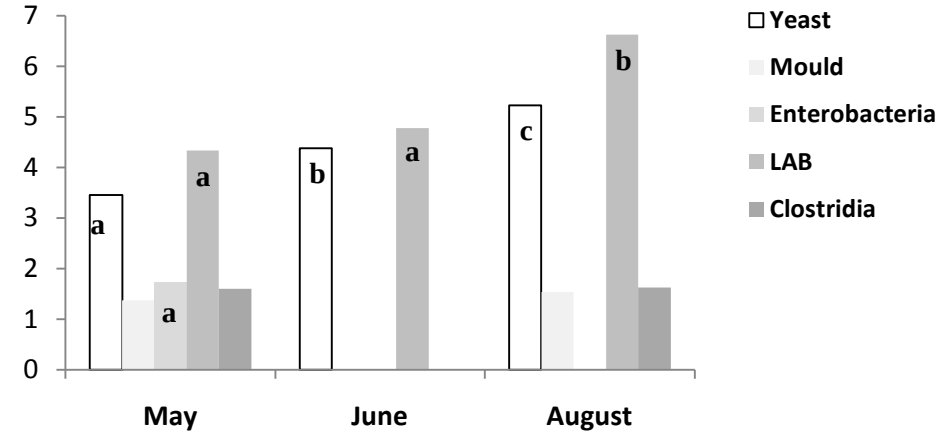

Figure 2 Microbial composition (log CFU/g) in primary growth haylages post-conservation. Different letters indicate differences between harvest dates at $P<0.0001$.

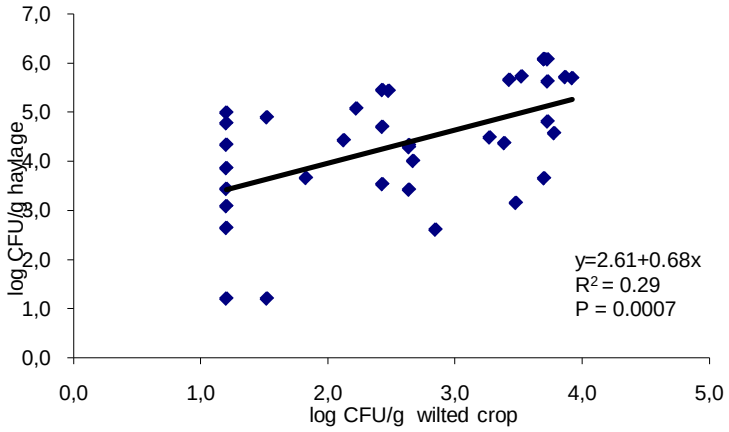

Figure 3 Linear regression between yeast counts in log CFU/g pre- (x-axis) and post- (y-axis) conservation of haylage.

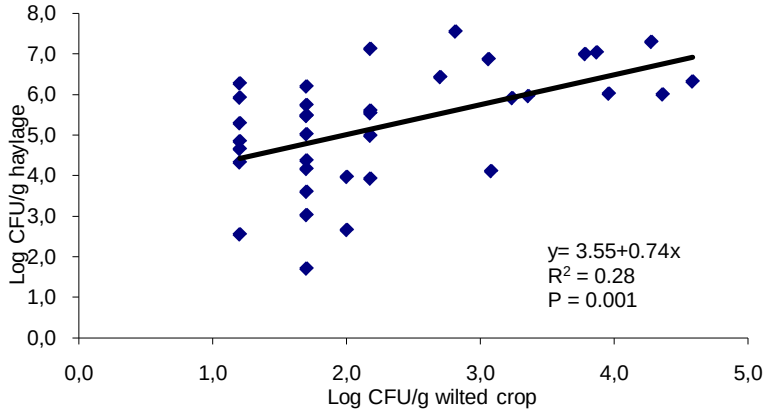

Figure 4 Linear regression between counts of lactic acid bacteria in log CFU/g pre- (x-axis) and post- (y-axis) conservation of haylage.

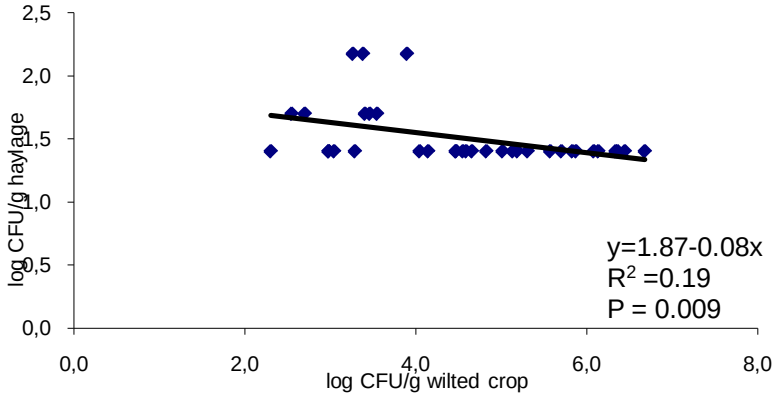

Figure 5 Linear regression between counts of enterobacteria in log CFU/g pre- (x-axis) and post (y-axis) conservation of haylage.

Conclusion

The harvest date of primary growth of grass swards influenced the microbial composition in the crop to a larger extent pre-conservation than post-conservation, with the exception of yeast and LAB counts which increased both pre- and post- conservation with increasing harvest dates.

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The ensiling capability of a mixture of sodium benzoate, potassium sorbate and sodium nitrite

M. Knicky and R. Spörndly
Dept. of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Kungsängen Research Centre, 75323, Uppsala, Sweden.

Introduction

Both clostridial growth and low aerobic stability are frequent problems in silage making. Clostridia are microorganisms that are responsible for the undesirable degradation of forage nutrients during fermentation in silages with low dry matter (DM) content (Pahlow et al., 2003). On the other hand, low aerobic stability is associated with silages with higher DM content where the growth of undesirable yeasts and moulds cause a reduction in crop nutrients (Woolford, 1990). These problems might be avoided by using selective additives, which promote good fermentation, improve aerobic stability and reduce hygienic risks.

The antimicrobial properties of sodium benzoate, potassium sorbate and sodium nitrite are well known and, as such, they are used as additives in the conservation of a variety of feeds and foods. In forage conservation, potassium sorbate and sodium benzoate are characterized as being effective in inhibiting spore-forming bacteria, yeasts and moulds at a pH range from 3 to 6 (Woolford, 1975). Sodium nitrite inhibits the growth of spore-forming bacteria, particularly at low pH levels (Woolford, 1975). The beneficial effect for silage quality of adding variety additive mixtures containing one or more of these compounds has been reported (Kleinschmit et al., 2005; Knicky and Lingvall, 2004). Knicky and Spörndly (2009) were, however, the first to demonstrate the promising results of from combining sodium nitrite with sodium benzoate and potassium sorbate in silage fermentation.

The objective of the study was to examine the potency of silage additive mixture, based on the combination of sodium benzoate, potassium sorbate and sodium nitrite, at preventing the growth of undesirable microflora in silages made from a wide range of different crops of various dry matter contents.

Materials and Methods

Thirteen types of crops were harvested during the period from June to October 2007 in Uppsala, Sweden or in the near surroundings. Crops were divided into 3 groups according to DM content and fermentation coefficient (FC = DM + (8 x WSC/BC), where WSC=water soluble carbohydrates, BC = buffering capacity). The first group included legume-based forages (red clover, lucern) at low DM content that were assumed difficult to ensile with FC below 35. The second group composed of easily ensilable forages composed mainly of grasses (timothy, meadow fescue) with DM content below 350 g/kg and FC above 35, while the third group represented easily or ensilable grasses with DM contents above 350 g/kg and FC above 35. Except for maize crop, which was harvested by a precision chopper (Claas-Jaguar), all forages were harvested manually with a scythe and chopped in a stationary cutter head to approximately 5 cm particle length. Forages in the third group were wilted for 4-8 hours prior to chopping. After chopping, the forages were mixed and divided into 2 fractions. One fraction was treated with a silage additive (sodium benzoate 200 g kg^{-1}, potassium sorbate 100 g kg^{-1}, sodium nitrite 50 g kg^{-1}) at the rate of 5 ml per kg FM on crops containg less than 350 g/kg FM, or 3 ml per kg FM on crops above 350 g/kg FM. The second fraction was left untreated and served as control. Silage additives were applied by hand with a spray bottle on the forage in a plastic bag and mixed thoroughly. Forage from each fraction was

then ensiled in 3 lab-silos (1.7 L volume with a fermentation lock). Directly after silo filling, water was added in the fermentation locks to achieve airtight seals. Six silos from each crop, in total 78 silos, were produced. Silos were stored in room temperature (20-24˚C) for at least 90 days.

Chemical and microbiological analyses were performed on both fresh forages and silages. Analyses of fresh forages included determination of DM, ash, crud protein (CP), WSC, nitrate, buffering capacity (BC), lactic acid bacteria (LAB), and clostridial spore count. The silage quality was assessed by determination of DM, pH, ammonia-N, fatty acids (lactic acid, acetic acid and butyric acid), ethanol, and 2.3-butanediol, clostridia spores and yeast counts. In addition, weight losses and aerobic stability of silages were determined.

Results and Discussion

In general, the results revealed considerably lower DM content and higher buffering capacity of fresh forages in the first group than in the other two groups (Table 1). Legumes are known to have relatively a high BC and low WSC content and when ensiled at a low DM content, it was therefore not surprising that the untreated silages in the first group displayed all signs of clostridial activity, such as higher formations of butyric acid ($P<0.001$) and ammonia-N ($P<0.002$), higher pH ($P<0.04$) and clostridia spore counts ($P<0.001$; Pahlow et al., 2003) in comparison with additive treated silages. A similar pattern of fermentation was also seen in silages from the second group.

Another situation was seen in the third group of crops, where silages were free of clostridia, and with no butyric acid present. This confirms earlier findings that wilting of forages above 400 g/kg is sufficient to eliminate clostridial growth in silages (Jonsson et al., 1990). Because of high DM content, activity of LAB in silages was also restricted, resulting in low production of fermentation acids and consequently low pH drop (McDonald et al., 1991). However, a problem of highly wilted silages can be low aerobic stability, associated with the growth of yeasts (Woolford, 1990), which is accompanied by an increase in temperature. This relationship was well demonstrated in our study, where untreated control silages contained high numbers of yeasts were also found to have low aerobic stability (Table 1). It was seen in three of four forages, where untreated silages contained higher yeast counts ($P<0.03$) than additive treated silages and consequently that it took less time for untreated silages to increase temperature more than 2°C than the treated silages ($P<0.04$). A similar pattern was observed with a 5°C increase ($P<0.05$) in two of the forages. Problems with low aerobic stability are not found in silages where clostridial fermentation is dominating, since high presence of butyric acid inhibits yeast growth (Weissbach & Haacker, 1988).

Therefore, it is predominately well fermented silages which are susceptible to aerobic deterioration. In this regard, results from the present study showed that the tested additive considerably restricted the growth of yeasts in all silages, which caused these silages to be aerobically stable during the whole examination time. The present investigation also confirmed the early finding (Knicky & Spörndly, 2006) that the mixture of sodium benzoate, potassium sorbate and sodium nitrite was efficient to improve silage quality in both high and low DM.

Table 1 Chemical and microbiological compositions of crops and silages at the end of storage. Values within rows with different superscripts are significantly different, P<0.05

	Group I		Group II		Group III		SEM	P value		
	Untreated	Treated[4]	Untreated	Treated	Untreated	Treated		T[2]	C[3]	TxC
Fresh forage										
DM	161±23		254±19		431±37					
CP	192±14		142±20		150±41					
WSC	78±24		143±22		90±57					
BC	76±2		45±8		45±5					
FC	25±3		51±7		59±13					
Silages										
DM	146	166	240	253	423	425				
pH	5.1^{a}	4.2^{b}	4.5^{ab}	4.0^{b}	4.8^{ab}	4.8^{ab}	0.23	**	NS	NS
NH_3-N	177.8^{a}	51.7^{bc}	105.8^{b}	60.4^{bc}	58.2^{bc}	24.0^{cd}	23.3	***	**	NS
LA	43.1^{bc}	122.2^{a}	56.7^{bc}	70.2^{b}	26.4^{c}	26.5^{c}	13.8	**	***	**
BA	55.0^{a}	0.4^{c}	20.7^{b}	0.4^{c}	0.4^{c}	0.4^{c}	7.69	***	**	**
Ethanol	12.9^{b}	3.5^{c}	21.2^{a}	6.1^{bc}	8.9^{bc}	5.1^{bc}	2.34	***	**	NS
Yeasts	1.7^{b}	1.7^{b}	2.1^{b}	1.7^{b}	3.8^{a}	1.7^{b}	0.43	**	NS	NS
Clost.	4.5^{a}	1.9^{b}	4.5^{a}	1.7^{b}	1.7^{b}	1.7^{b}	0.53	***	**	**
Stability[1]	6.1^{a}	6.1^{a}	5.4^{a}	6.0^{a}	3.2^{b}	6.1^{a}	0.67	**	NS	NS

DM=dry matter (g/kg); CP=crud protein (g/kg DM); WSC=water soluble carbohydrates (g/kg DM); BC=buffering capacity (g LA/kg DM); FC=fermentation coefficient; LA=lactic acid (g/kg DM); BA=butyric acid (g/kg DM). Yeasts and clostridia spores (log cfu/g forage).
[1] Stability express number of days until temperature of aerated silages increased by 2°C.
[2] T=effect of additive treatment. [3] C=effect of crop group. [4]Treated variants state silages treated with 5 ml (group 1, 2) or 3 ml (group 3) of silage additive/kg forage.

Conclusions

The application of a mixture of sodium benzoate, potassium sorbate and sodium nitrite considerably decreased the clostridia growth which resulted in a reduced ammonia-N and butyric acid formation in the crop at DM level below 350 g kg^{-1}. In the crop at DM level above 350 g kg^{-1}, the additive mixture was efficient to eliminate yeast activity in silages. The application of the additive mixture seems to be guarantee of prolonged storage stability of silages.

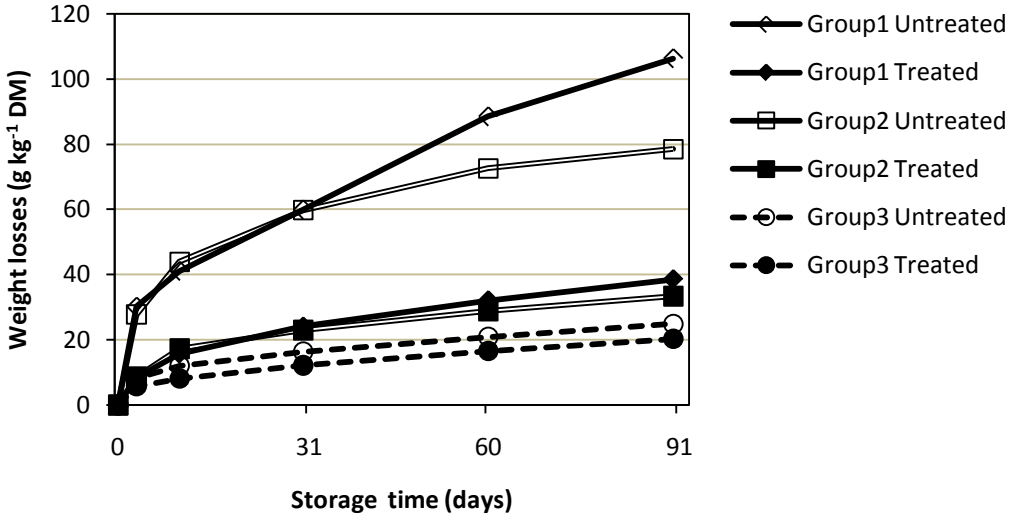

Fig. 1. Weight losses of silages during the storage period.

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The influence of different acidification levels and dry matter in ensiled sainfoin on the dissociation of tannin-protein complexes

M. Lorenz
Department of Animal Nutrition & Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences, S-753 23 Uppsala, Sweden

Introduction

Positive effects of tanniniferous plants on the efficiency of ruminal protein digestion and reduced environmental pollution in dairy production has drawn attention toward tannin containing legumes such as sainfoin (*Onobrychis viciifolia)* or birdsfoot trefoil (*Lotus corniculatus*). The binding of tannins to proteins is believed to reduce their susceptibility to degradation into non protein N-fractions in the rumen and hence increase the uptake of dietary protein in the intestinal tract.

Legumes are difficult to ensile and therefore acidifiers such as formic acid (FA) are used to quickly lower pH at the beginning of the ensiling. In light of known dissociative effects on the protein-tannin complex associated with low pH, coupled with current recommendations for increased FA application in legumes to improve silage storage quality, investigations into FA application limits are necessary. The objective of the experiment reported here was to test if the protein sparing effect of tannins in legume silage remains when high levels of FA are used as additive.

Materials and Methods

Samples of seventeen different sainfoin varieties were pooled and ensiled at different dry matter (DM; 18, 40, 50 and 60%) and acidification (0, 4 and 8 g FA/kg FM) levels in mini-silos, containing approximately 100 g plant material. Total N (N), buffer soluble N (BSN), non protein N (NPN), amino acid-N (AA-N), ammonia-N (NH3-N) and pH were measured before and after ensiling. An inhibited in-vitro (IIV) degradation method (Broderick, 1987) was used to test the influence of varying DM and pH on ruminal protein breakdown in fresh and ensiled material.

Proc REG of SAS was applied to test for differences among, and interaction between treatments for N-fractions. Proc Mixed was used for the IIV digestion data. Class variables were DM and FA. Response variables were the blank corrected sum of AAN and NH3-N at the respective time point with 0-h of AA-N and NH3-N included as covariates.

Results and Discussion

Table 1 shows the effects of FA at different DM on N-fractions. A linear relationship between FA and DM on BSN and NPN could be observed ($P<0.006$). The decreasing effect of formic acid on NH_3-N ($P<0.01$), as previously described in the literature (McDonald et al., 1991) was confirmed in this study with NH_3-N decreased to less than 50% as compared to non acidified silages. DM did not affect NH_3-N. A combination of DM and FA tended to decrease AAN ($P<0.095$).

Figure 1 depicts the effect of DM and acidification ($P<0.002$) on BSN. The effect of acidfication decreased as DM increased. No effect of acidification on BSN could be observed at 50 and 60% DM.

Table 1 Total N (N), buffer soluble N (BSN), non protein N (NPN), amino acid N (AAN), ammonia N (NH_3-N) and pH at different dry matter (DM) and formic acid (FA) in un-ensiled and ensiled sainfoin

Fresh herbage							
DM	FA	CP	BSN	NPN	AA-N	NH3-N	
%	mL/g FM	g/kg DM	g/kg N		g/kg BSN		pH
18	-	24.0	245	211	-	-	5.48
40	-	23.2	264	237	-	-	-
50	-	22.0	278	256	-	-	-
60	-	20.6	297	282	-	-	-
Ensiled Herbage							
18	0	21.1	539	472	330	8.7	3.90
	4	20.7	437	423	307	4.6	3.94
	8	20.4	422	411	254	3.5	3.70
40	0	21.7	360	356	411	12.1	4.16
	4	21.6	353	337	235	4.7	4.20
	8	21.7	321	312	280	5.5	3.92
50	0	22.6	322	307	340	11.7	4.34
	4	21.2	309	291	249	5.7	4.25
	8	20.7	306	294	235	5.3	3.94
60	0	22.8	285	263	303	7.9	4.76
	4	21.0	283	269	302	7.4	4.40
	8	21.4	284	269	277	5.8	4.04
Mean		21.4	352	334	294	6.91	4.13
SEM		0.17	15.6	13.89	11.07	0.56	0.06
Statistical significance:			P-values:				
DM	Linear	0.144	0.001	0.001	0.035	‡	‡
	Quadratic	0.283	0.020	0.068	‡	‡	0.001
FA	Linear	0.032	0.002	0.006	0.001	0.010	‡
	Quadratic	0.162	‡	‡	0.005	0.096	0.001
DM*FA		‡	‡	‡	0.095	‡	‡

‡ terms were removed to improve the fit of the model

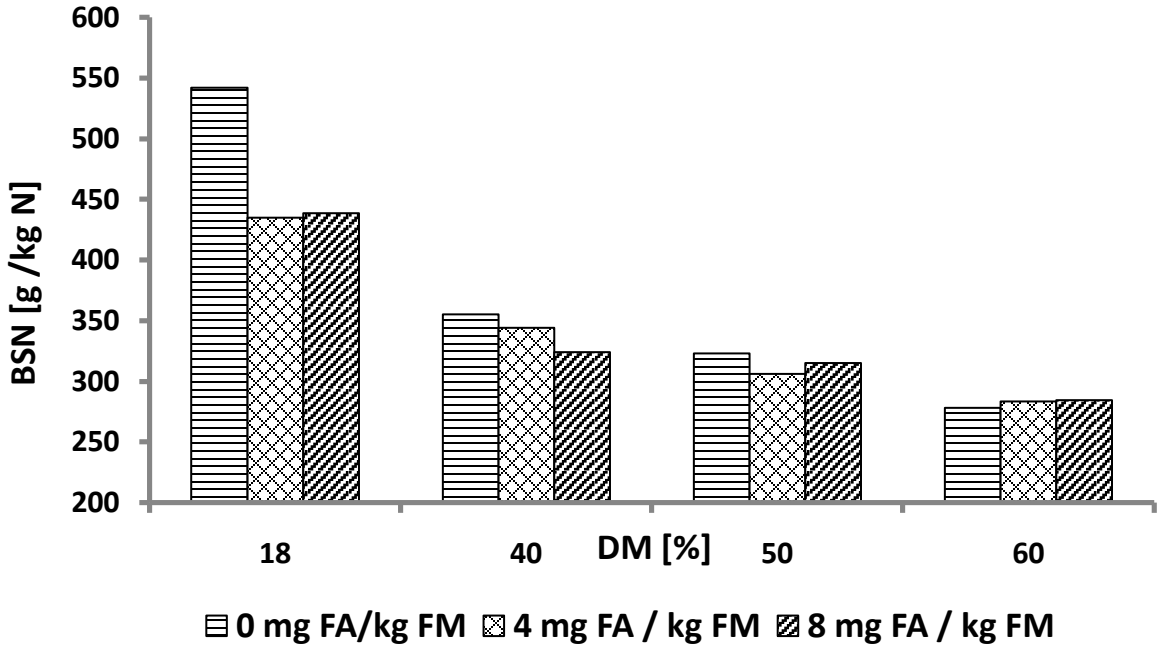

Figure 1 Buffer soluble N (BSN) in acidified and wilted silages (FA = formic acid; FM = fresh matter.

Table 2 Fraction-undegraded protein (FUD) at hour 0, 1, 2, 3 and 4 of incubation and rate of degradation (k_d)

DM	FA	Time (h)					
%	mL/g FM	0	1	2	3	4	k_d
18	0	0.64	0.61	0.53	0.51	0.47	4.4
	4	0.79	0.69	0.67	0.62	0.59	4.7
	8	0.80	0.70	0.69	0.64	0.60	4.6
40	0	0.76	0.64	0.64	0.62	0.59	3.6
	4	0.82	0.72	0.72	0.67	0.65	3.9
	8	0.83	0.71	0.72	0.69	0.66	3.6
50	0	0.79	0.67	0.71	0.65	0.65	3.0
	4	0.83	0.74	0.72	0.69	0.65	4.1
	8	0.83	0.75	0.70	0.69	0.67	3.8
60	0	0.83	0.75	0.73	0.70	0.65	4.1
	4	0.84	0.78	0.73	0.70	0.64	4.8
	8	0.83	0.77	0.72	0.70	0.67	3.9
Mean		0.80	0.71	0.69	0.658	0.62	
SEM		0.016	0.015	0.017	0.016	0.016	
Statistical significance:			P-values:				
		DM	<0.001				
		FA	<0.001				
		DM*FA	<0.001				

Dry matter (DM); formic acid (FA); fresh matter (FM)

Table 2 shows that DM, FA and their interaction (FA*DM) had an effect (P<0.001) on protein breakdown expressed as fraction-undegraded (FUD). Highest degradation was observed for silages with low DM and no FA addition. The obtained values for FUD were in the range of values from experiments by Broderick and Albrecht (1997) for various un-ensiled sainfoin cultivars that ranged from 50 to 96%.

Conclusions

High acidification with FA does not impair the protein sparing effect of tannin in legume silages, neither at low nor at high DM stages. The effect of FA on FUD was dependant on DM (Interaction P<0.001) and resulted in inhibited protein breakdown compared to non acidified silages.

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Minimum temperature for the successful fermentation of corn silage

T. Pauly
Department of Animal Nutrition & Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences, S-753 23 Uppsala, Sweden

Introduction

The cultivation of corn for silage is difficult in Scandinavia due to a short growing season, the frost risk after plant emergence or before harvest and the lack of varieties adapted to the climatic conditions in this area. Farmers are attracted by the prospects of corn silage such as one harvest instead of three with traditional grass silage, no wilting required, a high energy and low protein content that complements well grass silage and few if any negative effects on the fermentation characteristics (clostridial activity) after manure application. However, the cultivation of corn for silage gets increasingly risky the further north you go. Information on the minimum temperature for the successful ensilage of whole crop corn is not available in the literature. If ambient temperature at ensilage is too low, the fermentation process is impeded. As long as temperatures stay around 0°C or lower, the unfermented forage might not markedly deteriorate. However, when temperature rises again months later (spring) the forage usually starts to heat up and eventually gets mouldy. Low temperatures may also increase the risk of oxygen accumulation in the silage because oxygen influx is often larger than microbial consumption.

The goal with this project was to get an indication on the minimal temperature for the successful fermentation of whole-crop corn silage.

Materials and Methods

During two successive years, two lots of fresh whole crop corn were obtained from local growers. In 2008 the corn crop consisted of a mixture of 3 varieties (Avenir, Isberi, Eurostar), which were harvested on Nov. 5th close to Västerås (N 59° 27', E 16° 40') and in 2009 the corn variety Mas 09.A was harvested on Oct. 21th close to Uppsala (N 59° 58', E 17° 31'). The forage was chopped in a precision-chop harvester and ensiled in laboratory silos (1.7 L glass jars with water-filled air locks mounted on metal lids; see Picture 1). Silos were filled outdoors (2-6°C) within a period of approx. 1.5 hours. After homogenizing and sampling of the fresh forage, 10 or 14 silos per storage temperature were filled and moved into climate chambers or refrigerators set at 3 or 4 different temperatures. During the first year, mean storage temperatures were 6°, 12° and 18°C and during the second year, mean silage temperatures were 2.6°, 6.7°, 13.6° and 21.1°C. In the first year, 16 silos were moved from 6°C to 18°C after 45 days to see which effect that would have on silage pH compared to silages that had been stored at 18°C from the beginning.

Figure 1. Type of lab silos used in our experiments.

To study the pH course during silage fermentation, 2 silos from each storage temperature were opened and sampled at 5 or 7 occasions during a storage period of approx. 60 days. Only during the first year these silages were properly analyzed for dry matter (DM), ammonia-N, pH, lactic, acetic and butyric acid, ethanol and aerobic stability. Aerobic stability was determined only in the first year by moni-

toring the increase of silage temperatures during 7 days of aerobic storage at approx. 20°C ambient temperature (Honig, 1990).

Data from the silage composition and aerobic stability test were analyzed statistically by one factorial analysis of variance. The 'Proc GLM' statement of the PC program SAS (version 9.1.; SAS Institute Inc., Cary NC, USA) was used to calculate if differences between storage temperatures were statistically significant. Probabilities >0.05 were considered not significant. The smallest significant difference between treatment means was expressed as the minimum significant difference.

Results and Discussion

The composition of the fresh corn material is shown in Table 1. Cobs were more mature during the second that the first year (see starch content) and the epiphytic LAB counts were very high compared to grass-based forages.

Table 1 Composition of the fresh corn material. Values in g/kg DM if not stated otherwise

Year	Lot	DM	Ash	WSC	Starch	NDF	CP	pH	BC[a]	FC[b]	LAB[c]
2008	1	213	52	61	156	511	100	5.00	2.50	41	$1.3x10^7$
2009	2	293	39	75	199	475	86	4.54	1.93	60	$2.2x10^7$

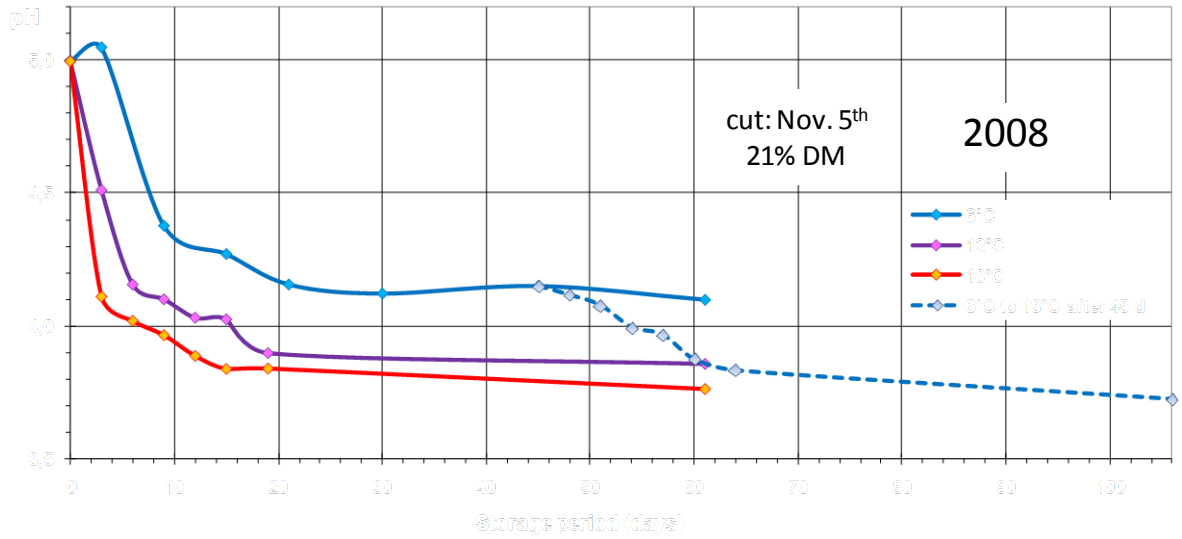
[a]BC = buffering capacity (g lactic acid to reduce pH in 100 g DM from 6.0 to 4.0); [c]FC = fermentability coefficient = DM/10 + (0.8 x WSC/BC) (Weissbach 1996); [c]LAB = lactic acid bacteria in the fresh crop (cfu/g FM).

Table 2 Composition of silages from 2008 after 61 days. Silos that were moved from 6° to 18°C after 45 days (6°➞18°C) were analyzed after 106 days (= 45+61)

Storage temp.	DM	Am-N[a] % of N	Lactic	Acetic	Butyric	Propionic	2,3-Butanediol	Ethanol
			g/kg DM					
6°C	219	6.1[a]	34[d]	16[d]	<0.6	0.3[c]	3.2[b]	19.7[b]
12°C	224	6.7[b]	50[c]	2.0[c]	<0.6	0.3[c]	3.2[b]	13.7[c]
18°C	218	7.0[c]	59[b]	29[b]	<0.6	1.3[b]	2.9[b]	10.4[d]
6°➞18°C	215	7.5[c]	61[a]	34[a]	<0.6	2.3[a]	4.6[a]	22.6[a]
Mean	219	6.8	51	25	<0.6	1.1	3.5	16.6
Prob.[c]	NS	**	***	***	NS	***	**	***
MSD[b]	183	0.81	1.8	1.8	-	0.6	0.7	2.8

[a] Am-N = ammonia-N in % of total N; [b]MSD = minimum significant difference between two means; [c]Prob. = Probability that all treatment means are equal; NS = not significant.

The fermentability coefficient (FC) was above the threshold value of 45 for easy fermentable silages only in the second year. However, first year silages were well fermented with the exception of high ethanol levels (see Table 2). Aerobic stability was high in all these silages irrespective of storage temperature. The course of the pH decrease in silages is shown in Figure 1 and 2. It was evident that even at temperatures as low as 2.6° or 6°C an acid formation and pH-decrease occurred. Final pH values leveled out at a much higher pH in silages stored at low as compared to high temperatures. When silages were moved after 45 days from 6° to 18°C ambient temperature, pH values dropped to approximately the same level as in silages stored at 18°C from the beginning. The initial pH increase in silages stored at 2.6° and 6°C might be explained by a longer aerobic phase due to a temperature-restricted plant respiration, which consumes oxygen. First after depletion of oxygen, lactic acid producing bacteria may be able to dominate the fermentation and reduce silage pH.



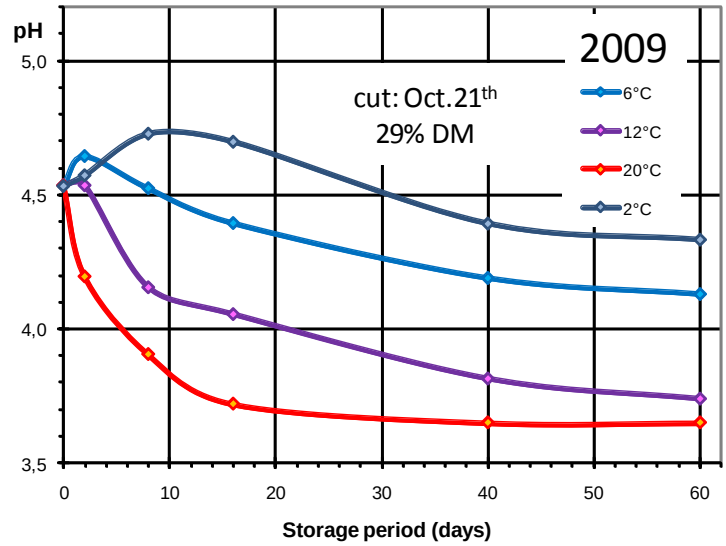


Figure 1+2. pH course of 2 whole crop corn silages stored at temperatures between 2° and 20°C.

Conclusions

This experiment indicates that a fermentation process (pH decrease) occurs even at temperatures as low as 2°C. The pH decrease was, however, much slower and final pH values leveled out at considerably higher values than in silages stored at higher temperatures. When the silage temperature increased later, silage pH dropped to the same level as in silage that was stored at the higher temperature from the beginning. Considering the lacking information on fermentation quality and aerobic stability of silages made at 2-3°C, a temperature of approximately 6°C appears to be the minimum temperature for the ensilage of whole-crop corn.

Acknowledgement

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Considered individually, some of our contributions to more effective agriculture may seem small, but even major rivers have many tributaries and these many small streams finally form a sizeable waterway. We call it Lean Farming and Lean Contracting.

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Phone: +46 371-345 00 • www.trioplast.com

Fibre content and physiochemical properties of various horse feed ingredients

C. Brøkner[a], K. E. Bach Knudsen[b], A. H. Tauson[a]
[a]*University of Copenhagen, Faculty of Life Sciences, Department of Basic Animal and Veterinary Sciences, Grønnegaardsvej 3, DK-1870 Frederiksberg C, Denmark.* [b]*Department of Animal Health and Bioscience, Faculty of Agricultural Sciences, Aarhus University, Research Centre Foulum, Blichers Allé 20, DK-8830 Tjele, Denmark.*

Introduction

There is an increasing need for identifying energy dense feed ingredients based on fibre, as starch has been shown to cause health problems in sports horses (Kronfeld et al., 2005). This experiment aimed at evaluating feeds considered to be suitable for horses by use of an enzymatic-chemical dietary fibre (DF) analytical method compared with conventional analytical methods of crude fibre (CF) and neutral detergent fibre (NDF). We expect the DF method to provide more detailed and useful information concerning the nutritional properties of feed ingredients for horses.

Materials and Methods

Fourteen different feeds of diverse botanical origin ranging from apple pulp, root crops, cereal grains, roughages, a commercial muesli feed (Equigard®, a loose chaff based concentrate composed of 24 % grass hay, 22 % apple pulp, 22 % sugar beet pulp from Mühle Ebert Dielheim GmbH, Germany), molassed sugar beet pulp (Betfor®, 1.5 cm long pulps from Danisco Sugar A/S, Denmark) and PreAlpin (PreAlpin Wiesencobs consisting of 4 % forbs and 95 % grasses from Agrobs GmbH, Münsing, Germany) were analysed in duplicates and the obtained mean values reported.

The dry matter content was determined on milled feed samples after drying at 105 °C for 24 h. Gross energy was analysed by bomb calorimetry (Parr instrument company, Illinois, USA). Crude fat content was determined by petroleum ether extraction in a Soxtec system after HCl hydrolysis according to Stoldt (1952). Nitrogen content was measured according to the Kjeldahl method (Tecator-Kjeltec system 1030, Tecator AB, Höganäs, Sweden) and a factor of 6.25 was used to calculate the crude protein content.

Total sugars and fructans were analysed by the enzymatic-colorimetric method of Larsson and Bengtsson (1983) and starch by an enzymatic-colorimetric method according to Bach Knudsen (1997). Total non-starch polysaccharides (NSP), divided into cellulose and insoluble (I) and soluble (S) non-cellulosic polysaccharides (NCP), were determined by gas-liquid chromatography for neutral sugars and by a colorimetric method for uronic acids in three parallel runs as described by Bach Knudsen (1997). In brief, starch was removed after incubation with thermostable α-amylase for 60 min. at 100°C and 120 min. incubation at 60°C with β-glucanase free amyloglucosidase. For the total NSP procedure, soluble fibres were precipitated with 80 % ethanol and the supernatant discarded. The starch free residue was treated with 12 M H_2SO_4 in order to swell cellulose which afterwards was hydrolysed to monosaccharides with 2 M H_2SO_4. The neutral sugars were reduced to alcohols and acetylated to alditol acetate derivates and quantified by gas-liquid chromatography. Uronic acids were measured separately by colorimetry (Scott 1979) and Klason lignin was measured gravimetrically. The procedure for total NCP was similar to total NSP except that the swelling of cellulose with 12 M H_2SO_4 was omitted and the starch-free residue was directly hydrolysed to monosaccharides with 2 M H_2SO_4. When analysing insoluble NSP, soluble

fibres were first extracted from the starch-free residue by a phosphate buffer and then analysed according to the procedure for total NSP. Klason lignin was measured gravimetrically as the sulphuric acid insoluble residue as described by Theander et al. (1986). The content of dietary fibre was calculated based on the following equations:

DF = Total non-cellulosic polysaccharides + Cellulose + Klason lignin

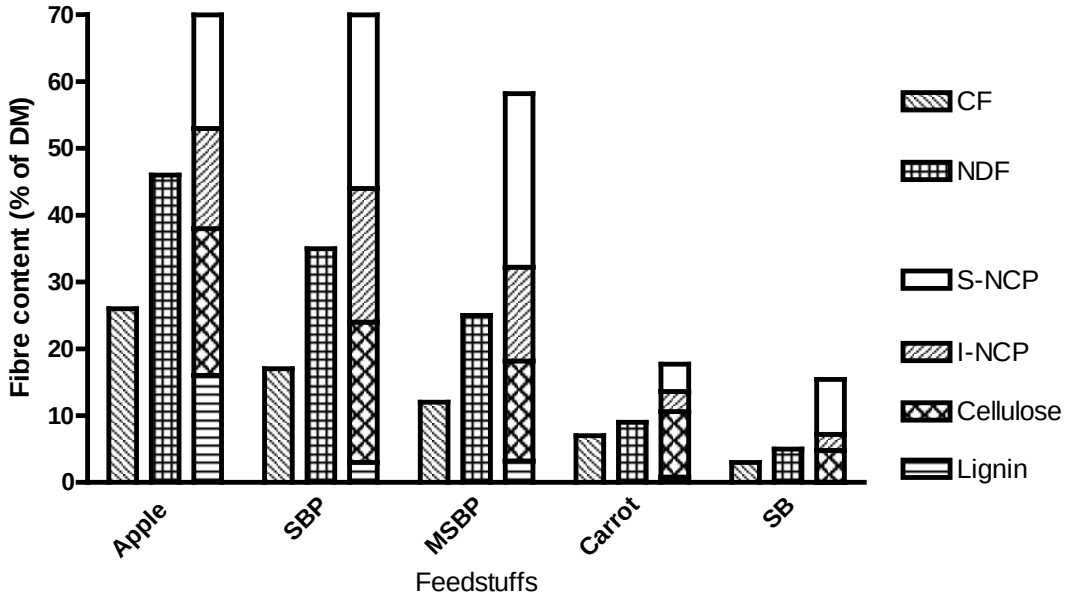
Crude fibre was analysed by a FiberCap system (FiberCap™ 2021/2023 Fibre Analysis System, Hillerød, Denmark) according to Mertens (2002). aNDF content with amylase pre-treatment was analyzed according to Van Soest (1967) and Mertens (2002) by the use of Fibertec system (Fibertec™ 2010 Auto Fibre Analysis System, Hillerød, Denmark). The physiochemical properties were quantified based on swelling and water binding capacity (WBC) (Canibe et al., 2002).

The amount of fibre detected according to the DF, NDF and CF methods was evaluated by analysis of variance using the PROC GLM procedure in SAS version 9.2 with feed and method as fixed effect. The feeds were grouped into root crops and apple pulp, roughages, cereal grains and Muesli and correlated to their physiochemical properties by use of PROC GLM procedure in SAS.

Results and Discussion

The DF method detected ($P < 0.001$) more NSP as compared to the NDF and CF methods (Figure 1 A – C). The greatest difference between the DF and NDF methods was found in root crops and apple pulp. The soluble NCP (S-NCP) fraction made up 24-55 % of total NSP of these feeds. The S-NCP fraction is lost in the NDF and CF methods due to solubilisation, which explains the higher recovery of total NSP by the DF method. This illustrates that a feed's potential as a fibre source may be underestimated depending on the analytical method. The S-NCP fraction is considered highly fermentable and potentially makes a greater contribution to the total energy supply in horses than the insoluble fraction (Hoffman et al., 2001).

A



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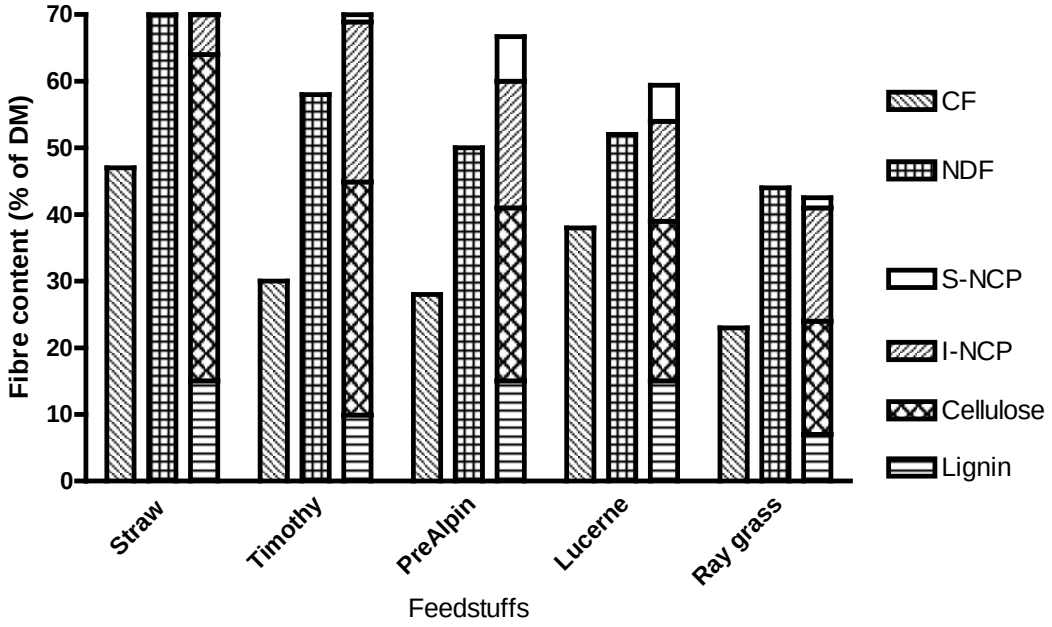

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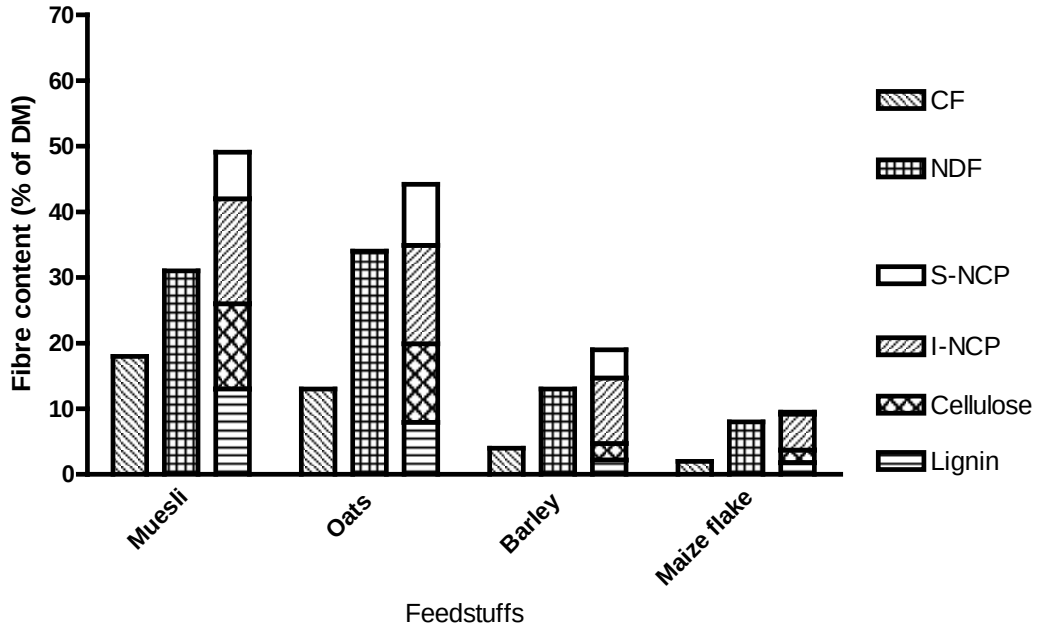

Figure 1 The content of fibre as percent of DM in root crops and apple pulp (A), roughages (B) and cereal grains and Muesli (C). Dietary fibre is divided into lignin, cellulose, soluble and insoluble non-cellulosice polysaccharides (S-NCP and I-NCP). Molassed sugar beet pulp (MSBP), Sugar beet pulp (SBP) and Sugar beet (SB).

The results are presented in Table 1. Between 91and 101 % of DM was detected. The highest total sugar content was measured in root crops with 76 % of DM in raw sugar beet followed by 56 % of DM in carrots. The starch content ranged from 34 % to 75 % in cereal grains. A systematic effect was measured for feed types and physiochemical properties (Table 1): Swelling ($r^2 = 0.68$, $P = 0.05$) and WBC ($r^2 = 0.77$, $P = 0.01$). Cereal grains differed ($P = 0.05$) from roughages, root crops and apple pulp in having lower WBC and swelling capacity.

Conclusions

These results clearly illustrate that soluble fibre fractions are lost depending on the analytical method and, thus, underestimated. This indicates the importance of using more detailed analytical procedures like the DF method in order to rank feed ingredients based on their fibre content and fibre quality. The physiochemical properties can be used to predict how a feed behaves in the digestive tract of horses.

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Table 1 Content of nutrients as percent of dry matter and the physicochemical properties of feeds of diverse botanical origin

	MSBP	SBP	SB	Carrot	Apple	Rye grass	PreAlpin	Timothy	Lucerne	Straw	Oats	Barley	Maize flake	Muesli
Chemical composition														
DM	91	92	96	89	92	89	96	94	95	96	92	90	90	90
Ash	7	8	2	7	2	9	11	6	8	2	3	3	1	10
CP	11	8	2	6	8	16	10	11	15	3	12	13	8	10
Crude fat	0.35	0.21	0.83	2.7	2.4	2.9	1.8	1.8	1.2	1.3	6.3	2.5	2.8	3.2
Total sugar	20.4	5.4	76	56	8.3	19	7.5	10	3.3	0.19	1.6	3.5	1.9	14
Starch	0.26	1.5	0.11	0.58	6.7	0.39	1.9	0.13	0.73	0.42	34	58	75	5.0
Fructans	nd	nd	nd	nd	nd	0.64	2.7	0.19	nd	nd	nd	nd	nd	0.13
NSP	54	70	15	17	57	36	52	60	44	79	36	17	7.6	36
Lignin	3.2	3.0	nd	0.80	16	7.0	15	9.9	15	15	7.9	2.2	1.8	13
DF	58	73	15	18	73	43	67	70	60	95	44	19	9.3	48
aNDF	25	35	5	9	46	44	50	58	52	81	34	13	8	31
CF	12	17	3	7	26	23	28	30	38	47	13	4	2	18
GE, MJ/g DM	17	17	17	17	19	19	18	19	19	19	19	18	18	18
Physicochemical properties														
Swelling, ml/g DM	8.3^{b}	13^{b}	7.1^{b}	35^{b}	6.7^{b}	11^{b}	8.2^{b}	10^{b}	8.4^{b}	9.4^{b}	5.0^{a}	3.7^{a}	5.7^{a}	7.7^{a}
WBC, g/g DM	4.5^{b}	5.3^{b}	3.3^{b}	8.9^{b}	4.3^{b}	9.5^{b}	4.5^{b}	6.1^{b}	5.1^{b}	7.8^{b}	1.7^{a}	1.3^{a}	2.2^{a}	2.9^{a}

Dry matter (DM), Crude protein (CP), Non-starch polysaccharides (NSP), Dietary fibre (DF), Neutral detergent fibre (aNDF), Crude fibre (CF), Gross energy (GE), Water binding capacity (WBC), Molasses sugar beet pulp (MSBP), Sugar beet pulp (SBP), Sugar beet (SB), Not detectable (nd). The values in rows with different superscripts differ at $P = 0.05$.

Form of α-tocopherol affects vitamin E bioavailability in Thoroughbred horses

J.D. Pagan, M. Lennox, L. Perry, L. Wood, L.J. Martin, C. Whitehouse and J. Lange
Kentucky Equine Research, Versailles, Kentucky 40383,USA

Introduction

Vitamin E functions as a biological antioxidant, preventing the oxidation of unsaturated lipid materials within cellular and subcellular membranes by neutralizing production of free radicals. Supplemental vitamin E may be beneficial in horses experiencing oxidative stress such as during parturition and exercise (Hargreaves et al., 2007) and for horses at risk of certain types of neurological diseases (Mayhew et al., 1987; Blythe and Craig, 1993).

Vitamin E can be obtained from natural or synthetic sources, but the chemical structure of each is different. Natural vitamin E is composed of one isomer (d-α-tocopherol [RRR α-tocopherol]), and it is the most bioactive form in human and animal tissue. Synthetic vitamin E is a mixture of eight isomers (dl-α-tocopherol [all-rac-α-tocopherol]), of which only one is identical to the natural isomer. These eight isomers vary greatly in relative biopotency. Synthetic or natural vitamin E is typically added to equine feeds in an esterifed form (α-tocopherol acetate) to prolong shelf life.

To account for differences in biopotency, the relative strengths of different forms of vitamin E are expressed as international units (IU) in which 1 mg of synthetic acetate equals 1 IU, 1 mg of natural acetate equals 1.36 IU, and 1 mg of natural alcohol equals 1.49 IU (Anon, 2000). These conversion factors were developed using laboratory animal models, and they may not be relevant for horses and humans. In fact, studies in humans have suggested that natural-source vitamin E is twice as bioavailable as the synthetic form (Acuff et al., 1998; Burton et al., 1998), and studies in horses have suggested that the relative bioavailability of natural-source vitamin E is greater than synthetic (Pagan et al., 2005; Hargreaves et al., 2007).

The following studies were conducted to determine if synthetic and natural-source vitamin E have similar bioavailabilities when administered at equal IU doses and to determine if water-dispersible forms of vitamin E are more bioavailable than lipid-soluble forms.

Materials and Methods

Two studies were conducted to assess the relative bioavailability of different forms of vitamin E. In study 1, single oral doses of three different forms of vitamin E were evaluated in eight Thoroughbred geldings (age 10.75 ± 2.2 years) during three one-week periods. The forms of vitamin E evaluated included synthetic vitamin E (dl-α-tocopheryl acetate) (SYN)[1], natural-source vitamin E acetate (d-α-tocopheryl acetate) (ACT)[2], and natural-source alcohol (d-α-tocopherol) (ALC)[3]. On the first day of each period, the horses were administered 5000-IU doses of vitamin E top-dressed on 1 kg of unfortified sweet feed at 7:00 AM. Baseline blood serum samples were collected immediately before dosing and at 3, 6, 9, 12, and 24 hours post-dosing.

In study 2, three Thoroughbred geldings (age 5.67 ± 1.2 years) were used in a replicated 3 x 3 Latin square design trial to assess the relative bioavailability of three forms of vitamin E. There were a total of six one-week periods with each horse receiving each form of vitamin E in two separate periods. The vitamin E forms studied were synthetic vitamin E (dl-α-tocopheryl acetate) (SYN)[1], a micellized d-α-tocopherol (Elevate WS)[4], and a d-α-tocopherol (Nano·E)[5] that had been nanodispersed into liposomes. Both of these processes render normally lipid-soluble vitamin E water dispersible. At the beginning of each period the horses

received a single 5000-IU dose of one of the vitamin forms top-dressed onto 1 kg of unfortified sweet feed. Baseline blood serum samples were collected immediately before dosing and at 3, 6, 9, 12, 24, 36, and 48 hours post-dosing. Throughout both studies the horses were maintained on an unfortified sweet feed plus grass hay.

Serum α-tocopherol was measured using high-performance liquid chromatography[6], and relative bioavailabilities were calculated from comparisons of magnitudes of responses measured by areas under the concentration versus time curves (AUC) and by comparisons of the peak concentrations of serum vitamin E following each dose. The AUC, baseline, peak, and maximal change from baseline data were analyzed by analysis of variance (ANOVA), and a Tukey-Kramer multiple comparison was used to examine differences between treatments.

Results and Discussion

In study 1, ACT and ALC had a significantly greater AUC than SYN ($P < 0.05$) (Table 1). There was no significant difference in AUC between ACT and ALC. Relative to SYN, the bioavailability of ACT and ALC equaled 197% and 252%, respectively. Time post dosing to peak vitamin E was not different between treatments and averaged 9.2 ± 1.2 (mean ± SE) hours. Although there was a trend towards higher peak levels and maximal change from baseline values for the ACT and ALC treatments compared to SYN, these differences were not significantly different ($P > 0.05$).

Table 1 Response in serum α-tocopherol to 5000-IU doses of synthetic, natural acetate, and natural alcohol forms of vitamin E

	Synthetic[1]	Natural acetate[2]	Natural alcohol[3]
n:	8	8	8
Area under curve (24 h)	5.9 ± 1.5[a]	11.6 ± 2.0[b]	14.9 ± 3.0[b]
Baseline vitamin E (µg/ml)	3.44 ± 1.80[a]	3.51 ± 0.09[a]	3.31 ± 0.13[a]
Peak vitamin E (µg/ml)	3.97 ± 0.08[a]	4.58 ± 0.39[a]	4.58 ± 0.39[a]
Δ vitamin E (µg/ml)	0.52 ±0 .12[a]	1.07 ± 0.39[a]	0.93 ± 0.18[a]

[ab]Means for the same item with the same letter are not different ($P > 0.05$)

In study 2, Elevate WS and Nano·E had a significantly greater AUC than SYN ($P < 0.05$) (Table 2). There was no difference in AUC between Elevate WS and Nano·E. Relative to SYN, the bioavailability of Elevate WS and Nano·E equaled 559% and 613%, respectively. Time post dosing to peak vitamin E was not different between treatments and averaged 12.0 ± 1.4 (mean ± SE) hours. Nano·E had higher peak and maximal change from baseline values compared to SYN ($P < 0.05$).

The results of these studies suggest that natural sources of vitamin E have a greater bioavailability than is accounted for in the current conversion factors of 1.36 and 1.49 used in the feed industry for natural acetate and alcohol, respectively. These differences should be taken into account when calculating the quantity of supplemental vitamin E required by horses.

Table 2 Response in serum α-tocopherol to 5000-IU doses of a synthetic and two water-dispersible forms of vitamin E

	Synthetic[1]	Elevate WS[4]	Nano·E[5]
n:	6	6	6
Area under curve (48 h)	15.62 ± 3.22[a]	87.36 ± 25.3[b]	95.85 ± 25.7[b]
Baseline vitamin E (µg/ml)	3.04 ± .30[a]	3.00 ± .39[a]	2.86 ± .42[a]
Peak vitamin E (µg/ml)	3.63 ± .36[a]	6.01 ± 1.26[ab]	6.69 ± 1.39[b]
Δ vitamin E (µg/ml)	.59 ± .08[a]	3.00 ± .89[ab]	3.83 ±1 .15[b]

[ab]Means for the same item with the same letter are not different ($P > 0.05$)

Natural-source water-dispersible forms of vitamin E were 5-6 times more bioavailable than synthetic vitamin E acetate, and a 5000-IU dose more than doubled serum vitamin E levels within 12 hr. These forms of vitamin E should be beneficial when a rapid increase in vitamin E is warranted such as during periods of oxidative stress (exercise or parturition) or for horses at risk of certain types of neurological disease.

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Footnotes

[1]ROVIMIX E-50 Adsorbate (dl-a-tocopheryl acetate), DSM Nutritional Products AG, Wurmisweg 576, CH-4303 Kaiseraugst, Switzerland

[2]KER Equine Ester™, Kentucky Equine Research, Versailles, KY 40383, USA

[3]NOVATOL™ 5-87 (d-α-tocopherol), Archer Daniels Midland Company, Decatur, IL 62526, USA.

[4]Elevate WS®, Kentucky Performance Products LLC, Versailles, KY 40383, USA

[5]Nano·E™, Kentucky Equine Research, Versailles, KY 40383, USA

[6]DCPAH Nutrition, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, USA

Fish oil and corn oil supplementation affect red blood cell and serum eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations in Thoroughbred horses

J.D. Pagan, T.L. Lawrence, and M.A. Lennox
Kentucky Equine Research, Versailles, KY 40383, USA

Introduction

Horses require both omega-3 and omega-6 fatty acids in their diets. The omega-3 family stems from alpha-linolenic acid (ALA), while the omega-6 family originates from linoleic acid (LA). Long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are intermediates in the formation of eicosanoids that have been shown to reduce inflammatory responses, support immune function, and enhance fertility (Curtis et al, 2000; Hall et al., 2004; Stelzleni et al., 2006; Vineyard et al. 2006). This study was conducted to compare the effect of supplementation with oil high in EPA and DHA (fish oil) or low in EPA and DHA (corn oil) on red blood cell (RBC) and serum EPA and DHA.

Materials and Methods

Twelve Thoroughbred geldings were supplemented for 127 d with 60 ml of either fish oil (EO·3)[a] or corn oil. They also received a basal diet of 8 kg of timothy hay and an unfortified sweet feed, soybean meal, sodium chloride, and calcium carbonate to meet NRC requirements. The horses were exercised three times weekly on a mechanical walker and turned out into small paddocks daily for 4-6 hours with muzzles to prevent grazing and housed overnight in 12 x 12 box stalls. Blood samples were taken at d 0, 29, 57, 92, and 127 in EDTA collection tubes before the morning feeding, placed immediately on ice, and analyzed for EPA and DHA.

Results and Discussion

By d 29, horses receiving fish oil had an average increase in serum EPA and DHA of 3.7-fold ($P \leq 0.05$) and 17.9-fold ($P \leq 0.01$), respectively (Figure 1 and 2). In horses receiving corn oil, serum EPA decreased 1.5-fold from baseline at d 57 ($P \leq 0.05$) and fourfold by d 92 ($P \leq 0.05$). By d 127, RBC DHA concentrations in the fish oil supplemented horses was over 1.9-fold greater ($P \leq 0.05$) than baseline (Figure 3), while there was no difference observed in RBC DHA from horses receiving corn oil. In the fish oil supplemented group, RBC EPA increased 11.5-fold ($P \leq 0.05$) by d 127 (Figure 4). Corn oil supplemented horses had lower than baseline RBC EPA at 57 d ($P \leq 0.05$), 92 d, and 127 d ($P \leq 0.01$).

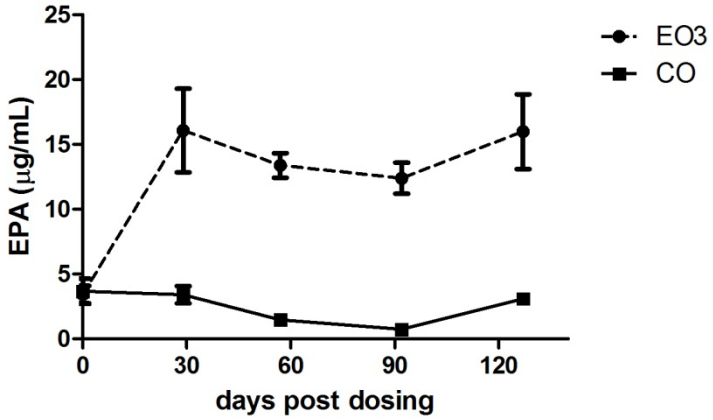

Figure 1. Serum EPA

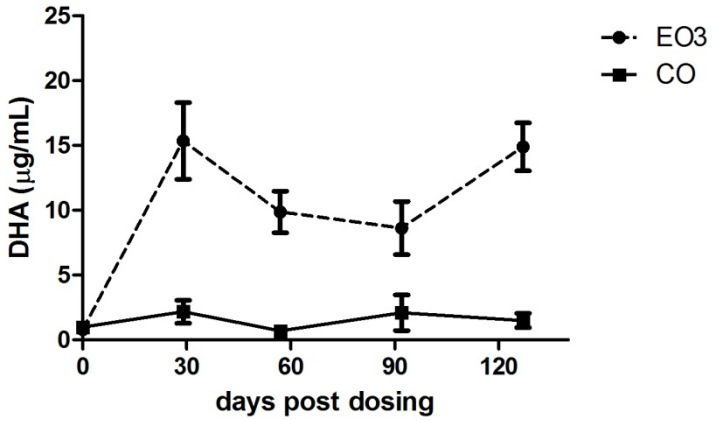

Figure 2. Serum DHA

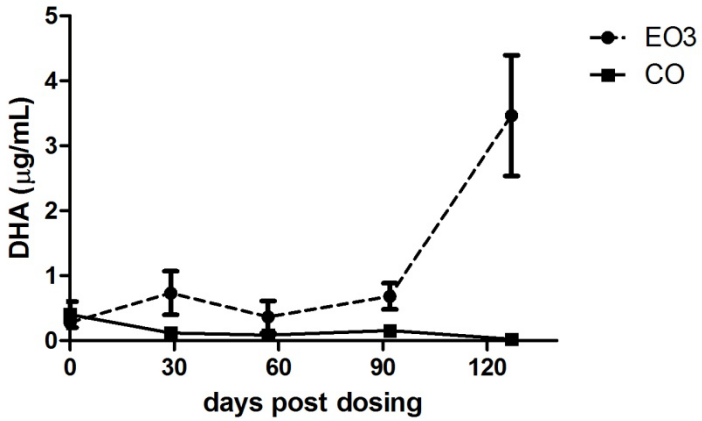

Figure 3. Red Blood Cell DHA

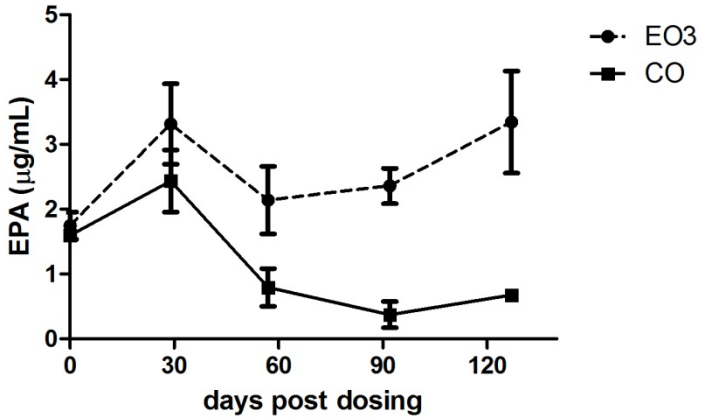

Figure 4. Red Blood Cell EPA

This study showed that 60 ml/d of fish oil supplementation increases serum and RBC EPA and DHA in horses. Corn oil supplementation resulted in a decrease in RBC EPA, which may affect RBC membrane fragility.

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Footnote

[a]EO·3™, Kentucky Equine Research, Versailles, KY 40383 USA

Effect of linseed based fibre feeds on diet digestibility in horses

M.T. Saastamoinen and S. Särkijärvi
MTT Agrifood Research Finland, Animal Production, Equines, Opistontie 10 a 1, FI-32100 Ypäjä, Finland

Introduction

Linseed (*Linum usitatissimum*) or by-products (groats, cakes, meals) of linseed oil pressing have been used in human and animal nutrition for a long time because of their believed beneficial effects on health. Linseed and its by-products are rich in fat, which is a good source of valuable fatty acids. It contains pectins and other dietary fibres which have been proved to promote the health of gastrointestinal tract in human and dogs (e.g. Reinhart *et al*., 1995). Linseed has also been evaluated for potential anti-inflammatory properties. Published research on linseed in horse nutrition is limited – however, Williams and Lamprecht (2008) has reviewed some studies. In a previous study we proved that when horses were fed plain linseed groats mixed to the concentrate portion, the diet digestibility decreased (Särkijärvi and Saastamoinen, unpublished). In the present study we examined the effect of two linseed based feed supplements on the diet digestibility. By including other raw materials (sugar-beet pulp, carrot, garlic) to the supplement the palatability and digestibility of the product can be improved. In addition, the ingredients used are offering good dietary fibres (e.g. Dogowski *et al*., 1998), and may have other health benefits as well (Särkijärvi *et al*., 2010; Saastamoinen *et al*., 2010). Especially sugar-beet pulp is well utilised by the horse (Lindberg and Jacobsson, 1992; Palmgren Karlsson *et al*., 2002), containing a lot of soluble and highly fermentable fibre (Bach Knudsen, 1997).

Materials and Methods

The influence of two linseed based feed supplements on the diet digestibility was tested with six Finnhorse mares (aged between 5 and 14 years, mean BW 636 kg). The horses were randomly allotted to three dietary treatments: A) Basal diet consisting of dried timothy-dominated hay and oats, B) Basal diet + Feed 1, and C) Basal diet + Feed 2. Feed 1 (F1) contained 70 % of linseed groats, 15 % dried carrot, 10 % dried garlic and 5 % molasses. Feed 2 (F2) contained 65 % of linseed groats, 15 % sugar-beet pulp, 10 % dried garlic, 5 % dried carrot and 5 % molasses. The chemical composition of the feeds is presented in Table 1.

The horses were individually fed at the maintenance level according to the Finnish feeding recommendations, the forage-to-concentrate ratio being 70:30. Feeds were offered three times a day at 0630, 1230 and 1730. The grain ration was given about 30 minutes after the hay ration. The experimental feeds (F1, F2) were fed at a level of 10 % of the dry matter (DM) intake, the average daily portion being on average 850 g/horse divided to three equal portions fed separately after the intake of oats. The experimental feeds were soaked before feeding. Mineral intakes were balanced with a commercial mineral mixture during the days between the collecting periods.

The mares were individually housed in pens (3 x 3 m) with free access to water and salt block. The horses were exercised daily in outdoor paddocks for 4 hours and 1 hour by riding. They were de-wormed before the experiment and dental care and vaccinations were carried out regularly. Blood samples were collected at the beginning of each period and analyzed for white and red blood cells, mean corpuscular volume, packed cell volume, hemoglobin, urine, serum alanine transminase, serum gamma-glutamyl transpeptidase to evaluate possible effects of the diets to the health of the horses.

The experimental design was arranged as two balanced 3 x 3 Latin Squares. Each experimental period consisted of 21 days: 16 days of adaptation to the new diet followed by a five-day period of representative faeces sample collection. The change in rations between periods was made gradually during the first five days of the adaptation period. The horses were weighed after each collection period.

Table 1 Chemical composition of the experimental feeds (g/kg DM)

	Hay	Oats	Linseed feed 1	Linseed feed 2
Dry matter g kg^{-1}	870.7	883.1	905.2	885.4
Organic matter	934.7	971.4	930.6	924.3
Crude protein	95.0	112.8	211.5	209.8
Ether extract	15.6	61.0	185.7	172.7
NDF	687.7	263.3	183.3	228.0
Crude fibre	339.0	89.0	96.0	105.3
Ash	63.5	28.6	69.4	75.7
NFE	485.1	708.6	437.5	436.5

The data was analysed with a linear mixed model using the MIXED procedure of the SAS system using the REML estimation. The statistical model was: $Y_{ijk} = \mu + a_i + t_j + p(sq)_k + \varepsilon_{ijk}$, where Y_{ijk} is the observation, μ is the overall mean, a_i is the random effect of i^{th} animal (i=1,…6), t_j is the fixed effect of j^{th} dietary treatment (j=1,…3), $p(sq)_k$ is the fixed effect of k^{th} period within the square (k=1,…3), and ε_{ijk} is the normally distributed error with a mean of 0 and the variance δ^2.

Digestibility data were obtained by using chromium mordanted straw (68 mg Cr/g DM) with a dalily dose of 1.6 g/kg feed DM as an indigestible external marker for the estimation of apparent digestibility, administrated as described by Särkijärvi and Saastamoinen (2006). Palmgren Karlsson (2001) suggested that chromium mordanted fibre could be an alternative for the administration of chromium, but may result in underestimated digestibility values. In Särkijärvi *et al.* (2010), however, chromium mordanted silage gave quite precise digestibility values in horses. Chromium mordanted straw was prepared according to Udén *et al*. (1980). Faecal grab samples were taken from each horse twice a day, after the morning and mid-day feeding, during five-day collection period. Daily faecal samples were stored at -24°C until mixed, sub-sampled and dried for laboratory analysis. Samples of hay and oats were collected for analysis over the last seven days of each period. The feed samples were stored until the end of the five-day collection period and handled in the same manner as the faecal samples. Prior to morning feeding, feed refusals from the previous 24 h, if any, were collected and weighed. Feed and faeces samples were analysed for DM, ash, crude protein (CP), ether extract (EE), crude fibre (CF), neutral detergent fibre (NDF), and, in addition, faeces samples also for pH, as described by Särkijärvi and Saastamoinen (2006). Also the treatment and analysis of the blood samples was described in Särkijärvi and Saastamoinen (2006).

Results and Discussion

Both supplemented diets (F1 and F2) had higher digestibility of crude protein (CP) compared to the basal diet ($P<0.05$) (Table 2). Also, the digestibility of fat (ether extract, EE) was higher in the supplemented diets than in the basal feeding ($p<0.01$), being on average 68.8 % and 55.5 % for the supplemented and basal diets, respectively. The improvement of the digestibilities is due to the higher concentration of those nutrients in the supplemented diets compared to the basal diet, as reported previously e.g. by Gibbs *et al.* (1988), Särkijärvi and

Saastamoinen (2006) and Ragnarsson and Lindberg (2008) for CP, and Takagi *et al*. (2003) and Lindberg *et al*. (2006) for EE.

The digestibilities of NDF and CF did not differ between the diets, but the CF digestibility was numerically somewhat lower in F1 diet compared to F2 and basal diets. This may be due to the larger proportion of linseed groats and smaller proportion of other fibre sources in the F1 diet. In a previous study (Särkijärvi and Saastamoinen, unpublished) we found that plain linseed groats supplementation from 6 to 10 % in the diet DM gradually decreased the diet digestibility. This has also been reported in dogs (Kempe and Saastamoinen, 2007), and may be due to poor digestibility of linseed husks and hull's mucilage content. On the other hand, F2 diet provided more dietary fibres from sugar-beet pulp and carrot having possible a prebiotic effect on intestinal bacteria (e.g. Snel *et a*l., 2002; Dongowski *et al*., 1998).

The digestibility of ash (minerals) was higher in the linseed groats supplemented diets (F1 and F2) than in the basal diet, maybe again due to their higher mineral (ash) content. There was no difference in the digestibility of nitrogen free extracts (NFE) between the diets

The highest digestibility coefficient of the diet DM was observed for the F2-supplemented diet, being 57.0 %. Also the OM digestibility was somewhat higher in the F2 diet than in other diets. However, the differences were not statistically significant.

Table 2 Digestibility coefficients (%) of the total diet nutrients

	Basal diet	F1 supplemented	F2 supplemented
Dry matter	54.8	55.3	57.0
Organic matter	56.9	57.2	58.9
Crude protein	61.4	64.0	65.7
Ether extract	56.2	68.0	68.8
NDF	47.2	47.8	47.9
Crude fibre	44.6	43.9	46.3
Ash	19.7	25.1	27.4
NFE	62.8	62.0	63.5

Based on the blood analyses no adverse health effects were observed due to the supplementation with linseed based feeds during the nine-week experimental period. This was in agreement with Särkijärvi & Saastamoinen (unpublished) and Saastamoinen *et al*. (2010). In literature, linseed is claimed to have toxic and adverse health effects because of enzyme linase which would release HCN from a glycoside of the seeds (e.g. Frape, 1998). However, the current *Linum usitatissimum* varieties have quite low concentrations of these health detrimental compounds. The palatability of the supplemented diets were good and no refusals were left. In a previous study, we have observed that the palatability of plain linseed groats was not very good when fed large (more than 6 % in DM) portions (Särkijärvi & Saastamoinen, unpublished).

Conclusions

Linseed based supplements used in this study improved crude protein and fat digestibility of hay-oats diets of horses. No adverse effects on the digestibility of any component of the diet or on the health of the horses were observed. Linseed acts as a good source of extra protein and fat which result in improvement of digestibility of those nutrients. Supplemental feeds with ingredients suitable for horses and offering dietary and highly soluble fibres may promote the function and health of the digestive tract of the horse.

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Effects of a forage-only diet on body weight, microflora and V_{La4} on Standardbred horses in training

A. Jansson and J. E. Lindberg
Dept of Animal Nutrition and Management, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

Introduction

Horses are grass eaters and accordingly adapted to high fibre diets. However, most athletic horses are offered starch-rich diets although horses have limited starch digestion capacity (Kienzle, 1994) and such diets may increase the risk for colic (Tinker et al., 1997; Hudson et al., 2001). A major reason for high starch feeding strategies might be that forages with a high enough energy density are not readily available on the market. However, high energy forages can easily be produced, especially during Nordic conditions where the climate favours high sugar content and high fibre digestibility in forages (Ragnarsson, 2009). A drawback with high forage diets might be an increased body weight (BW) (Ellis et al., 2002) which might increase the work load in exercising horses and thereby possibly impair performance. The aim of this study was to investigate the effect of a forage-only diet compared to a 50:50 mixed diet (forage and starch-rich concentrate) on BW, plasma lactate threshold (V_{La4}) and faecal microflora in Standardbred horses in training.

Material and methods

Six geldings in race condition were used (27 ± 8 races, record 77.3 ± 0.8 s/km, age 6.5 ± 0.4 years, initial body weight 515 ± 21 kg). The horses were fed a forage-only diet (F, early cut timothy/meadow fescue haylage) and a 50:50 mixed diet (C) including haylage (late cut) and concentrate in a change-over design for 29 days, respectively. The concentrate included 82 % oats, 14 % soy bean meal, 2.7 % wheat bran and 1.4 % sugar. Both diets were supplemented daily with 51 ± 2 g of a mineral and vitamin supplement (Miner Röd, Krafft, Sweden), 36 ± 1 g sodium chloride and diet C with 34 ± 1 g ground lime stone. The feed allowances were estimated to be iso-caloric (116 ± 5 in diet F *vs* 117 ± 5 MJ ME/day in diet C) and iso-nitrogenous (1002 ± 45 in diet F *vs* 1008 ± 45 g digestible crude protein/day).

The horses were trained on a track at heart rates above 200 beats/min (interval training or 1600-2000 m heats) every third or fourth day (same days in both periods). On day 25, the horses were transported to a clinic and performed an incremental exercise test on a treadmill and the velocity at plasma lactate concentration 4 mmol/L (V_{La4})was estimated. The BW was registered before each training session. Faecal samples were collected on day 29, DNA was extracted and bacterial 16S rRNA genes were amplified and bacterial community composition assessed by terminal-restriction fragment length polymorphism and cloning and sequencing (Willing et al., 2009). A total of 86 clones were sent for bidirectional sequencing and resulting quality sequences were compared to the GenBank database using BLAST search. Sequences were aligned using the hidden markov model alignment algorithm implemented in the ARB phylogenetic analysis program (Ludwig et al. 2004). The alignment model was generated from all available full-length 16S rRNA sequences from type strains (5160 as of August 2008) that were aligned by secondary structure in the Ribosomal Database Project server (Cole et al. 2007), then imported into ARB. Cloned sequences were then aligned to the model and inspected manually for alignment errors.

Values are presented as means ± standard error of the mean. All data were subjected to analysis of variance (GLM procedure in the Statistical Analysis Systems package, SAS

Institute Inc. Cary, NC, USA). The *P* value for significant difference between treatments was < 0.05.

Results and Discussion

The mean BW was higher on diet F (519.0 ± 0.5 kg) compared to diet FC (516.0 ± 0.5 kg). There was no difference in V_{La4} between diets although there was a tendency for a higher V_{La4} on diet F (8.0 ± 0.1 *vs* 7.6 ± 0.1 m/s, P<0.1). There was a reduced microbial stability and an increase of lactic acid bacteria and members of the *Streptococcus bovis/equis* complex in the faecal flora on diet C compared to diet F. Diet C also resulted in the increase in members of *Clostridiaceae* cluster III and a concomitant reduction in an unknown group of *Bacteroidales*.

Although this study indicates that horses on a forage only diet might be heavier than horses on a concentrate rich diet, the increased weight seemed not to negatively affect the response to exercise. In contrast, V_{La4} tended to increase on diet F. It was expected, due to the water holding capacity of fibres, that the horses would gain BW on diet F. However, the weight gain on diet F was small (3 kg) and it is possible that this extra weight had been lost at the time of the exercise test, due to lack of feed intake during the hours preceding the test (Connysson, 2009). This small increase in body weight is in contrast to observations on low intensity trained horses where the weight gain was 10 kg on a high fibre diet (Ellis *et al.*, 2002). It is likely that differences in the chemical composition between forages are the reason for the differences in weight gain in these studies.

The changes in the microflora (Willing et al., 2009), suggest lower relative abundance of specific bacterial populations that have been associated with the induction of laminitis on the forage diet. Although there was no effect of diet on the diversity of the bacterial population, the absence of concentrate from the diet resulted in a more stable microbial population. It should be noted that the starch intake level in the current study is considered to be a safe level of dietary starch inclusion in horses with respect to digestive disturbances (Kienzle, 1994). This indicates that the previously suggested limit for starch intake (2 g starch/kg BW) to avoid digestive problems in horses and support equine health and welfare, may have to be changed.

Conclusions

A high energy forage-only diet might increase BW but seemed not to affect VLa4 negatively. The changes observed in the faecal microflora indicate an increased availability of starch and soluble carbohydrates in the large intestine.

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Prediction of energy content in forage for horses

J. E. Lindberg[1] & S. Ragnarsson[2]
[1]*Department of Animal Nutrition & Management, P.O. Box 7024, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden;* [2]*Holar University Collage, 551 Saudárkrókur, Iceland*

Introduction

Currently, the content of energy in forage for horses in Sweden is predicted from ruminant feed evaluation data (Lindgren, 1979) that have been adapted to horses. However, these adaptations are not based on *in vivo* data from horses. Therefore, it is of interest to find ways to predict the energy value in forage for horses from *in vivo* measurements in horses.

In the present study the possibility to predict the content (MJ/kg DM) of digestible energy (DE) in forages fed to horses from the dietary content of neutral detergent fiber (NDF), acid detergent fiber (ADF) and dietary fiber (DF), and the coefficient of *in vitro* digestibility of organic matter (IVDOM) was evaluated.

Materials and Methods

Energy balance data (average of four observations) from eight batches of preserved forage, produced in the north of Iceland and fed at maintenance level of energy intake to mature Icelandic horses in balance trials, were used (Ragnarsson, 2009). All forages were plastic wrapped and wilted, and baled in large (250-450 kg) round or square bales and fed straight out of the bales in long form. The forage-only diets were fed for a total of 20 days, comprising 14 days of adaptation and 6 days for total collection of faeces, to determine the total tract apparent digestibility of energy. During adaptation periods horses had access to salt lick stones and were fed minerals during non-collection periods.

NDF was analyzed according to Chai and Udén (1998) using undiluted ND solution, sodium sulphate and amylase. ADF was analyzed according to Goering and van Soest (1970) and DF as described by Högberg and Lindberg (2006). The IVDOM was analyzed according to Lindgren (1979) using rumen fluid as inoculum. Gross energy was measured with a bomb calorimeter (Parr 1241 Oxygen Bomb Calorimeter, Illinois, USA).

The effect of fiber content and IVDOM on the digestible energy (DE) content in preserved forage was subjected to linear regression analysis.

Results and Discussion

The range in content (g/kg DM) of dietary components in the forages were: crude protein 93-200, NDF 503-639, ADF 270-411 and DF 534-645. The IVDOM (%) ranged from 62-83, and the range in GE and DE (MJ/kg DM) was 19.2-20.1 and 9.0-14.1, respectively.

The DE of the forages were linearly related ($P<0.05$) to the content of NDF, ADF and DF, and the IVDOM (Table 1). This was in line with earlier studies showing a linear decline in the digestibility of organic matter (dOM) with increasing dietary fiber content in different feedstuffs fed to horses (Smolders et al., 1990). In the current study, the best prediction of DE in the forages was found for the IVDOM ($r^2=0.95$). Similarly, Smolders et al. (1990) reported that an *in vitro* assay gave the best predictions of dOM in various roughages. As shown in Table 1, neither of the fiber measures used (NDF, ADF or DF) were able to predict the variation in DE ($r^2=0.53$-0.63) in the forages with a high accuracy.

Table 1 Relationship between the content (MJ/kg DM) of digestible energy (DE), and the dietary content (g/kg DM) of NDF, ADF and DF, and the coefficient of *in vitro* digestibility of organic matter (IVDOM) in preserved forage [#]

	NDF	ADF	DF	IVDOM
Intercept	25.94	18.97	28.92	-5.65
SE	4.844	2.849	5.406	1.545
Slope	-0.0256	-0.0222	-0.0299	0.2335
SE	0.0086	0.0085	0.0093	0.0208
R^2	0.59	0.53	0.63	0.95
RSD	1.36	1.57	1.23	0.15
P-value	0.025	0.040	0.018	0.0001

[#] SE=standard error; RSD=residual standard deviation

Conclusions

Neither of the common fiber measures used to evaluate the nutritional value of feedstuffs, such as NDF and ADF, nor the more advanced DF measure, was able to predict the variation in DE in preserved forage for horses with an acceptable accuracy as reflected in the low R^2 values. In contrast, IVDOM appears to be an acceptable method to predict DE content in preserved forage for horses.

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The effect of short-term adaptation to a high-fat diet on insulin sensitivity in aged Thoroughbred horses

L. Perry, J.D. Pagan, and L. Wood
Kentucky Equine Research, Versailles, Kentucky 40383, USA

Introduction

Insulin resistance is an associated risk factor in laminitis, metabolic syndrome, and equine Cushing's disease (Andrews and Frank, 2009), especially among aged horses. Management strategies to reduce the incidence and severity of these diseases include exercise, weight loss, and dietary changes. Diets high in nonstructural carbohydrates have been suggested to negatively affect insulin sensitivity in horses (Hoffman et al., 2003; Treiber et al., 2005), while it is unclear how high-fat diets affect insulin sensitivity. Therefore, the objective of this study was to examine whether short-term adaptation to a high-fat diet would affect insulin sensitivity in aged horses.

Materials and Methods

Three Thoroughbred geldings (18-24 yr; BCS 6.5-7; weight 591.0 ± 51.0 kg) were used in a three-period longitudinal study with each period lasting 35 days. During the first and third periods, the horses were fed 9.09 ± 1.90 kg/d of mixed grass/legume hay and 2.5 kg of an unfortified sweet feed (CHO 1 and 2) (Table 1). During the second period, the horses received the same amount of mixed grass/legume hay and 1.5 kg of a grass/legume hay cube and 600 ml of soybean oil (FAT) (Table 1). The CHO and FAT diets were formulated to be isocaloric. In the FAT ration, 21% of daily DE was supplied from fat and 10% of DE came from nonstructural carbohydrates (NSC). In the CHO ration, 9% of daily DE was from fat and 26% of DE was from NSC.

Table 1 Nutrient composition of experimental diets and hay

Nutrient % (DM basis)	CHO	FAT	Grass/Legume Hay
DM	88.30	93.50	89.50
CP	10.75	15.60	22.45
ADF	5.20	19.10	37.45
NDF	13.90	32.90	51.00
Fat	5.40	25.0	1.20
Starch	55.20	1.10	0.60
WSC	6.70	5.00	6.00

Fasting blood samples were taken on d 0, 7, 14, 21, 28 and 35 of each period. An oral (OGTT) and intravenous (IVGTT) glucose tolerance test was conducted each period on d 28 and d 35, respectively. For the OGTT, a 50% dextrose solution was administered at a rate of 1 g glucose/kg BW via nasogastric tube. Blood samples were taken via jugular catheter immediately before (0 min) and at 30, 60, 90, 120, 180, 240, 300, and 360 min post administration. For the IVGTT, a 50% dextrose solution was administered intravenously at a rate of 0.5 g glucose/kg BW over 10 min and blood samples were collected immediately before (0 min) and at 5, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min post administration. Weekly fasting blood samples were analyzed for glucose, insulin, and triglycerides. Samples

taken during the OGTT and IVGTT were analyzed for insulin, glucose, and nonesterified fatty acids (NEFA).

Results and Discussion

During the OGTT, blood glucose was higher in the FAT group at 120 min ($P < 0.05$) compared to CHO1 and was higher at 120 min ($P < 0.01$) and 180 min compared to CHO2 (Figure 1). Insulin during the OGTT was not different (Figure 2). During the IVGTT, the area under the curve (AUC) for glucose concentration vs. time was higher for the FAT group compared to both CHO1 ($P < 0.01$) and CHO2 ($P < 0.001$). Glucose during the IVGTT was higher ($P < 0.05$) in the FAT group at 5, 30, 90, 120, 180, and 240 min compared to CHO1 and at 5, 15, 30, 60, 90, 120, 180, and 240 min compared to CHO2 (Figure 3).

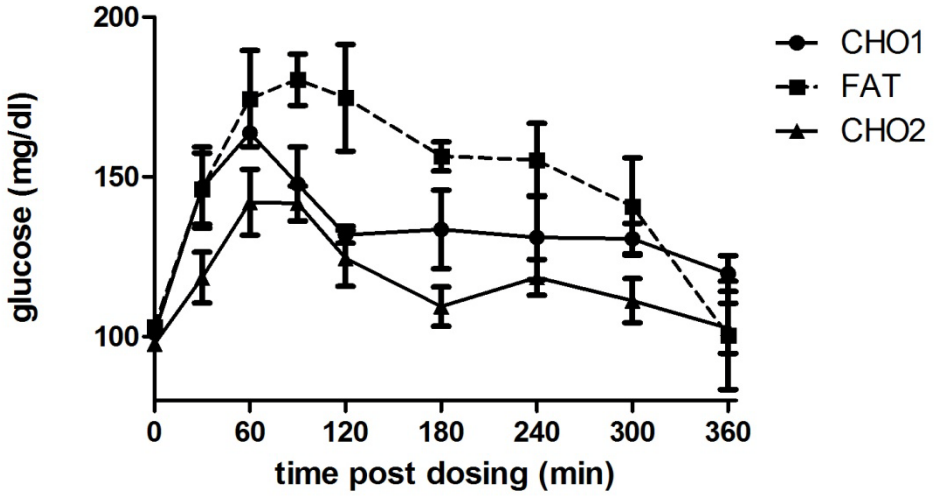

Figure 1 Glucose response during an oral glucose tolerance test (OGTT).

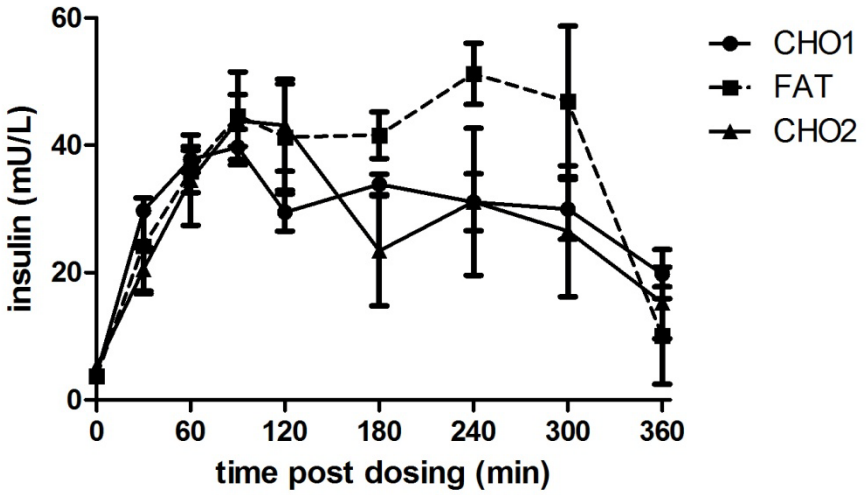

Figure 2 Insulin response during an oral glucose tolerance test (OGTT).

The AUC for insulin during the IVGTT was not different between treatments, but insulin in the FAT group was lower ($P < 0.05$) at 5, 15, 30, 60, 90, and 120 min compared to CHO1 and at 5, 60, 90, and 120 min compared to CHO2 (Figure 4). The results of this study suggest that feeding a high-fat diet to aged horses reduces insulin sensitivity compared to a moderately

high-carbohydrate diet. During the IVGTT, horses on the high-fat diet produced less insulin and took longer to clear glucose from their blood. Further research is needed to determine if these differences were due to high fat in the ration or a lack of readily digestible carbohydrate.

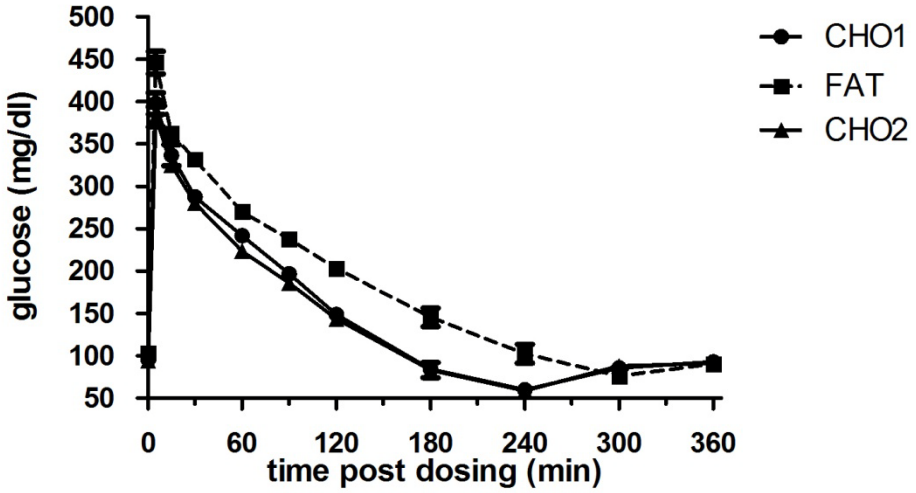

Figure 3 Glucose response during an intravenous glucose tolerance test (IGTT).

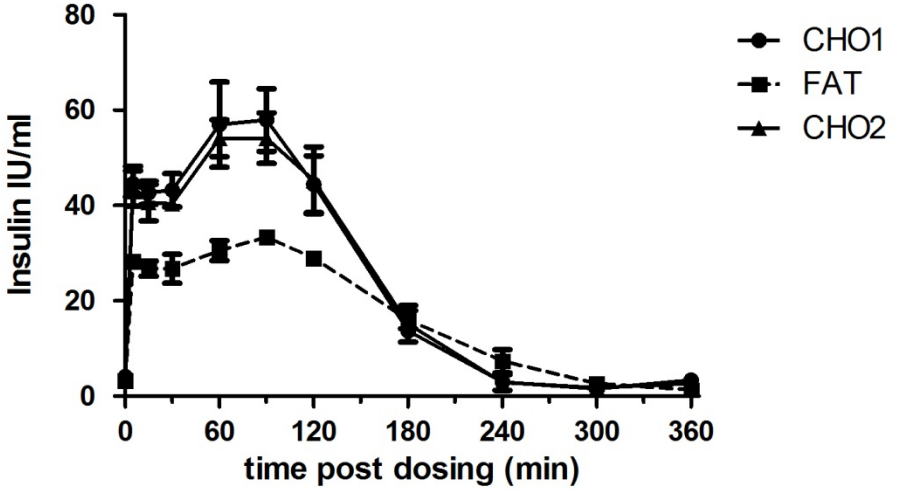

Figure 4 Insulin response during an intravenous glucose tolerance test (IGTT).

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Effect of equine characteristics and dietary factors on mean retention time of digesta in the gastrointestinal tract - a meta-analysis

P. Nørgaard and J. S. Jensen
Department of Basic Animal and Veterinary Sciences, Faculty of Life Sciences, University of Copenhagen, DK-1870 Frederiksberg, Denmark

Introduction

The relative short mean retention time (MRT) of digesta in the gastrointestinal tract in equines limits the digestion of neutral detergent fibre (NDF) in the hindgut of equines (Hyslop et al. 1997; Weyenberg et al. 2006). The published MRT values range from 21 h in Welsh ponies fed dehydrated alfalfa ad libitum (1.7% NDF of body weight (BW)) (Pearson et al. 2001) to 64 h in Highland ponies restrictively fed dehydrated alfalfa (0.4% NDF of BW) (Cuddeford et al. 1995). According to a review by Weyenberg et al. (2006), the MRT value can be affected by several animal and feed related factors like BW, physiological state, type of diet and feeding level. The MRT values have been found to be dependent on breed (Cuddeford et al. 1995; Miraglia et al. 1992; Todd et al. 1995; Pearson et al. 2006; Drogoul et al. 2000; Pearson and Merritt, 1991), exercise (Pagan et al. 1998; Orton et al. 1985b), diet (Cuddeford et al. 1995; Pearson et al. 2006; Drogoul et al. 2000, 2001; Pearson and Merritt, 1991; Moore-Colyer et al. 2003; Rosenfeld et al. 2009) and cannulation of cecum plus colon (Drogoul et al. 2000).

Pagan et al. (1998) and Orton et al. 1985 observed a lower MRT in exercised compared with non exercised horses. Drogoul et al. (2000) observed a lower MRT value in cross bred gelded ponies fed ground and pelleted hay compared with chopped hay, but Miyaji et al. (2008) observed no different MRT value by feeding either hay or silage to Thoroughbred horses. Drogoul et al. (2001) also found in mature ponies the same MRT of forages particles as for Yb labelled barley grain. Pearson et al. (2006) observed that 25% feed restriction caused a 9 to 18% higher MRT in ponies fed low and high quality forages ad libitum, but oppositely, across the four forages the MRT values increased by 0.12 h per increased intake of forage DM per $BW^{0.75}$.

The MRT of digesta particles has been measured by use of different markers like Ru-P (Orton et al. 1985a,b), ytterbium chloride hexahydrate (Yb) (Pagan et al. 1998; Drogoul et al. 2000; Moore-Colyer et al. 2003), chromium mordanted fibre (Cr) (Cuddeford et al. 1995; Pearson and Merritt, 1991; Pearson et al. 2001, 2006; Uden et al. 1982, europion bond to forage cell wall (Eu) (Drogoul et al. 2001) and samarium (Sm) by Miyaji et al. 2008). The objectives of the present study was to study effect of marker type, dietary and equine characteristics on MRT of digesta in the gastrointestinal tract of by use of a meta-analyze of published MRT values.

Materials and Methods

The 46 different MRT values from 10 published studies were analyzed by a meta-analysis including fixed effects of type of breed, levels of exercise, forage type, level of feeding and type of marker as class variables and BW, $BW^{0.75}$, DM intake per $BW^{0.75}$, NDF intake per BW, diet crude protein (CP) content and CP*CP as continuous variables in the full model 1, and studies as random effects. The animals were classified as Shetland ponies (Cuddeford et al. 1995), Welsh ponies (Moore-Colyer et al. 2003; Pearson and Merritt, 1991; Pearson et al. 2001, 2006), Welsh cross ponies (Drogoul et al. 1995), Highland ponies (Cuddeford et al. 1995), Stock horses (Orton et al. 1985, p386) and Thoroughbreds (Cuddeford et al. 1995;

Miraglia et al. 1992; Miyaji et al. 2008). The exercises were classified into three levels and included horses tied or housed loose in small boxes with no additional exercise (Cuddeford et al. 1995; Drogoul et al. 2000; Miraglia et al. 1992; Orton et al. 1985; Pearson and Merritt, 2003), horses walked or with access to a pen (Moore-Colyer et al. 2003; Pagan et al. 1998; Pearson et al. 2001,2006) or exercised at least ½ h on a rotunda/treadmill (Orton et al. 1985; Pagan et al. 1998). The forage supply was classified into six different types and included grass hay (Drogoul et al. 2000; Miraglia et al. 1992; Moore-Colyer et al. 2003; Pearson et al. 2006), dehydrated alfalfa (DA) (Cuddeford et al. 1995; Pagan et al. 1998; Pearson et al. 2001,2006), molassed and chopped oat straw (MOS) (Cuddeford et al. 1995), a mixture of DA and MOS (Cuddeford et al. 1995), whole crop oat hay (Orton et al. 1985), and barley straw (Pearson et al. 2006). The content of NDF in whole crop oat hay was assumed to be 60% in DM according (Khorasani et al. 1993).

The horses were fed ad libitum at 14 treatments with a mean intake of 1.33±0.33 NDF/BW,% (Miraglia et al. 1992; Orton et al. 1985; Pearson and Merritt, 1992; Pearson et al. 2001, 2006) and restrictively at 32 treatments with a mean intake of 0.82±0.24 NDF/BW,% (Cuddeford et al. 1995; Drogoul et al. 2000; Miraglia et al. 1992; Miyaji et al. 2008; Moore-Colyer et al. 2003; Pagan et al. 1998; Pearson et al. 2001; 2006). The content of dietary crude protein was 10.9% (SD 4.7) of DM.

MRT values from donkeys, horses with cannulation of the lower gut or studies with insufficient animal or dietary characteristics were not included into the meta-analysis.

The meta-analysis was done by use of the full model shown below by use of the mixed procedure in SAS version 9.1 and stepwise *Backwards Elimination* of fixed effects (P>5%) in order to get the reduced model according to Upton and Stoke (2008) and with the number of horses per treatment as weight.

Full model: $MRT_{ijedkmnptyzxw}$ (h) = $\mu + \alpha(DMI_i/BW^{0.75}) + \kappa(NDF/BW_j) + \beta_y(NDF/BW_j) + \gamma(NDFf/BW_e) + \upsilon(BW_d) + \sigma(BW^{0.75}{}_k) + \lambda_m + \varphi_n + \eta_p + \delta_t + \tau(CP_z) + \psi(CP_z*CP_z) + \xi_x + \varepsilon_w$

Reduced model: $MRT_{jdkmyzw}$ (h) = $\mu + \beta_y(NDF/BW_j) + \upsilon(BW_d) + \lambda_m + \psi(CP_z*CP_z) + \varepsilon_w$

μ = mean MRT

α = fixed effect of intake of $DMI/BW^{0.75}$, %, i, i = [4-15.3]

κ = fixed effect of fixed effect of intake of NDF/BW

β_y = fixed effect of intake of NDF/BW,% j, j = [0,38-2,03] for y different forage type y=1,2…6

γ = fixed effect of intake of forage NDFf/BW,% e, e = [0.38-2.3]

υ = fixed effect of body weight (BW) kg d, d = [108-786]

σ = fixed effect of metabolic $BW^{0.75}$ kg, k, k = [34-148]

λ_m = fixed effect of breed m, m = {Thorough, Stockhorse, Shetland, Highland or Welsh pony}

φ_n = fixed effect of exercise n, n =1, 2, 3

η_p = fixed effect of mark p, p = {Ru-P, Yb, Sm, Cr}

δ_t = fixed effect of feeding level t, t = {restrictive, ad libitum}

τ = fixed effect of crude protein content (CP), % in DM, z, z = [2.9-17.2]

ψ = fixed effect of CP*CP

ξ_w = random effect of study x, x =1, 2,…. 10

ε_i = random residual variation

Results and Discussion

The results from the meta-analysis of the MRT values by use of the reduced model are shown in Table 1. Breed type (P<0.0001), the BW (P<0.03), intake of NDF/BW (forage type) (P<0.001), and CP*CP (P<0.003) had an effect on the MRT values. The model predicted BW corrected MRT values to be highest in cross breed ponies and the lowest in Stock horses and Welsh ponies.

Table 1 Effects of breed, body size (BW), intake of neutral detergent fibre (NDF) and dietary protein content on MRT (h) values from 10 different studies including 46 different treatments

	degree of freedom	Estimate	SE	P<
Overall reduced model RMSE	26		6.5	
Intercept	26	29.4	6.1	0.003
Breed	4			0.001
Thoroughbreds, BW=562±88 kg		-8.8	6.4	
Highland pony, BW=505 kg		2.5	6.6	
Welsh pony, BW=233±39		0	.	
Cross breed pony, BW=230 kg		13.3	9.3	
Stock horse, BW=225±10 kg		-1.4	13.5	
Shetland pony, BW=108 kg		8.6	6.1	
Body size (BW), kg	1	0.033	0.009	0.002
NDF intake, NDF/BW,% from:	6			0.001
Barley straw		-13.7	4.4	
Molassed chopped oat straw (MOS)		-3.6	3.2	
Whole crop oat hay		-13.0	8.9	
Grass hay		-7.0	2.6	
Dehydrate alfalfa (DA)		-12.1	3.0	
Mixture of DA and MOS		-9.7	4.2	
Dietary crude protein (CP), % DM, CP*CP	1	0.027	0.01	0.02

Meyer et al. (1993) found that the mass of the gastrointestinal tract increased linearly with increased BW. The reduced model predicted the MRT value to decrease at increase intake of NDF/BW (P<0.006) with different effects of NDF for the different type of forage (P<0.007). The model predict the MRT value to decrease by 14 h per NDF/BW, % by feeding barley straw, to decrease by 4 h per NDF/BW, % by feeding molasses oat straw. The reduced model predicts the MRT value to increase at increasing content of dietary protein.

The metabolic body weight, intake of DM/$BW^{0.75}$, feeding level (restrictive vs ad libitum), the CP value or exercise did not affect the MRT value. The analysis of the effect of exercise on MRT was complicated by non-uniform exercise description in the different studies. The lower MRT values observed by Orton et al. (1985) in exercised equines might be caused by a higher intake of NDF/BW in the exercised compared with non exercised horses.

Exclusion of breed, BW, CP*CP and studies from the reduced model lead to a simple model: MRT (h) = 44.5 - 9.7*NDF/BW,%, R^2=0.26, P<0.003, which predicts the MRT value to decrease by 9.7 h per increased intake of NDF/BW,%. There is a negative correlation of -0.46, n=46, P<0.002, between the CP content and NDF/BW within the present dataset.

Exclusion of CP*CP as a fixed effect in the reduced model did not affect the significance of breed, BW and NDF/BW(forage type), but it affects the estimated parameter values of increased NDF intake per BW from the different types of forages and it increased the RMSE value from 6.5 to 7.2 h. The effect negative effect on MRT at increased NDF intake is in agreements with the finding in cattle (Huhtanen et al. 2006). The intake of forage NDF/BW did not effect MRT value when total NDF/BW was in the model, even Drogoul et al. (2000) observed a 3 h higher MRT time in cross bred ponies fed restrictively, ground and pelleted hay compared with chopped hay at the same intake.

Conclusions

The meta-analysis shows that the MRT in equine is affected by breed type, body weight, and intake of NDF per BW and by the dietary protein content. The reduced model predicted the MRT value to increase at increasing body weight and content of protein in the diet, but to decrease at increasing intake of NDF/BW depending on the kind of forage.

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Influence of sea buckthorn by-products premix feeding on the mare and foal blood biochemical indices

D. Ositis[1], D. Seglina[2], S. Strikauska[3], S. Bula[1]
[1] Latvia University of Agriculture (LUA), Faculty of Agriculture LUA, Latvia; [2] State Dobele Horticultural Plant Breeding Experimental Station, Latvia; [3] Scientific Laboratory of Agrobiochemical Analyses of LUA

Introduction

In the last few years, seabuckthorn (*Hippophae rhammoids L.*) has become a very popular plant to prevent soil erosion and serve as an economic resource for food, medicine products, and feedstuffs. As a legend says, the leaves of sea buckhorn were used in Ancient Greece for curing of horses. The horses fed with leaves were known to recover very rapidly and to acquire a smooth and glossy hair coat. Hence, the name of this plant *Hippophae* originates from *hippos* – horse, *phae* – shedding lustre. Seabuckthorn develops an extensive root system rapidly, and is therefore an ideal plant for soil erosion control (Li et al., 1996).

The information on nutritional and medicine value of seabuckthorn is relatively new. The seabuckthorn industry has been thriving in Russia since 1940s when scientists there began investigating the biologically active substances found in the berries, leaves, and bark. These products were utilized in the diets of Russian cosmonauts and as a cream for protection from cosmic radiation (Xu et al., 2001).

A number of vitamins, flavonoids and sterols present in the plant are thought to be responsible for its versatile pharmacological activities such as anti-inflammatory, chemical or physical burn wound healing ability, anti-gastroulcerative activity, hepatoprotective, anti-cancerous and anti-atherosclerotic properties (Varshney et al., 2005). It has been reported that seabuckthorn contains more than 190 bioactive compounds in the leaves, sprigs, seed pulp, and juice. These compounds include fat soluble vitamins (A, K and E), water soluble vitamins (C, B_1, B_2, folic acid, etc.), 22 fatty acids, 42 lipids, organic acids, amino acids, carbohydrates, tocopherols, flavonoids, phenols, terpenes and tannins (Zhang, 1990). Most of the research on seabuckthorn was done on small laboratory animals such as guinea-pigs, rats and rabbits, and directly on humans. As far as large domestic animals are concerned, not much work has been done. Therefore, the aim of our research project was to evaluate the influence of feeding seabuckthorn by-products on blood biochemical indices of mares and foals.

Materials and Methods

The field trial was conducted on the studhorse farm”Burtnieku zirgaudzētava” Ltd (Burtnieku pagasts of Valmiera region) from January to May 2007. In trial, 15 Latvian pedigree mares in foal were used. They were split into three groups (5 mares per group), similar genetic and production parameters, and randomly assigned to one of the three experimental diets. Mares of all three groups were fed a basal diet which consisted of 8 kg meadow hay and 5 kg of rolled oats. Besides, in the diet of the experimental group II, 0.3 kg of dried seabuckthorn leaves and sprigs were added, but in the diet of the experimental group III, 0.3 kg of dried berry residues were added. All mares were in foal since 2.5 – 3 months.

The chemical composition of hay, oats, seabuckthorn leaves and sprigs, and dried berry residues were analyzed in the Scientific Agrochemical laboratory of Latvia University of Agriculture to evaluate nutritional value of the experimental diets (Table 1).

Table 1 Nutrient content of feed dry matter

Nutrients	Meadow hay	Oats	Seabuckthorn hay	Berry residues
Dry matter,%	90.8 ± 1.25	90.0 ± 1.25	91.4 ± 1.25	90.8 ± 1.25
Crude protein, %	8.5 ± 0.30	12.4 ± 0.30	18.8 ± 0.30	18.0 ± 0.30
Crude fibre, %	35.6 ± 0.50	11.6 ± 0.50	17.3 ± 0.50	13.9 ± 0.50
Ca, %	0.53 ± 0.050	0.16 ± 0.050	1.18 ± 0.050	1.23 ± 0.050
P, %	0.19 ± 0.030	0.21 ± 0.030	0.19 ± 0.03	0.18 ± 0.03
Zn, mg kg^{-1}	3.7 ± 0.50	35.3 ± 0.10	15.7 ± 0.10	11.5 ± 0.10
Cu, mg kg^{-1}	8.8 ± 0.50	7.4 ± 0.50	3.9 ± 0.50	3.0 ± 0.50
Mn, mg kg^{-1}	70.0 ± 0.80	52.6 ± 0.80	63.9 ± 0.80	65.1 ± 0.80
Fe, mg kg^{-1}	175.0 ± 1.00	123.0 ± 1.00	112.8 ± 1.00	101.0 ± 1.00

At the beginning of trial, blood samples of all mares were analyzed for blood hematological indices: red blood cell count (erythrocytes), hemoglobin, hematocrit, mean corpuscular volume or mean red blood cell volume (MCV), mean corpuscular or cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution range(RWD), thrombocytes (anucleated cell fragments or platelets), leucocytes or "white blood cells" and different types of leucocytes (granulocytes − neutrophils, eosinophils, basophils; and agranulocytes − lymphocytes and monocytes), erythrocyte sedimentation rate (ESR), as well as content of Mg, Mn, Zn, Cu, and units of glutatione peroxidase. The same analyses were done on mares and foals after foaling in the laboratory "E. Gulbja laboratorija" Ltd in Riga.

The fruit branches had first been frozen and berries removed.. Juice had been extracted by pressing or centrifugal techniques, leaving a residue for feeding. Fruit branches with a diameter of 0.5 to 1.0 cm with leaves and sprigs and berry residues were dried separately, grind on 1 mm sieve and used as fodder additive.

The obtained results were statistically processed by MS Excel program package using methods for descriptive statistics, analysis of variance and t-test.

Results and Discussion

At the beginning of trial, in January 2007, no remarkable differences in mare blood hematological indices in experimental groups were observed (Table 2). Only exception was a difference between control and experimental group III in MCHC ($p < 0.05$), between control and experimental group II ($p < 0.05$) in glutatione peroxidase, and between control and experimental group III ($p < 0.05$) in the content of Cu (μg L^{-1}). The number of animals in the trial and analyzed blood samples was too small to explain this difference.

Substantial alterations in mare blood hematological indices between the control and experimental group II were observed after foaling (Table 3). In group II, which had received 0.3 kg of ground dried seabuckthorn leaves and sprigs, differences in several indices were seen, relative to the control group ($p < 0.05$). Group II had lower RWD, reduced count of leucocytes, lower eosinophil:basophil ratio, reduced count of lymphocytes and monocytes and increased content of Zn (μg dL^{-1}). All these changes are preferable for the health of mares.

Significant changes ($p < 0.05$) were detected in neonate foal blood hematological indices between the control and experimental groups II and III (Table 4). Erythrocytes count, hemoglobin, and MCHC concentrations were lower in groups II and III, but leucocytes count higher. ESR in experimental group II was higher; hematocrit, and content of Zn (μg dL^{-1}) were lower but thrombocytes count higher in experimental group III than in control group.

This suggests that seabuckthorn by-products have a potential for improving blood hematological indices in both mares and foals.

Table 2 Mare blood hematological indices at the beginning of the trial

Indices	Control group	Group II	Group III
Erythrocytes, 10^{-12} L^{-1}	7676 ± 629	7958 ± 396	8598 ± 312
Hemoglobin, g L^{-1}	128.4 ± 9.10	134.2 ± 3.42	137.6 ± 5.15
Hematocrit, %	34.0 ± 2.30	35.2 ± 1.14	37.2 ± 1.25
MCV, f L/cell	44.2 ± 0.74	44.6 ± 0.95	43.2 ± 0.63
MCH, pg/cell	16.4 ± 0.22	17.0 ± 0.35	16.0 ± 0.37
MCHC, g L^{-1}	379.2 ± 1.52[a]	380.6 ± 1.82	369.6 ± 2.44[b]
RDW, %	19.9 ± 0.44	20.1 ± 0.32	20.3 ± 0.42
Thrombocytes, L^{-1}	1102 ± 138	1060 ± 112	1606 ± 300
Leucocytes, L^{-1}	8568 ± 711	8520 ± 814	9254 ± 541
Neutrophils, %	58.0 ± 4.10	57.8 ± 4.32	53.6 ± 3.53
Eosinophils, %	2.2 ± 0.96	3.2 ± 0.22	2.0 ± 0.51
Basophils, %	0 ± 0	0.4 ± 0.02	0.2 ± 0.02
Lymphocytes, %	33.6 ± 4.32	33.8 ± 4.41	40.4 ± 3.54
Monocytes, %	6.2 ± 1.32	4.8 ± 0.60	3.8 ± 0.74
ESR, mm h^{-1}	14.2 ± 1.24	15.8 ± 1.67	19.8 ± 0.68
Mg, mmol L^{-1}	0.790 ± 0.0231	0.768 ± 0.0411	1.006 ± 0.0624
Zn, µg dL^{-1}	91.0 ± 5.51	89.6 ± 4.44	92.2 ± 5.28
Cu in blood serum µg L^{-1}	97.2 ± 9.28[a]	107.0 ± 7.67	117.2 ± 7.52[b]
Glutatione peroxidase, U L^{-1}	9385 ± 1797[a]	22714 ± 933[b]	14342 ± 3374

a-b figures with different letter superscripts is different significantly ($p<0.05$); other abbreviations in tables, see materials and methods. fL – abbreviation of femtolitre; femto = 10^{-15} part of whole; pg – abbreviation of pictogram; pictogram = 10^{-12} part of gram.

Significant changes ($p < 0.05$) were detected in neonate foal blood hematological indices between the control and experimental groups II and III (Table 4). Erythrocytes count, hemoglobin, and MCHC concentrations were lower in groups II and III, but leucocytes count higher. ESR in experimental group II was higher; hematocrit, and content of Zn (µg dL^{-1}) were lower but thrombocytes count higher in experimental group III than in control group. This suggests that seabuckthorn by-products have a potential for improving blood hematological indices in both mares and foals.

Table 3 Mare blood hematological indices after foaling

Indices	Control group	Group II	Group III
Erythrocytes, 10^{-12} L^{-1}	9432 ± 1040[a]	7910 ± 303[b]	8142 ± 387[b]
Hemoglobin, g L^{-1}	136.6 ± 8.11[a]	127.2 ± 5.94[b]	128.0 ± 3.27[b]
Hematocrit, %	37.2 ± 1.75[a]	35.6 ± 1.54	35.6 ± 1.27[b]
MCV, f L/cell	41.0 ± 2.67	45.4 ± 0.88	43.8 ± 0.75
MCH, pg/cell	15.0 ± 0.85	16.4 ± 0.24	15.6 ± 0.47
MCHC, g L^{-1}	365.4 ± 6.88[a]	356.0 ± 3.67[b]	359.4 ± 3.12[b]
RDW, %	21.1 ± 0.61[a]	19.1 ± 0.25[b]	20.2 ± 0.33
Thrombocytes, L^{-1}	1724 ± 106[a]	1586 ± 286	1572 ± 190[b]
Leucocytes, L^{-1}	11956 ± 1100[a]	9192 ± 823[b]	10114 ± 1189[b]
Neutrophils, %	28.9 ± 2.24	32.84 ± 2.42	35.2 ± 3.86
Eosinophils, %	4.3 ± 0.41[a]	7.8 ± 1.22[b]	4.8 ± 0.28
Basophils, %	1.2 ± 0.22[a]	2.5 ± 0.58[b]	1.1 ± 0.44
Lymphocytes, %	59.3 ± 2.55[a]	52.2 ± 3.74[b]	53.3 ± 4.45
Monocytes, %	6.3 ± 0.54[a]	4.6 ± 1.02[b]	5.5 ± 1.08
ESR, mm h^{-1}	17.2 ± 2.82	18.6 ± 1.25	24.2 ± 5.74
Mg, mmol L^{-1}	0.924 ± 0.0660	0.986 ± 0.0741	0.804 ± 0.0080
Zn, µg dL^{-1}	56.0 ± 1.52[a]	68.4 ± 3.55[b]	50.2 ± 5.64
Cu in blood serum µg L^{-1}	90.0 ± 12.22	104.6 ± 7.27	91.4 ± 5.45
Glutatione peroxidase, U L^{-1}	20203 ± 1798	23453 ± 1673	16446 ± 3379

a-b figures with different letter superscripts is different significantly ($p<0.05$) ; other abbreviations in tables, see materials and methods. fL – abbreviation of femtolitre; femto = 10^{-15} part of whole; pg – abbreviation of pictogram; pictogram = 10^{-12} part of gram.

Conclusions

Seabuckthorn (*Hippophae rhamnoides*) hay of leaves, sprigs and dried berry residues has high crude protein content in the dry matter – 18.8 and 18.0%, respectively and the contents of microelements Zn, Cu, Mn and Fe in hay are comparable with those in meadow hay and oats. Mare blood indices after foaling in the group receiving 0.3 kg of ground dried seabuckthorn leaves and sprigs (group II) had lower RWD, reduced count of leucocytes, lower eosinophil:basophil ratio, reduced count of lymphocytes and monocytes and increased content of Zn (µg dL^{-1}). Changes were also detected in neonate foal blood hematalogical indices between control and experimental groups II and III − erythrocytes count, hemoglobin, and MCHC concentrations were lower in groups II and III, but leucocytes count higher. Hematocrit and Zn (µg dL^{-1}) content were lower but thrombocytes count higher in experimental group III than in control group.

Table 4 Foal blood hematological indices

Indices	Control group	Group II	Group III
Erythrocytes, 10^{-12} L^{-1}	1143 ± 151[a]	1074 ± 389[b]	992 ± 312[b]
Hemoglobin, g L^{-1}	145 ± 1.3[a]	136 ± 3.8[b]	119 ± 3.2[b]
Hematocrit, %	39 ± 0.4[a]	37 ± 1.1	32 ± 0.8[b]
MCV, f L/cell	34.0 ± 0.91	34.2 ± 0.52	32.5 ± 0.75
MCH, pg/cell	12.4 ± 0.22	12.4 ± 0.21	12.0 ± 0.35
MCHC, g L^{-1}	373 ± 0.9[a]	367 ± 1.8[b]	369 ± 1.1[b]
RDW, %	24.2 ± 0.62	22.3 ± 0.45	22.9 ± 0.44
Thrombocytes, L^{-1}	4925 ± 812[a]	5524 ± 510	7950 ± 1428[b]
Leucocytes, L^{-1}	10014 ± 745[a]	11832 ± 678[b]	13042 ± 1444[b]
Neutrophils, %	32.1 ± 0.07	31.3 ± 3.44	30.0 ± 3.45
Eosinophils, %	1.2 ± 0.41	0.6 ± 0.18	2.6 ± 1.15
Basophils, %	1.1 ± 0.04	1.0 ± 0.06	1.1 ± 0.35
Lymphocytes, %	59.9 ± 1.25	61.5 ± 3.36	61.0 ± 2.41
Monocytes, %	5.7 ± 0.84	5.5 ± 0.62	5.2 ± 0.75
ESR, mm h^{-1}	7.0 ± 1.33	11.6 ± 1.28	10.0 ± 1.46
Mg, mmol L^{-1}	0.850 ± 0.0022	0.861 ± 0.0256	0.852 ± 0.0336
Zn, µg dL^{-1}	68.3 ± 1.82[a]	71.5 ± 3.72	61.6 ± 2.75[b]
Cu in blood serum µg L^{-1}	93.3 ± 5.44	87.4 ± 14.72	86.4 ± 8.26
Glutatione peroxidase, U L^{-1}	15621 ± 1650	16285 ± 1740	15924 ± 4707

a-b figures with different letter superscripts is different significantly ($p<0.05$) ; other abbreviations in tables, see materials and methods. . fL – abbreviation of femtolitre; femto = 10^{-15} part of whole; pg – abbreviation of pictogram; pictogram = 10^{-12} part of gram.

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The effect of maturity of haylage on the apparent total tract digestibility of dietary fibre in horses

R. B. Jensen[a], C. Brøkner[a], K.E. Bach Knudsen[b] and A.H. Tauson[a]
[a]*Department of Basic Animal and Veterinary Sciences, Faculty of Life Sciences, University of Copenhagen Denmark*
[b]*Department of Animal Health and Bioscience, Faculty of Agricultural Sciences, University of Aarhus, Denmark*

Introduction

Starch rich concentrates are commonly fed to horses, especially performance horses with elevated energy requirements. However, feeding large amounts of starch might increase the risk of developing disease like colic, laminitis and gastric ulcers. Alternatives to starch rich concentrates could be highly fermentable fibrous feeds like sugar beet pulp (Moore-Coyler *et al.* 2002) and soya bean hulls (Coverdale *et al.* 2004). Accurate analytical methods recovering the fibre fraction of feedstuffs are required to fully understand the digestion of this fraction in horses.

Different analytical procedures have been used to characterise the fibre fraction of feedstuffs, with the crude fibre (CF), acid (ADF) and neutral detergent fibre (NDF) methods commonly used in studies with horses. The CF method only recovers a small amount of the total fibre fraction and the detergent methods do not fully recover soluble fibres; the actual fibre content is therefore underestimated in a number of feedstuffs. The dietary fibre (DF) analysis method gives a more complete description of the fibre components; hence, enables a more detailed description of the fibre fraction of feedstuffs and their digestibility (Bach Knudsen, 2001). Implication of this method has unfortunately only been done in limited studies regarding equine nutrition (Moore-Coyler *et al.* 2002; Moore-Coyler and Longland 2000).

The aim of the present study was to measure the apparent total tract digestibility (ATTD) of fibre in horses where traditional fibre analyses were extended with the dietary fibre analysis.

Material and methods

Eight horses (4 Icelandic and 4 Danish Warmblood) were used to investigate the effect of stage of maturity of haylage on the ATTD of a diet consisting of sugar beet pulp, black oats and early or late cut grass haylage. The experiment was designed as a crossover study consisting of two experimental periods. Each 21 day period consisted of 17 days of adaption to a diet and four consecutive days of total collection of faeces. The horses were housed in stalls with rubber mats and faecal collection was performed by collecting faeces from the rubber mats frequently.

The feed rations were balanced to provide the same amount of dry matter/kg body weight on each diet, with 80:20 forage to concentrate ratio. The daily ration of haylage was divided into 5 meals and the concentrate, consisting of SBP and black oats, was fed twice daily. Two different 1st cut haylages from 2 different fields sown with the same grass seed mixture consisting of 65 % rye grass (*Lolium perenne*), 20 % meadow-grass (*Poa pratensis*) and 15 % timothy (*Phleum pratense)* were cut on 25th of May and on 14th of June. The crops were cut with a mower conditioner and left for wilting until they were baled two days later. Tedding was done two times during wilting, and the bales were wrapped immediately after baling.

Detailed information on all the chemical analyses can be found elsewhere, and only fibre analyses are presented here. Fibre in feedstuffs and faeces were analyzed as CF, ADF, NDF

and DF. The DF analysis determines cellulose, soluble and insoluble non-cellulose polysaccharides (NCP) including the constituent sugars, total NSP (cellulose + NCP) and DF (total NSP + lignin) (Bach Knudsen 2001).

All statistical analyses were performed using the general linear models procedure (GLM) in SAS® (Version 9.1, SAS Institute Inc. Cary, North Carolina, USA) according to the following model, where Y denotes the response variable analyzed: Y_{ijk} = μ + fixed effect of $diet_i$ + fixed effect of $breed_j$ + random effect of horse within $breed_k$ + residual $error_{ijk}$. The interaction of diet x breed was not significant and it was removed from the model. Results are presented as least square means (LS means) with root mean square error (RMSE) as a measure of variance. Effects were considered significant if $P<0.05$ and a tendency if $P<0.10$.

Results and discussion

The fibre analyses of the feedstuffs are presented in Figure 1, and it is clear that the different methodologies (CF, ADF, NDF and DF) gave different results. The amount of fibre recovered in the ADF analysis agreed with the amount of fibre recovered in the CF analysis. However, CF and ADF represent only a small and variable part of the total amount of fibre (Van Soest 1994). Soluble fibre is not recovered in the NDF analysis (Van Soest *et al.* 1991) justifying the good agreement found between insoluble NCP, cellulose + lignin and NDF. The amount of soluble NCP in SBP is approximately 40 % of total NSP, and a satisfactory recovery of the fibre fraction of feedstuffs with a high amount of soluble fibre can only be obtained with the DF analysis.

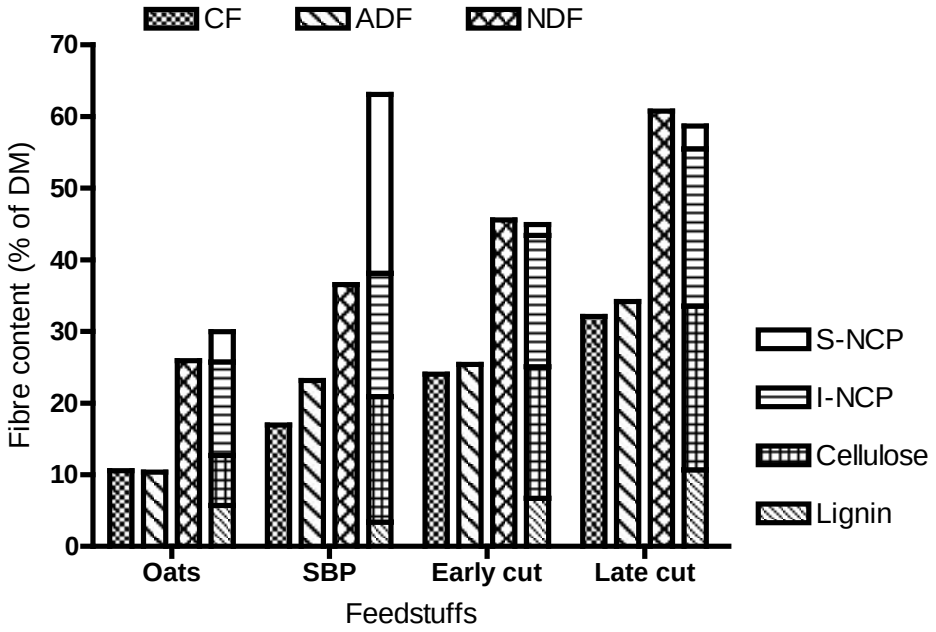

Figure 1 The fibre content (in % of DM) of the different feedstuffs (oats, sugar beet pulp (SBP) and haylage (early or late cut)) analyzed as CF, ADF, NDF and DF. Dietary fibre is divided into lignin, cellulose, insoluble (I-NCP) and soluble (S-NCP) non-cellulose polysaccharides.

Stage of maturity at cutting is the main factor influencing digestibility of forage, because of the changes occurring in the cell walls as the plant matures (lignification of cell walls) (Ragnarsson and Lindberg 2008; Van Soest 1994). In haylage all analyzed fibre fractions

increased with advancing stage of maturity and the cell wall components cellulose, NCP and lignin caused this increase (Figure 1).

Feeding early cut haylage resulted in a significantly (P<0.05) higher ATTD of all analyzed fibre fractions than feeding late cut haylage, except CF and ADF for which only a tendency was found (Table 1). The composition of the constituent sugars of the NCP fraction is determined in the DF analysis. High ATTD of arabinose and uronic acids as well as a lower digestibility of xylose has been found by Moore-Colyer *et al.* (2002) and Moore-Colyer & Longland (2000), in agreement with the present results. Arabinose and uronic acids are mainly found in the soluble fibre fraction whereas xylose is found in the insoluble fibre fraction, supporting the general view that soluble fibres are more fermentable than insoluble fibres.

Table 1 The apparent total tract digestibilities of fibre of the experimental diets consisting of black oats, SBP, and either early or late cut haylage fed horses

	Diet			*P*-values	
	Early cut haylage	Late cut haylage	RMSE	Diet	Horse
Traditional fibre analysis					
CF	52	47	4.28	0.07	0.01
ADF	51	46	4.31	0.06	0.01
NDF	60[a]	51[b]	3.11	0.001	0.01
Dietary fibre analysis					
Cellulose	60[a]	50[b]	3.98	0.002	0.02
NCP	71[a]	61[b]	2.48	<0.001	0.01
Xylose	62[a]	49[b]	2.88	<0.001	0.01
Arabinose	87[a]	80[b]	1.37	<0.001	0.06
Uronic acids	89[a]	83[b]	1.58	<0.001	0.04
T-NSP	66[a]	56[b]	3.05	<0.001	0.01
DF	55[a]	46[b]	3.38	<0.001	0.01

The soluble fibre fraction is of special interest in nutrition of horses as it is highly fermentable and therefore might be a good alternative as energy source for performance horses with high energy requirements. The DF analysis is the only method recovering soluble fibre. Detailed information on fermentation of the different fibre fractions can be obtained, when the DF analysis is applied to digestibility studies, and this method is superior to traditional fibre analytical methods.

Conclusions

Stage of maturity of haylage affects the fibre composition and this influences the ATTD of the fibre fraction negatively as the grass matures. Detailed information of fibre in feedstuffs and the fermentation of the different fibre fractions can be obtained, when the DF analysis is applied to digestibility studies with horses.

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Effect of grass species and time of cutting on digestibility in horses – comparison of methods

S. Särkijärvi[1], R. Sormunen-Cristian[2], T. Heikkilä[2], M. Rinne[2], M. Saastamoinen[1] and L. Jauhiainen[2]
[1]*MTT Agrifood Research Finland, Opistontie 10 A 1, FI-32100 Ypäjä, Finland*
[2]*MTT Agrifood Research Finland, FI-31600 Jokioinen, Finland*

Introduction

Knowledge of diet digestibility is a basic requirement for the evaluation of diet quality and the elaboration of well-balanced diets. Digestibility determination by a total faecal collection is exact, but time-consuming and expensive. Digestibility coefficients obtained using sheep are used in the feed evaluation systems for horses (e.g. MTT 2006). Development of different marker methods has been slow and the information on the relationships between total faecal collection and other methods is scarce. The comparative studies are done mainly on acid-insoluble ash as a marker (Goachet *et al.* 2009, Bergero *et al.* 2004, Miraglia *et al.* 1999). In addition, the comparisons are rarely done in trials where silage has been used as feed. The aim of this study was to compare the apparent digestibility of silages determined with total collection with the values obtained using marker methods. Relation of organic matter pepsin-cellulase solubility (*in vitro* digestibility) and *in vivo* digestibility was also studied. Silages were made of timothy/meadow fescue and tall fescue grass at three different cutting times in the first cut to produce silages that varied markedly in digestibility.

Material and Methods

A grass mixture of timothy (*Phleum pratense* L., 2/3) and meadow fescue (*Festuca pratensis* Huds., 1/3) (TMF) and pure tall fescue grass (*Festuca arundinacea* Schreb.) (TF) were cut from primary growth at three different dates (19 June, 26 June and 3 July) in 2006 at Jokioinen Finland. After wilting for 8 - 24 h, the grass was round-baled using an acid based additive (formic acid 76% + ammonium formiate 5.5%, 5 l/t).

In vivo apparent digestibility of silages was determined by the total faecal collection method in MTT Agrifood Research Finland in Ypäjä. Six Finnhorse mares, 5 to 13 years old, weighing 624 ± 36 kg were randomly allotted to treatments in an unbalanced 6 × 4 Latin square design. Each mare was placed in an individual stall. The mares had a 16-day acclimation period followed by a 5-day collection period. To avoid contamination of faeces with urine, the mares were harnessed with urine collection device originally designed for pregnant mare urine collection (Equisan Marketing Ltd, Australia). The diets during the treatment period were designed to meet daily allowance of 60 g dry matter (DM)/kg metabolic live weight ($kgW^{0.75}$) for horses. Actual silage intake was measured by weighing (offered minus refused silage).

Silage and faecal samples were analysed for DM, crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, indigestible NDF and for silage fermentation quality (pH, volatile fatty acids, lactic acid, water-soluble carbohydrates, ammonia N and soluble N) according to the methods described by Kuoppala *et al*. (2007). Chromium mordanted silage (27 mg Cr g^{-1} DM), daily dose of 4 g kg^{-1} feed DM administered orally, was used as a marker to determine total DM digestibility. The Cr marker was prepared according to Udén *et al.* (1980) and measurement of marker concentration was performed with ICP emission spectrophotometer (Thermo Jarrel Ash/Baird, Franklin, USA). *In vitro* organic matter digestibility was based on cellulase solubility method, which was a

modification of the method described by Nousiainen *et al.* (2003). The results were calculated with correction equations to convert pepsin-cellulase solubility values into *in vivo* digestibility by equations based on a data set comprising of Finnish *in vivo* digestibility trials with sheep (Huhtanen *et al.* 2006).

Statistical analyses were carried out using the MIXED procedure of the SAS system (SAS 9.2). The response variable (Y) was analysed according to the following statistical model:

$Y_{ijkl} = \mu + animal_i + period_j + feed_k + cutting\ time_l + feed*cutting\ time_m + \varepsilon_{ijklmn}$.

Results and Discussion

The DM content of silages ranged from 357 to 550 g/kg (Table 1). The TF silages contained more ash (95 *vs.* 76 g/kg DM), but less CP (116 *vs.* 134 g/kg DM) than TMF silages. The silages cut at the same time did not differ in CF and NDF contents. Fermentation quality of all silages was good.

Table 1 Chemical composition and fermentation quality of the experimental silages

	Timothy-meadow fescue			Tall fescue		
Cutting date in 2006	June 19	June 26	July 3	June 19	June 26	July 3
Dry matter (DM), g/kg	478	421	550	498	357	479
Composition, g/kg DM						
Ash	81	76	72	97	97	89
Crude protein	152	136	113	130	118	101
Crude fibre	297	318	336	290	320	342
Neutral detergent fibre (NDF)	558	582	623	543	579	625
Indigestible NDF	73	106	157	63	121	188
Acid detergent fibre	297	310	332	293	319	344
Lignin	28	31	40	20	29	38
Water soluble carbohydrates	82	82	91	105	76	87
Lactic acid	29	31	2	28	50	5
Acetic acid	7	8	5	8	9	5
pH	4.48	4.50	5.16	4.65	4.39	5.23
Soluble nitrogen (N), g/kg N	583	679	539	530	650	577
Ammonium N, g/kg N	38	46	33	32	51	38

The organic matter digestibility (OMD) of each silage, calculated on the basis of total faecal collection, marker and *in vitro* methods, are summarised in Table 2. As expected, the digestibility of organic matter in both grass species was influenced by date of cutting ($P<0.01$). The silages made at the early cutting time were the most digestible. The TMF had higher OM digestibility compared to TF (Grass; $P<0.1$) according to all methods.

The digestibility methods resulted in different values. The *in vitro* method overestimated the digestibility compared to the values using *in vivo* faecal collection, the method being calibrated to present digestibility in sheep. We have previously reported that the digestibilities of TMF and TF silages were 18 and 26% higher in rams than mares, respectively (Särkijärvi *et al.* 2008). The iNDF method resulted in an underestimation while the chromium marker method was the most precise compared to *in vivo* digestibility. When the achieved values were fitted against *in vivo* values (Fig. 1), the coefficients of determination were 0.949, 0.763, 0.960 and 0.948 for i*n vitro*, iNDF, Cr and cellulase solubility, respectively.

Table 2 Organic matter (OM) digestibility, cellulase solubility and D value[1] of the experimental silages

Cutting date 2006	Timothy-meadow fescue			Tall fescue			SEM[3]	Significance	
	June 19	June 26	July 3	June 19	June 26	July 3		Grass	Cut
OM digestibility									
In vivo, total collection	0.673	0.567	0.517	0.609	0.527	0.457	0.0132	<0.001	<0.001
In vivo, chromium	0.653	0.578	0.551	0.613	0.527	0.466	0.0174	<0.01	<0.001
In vivo, iNDF[1]	0.567	0.532	0.428	0.522	0.502	0.301	0.0380	<0.10	<0.01
In vitro, cellulase	0.741	0.698	0.648	0.730	0.664	0.616	0.0038	<0.001	<0.001
Cellulase solubility, g kg OM^{-1}	787	739	683	775	701	647	4.2	<0.001	<0.001
D value[2], DOM g kg DM^{-1}									
In vivo, total collection	619	524	480	549	476	416	13.1	<0.001	<0.001
In vivo, chromium	601	516	511	553	476	423	16.8	<0.01	<0.001
In vivo, iNDF	522	492	397	471	453	273	34.8	<0.05	<0.01
In vitro, cellulase	681	645	602	659	599	560	3.9	<0.001	<0.001

[1]Indigestible neutral detergent fibre; [2]Digestible organic matter (DOM) in dry matter (DM); [3]Standard error of means.

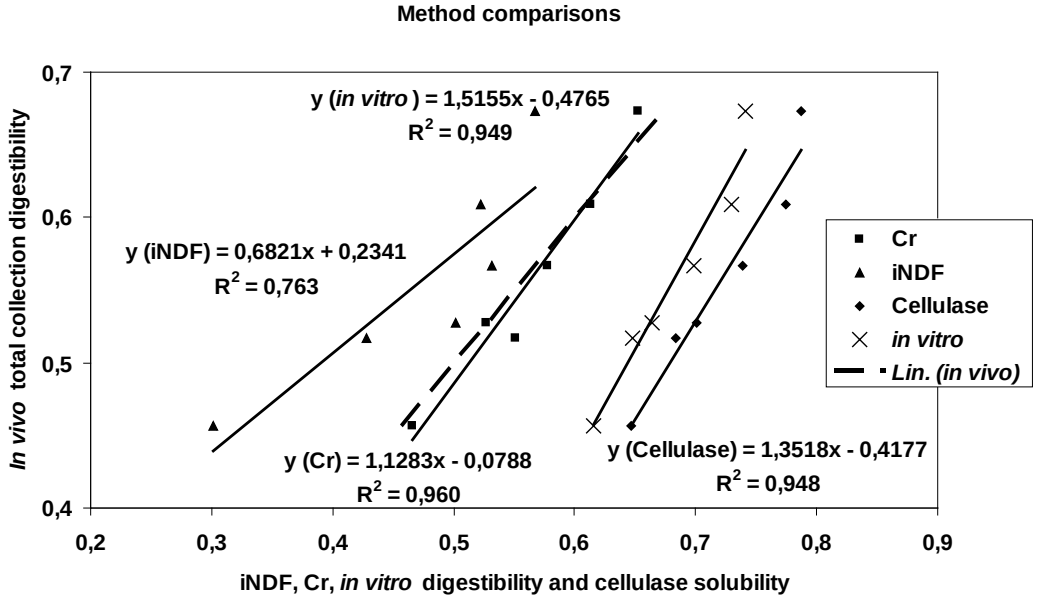

Figure 1 Digestibilities determined with different methods and cellulase solubility, fitted against *in vivo* total collection values. Respective equations and coefficients of determination (R^2).

Conclusions

The digestibility of silages in horses was influenced by cutting date, grass species and digestibility determination method. Timothy/meadow fescue silages were more digestible than tall fescue silages. The silages cut at early stage were more digestible compared to silages cut at later stages. Chromium mordant and cellulase solubility predicted silage organic matter digestibility accurately while iNDF was somewhat less accurate. The average digestibilities of the six silages determined with Cr, *in vitro* and iNDF, were 0.561, 0.683,

0.476, respectively, while the digestibility using total faecal collection was 0.559. This indicates that most precise values were achieved when chromium mordanted silage was used as a marker. The present values of *in vitro* digestibilities are for sheep, which explains the overestimation of horse digestibilities. Cellulase solubility had high R^2 –value with *in vivo* OMD in horses, which makes it an interesting tool to predict digestibilities of horse feeds.

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Dietary Protein Limbo Bar: How low can we go?[1]

G. A. Broderick
Research Dairy Scientist, US Dairy Forage Research Center, Agricultural Research Service, USDA, Madison, Wisconsin 53706

Introduction

Ruminants make efficient use of diets that are poor in true protein content because microbes in the rumen are able to synthesize a large proportion of the animal's required protein. The amino acid (AA) pattern of this protein is of better quality than most dietary ingredients commonly fed to domestic ruminants (Schwab, 1996). Further, ammonia utilization by ruminal microbes allows the feeding of nonprotein N (NPN) as well as capture of recycled urea N that would otherwise be excreted in the urine. Van Vuuren and Meijs (1987) estimated a theoretical maximum for N utilization in lactating cows of about 43%; using their assumptions in the NRC (2001) model yielded a slightly higher estimate. However, conversion of dietary N to milk N has been 32-34% under experimental conditions and typically much lower under normal feeding and management (Broderick et al., 2008). Inefficient N utilization necessitates feeding supplemental protein, increasing production costs and contributing to environmental pollution. An estimated 25% of dairy manure N is lost as ammonia under current U.S. practices (NRC, 2003). Similar losses likely occur in the Nordic countries. Trends for increasing animals per farmstead further contribute to nutrient accumulation on dairy farmland and to greater environmental impacts.

Optimizing formation of microbial protein in the rumen

A number of experiments have shown that ruminants can be moderately productive on diets in which virtually all of the crude protein (CP) is supplied from NPN (e.g., Virtanen, 1966). Bryant and Robinson (1962) suggested that ammonia-N was more important than N from AA and peptides for growth of many pure cultures of ruminal bacteria. However, Satter and Slyter (1974) fed diets to mixed ruminal organisms in continuous culture fermenters in which CP content was increased above a basal level of 4% (dry matter (DM) basis) by adding only urea. In 3 experiments, ammonia concentrations remained at ~1 m*M*, and microbial protein yield increased linearly, until dietary CP reached ~13%. At that point, fermenter protein outflow stopped increasing and ammonia concentration climbed rapidly; microbial protein yield did not increase above a mean ammonia concentration of ~2 m*M* (Satter and Slyter, 1974). This value was adjusted to about 3.6 m*M* (5 mg ammonia-N/dL) for a safety margin. Schaefer et al. (1980) found that ≤1 m*M* ammonia (1.4 mg N/dL) gave 95% of maximum growth for 9 of 10 pure cultures of ruminal bacteria studied. Results reported in both studies called into question the value of feeding NPN in many situations.

There has been much disagreement over the past 36 years about whether 5 mg N/dL ("5 milligram percent") was in fact the upper limit for ammonia utilization in the in vivo rumen. For example, Mehrez et al. (1977) infused urea into the sheep rumen and found that in situ barley digestion increased with increasing ammonia until reaching ~20 mg ammonia-N/dL. Odle and Schaefer (1987) conducted similar studies in cattle and observed maximal rates of in situ DM disappearance at 12 and 6 mg ammonia-N/dL for barley and corn DM. Ruminal

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concentration of diaminopimelic acid, now a rarely used marker for bacterial protein, increased with urea addition to a high-corn diet until ammonia-N reached 8.5 mg/dL (Kang-Meznarich and Broderick, 1980). It has been speculated that higher optimal ammonia concentrations are required in situ and in vivo under some circumstances because of physical association of bacteria with particulate substrates and to very low ammonia levels in these localized niches (Odle and Schaefer, 1987).

Part of the confusion about the ammonia "requirement" of ruminal microbes may stem from the confounded relationship of ammonia concentration to protein degradation. Ammonia is formed partly from deamination of AA resulting from ruminal protein degradation and ammonia production parallels formation of peptides and free AA. It has been known for some time that protein breakdown products other than ammonia stimulate microbial protein synthesis in the rumen. For example, Argyle and Baldwin (1989) showed that adding only 1 mg/L of a blend of protein AA plus 1 mg/L of peptides (trypsin-digested casein) more than doubled in vitro cell yield of mixed ruminal organisms. They also found progressively lower response to 10 and 100 times greater addition of AA and peptides.

Dramatic responses to rumen degradable protein (RDP) supplementation also have been observed in vivo. Kalscheur et al. (2003) held rumen-undegraded protein (RUP) supply constant but increased RDP by replacing treated soybean meal with solvent soybean meal; they observed significant increases in yield of milk, fat and protein at equal DM intake, although N efficiency declined. Recent in vivo results demonstrated significant linear depression in yield of milk, fat and protein when RDP from urea replaced RDP from soybean meal; these production effects appeared to be caused largely by depressed ruminal outflow of nonammonia N (NAN), essential AA and total AA due to reduced efficiency of microbial protein synthesis (Broderick and Reynal, 2009). We believe that, at least under some conditions, NPN sources cannot provide all of the RDP and that RDP from true protein is required to optimize microbial protein formation in the in vivo rumen.

Matching carbohydrates with rumen-degraded protein

The approach of the NRC (2001) is to first match RDP supply to ruminal carbohydrate fermentation, and then to provide sufficient RUP to meet any shortfall in metabolizable protein. Processing affects extent of ruminal cornstarch digestion much more than total tract digestibility (Owens et al., 1986). Feeding cows high moisture corn that was hammer-milled through a 1-cm screen reduced mean particle-size from 4.3 mm to 1.7 mm, reduced in vivo ruminal ammonia concentration, and increased yield of milk (2.4 kg/d) and protein (120 g/d) (Ekinci and Broderick, 1997). Processing dry shelled corn to reduce mean particle size from 3.5 to 0.6 mm increased ruminal starch digestibility from 54 to 70% (Remond et al., 2004). Charbonneau et al. (2006) observed that replacing cracked corn with ground corn or ground corn plus corn starch increased milk yield 10% and protein yield 14% in dairy cows. Metabolic problems associated with ruminal acidosis limit the feeding of readily fermented carbohydrates for stimulating formation of microbial protein; thus, it is important to know the degree to which grain feeding or processing will optimize performance without hurting the cow. The lactating cow's demand for energy is substantial and optimal dietary concentrate is dictated more by long-term rumen and animal health than by maximum milk production.

Corn silage is commonly fed to provide a high-energy "forage" and corn silage may also be fed to dilute the highly degradable protein in hay-crop silages. Dhiman and Satter (1997) replaced 1/3 or 2/3 of dietary alfalfa silage with corn silage. Compared to 100% of the forage from alfalfa, milk yield was 6% higher over the whole lactation when 2/3 of the dietary forage was alfalfa silage and 1/3 was corn silage; there also were comparable improvements

in N efficiency. Brito and Broderick (2006) assessed the effects of step-wise replacement of alfalfa silage with corn silage. Greatest improvement in N efficiency, without loss of production of milk, fat and protein, occurred when about 50% of the forage came from alfalfa silage and 50% from corn silage. Optimizing dietary ratios of alfalfa silage to corn silage resulted in similar or greater productivity at lower levels of protein supplementation.

Dairy cows often fed excessive crude protein

Dietary CP that is not utilized by the cow is excreted largely as urinary N, regardless of whether or not it contributes absorbed AA. In the first of several trials testing effects of altering dietary CP levels, 3 energy densities (36, 32, and 28% neutral detergent fiber (NDF)) were fed at each of 3 levels of CP (15.1, 16.7, and 18.4%, added as solvent soybean meal) (Broderick, 2003). There were no interactions—cows responded to CP the same at all 3 energy densities. Milk and protein yield both increased with the first CP increment, but there was no production difference between 16.7 and 18.4% CP. However, there was a marked increase in urinary N excretion (virtually all of which was urea) with increased dietary CP. In a second study, dietary CP was increased in steps of 1.5 percentage units, from 13.5 to 19.4% CP (Olmos Colmenero and Broderick, 2006); urinary N excretion and MUN reflected the linear decline in milk N/N intake with increasing CP. There were quadratic responses indicating maximal milk and protein yields at, respectively, 16.7 and 17.1% CP. Feeding more than these amounts of CP actually appeared to depress production.

Wu and Satter (2000) found that the dietary CP regime supporting optimal yield of fat corrected milk (3.5%; FCM) for the whole lactation with greatest N-efficiency was to feed 17.4% CP for the first 16-weeks after calving, followed by 16.0% CP for the remaining 28 weeks. Increasing dietary CP to as much as 19.3% during the first-phase, or to 17.9% CP during the second phase, did not improve FCM yield but only increased N excretion. Laboratory studies with the urine and feces excreted by cows fed the lowest and highest of the 5 CP levels in Olmos Colmenero and Broderick (2006) indicated that ammonia volatilization was more rapid from both fresh and stored manure produced when cows were fed higher CP (Misselbrook et al., 2005).

A caution regarding the minimizing of dietary CP and RDP relates to potential effects on ruminal fiber digestion. Similar to the effect on milk production discussed above, we found a quadratic response to dietary CP indicating maximal total tract NDF digestion occurred at 16.7% CP (Olmos Colmenero and Broderick, 2006). Moreover, replacing RDP from soybean meal with RDP from urea resulted in a small linear improvement in NDF digestion, even though the lowest mean ruminal ammonia concentration (when no urea was fed) was 8.2 mg N/dL (Broderick and Reynal, 2009). As suggested for starch digestion (Odle and Schaefer, 1987), there may be niches within the rumen where inadequate ammonia limits growth or activity of cellulolytic organisms.

Feeding Rumen-Undegraded Protein and Protected Amino Acids

Dairy diets often contain high CP and high NPN (from hay-crop silages such as alfalfa) and frequently there are substantial responses to RUP supplements, such as heat-treated soy protein (e.g., Faldet and Satter, 1991) or fishmeal (Broderick, 1992). Compared to an equal CP diet containing urea, we found a dramatic response to feeding 1 of 3 true proteins differing in RUP content and AA pattern (Brito and Broderick, 2007). Among the true proteins, flow of RUP and total protein from the rumen was greatest on cottonseed meal, intermediate on canola meal and lowest on solvent soybean meal; however, protein and fat

yield were highest on canola meal, intermediate on soybean meal, and lowest on cottonseed meal. Lower protein and fat yields probably resulted from the poorer quality of RUP in cottonseed meal (Brito et al., 2007).

Methionine and lysine are often cited as the limiting essential AA for lactating dairy cows (e.g., Schwab, 1996), although Nordic feeding trials indicated that histidine was first-limiting under conditions where cows consumed diets based on grass silage plus cereal concentrates (Korhonen et al., 2000). Enhanced production with increased RUP in the trials just discussed may have been due to the AA pattern of RUP supplied by fishmeal and canola meal being more complementary to microbial protein. Responses to rumen-protected methionine (RPM) have been more consistent than to rumen-protected lysine (Armentano et al., 1997). The advantage of post-ruminal supplementation of a limiting AA is that requirement can be met with little N input. Potential value for exploiting this strategy was shown in German studies where supplementing RPM at 14.7% CP resulted in milk protein secretion equal to that at 17.5% CP, and 31 versus 27% conversion of dietary N to milk N (Kröber et al., 2000). We obtained similar protein yield, and even greater yield of milk and FCM, when RPM was fed in diets containing 17.3 and 16.1% CP versus an 18.6% CP diet without RPM (Broderick et al., 2008). Moreover, production on 15.8% CP plus RPM was about equal to that on 17.1% CP without RPM in a later experiment (Broderick et al., 2009). Rulquin et al. (2006) and Chen et al. (2009) both reported increased yields of milk protein when supplementing with 2 different sources of RPM. Recently, Balchem Company began marketing rumen-protected lysine. Availability of this supplement has become more important in North America because of the large amounts of distillers grains coming from ethanol production from corn; corn protein is remarkably low in lysine (NRC, 2001).

Summary

Dairy cows excrete 2 to 3 times more N in manure than they secrete in milk, increasing milk production costs and environmental N pollution. Optimizing microbial protein formation in the rumen will improve the protein status of the lactating cow. NPN can replace only part of the dietary RDP because RDP from peptides and AA stimulates microbial protein synthesis. Ammonia is best utilized in diets that are high in starch and other nonfiber carbohydrates. Reducing particle size increases ruminal digestion of grain starch and increases microbial protein formation, so long as ruminal pH is not depressed. Dietary CP not utilized for production is lost mainly in the urine, the most polluting form of excretory N. Reversal trials testing typical diets showed no increase in yield of milk, FCM or protein with more than about 16.5% dietary CP. Another study showed that cows fed 15.8% CP plus RPM yielded as much milk, fat and protein as cows fed 17.1% CP without RPM. Substantial differences in the effectiveness of different sources of RUP for lactating cows likely are due to differences in AA profile.

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Residual feed intake in beef cattle – a literature review

M. Pesonen

MTT Agrifood Research Finland, Animal Production Research, Tutkimusasemantie 15, 92400 Ruukki, Finland

Introduction

Feed constitutes ¾ of variable expenses in beef cattle production. A 5 % improvement in feed efficiency can have an economic effect four times greater than 5 % improvement in average daily gain (Okine et al. 2003). The simplest and most widely used measure of feed efficiency is feed conversion ratio (feed:gain). Feed conversion ratio is highly correlated with production traits (Arthur et al. 2001, Nkrumah et al. 2004). Improved feed conversion and increased growth rate used as a breeding tool can increase beef cow size (Archer et al. 1999). Residual feed intake is one possible way of measuring feed efficiency in beef cattle. Marker assisted selection (MAS) might reduce the costs in selecting more efficient beef cattle. Heritably estimates of residual feed intake indicate that selection programs can be effective in lowering the feed consumption both in growing and mature animals without detrimental effects on production level.

Residual feed intake

Koch et al. (1963) first proposed the concept of residual feed intake (RFI). Residual feed intake is defined as the difference between actual feed intake and predicted feed intake required for the observed rate of gain and body weight. Efficient animals are those that consume less feed than expected based on their size and growth rate, therefore they will have negative residual feed intake (-RFI). Conversely, inefficient animals will consume more feed than expected and have positive residual feed intake (+RFI).

Residual feed intake is a trait that has a moderate heritability (h=0.39) (Schenkel et al. 2003). A notable feature that distinguishes residual feed intake from other feed efficiency traits is the small genetic relationships with production traits as growth, size, fatness and muscling (Archer et al. 1999). The correlation between residual feed intake in mature and post-weaning animals is high (0.98) (Herd et al. 2003). Selection for residual feed intake post-weaning may result in improved residual feed intake in mature animals without influence on mature size or growth rate of the animals (Herd & Bishop 2000).

Physiology behind residual feed intake

Mechanisms contributing to the variation in residual feed intake in cattle are: Protein turnover, tissue metabolism and stress 37 %, digestibility 10 %, heat increment of fermentation 9 %, physical activity 9 %, body composition 5 %, feeding patterns 2 % and other yet unknown 27 % (Herd & Arthur 2009). There are probably several unknown factors associated which are likely to be divided equally between already found mechanisms (Herd & Arthur 2009).

Body composition is an important factor regulating feed efficiency. Research has indicated a relationship between residual feed intake and body composition, mainly fatness. Energetically efficient, animals with low residual feed intake, deposit less fat (Schenkel et al. 2003, Nkrumah et al. 2004).

Variation in feeding behavior and feeding patterns has been associated with residual feed intake. Higher residual feed intake (less efficient) cattle have faster decline in average daily feeding session times and they spend more time eating in the beginning of the growing period

compared to lower residual feed intake cattle. Lower residual feed intake (more efficient) cattle seem to quickly settle into regular feed intake cycle (Richardson & Herd 2004, Dobos & Herd 2008). Higher residual feed intake has been associated with longer time feeding and more eating sessions per day and faster rate of eating (g/min). Feeding behavior traits had moderate heritability and were positively correlated with residual feed intake (Robinson & Oddy 2004, Lancaster et al. 2009).

Genetic makeup affects animal's susceptibility to stress. Physiological symptoms of stress include elevated metabolic rate and increased energy consumption. Cattle with high residual feed intake have been shown to have number of parameters that indicate their greater susceptibility stress or having less-effective mechanism to cope and adapt to different stressors, as a consequence they metabolized more feed energy than predicted (Richardson & Herd 2004, Richardson et al. 2004).

Derno et al. (2005) found that an animal which produces less heat with same diet has also reduced maintenance energy requirement. The genetic variation between residual feed intake and maintenance energy requirement has been shown to be similar (Herd & Bishop 2000). Animal's temperature measured from eye, cheek and feet has also been associated with feed efficiency. Lower surface temperature has indicated lower residual feed intake (Montanholi et al. 2009).

Residual feed intake is a composite trait which is influenced by many physiological processes. It has been estimated that over 100 genes are involved with the control of the efficiency of nutrient utilization (Herd & Arthur 2009). Although multiple markers have been described, no major gene affecting residual feed intake has been found (Moore et al. 2009). Residual feed intake is a trait that will benefit from selection through gene or marker-assisted selection, as more traditional approaches to genetic improvement are hindered by the high cost and/or labor-intensity of measurement.

Production impact of residual feed intake

Most research on residual feed intake has been done in growing cattle. The average dry matter intake has been 1,5 – 3,0 kg/d lower in low residual feed intake animals than in high residual feed intake animals. The average daily gain does not differ between divergent residual feed intake cattle (Nkrumah et al. 2004, 2007, Kelly et al. 2009, Lawrence et al. 2009). There is evidence of genetic relationship between residual feed intake and different carcass qualities (Richardson et al. 2001). Subcutaneous fat depth is decreased with low residual feed intake animals; they are over all leaner than high residual feed intake animals (Herd et al. 2003, Nkrumah et al. 2004, Lancaster et al. 2009).

In mature beef cows residual feed intake affects the most the maintenance energy requirement. Low residual feed intake beef cows have consumed 0,7 – 1,5 kg/d less feed dry matter than high residual feed intake animals (Arthur et al. 2005, Basarab et al. 2007, Lawrence et al. 2009). Significant differences in beef cow weights, condition scores or maternal productivity have not been found between high and low residual beef cows (Arthur et al. 2005, Basarab et al. 2007, Lawrence et al. 2009). Low residual feed intake beef cows tend to calve 5 – 6 days later than high residual feed intake beef cows, presumably because they fell pregnant later in the previous mating season (Arthur et al. 2005, Basarab et al. 2007). Such an association with residual feed intake is unfavorable in herds with restricted 60 – 80 days breeding season. Low residual feed intake beef cows also produced less twin calves (Basarab et al. 2007). The relationship between residual feed intake and fatness both fat deposition and fat maintenance should be accounted to overall productivity of mature animals. An ultrasound scan of back fat thickness should be accounted to genetic

improvement breeding programs for residual feed intake (Nkrumah et al. 2004, Arthur et al. 2005, Basarab et al. 2007).

Environmental benefit

Australian and North American studies have shown that selection for low residual feed intake cattle will reduce methane emissions by 15 - 30 % and manure production 15 – 20 % relative to selection for high residual feed intake cattle. Manure nitrogen, phosphorous and potassium levels have been 15 – 17 % lower in low residual feed intake cattle (Nkrumah et al. 2006, Hegarty et al. 2007). Although McDonnell et al. (2009) did not get any differences in methane production with cattle selected divergently for residual feed intake. The mechanisms behind differences in methane emissions, independent of feed intake, are unknown (Nkrumah et al. 2006). The lower feed intake is the major factor for lower manure production in low residual feed intake cattle (Nkrumah et al. 2006, Hegarty et al. 2007). Selection for reduced residual feed intake allows maintaining the same level of production with less plant biomass. This provides the farmer more flexibility to develop a property management plan (Herd et al. 2003, Herd & Arthur 2009).

Potential for animal breeding programs

Traditionally, cattle breeding and genetic improvement programs have been aimed at growth, carcass and reproductive traits. Less attention has been directed towards selecting animals with improved feed efficiency. Residual feed intake is moderately heritable. It can be used in cattle breeding programs to improve feed efficiency of beef cattle (Archer et al. 1999, Arthur & Herd 2005). Divergent selection lines have shown clear difference in feed consumption between low and high residual feed intake animals. After a single generation of divergent selection the difference in feed intake was 0,6 kg feed dry matter/day (Richardson et al. 2001) and after five generations 1,2 kg feed dry matter/day between low and high residual feed intake animals (Arthur et al. 2001). Average daily gain was equal 1,4 kg/d between the selection lines (Arthur et al. 2001).

Tools for easier and more accurate identification of inherited traits are developed. Marker assisted selection uses the results of DNA testing to assist in the selection of individuals and improve the accuracy of selection. The asset of marker assisted selection is that animal can be tested early in its life and environment does not affect the result (Moore et al. 2009). Residual feed intake is prime candidate for marker assisted selection. Commercial gene-test from Pfizer Animal Genetics and IGENITY has been available for three years. The commercial gene-tests for residual feed intake are based on SNPmarker-panels and validation in different reference cattle populations. The accuracy of gene-test is still fairly low, about 30 %.

Animal's temperament and susceptibility to stress may be a key driver behind beef cattle energetic efficiency (Richardson & Herd 2004, Nkrumah et al. 2007). Temperament may need to be included in the beef cattle breeding goals (Nkrumah et al. 2007).

Selection for low residual feed intake will reduce the amount of feed needed for maintenance and growth. Productions traits are not affected. Residual feed intake may be a trait which could provide some help for reducing beef cattle feeding costs (Herd et al. 2003, Kelly et al. 2009, Lawrence et al. 2009).

Conclusions

Genetic variability can be clearly found in feed intake and feed efficiency of beef cattle. All of the physiological factors affecting feed efficiency are not known. Currently it is estimated that one of the major factors is animal temperament, easily handled cattle grow and produce

more efficiently than unmanageable cattle. Improvement in feed efficiency can improve profitability and reduce environmental impact of beef production. More efficient cattle will achieve same level of production with less feed, thus excretion of nutrients will be lower. Residual feed intake is animal's individual result of feed consumption. In growing animals greater genetic potential for lean growth is associated with desirable residual feed intake. Breeding selection for residual feed intake may have a positive effect on beef cattle carcass traits as low residual feed intake cattle are leaner and slightly more muscular. The relationship between residual feed intake and mature animal fertility is unclear. Special attention should be given to fertility traits in replacement animals. Traditional animal breeding programs, along with marker-assisted selection, should improve the ability to select animals for improved feed efficiency.

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The effect of diet and intrinsic characteristics of feed particles on passage kinetics in dairy cows

S. Ahvenjärvi, M. Rinne, T. Heikkilä, and P. Huhtanen*
MTT Agrifood Research Finland, 31600 Jokioinen, Finland
**Current address: Swedish University of Agricultural Sciences, Umeå, Sweden*

Introduction

Dynamic models describing the digestive processes in the rumen require information on the passage and digestion kinetics of feed components. Previous studies have established that the passage rate of feed particles is determined by factors related to the level of forage intake and the diet composition (Cannas and Van Soest, 2000). Evaluation of the role of intrinsic feed particle characteristics on passage kinetics has been less extensively studied.

The objective of the current experiment was to assess the following hypothesis 1) the passage rate of forage and concentrate particles is dependent on the diet fed to the animal; i.e. consistency of the raft of partly digested and masticated forage particles that occupy the rumen and reticulum, and 2) the passage rate of forage and concentrate particles is dependent on the particle intrinsic characteristics that influence the physical dimensions and functional specific gravity of the particles.

Materials and Methods

The experimental treatments consisted of four different forages, early harvested grass silage (GE), late harvested grass silage (GL), red clover silage (RC), and whole crop barley silage (WCB). Grass was a mixture of timothy and meadow fescue. GE, GL, RC and WCB were cut on 13 June, 3 July, 29 June and 2 August 2006, respectively. Grass and clover silages were wilted for approximately 24 h. All silages were ensiled with a formic acid based additive and ensiled in round bales.

Forages were offered at ad libitum intake allowing for at least 5% refusals and the amount of concentrates offered was adjusted to 40% of total dry matter (DM) intake. Animals were multiparous Finnish Ayrshire dairy cows equipped with rumen cannulas. The cows were on average 74 days in milk (SD 53 d) at the beginning of the experiment and weighed 642 kg (SD 35 kg). The treatments were allocated to the experimental animals according to a 4×4 Latin square design with four 21-d periods. Feed intake, milk yield, rumen pool sizes and nutrient digestibility were determined to characterize the nutritional differences between forage diets.

To assess the effect of the diet on particle passage kinetics, each forage was labelled with one of four rare earth elements Yb, Dy, Er, and Sm and in addition rapeseed meal (RSM) particles were labelled with La. To avoid confounding effects due to different markers each marker was labelled onto different forage on each period. Cr-EDTA was used to determine the liquid passage rate. Labelled forage and RSM particles and Cr-EDTA were administered as a pulse dose into the rumen and the passage kinetics were determined based on marker excretion pattern determined in faeces over 96 h. Compartmental mean retention time (CMRT) and total mean retention time (TMRT) were estimated using a two-compartment model assuming a time-dependent and time independent retention time in the first and second compartment, respectively (Pond et al., 1988).

Results and Discussion

The forages were selected to represent a wide range of nutritional quality although, in practice, RC and WCB are seldom fed as sole forage to dairy cows but are often mixed with grass species. GE was of high nutritional quality as indicated by low neutral detergent fibre (NDF) and indigestible NDF (iNDF) concentration and high organic matter (OM) digestibility (Table 1). GL was harvested at a more advanced stage of maturity that was reflected in higher NDF and iNDF concentration and lower OM digestibility. Compared with the grasses, RC was characterized by low NDF concentration but proportionally much higher concentration of iNDF (36% of NDF). Also the NDF fraction of WCB consisted of a large proportion of iNDF but the fibre concentration was diluted by starch (171 g/kg DM) from the grain.

Table 1 Chemical composition, fermentation quality and organic matter digestibility of early cut grass (EG), late cut grass (LG), red clover (RC) and whole crop barley (WCB) silages.

	Experimental forages				
Item	GE	GL	RC	WCB	Concentrate
Dry matter, g/kg	395	567	387	402	887
pH	4.9	5.0	4.5	4.4	
In dry matter, g/kg					
Ash	88	70	97	62	70
Nitrogen	28.8	18.1	26.2	11.5	35.0
NDF[1]	509	623	385	517	133
iNDF[2]	49	177	137	161	20
Total acids	10.2	6.2	32.2	25.2	10.2
Sugars	117	107	82	102	ND[4]
Ethanol	7.3	4.7	2.1	8.4	ND
N-fractions, g/kg N					
Ammonia-N	36	30	42	47	ND
Soluble N	674	508	328	624	ND
Organic matter digestibility[3]	0.769	0.613	0.648	0.631	ND

[1]Neutral detergent fibre; [2]Indigestible neutral detergent fibre; [3]Measured in vivo with sheep at maintenance level of feeding using total faecal collection method; [4]Not determined.

The GE and RC diets promoted the highest feed intake and milk yield (Table 2). The OM and NDF digestibilities of the GE diet were higher than those on the RC diet and the passage rate of indigestible NDF (iNDF) and digestion rate of potentially digestible NDF (pdNDF) were higher for the GE diet compared with the RC.

Feed intake and milk yield recorded on diets GL and WCB were lower than those observed on the GE and RC diets. The reasons for the lower nutritional quality of GL and WCB forages compared with the GE were related to higher NDF and iNDF concentration that were reflected in lower pdNDF digestion rate in the rumen and lower total tract OM and NDF digestibility. High nutritional quality of RC seemed to be related to low NDF concentration that promoted high feed intake because the NDF digestibility of RC was low owing to high iNDF concentration and low digestion rate of pdNDF.

Table 2 Effect of forage on feed intake, milk production, rumen passage and digestion kinetics, and total tract digestibility.

Item	Diet[1]				
	GE	GL	RC	WCB	SEM[2]
Dry matter intake, kg/d	22.6[a]	20.0[b]	22.8[a]	21.2[ab]	0.47
Milk, kg/d	35.1[a]	31.4[b]	34.1[a]	31.1[b]	0.69
Rumen NDF[3] pool, kg	6.4[b]	7.4[a]	6.7[ab]	7.6[a]	0.28
iNDF k_p, 1/h[4]	0.032[a]	0.029[ab]	0.024[c]	0.028[bc]	0.001
pdNDF k_d, 1/h[5]	0.040[a]	0.040[a]	0.031[b]	0.027[b]	0.002
Digestibility					
Organic matter	0.73[a]	0.67[b]	0.68[b]	0.63[c]	0.005
NDF	0.62[a]	0.49[b]	0.36[c]	0.36[c]	0.012

[1]Diets consisted of four different forages, early harvested grass silage (GE), late harvested grass silage (GL), red clover silage (RC), and whole crop barley silage (WCB) supplemented with concentrate mixture; [2]Standard error of the mean; [3]Neutral detergent fibre; [4]Passage rate of indigestible NDF from the rumen and reticulum; [5]Digestion rate of potentially digestible NDF from the rumen and reticulum; [a,b,c]Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 3 Effect of forage diet on compartmental and total mean retention time of Cr-EDTA, forage and rapeseed meal particles

Item	Diet[1]				
	GE	GL	RC	WCB	SEM[2]
Cr-EDTA[3]					
CMRT[4]	10.5[c]	12.3[ab]	10.7[bc]	12.5[a]	0.48
TMRT[5]	16.5	18.0	16.7	18.4	0.87
GE particles					
CMRT	31.7[b]	30.5[b]	35.0[a]	34.4[a]	0.67
TMRT	35.9[b]	38.1[ab]	38.8[a]	39.0[a]	0.77
GL particles					
CMRT	36.7[b]	35.3[b]	40.6[a]	38.8[ab]	1.00
TMRT	39.7	41.3	43.2	42.0	1.15
RC particles					
CMRT	30.7[ab]	29.0[b]	30.8[ab]	32.7[a]	0.75
TMRT	36.0[b]	37.9[ab]	36.6[ab]	38.7[a]	0.63
WCB particles					
CMRT	36.2[bc]	34.0[c]	40.5[a]	39.3[ab]	1.19
TMRT	40.2	40.4	43.6	43.0	1.18
RSM particles[6]					
CMRT	16.6	17.6	16.5	17.5	0.61
TMRT	24.0[b]	25.8[a]	23.8[b]	25.2[ab]	0.44

[1]Diets consisted of four different forages, early harvested grass silage (GE), late harvested grass silage (GL), red clover silage (RC), and whole crop barley silage (WCB) supplemented with concentrate mixture; [2]Standard error of the mean; [3]Chromium ethylenediaminetetraacetic acid used as a liquid marker; [4]Compartmental mean retention time; [5]Total mean retention time; [6]Rapeseed meal particles; [a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).

The CMRT of all forage particles and Cr-EDTA was dependent on the type of forage diet (Table 3). In contrast, no significant differences were noted in the CMRT of RSM particles. The effect of the diet on the TMRT of feed particles was significant for GE, RC, and RSM particles but not for GL and WCB particles or Cr-EDTA. The difference between diets in

TMRT of forage particles was in excess of 3 h and tended to be shortest for the GE diet with smaller differences between the other forages. This observation was consistent with the highest iNDF passage rate from the rumen observed on the diet GE. When the TMRT of forage particles was calculated across the diets it was 38.0 h for GE, 41.6 h for GL, 37.3 h for RC, 41.8 h for WCB, and 24.7 h for RSM particles; i.e. the difference between RC and WCB was 4.5 h. These results indicate that the passage kinetics of forage particles is affected by the intrinsic characteristics of feed particles and the type of diet fed to the animal and quantitatively these effects are of similar magnitude.

Conclusions

The passage kinetics of forage particles is affected by the intrinsic characteristics of feed particles and the type of diet fed to the animal. Quantitatively these effects are of similar magnitude. The effect of a diet on RSM particles was significant but quantitatively smaller owing to faster passage rate of RSM particles.

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The effect of grass clover silage on faecal characteristics

L. Nielsen, M. Riis Weisbjerg and K. Søegaard[1]
Danish Institute of Agricultural Sciences, Department of Animal Health and Bioscience Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark. [1]Department of Agroecology and Environment

Introduction

It has long been known that there is a relationship between the composition of the diet and the faecal consistency in cattle (Ireland-Perry & Stallings 1993). In dairy cow production liquid faecal consistency is considered to be a problem (Bligaard *et al.* 2010). The liquid consistency affects the hygiene in the stable and therefore it can have a negative effect on for example health of udder and hoofs and on milk production and composition. Further, the digestibility of feed and thereby feed efficiency is assumed to be negatively correlated with liquid faeces (Sehested *et al.* 2006). Nadeau *et al.* (2007) found a correlation between DM content in faeces and faecal consistency score after visual assessment. Further, they found a significant positive correlation between the content of NDF in the diet and the consistency of the faeces. Moreover, Sehested *et al.* (2006) concluded that the consistency of faeces is determined by the water content and the water holding capacity. Previous findings suggest that the level of protein in the diet is related to the faecal consistency (Ireland-Perry & Stallings 1993), but this is in contrast with more recent research, where the content of starch and electrolytes such as sodium, potassium and chloride are in focus (Sehested *et al.* 2006, Bligaard *et al.* 2010). In addition Bligaard *et al.* (2010) found that feed intake had a positive correlation to DM concentration in faeces, but this could derive from the composition of the diet, as a positive correlation was also seen between the feed intake and the dietary content of starch. Bligaard *et al.* (2010) also found a negative correlation between forage proportion of total ration DM and DM concentration in faeces.

The aim of the present study was to examine the effect of different grass clover silages and forage:concentrate ratios on the faecal consistency. The comparison included two forage:concentrate ratios (80:20 vs. 50:50), and two cuts (early first cut and late second cut) in spring season and 2 cuts (early fourth cut and late fourth cut) in summer season. This production experiment included registrations on feed composition, feed intake and digestibility of feed, milk production and composition, chewing activities and faecal dry matter concentration and consistency scores. In this paper, preliminary results on feed intake, faecal dry matter concentration and faecal scores will be presented.

Abbreviation key: CP = crude protein, DM = dry matter, DMI = dry matter intake, F:C = forage:concentrate ratio, FU = feed unit, NDF = neutral detergent fiber, NELp20 = Norfor net energy lactation, with DMI at 20 kg.

Materials and Methods

Silage Preparation

Grass clover mixed leys were harvested in 4 different cuts for silage. The 4 different cuts were an early first cut (9th of May), late second cut (22nd of June), early fourth cut (7th of August) and a late fourth cut (23rd of August). After prewilting 1-2 days to approximately 40 % DM, cuts were precision chopped with a forage harvester (John Deere 6750) equipped with 28 knives, to obtain a theoretical length of cutting of 19.2 mm. The chopped material was transported to a clean area and mixed, before round bales were made with an Orkel MP 2000

Compactor and wrapped with 11 layers of 25 µm plastic. No ensiling additives were used. The bales were placed together and covered with a net to protect from birds.

Maize whole crop was harvested on 25th of October and precision chopped to a theoretical length of cutting of 13.5 mm with a kernel processor set at 3.5 mm, before ensiling in an indoor bunker silo without additives.

Cow and Diets

Twenty-four lactating Holstein Friesian cows in first half of lactation were used in this study, consisting of two separate experiments. The experiments were set up as 4x4 Latin square designs, with four 21-days periods and a 2x2 factorial arrangement of treatments within each experiment. The cows were assigned to three blocks, according to parity, giving 3 cows on each treatment in each period. Cows were tied up individually in beds covered with mattresses and sawdust bedding. Cows had free access to drinking water and were milked twice daily. In both experiments the forage consisted of 2/3 grass/clover (rye grass, red clover, white clover) silage and 1/3 maize silage. In experiment 1 the forage:concentrate (F:C) ratio was 80:20 and in experiment 2 50:50. Concentrate composition is in Table 1.

Table 1 Composition of concentrate

	Experiment 1	Experiment 2
	Concentrate premix A	Concentrate premix B
	Kg DM/100 kg DM	Kg DM/100 kg DM
Soybean meal	42.4	27.0
Wheat	48.2	68.3
Minerals and Vitamins	9.35	4.51

Total Mixed Ration (TMR was prepared every day in a feed mixer (Cormall Horizontal Screw Mixer) for 12 minutes and offered twice daily (07:30 and 14:00 h) ad libitum (a minimum of 5% residuals) in equal size portions. Residuals were removed and weight recorded before morning feeding on an individual level. DM concentrations in rations were measured weekly. In Table 2 the chemical composition of the four different silages is shown and Table 3 shows the composition of the different diets. Table 2 shows that the protein level was relatively high in the two fourth cuts, compared to the others. The botanical composition of the four cuts divided into grass and clover showed a ratio of 70:30 in the two early cuts and 60:40 in the two late cuts. Leaves and stem showed a ratio of 80:20 in the early 1st cut, 45:55 in late 2nd cut, and 95:5 in the two 4th cuts. Feed fraction and calculated protein concentrations in the rations are shown in Table 3.

Table 2 Chemical compositions of silages[1] per kg dry matter (DM)

Cuts:	Early 1st	Late 2nd	Early 4th	Late 4th	Maize
DM, %	46.6	40.0	43.8	35.0	35.6
Ash, % of DM	8.7	10.0	10.7	10.6	2.9
CP, % of DM	18.6	14.5	27.7	23.3	9.1
NDF, % of DM	30.1	42.0	33.1	34.7	37.0
Kg DM/FU	0.97	1.20	1.04	1.15	1.08
NELp20, MJ/kg DM	6.61	5.95	6.51	6.06	6.88

[1]Chemical composition parameters were determined by NIRS in bore samples taken before experiment start (Eurofins Steins Laboratories A/S, Holstebro, Denmark).

Table 3 Feed fractions and protein concentration[1] in experiment 1 and 2

	Cuts	Early 1st	Late 2nd	Early 4th	Late 4th
Experiment 1, ratio 80:20	Concentrate premix A	20	20	20	20
	Maize silage	26.7	26.7	26.7	26.7
	Grass clover silage	53.3	53.3	53.3	53.3
	CP, % of DM	17.9	15.7	22.3	20.0
Experiment 2, ratio 50:50	Concentrate premix B	50	50	50	50
	Maize silage	16.7	16.7	16.7	16.7
	Grass clover silage	33.3	33.3	33.3	33.3
	CP, % of DM	18.9	17.5	21.6	20.1

[1] Protein concentration estimated based on pre-experimental forage analysis and table values for concentrates

Faecal sampling

During the last three days of each period, faecal grab samples of approximately 200 mL were collected twice daily at 0900 h and 1500 h and stored at -20°C. After last sampling in each period, samples were thawed, mixed and scored for consistency by two persons (scale 1-5, where 1 is liquid faeces) and dry matter concentrations were determined by drying approximately 2x200 mL of each sample in a forced-air oven at 60°C for 48 h.

Statistical analysis

Data were analyzed (SAS version 9.2, 2010) within each experiment by analysis of variance using the GLM procedure for faeces DM and score, and Mixed procedure for feed intake to account for repeated measurements.

Results and Discussion

Feed intake

Effects of forage source and forage:concentrate ratio on dry matter intake (DMI) are presented in Table 4. A significant effect of treatment was found in experiment 1 ($p < 0.0001$).

Table 4 Means of dry matter intake showed in kg per day and influence of forage source on faecal dry matter (DM) percentage and consistency score

Cut	Early 1st	Late 2nd	Early 4th	Late 4th	*SE*	*P-value*
Experiment 1, F:C 80:20						
DMI, kg/day	21.5	19.2	21.3	21.2	0.22	< 0.0001
Faecal DM, %	13	11.9	12.4	12	0.27	0.0449
Faecal consistency score	3.14	3.31	3.09	3.02	0.08	0.0383
Experiment 2, F:C 50:50						
DMI, kg/day	21.5	21.6	22.1	21.5	0.26	0.44
Faecal DM, %	13.9	12.6	14.2	13.3	0.2	< 0.0001
Faecal consistency score	3.04	3.1	3.25	3.1	0.1	0.4808

The cows with forage:concentrate ratio 80:20 consumed less DM than the cows with forage:concentrate ratio 50:50. The ration with 80% forage and late 2 cut grass/clover silage resulted in the lowest intake. A tendency towards higher DMI of early cut was seen, especially in experiment 1, and this could be explained by higher digestibility.

Faeces

Cows consuming diets with higher ratio of forage had lower faecal DM (Table 4). In both experiments a significant effect of treatment was found on the faecal DM, but on faecal score

only in experiment 1. Further, a strong effect of cow was seen in both experiments on both faecal DM and score.

The results support the assumption (Bligaard *et al.* 2010) that an increasing digestibility is correlated with an increased faecal DM concentration. The higher DM concentration in faeces was found to be induced both by increasing digestibility caused by increasing ratio of concentrate and by higher digestibility induced by the more easily digestible silage. Higher protein concentration in especially the early fourth cut silage did not seem to affect the consistency negatively, as the faeces did not appear liquid as a result of high protein level as stated in previous studies (Ireland-Perry & Stallings 1993), and DM concentration was even highest on these protein-rich treatment rations.

There was a general positive correlation between DM concentration and score (Figure 1). However, treatment effects on DM concentration, like increased DM concentration with increased F:C ratio, and with increased silage digestibility, was not mirrored in the score observations, indicating that although there is an overall positive correlation between DM and score, nutritional strategies increasing DM concentration do not necessarily increase score.

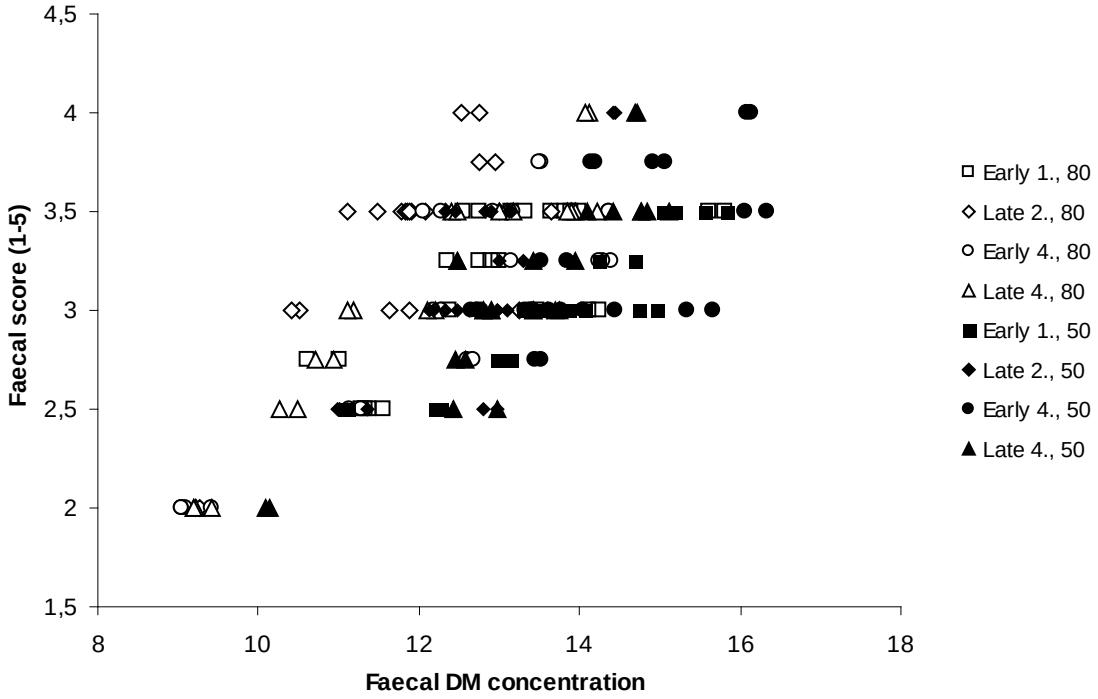

Figure 1 Correlation between faecal dry matter (DM) concentration and faecal consistency score

Ireland-Perry & Stallings (1993) suggested that higher DM concentration in low forage diets (17 % ADF) could have been composed by more non-fiber components, such as escaped dietary starch, and greater proportion of microbial matter due to higher hindgut fermentation. The lower DM concentration and higher faecal score for the high forage diets (25% ADF) they explained by greater proportion of fiber, assuming fibrous material has a high water holding capacity.

Bligaard *et al.* (2010) found an indication of a positive correlation between DMI and faecal DM concentration. Though, the proportion of DMI could probably be explained by the composition of the ration and therefore the correlation between DMI and faecal DM is not "direct". Figure 2 shows the correlation between DMI and faecal DM in this present study.

In general there was a weak tendency towards a positive linear correlation between faeces DM concentration and feed intake. However, the results from experiment 2 seemed to have a negative correlation, and this might be explained by a higher faecal water loss caused by a greater DMI in experiment 2, as increased DMI increase the rate of passage and the passage of undigested lignified matter and thereby the faecal water loss (Ireland-Perry & Stallings 1993).

Conclusions

Level of DMI was found to be higher for the cows consuming the higher level of concentrate (50 % vs. 20 %) and moreover a tendency towards higher DMI of early cuts was seen.

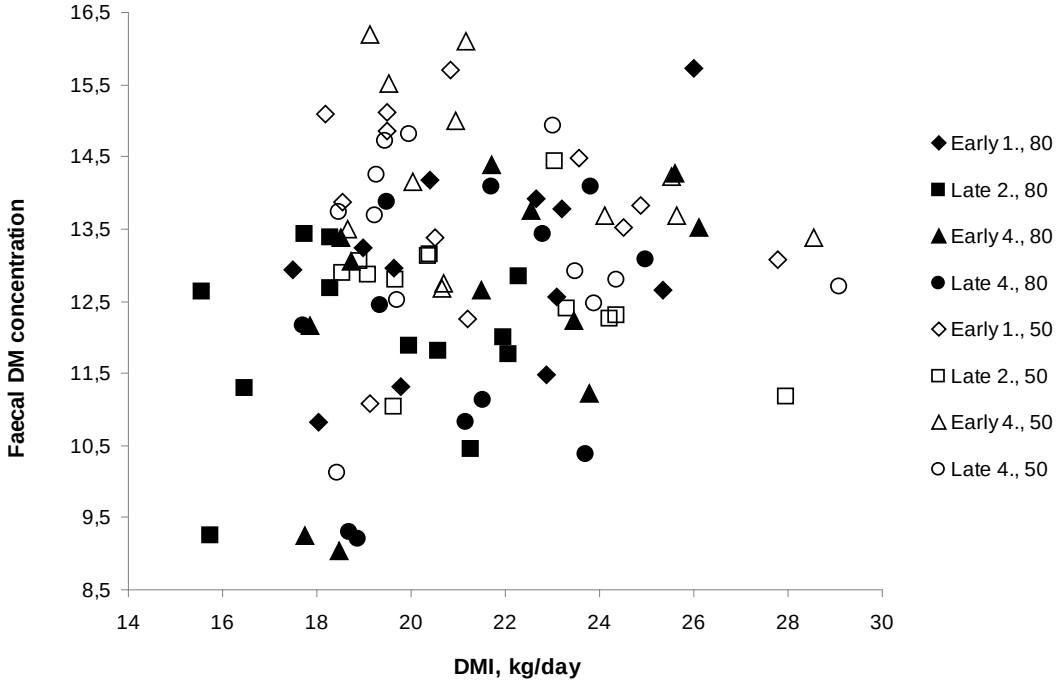

Figure 2 Correlation between dry matter intake (DMI) and faecal DM concentration

Digestibility of rations, both induced by higher concentrate ratio and by more easily digestible forage, was found to be positively correlated with faecal DM concentration. The rations with a higher protein level did not affect the consistency negatively. A general positive correlation between faecal DM concentration and consistency score was found. The correlation between DMI and faecal DM concentration was not clear, though a tendency of a positive linear relationship was seen.

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Effect of chopping on diet selection by young dairy steers fed whole-crop barley silage

B.-O. Rustas[1] and E. Nadeau[2]

[1]*Department of Animal Nutrition & Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences, S-753 23 Uppsala, Sweden*

[2]*Department of Animal Environment and Health, Swedish University of Agricultural Sciences, P.O. Box 234, SE-532 23 Skara, Sweden*

Introduction

Diet selection can affect nutrient intake in cattle fed diets of forage and concentrate. Whole-crop cereals, when harvested at late stages of maturity, can be considered as a mix of grain and straw and as such might be sorted when fed to growing cattle. Wallsten et al. (2009) reported sorting against NDF in dairy heifers, indicating a preference for grain, when fed whole-crop oat and two-rowed barley but no sorting when fed six-rowed barley. They attributed the lack of sorting in the six-rowed barley to the rough awns of the crop which presumably constrained intake. Rustas et al. (2010) observed selection for starch by dairy steers fed chopped whole-crop barley silage but no selection when fed the same silage unchopped. The difference in sorting behaviour probably was due to more intact awns on the grain in the unchopped silage (Rustas et al., 2010). Hence, sorting for grain may be constrained by the presence of awns in whole-crop cereals but the effect might be minimized by chopping. However, there are no reports on diet selection by young cattle, which might be expected to be more sensitive than older animals to the bristly awns, when fed whole-crop cereals. The aim of this study was to examine the effect of chopping whole-crop barley silage on diet selection by young dairy steers.

Materials and Methods

Whole-crop barley silage (WCBS) was produced from spring barley harvested at the mid-dough stage of maturity (growth stage 85 according to Zadoks et al., 1974) outside Skara in south-west Sweden. The barley crop was cut with a mower conditioner, baled and wrapped with eight layers of plastic film using an integrated round baler and wrapping machine. Knives were not engaged in the baler. Bales were conserved for at least 90 days before feed-out. The WCBS was either fed unchopped or after being chopped to a theoretical length of cut of 20 mm, using a precision chop forage wagon. In a 63 day long experiment, 63 dairy steers (30 Swedish Holstein, 33 Swedish Red) were used in a randomised block design with pen as the experimental unit. The steers were blocked according to their live weight and there were two pens with heavy steers (216 kg) and three pens with light steers (149 kg) in each of the treatments. The steers were fed WCBS at 105-110% of their ad libitum intake and each steer was supplied with a daily allowance of 0.4 kg rolled barley, 0.6 kg soybean meal and 80 g mineral feed. Orts were collected and weighed three times a week, except for the experimental weeks 2, 5 and 8 when the orts were collected daily and stored frozen. Frozen orts were later pooled to 1 sample for each collection week. The concentrates were supplied on top of the silage in the morning during all experimental weeks except weeks 2, 5 and 8, when concentrates were supplied after removal of the orts and were eaten up before the WCBS was fed. Silage samples were collected every day at feed-out, frozen immediately and later pooled to one sample per week for dry-matter (DM) determination and to three samples in total for determination of nutrient composition. Chemical analyses of silages and orts were performed according to Rustas et al. (2010).

The extent of selection was evaluated by relating the average composition of the orts from experimental weeks 2, 5 and 8 to the corresponding pooled samples of WCBS. The composition of silages and their corresponding orts were analysed with paired t-tests considering means of offered WCBS and corresponding orts as pairs in the analysis. Feed intake, liveweight gain (LWG) and sorting were analysed with the GLM procedure of Minitab (Minitab 15; Minitab Inc., State College, PA, USA) using a model with the effect of block and treatment.

Results and Discussion

Feeding steers with unchopped WCBS resulted in increased concentrations of DM, ash, starch and in vitro digestible organic matter (IVDOM) and decreased concentrations of neutral detergent fibre (NDF) and crude protein (CP) in the orts compared with the offered silage (Table 1).

Table 1 Composition of whole-crop barley silage (WCBS) and corresponding orts from dairy steers. All values are in g kg^{-1} DM, unless stated otherwise.

	Unchopped				Chopped			
	WCBS	Orts	SEM	Sign.	WCBS	Orts	SEM	Sign.
Dry matter, g kg^{-1} fresh weight	329	422	3.81	***	314	347	2.70	ns
Ash	50.5	93.7	1.62	***	61.6	71.8	6.13	ns
Crude protein	83.0	77.8	0.94	**	83.4	82.2	0.30	*
Starch	173	207	7.06	**	157	145	9.96	ns
Neutral detergent fibre	449	404	5.33	**	460	466	3.75	ns
IVDOM[1], g kg^{-1} OM	756	827	3.23	***	752	742	9.46	ns

[1] *In vitro* digestible organic matter (g kg^{-1} OM) after 96 hours incubation in buffer and rumen liquid.

The higher starch concentrations of the orts indicate that the steers avoided the grain during eating. This was supported in visual examination of the orts, as grain and awns were accumulated on the top of the WCBS in the manger. Awns were present in small clusters either attached to the grain or detached and this seemed to be the result of the animals actively avoiding the awns during eating. Awns are bristly and scabrous and are thought to affect eating behaviour and decrease feed intake by being noxious to animals (Laca et al., 2001). Feeding steers with chopped WCBS resulted in decreased concentration of CP in the orts compared with the offered silage (Table 1). The lack of difference in starch concentration between orts and chopped WCBS offered to animals indicates that the steers receiving chopped silage did not actively select with respect to grain in the diet. The sorting behaviour affected the composition of ingested silages in accordance with the changes in orts compared with offered silages (Table 2). From Table 2 it is, however, evident that the magnitude of the change caused by selection was rather small and rarely of importance from a nutritional point of view.

Chopping increased the intakes of DM and NDF from WCBS by 21 and 23% when expressed as g kg^{-1} LW which resulted in an increased LWG by 20% (Table 3). The increased intake due to chopping indicates that chopping cleared some constraints on intake that were present in the unchopped WCBS. Rustas et al. (2010) also found increased intake after chopping of WCBS harvested at the dough stage of maturity when fed to dairy steers of an initial LW of 350 kg. They attributed the differences in intake to selective intake of grain which occurred in

the chopped but not in the unchopped silage and proposed the difference being due to long awns in the unchopped WCBS.

Table 2 Effect of physical form (unchopped or chopped) on sorting (%) by steers fed whole-crop barley silage (WCBS)[1]. All values are in g kg^{-1} DM, unless stated otherwise

Item	Unchopped WCBS	Chopped WCBS	SEM	*P*-value
Dry matter, g kg^{-1} fresh weight	95.9***[2]	99.3*	0.18	<0.001
Ash	87.6**	99.2	1.02	<0.001
Crude protein	100.8**	100.2**	0.10	0.003
Starch	97.4**	100.4	0.34	<0.001
Neutral detergent fibre	101.3**	99.9	0.12	<0.001
IVDOM[3], g kg^{-1} OM	98.7***	100.0	0.08	<0.001

[1] Sorting % = 100 × (concentration of item in consumed WCBS/concentration of item in offered WCBS). Sorting values equal to 100% indicate no sorting, <100% indicate selective refusals (sorting against), and >100% indicate preferential consumption (sorting for).
[2]Difference in sorting values from 100% expressed as: *P < 0.05, **P < 0.01, ***P < 0.001.
[3] *In vitro* digestible organic matter (g kg^{-1} OM) after 96 hours incubation in buffer and rumen liquid.

Table 3. Effect of physical form (unchopped or chopped) on ad libitum intake of whole-crop barley silage (WCBS) and live-weight gain of dairy steers.

	Unchopped	Chopped	SED	*P*-value
Dry matter, g kg^{-1} live-weight day^{-1}	16.3	19.7	0.51	<0.001
Neutral detergent fibre, g kg^{-1} live-weight day^{-1}	7.38	9.06	0.229	<0.001
Live-weight gain, kg day^{-1}	0.855	1.028	0.0230	<0.001

The increased DM intake due to chopping in this study (21%) was greater than the 6% increase reported by Rustas et al. (2010), who used older and heavier steers. The effect of chopping on the direction of sorting, with decreased concentration of starch in the orts due to chopping, was the same in this study as in the study by Rustas et al. (2010). The fact that the younger and smaller steers fed unchopped WCBS in this study sorted against starch, which did not occur in the study by Rustas et al. (2010), who used older and heavier steers, could be explained by differences in sensitivity to awns due to size of the animals. The greater sensitivity to awns by the smaller steers might also explain the greater improvement in intake due to chopping in this experiment compared to the results by Rustas et al. (2010).

Conclusions

Chopping of WCBS harvested at the dough stage of maturity substantially increased intake and LWG in young dairy steers, which was related to the negative effects of awns in the unchopped silage on intake.

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Feed intake and faecal particle size distribution in ewes fed grass silage mixed with concentrate or fed separately at two particle lengths pre- and post partum

M. Brun-Rasmussen[1], E. Nadeau[2], P. Nørgaard[1], C. Helander[2], and A. Arnesson[2]
[1]Department of Basic Animal and Veterinary Sciences, Faculty of Life Sciences, University of Copenhagen, Frederiksberg C, Denmark; [2]Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, Sweden

Introduction

The transition period is a challenging time for the female ruminant with severe feed related disorders (Drackley, 1999). A great interest has been displayed for dry matter intake (DMI) at this time to improve animal health and welfare and also to promote greater performance. Chopping of grass silage from a particle length of 9 to 1.8 cm resulted in 25% higher feed intake in sheep (Deswysen et al., 1978). Poppi et al. (1980) fed sheep with grass and hay of different maturity stages and found that 1-3% of the particles in faeces were retained on a 1.18 mm-sieve and named it the Critical Particle Size (CPS). The CPS can be used for evaluation of the rumen function and environment, where 5% or less of large particles (>1.18 mm) in faeces indicate that it is a well working rumen (Nørgaard et al., 2007). The objective of this experiment was to study the effects of cutting grass silage and a separate vs. mixed allocation of concentrate and forage on feed intake and faecal particle size distribution in ewes during late gestation and early lactation.

Materials and Methods

The experiment was conducted at Götala Research Station, Skara, Sweden in 2008/2009. The experimental design was a quantitative split plot design with three dietary treatments tested during two periods; gestation and lactation. The dietary treatments; LS; long grass silage (33±17 cm) and 0.8 kg concentrate fed separately. CS; cut grass silage (60-85% retained on a 19 mm-sieve) and 0.8 kg concentrate fed separately and MS; cut grass silage and concentrate mixed and fed as a total mixed ration to 21 Swedish Finewool/Dorset twin-bearing ewes in their second or later gestation. The grass species in the silage included timothy (*Phlenum pratense* L.), meadow fescue (*Festuca pratensis* L.) and perennial ryegrass (*Lolium perenne* L.). The forage was harvested during the first regrowth in July 2008 and was conserved as round bales and offered to meet 110% of ad libitum intake. The ingredient composition of the pelleted concentrate included 23% wheat, 17% extracted rapeseed meal, 14% dried beet pulp, 10% dried distiller's grain, 7% wheat flour, 7% barley, 6% wheat bran, 4% soy meal, 4% oat grain and 2% molasses (*Lantmännen Lantbruk*, Sweden). The intake was recorded and the faeces were collected daily during four continuous days within 7-40 days before- (pre partum) and 13-31 days after parturition (post partum). The ewes were kept in individual pens (2x2 m) with free access to water, vitaminized mineral mixture and with straw as bedding. The body weight (BW) and body condition score (BCS) of the ewes were recorded weekly. The mean BW and BCS values before- and after the faeces collection period were included in the statistical analysis. Composited fresh silage, orts and concentrate were analyzed for crude protein by the Kjeldahl method. The dry matter (DM) content in feeds and refusals were determined daily by oven drying at 60° C for a minimum of 24 hours. Oven dried samples of silages and orts were pooled and ground for analysis of NDF and ADF by using the Ankom^{220} fibre analyzer (Ankom Technology, Macedon, NY, USA) described by Van Soest et al. (1991). Heat-stable α-amylase was added and sulphite omitted. The in vitro digestibility coefficients of organic matter (OM) in silage were determined by VOS according to Lindgren

(1979). Metabolizable energy (ME, MJ/kg DM) can then be calculated from the VOS value by the equation described by Lindgren (1983; 1988). The ME (MJ/kg DM) in concentrate was calculated according to Axelsson (1941). The estimations of DMI, ME intake and NDF intake per kg $BW^{0.75}$ or per kg BW were based on the BW values recorded within the first week after parturition. Samples of faeces for dry-sieving were washed in nylon bags with mesh size of 0.01 mm and frozen for 24 hours followed by freeze drying for 48 hours at -20°C. The particles were sorted by automatic separation (Retsch AS 200 control) followed by manual separation of clustering particles into six sieving fractions with pore sizes 2.36 (O), 1 (M), 0.5 (S), 0.212 (D) and 0.106 mm (C) and a bottom bowl (B). The arithmetic mean particle size (APS), the geometric mean particle size (GPS), the median and the 95% percentile were estimated as described by Nørgaard (2006). Twelve faecal samples in total were analyzed by image analysis. From each treatment group, two samples were analyzed from both periods. The arithmetic mean particle length and width (APL and APW), the geometric mean particle length and width (GPL and GPW), the median and the 95% percentile were estimated according to Nørgaard (2006). Data on feed intake, BW, BCS and faecal characteristics were analyzed statistically using the mixed procedure (PROC MIXED) of SAS (ver. 9.01, 2002).

Results and Discussion

Chemical composition of silages and concentrate is shown in Table 1.

Table 1 Chemical composition of long grass silage and cut grass silage (± standard deviation)

		Late gestation		Early lactation	
	Concentrate	Long silage	Cut silage	Long silage	Cut silage
Number of samples	n=2	n=3	n=3	n=3	n=3
DM, %	87	44 ± 2.5	42 ± 0.7	44 ± 2.6	42 ± 1.5
Crude protein, g/kg DM	175 ± 0.4	202 ± 15.1	207 ± 8.7	187 ± 4.5	180 ± 10.5
NDF, g/kg DM	253 ± 1.0	462 ± 0.1	470 ± 0.5	445 ± 1.1	476 ± 1.1
ADF, g/kg DM	117 ± 0.4	289 ± 2.8	267 ± 0.7	275 ± 1.7	269 ± 0.5
Starch, g/kg DM	232	-	-	-	-
ME, MJ/kg DM	12.8	11.6 ± 0.3	11.4 ± 0.2	11.8 ± 0.1	11.4 ± 0.2
VOS[a], %	-	93 ± 3.5	91 ± 1.2	95 ± 2.1	91 ± 2.6

[a]VOS = rumen soluble organic matter.

Daily intakes of silage DM, silage NDF, total DM and of ME were higher ($P<0.001$) three weeks post partum compared with pre partum, with no differences between the dietary treatments. Daily intakes of silage NDF and silage ME per kg BW and per kg $BW^{0.75}$, respectively, were 90% higher post partum compared with pre partum. The daily total DMI pre partum and post partum was 2.5 kg and 4.3 kg, respectively, which was close to the intake recorded, in twin-bearing ewes by Nadeau & Arnesson (2008) and Jalali et al. (2008). Neither chopping nor a mixed ration (MS) provided a positive effect on feed intake. However, a tendency to an effect of the interaction between status and feed was detected for DMI (kg/day) and (g DM/kg $BW^{0.75}$) and ME intake (MJ/day) and (MJ/kg $BW^{0.75}$) with the MS diet fed to lactating ewes to induce the highest intake. Cutting the grass silage did not increase intake in this study (Table 2), which contradicts with results by Deswysen et al. (1978).

A higher ($P<0.05$) proportion of faecal particles was retained on the upper sieve fractions post partum (Table 3). The higher proportion of large faecal particles in early lactation compared to late gestation might be related to the higher DMI (Table 2). Van Soest (1982) reported a

linear relationship between increased DMI and the amount of large particles in faeces. This was obtained by wet sieving of faeces from cattle. In addition, the DM content in faeces and the proportion of particle DM (PDM) was higher (P<0.001) in ewes pre partum compared to ewes in post partum.

Table 2 Intake, body weight and body condition score of ewes fed long silage (LS) or cut silage (CS) with concentrate fed separately or as a total mixed ration (MS) during gestation and lactation

	Late gestation			Early lactation				p-value		
	LS	CS	MS	LS	CS	MS	SE	Status	Feed	SxF
Number of samples	n=7	n=7	n=7	n=6	n=7	n=7				
DM intake[a] (kg/d)	2.6	2.5	2.4	4.4	4.1	4.6	0.22	***	NS	T
DM intake silage (kg/d)	1.9	1.8	1.9	3.7	3.4	3.7	0.21	***	NS	NS
DM intake (g/kg $BW^{0.75}$)	90	84	79	151	135	154	6.38	***	NS	T
NDF intake (kg/d)	1.1	1.0	1.2	1.8	1.7	1.9	0.10	***	NS	NS
NDF intake (g/kg BW)	12	11	11	20	19	20	1.1	***	NS	NS
NDF intake silage (g/kg BW)	10	9	10	19	17	19	0.9	***	NS	NS
ME intake (MJ/d)	32	30	28	52	47	54	2.7	***	NS	T
ME intake (MJ/kg $BW^{0.75}$)	1.1	1.0	0.9	1.8	1.6	1.8	0.07	***	NS	T
ME intake silage (MJ/kg $BW^{0.75}$)	0.8	0.7	0.7	1.5	1.3	1.4	0.07	***	NS	NS
Body Weight (kg)	92.1	96.2	96.7	91.1	96.4	95.2	3.2	NS	NS	NS
Body Condition Score	3.3	3.8	3.7	2.7	2.9	3.1	0.1	***	*	NS

[a] recorded 7-40 days before- (pre partum) and 13-31 days after parturition during four days; NS = not significant (P>0.1); T = tendency to significance (0.05<P<0.10); *P<0.05; ***P<0.001; Status = gestation or lactation.

The dietary treatments did not affect the distribution of particles in the individual sieving fractions, nor the mean particle size. The overall length and width values of faecal particles were not different pre- and post partum, nor did the dietary treatments affect the particle length or width values. The proportion of large faecal particles (>1mm) was 1.14% pre partum (Table 3) and considerably lower than the proportion of 2.9% in twin-bearing ewes four weeks pre partum fed grass silage at a similar NDF content (449 g NDF/kg DM) as reported by Jalali et al. (2008). The different proportions of large faecal particles between the studies could depend on differences in botanical composition and in morphology of the grasses as a first harvest in early June 2007 was used in the study by Jalali et al. (2008), whereas the first regrowth, harvested in July 2008, was used in this study. In addition, different ewes were used in the studies although the animals were from the same herd. Five percentages of the faeces particles were found to be longer than 3.6-4.7 mm, when using the image analysis technique, which was 9.6 times higher than the 95% percentile value obtained by the dry sieving technique (0.38-0.46 mm) (see Table 3). Dry sieving allows thin particles to pass through the sieve with a mesh of 1 mm. As seen in Table 3, the 95%-percentile for width by image analysis is more alike the 95%-percentile by dry sieving and could be an indication of separation of the particles by width more than the length. Nørgaard (2006) observed the same tendency when he obtained particle lengths at 6 mm with image analysis in faeces from Jersey cows fed grass silage. This was 5 times longer than CPS of 1.18 mm proposed by other authors (Poppi et al., 1981) and was explained by particles having lengths 5 times the width.

Table 3 Faecal particle size proportions in the sieving fractions (%), overall particle size obtained by dry sieving and overall particle length and width obtained by image analysis of washed faecal particles from ewes in gestation or lactation fed long silage (LS) or cut silage (CS) with concentrate fed separately or as a total mixed ration (MS) during gestation and lactation

	Late gestation			Early lactation				p-value
	LS	CS	MS	LS	CS	MS	SEM	Status
Dry sieving	n=7	n=7	n=7	n=7	n=7	n=7		
O+M, %	1.11	1.2	1.1	1.44	1.81	1.73	0.2	**
O, 2.36 mm, %	0.27	0.4	0.3	0.5	0.6	0.4	0.1	*
M, 1.0 mm, %	0.8	0.8	0.8	1	1.2	1.3	0.1	***
S, 0.5 mm, %	8	8	8	7	7	8	0.6	NS
D, 0.212 mm, %	34	32	33	27	29	29	1.4	**
C, 0.106 mm, %	38	40	38	41	41	42	1.2	**
B, 0.0 mm, %	18	19	20	23	21	20	1	*
Mode, mm	0.24	0.23	0.23	0.21	0.21	0.22	0.009	**
median, mm	0.25	0.25	0.25	0.23	0.24	0.24	0.007	NS
APS, mm	0.26	0.25	0.25	0.24	0.26	0.26	0.009	NS
GPS, mm	0.19	0.18	0.19	0.17	0.18	0.18	0.006	T
95% percentile, mm	0.39	0.46	0.38	0.41	0.45	0.43	0.03	T
DM, %	32	31	32	24	23	25	1.09	***
Particle DM, %	14	16	17	11	11	13	0.7	***
Image analysis	n=2	n=2	n=2	n=2	n=2	n=2		
Overall particle length	(L) and	Width	(W)					
Mode_PL, mm	0.28	0.24	0.30	0.28	0.30	0.32	0.05	NS
APL, mm	1.23	1.42	1.28	1.29	1.36	1.35	0.16	NS
MPL, mm	0.88	0.84	0.81	0.86	0.87	0.88	0.10	NS
GPL, mm	0.81	0.82	0.79	0.81	0.84	0.86	0.08	NS
95% percentile	3.6	4.7	3.7	4.0	4.2	4.1	0.5	NS
Mode_PW, mm	0.064	0.058	0.063	0.062	0.071	0.072	0.004	NS
APW, mm	0.20	0.21	0.21	0.19	0.19	0.20	0.01	NS
MPW, mm	0.14	0.14	0.13	0.13	0.12	0.14	0.01	NS
GPW, mm	0.14	0.14	0.14	0.13	0.13	0.14	0.009	NS
95% percentile, mm	0.58	0.61	0.58	0.55	0.54	0.58	0.03	NS

NS = not significant (P>0.1), T = tendency to significance (0.05<P<0.10), *P<0.05, **P<0.01, ***P<0.001. Status = gestation or lactation.

Conclusions

Intakes of DM and NDF were 70% higher in ewes during early lactation than during late gestation. Chopping silage and feeding a mixed ration (MS) did not affect DMI either pre- or post partum, except for a tendency for the MS diet to increase DMI post partum. Faecal particle size distribution was affected by physiological status of the ewes. More large particles (>1 mm) were found in faeces from ewes post partum compared to pre partum. Furthermore, the 95% percentile value tended to be higher post partum compared with pre partum. No effects were observed for any of the dietary treatments on the distribution of faecal particles in the individual sieve fraction, on the overall mean particle size values or on the overall particle lengths and widths measured by image analysis. The faecal particles appear to have lengths 6-7 times longer than the widths.

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Slaughter weight and chest girth measurement data to estimate dairy cow live weight change during lactation

I. Schei and H. Volden
TINE Norwegian Dairy Association, P.O. Box 58, 1430 Ås, Norway and
Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway

Introduction

The body size of the dairy cows is highly variable not only between breeds but also among animals within breed. Large cows have a higher feed intake and higher milk production than smaller cows. Moreover, the feed efficiency also increases in larger animals due to a lower maintenance requirement per kg milk produced. This has indirectly led to increased body weight (BW) of dairy cows with high genetic merit for milk production (Veerkamp, 1998). Moreover, the variation in the cows' BW between herds is large (Koenen and Groen, 1998) due to that high milk yield per cow is not always a goal on all farms. In Norway, every farmer is restricted to a milk quota, and how they do the production; high milk yield on fewer animals or lower milk yield on more animals, depend on the feed and housing resources on each farm. Information about the cows' BW is important for calculating nutritional requirement for maintenance and growth. However, few commercial dairy farms have handling systems for determining BW. Therefore, chest girth measurement (CG) has been the traditional way of measuring BW on farms. In Norway, information of every cow from each herd that is a member of the Norwegian Dairy Herd Recording system can be connected to the feed evaluation system, TINE Optifôr /NorFôr system. This makes it possible to optimize feed rations for every cow throughout lactation based on information of the farm and the animal. However, monitoring CG is a voluntary registration in the dairy herd recording system, and therefore, when optimizing feed rations farms may lack information about the cows' BW. Instead, information of slaughter weight (SW) from the last 20 slaughtered cows for every lactation number in the herd is used to estimate BW. The objective of this study was to estimate BW change during lactation of dairy cows based on SW and CG data from the Norwegian heard recording system.

Materials and Methods

Individual data for Norwegian Red cattle (NRF), comprising three years (2007 to 2009) from the Norwegian Dairy Herd Recording system, were used. Cows registered as lactating dairy cows and that have a calving date less than 305 days before the slaughter date or date of CG measurement were selected. For cows with CG measurement a request of at least one test-day milk recording was required. Data set consisted of a total of 197 827 SW from 13 016 different farms, of which 55 010 cows were in 1st lactation, 48 234 were in 2nd lactation and 94 583 were older than 2nd lactating cows. To estimate BW of the cow, a slaughter percentage of, 47 and 46 for 1.lactation and older cows were used, respectively. The CG data comprised a total of 94 469 records from 9009 different herds. Of the cows, 69 925 were in 1st lactation, 12 158 were in 2nd lactation and 14 204 were older than 2nd lactation cows. Body weight based on CG measurement was estimated according to the following function found for NRF heifers and cows (Bekkevold and Helberg, 2009): $BW=0.0309CG^2-3.9239CG+189.22$ where CG is measured in cm. The data were divided into three datasets; 1st lactation cows, 2nd lactation cows and older cows (3 or more lactations). The datasets were run separately and analysed for fixed effects of BW estimating method, weeks in milk (WIM), calving month, region and their two-way interactions. 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 BW estimated from SW and CG for lactation number 1, 2 and >=3 are presented in Table 1. The corresponding values during the lactation period (BW*WIM interaction) are shown in Figure 1. In general, BW estimated from SW data gave lower (P<0.01) values than those estimated from CG data for all lactation numbers, in average 44, 24 and 25 kg for 1st, 2nd , and >=3rd lactation, respectively. The interaction of BW method*WIM showed that the trajectory between the methods differed (P<0.01) for all lactation numbers. Despite significant interaction, the visible shape of the curves estimated for 1st lactation cows were almost similar for the two methods, and the difference between the methods was the same throughout lactation (Figure 1). However, for 2nd lactation and older cows, the drop in BW in early lactation was larger and more prolonged using the SW data than using the CG data. This resulted in a higher difference in BW between the methods in early lactation, whereas later in lactation the curves met as lactation progressed. These results showed that estimation of BW based on SW and BW gave different result. The mathematical function used to estimate BW from CG was developed on 40 NRF heifers and cows ranging from 108 to 633 kg (Bekkevold and Helberg, 2009). These authors found a high curved correlation between the CG tape and BW giving a R^2 of 0.99. Others scientists have observed similar relationship between CG tapes and BW on NRF (Berge, 1977) and on other breeds (Heinrichs et al, 1992; Koenen and Groen, 1998), indicating that CG should give a good prediction of the cows' BW. The slaughter percentage used here (47 and 46% for 1st lactation and older cows, respectively) were recommended for NRF assuming a body condition score (BCS) of 3.0-3.5 (1 is extremely thin, 5 is extremely fat; Havrevoll, 2010, personal communication). The slaughter percentages used are lower or similar to those used for comparable breeds (Vestergaard et al., 2007). No correction of BCS was done because of lack of available information. In particular for cows older than 1st lactation, the BCS might be an important factor due to that the drop in BW in early lactation could be a result of a higher frequency of sick cows and cows in bad condition. Normally, cows are in high metabolic stress in this period of lactation by high milk production and mobilization of body reserves and, consequently, they are more exposed to diseases. However, in Norway, cows are normally not slaughtered in this period of lactation, which indicate that slaughtered

Table 1 Least-square means of body weight (BW) estimated from slaughter weight and from chest girth measurements during lactation of 1st, 2nd, and >=3rd lactating cows

	Slaughter data		Chest girth data		BW method		BW method*WIM[1]	
	Mean	SEM	Mean	SEM	F	P	F	P
1st lactation	489	2.7	533	2.7	9809	***	2.53	***
2nd lactation	553	1.8	575	1.9	488	***	3.95	***
>=3rdlactation	587	3.5	611	3.6	703	***	4.91	***

Weeks in milk[1]

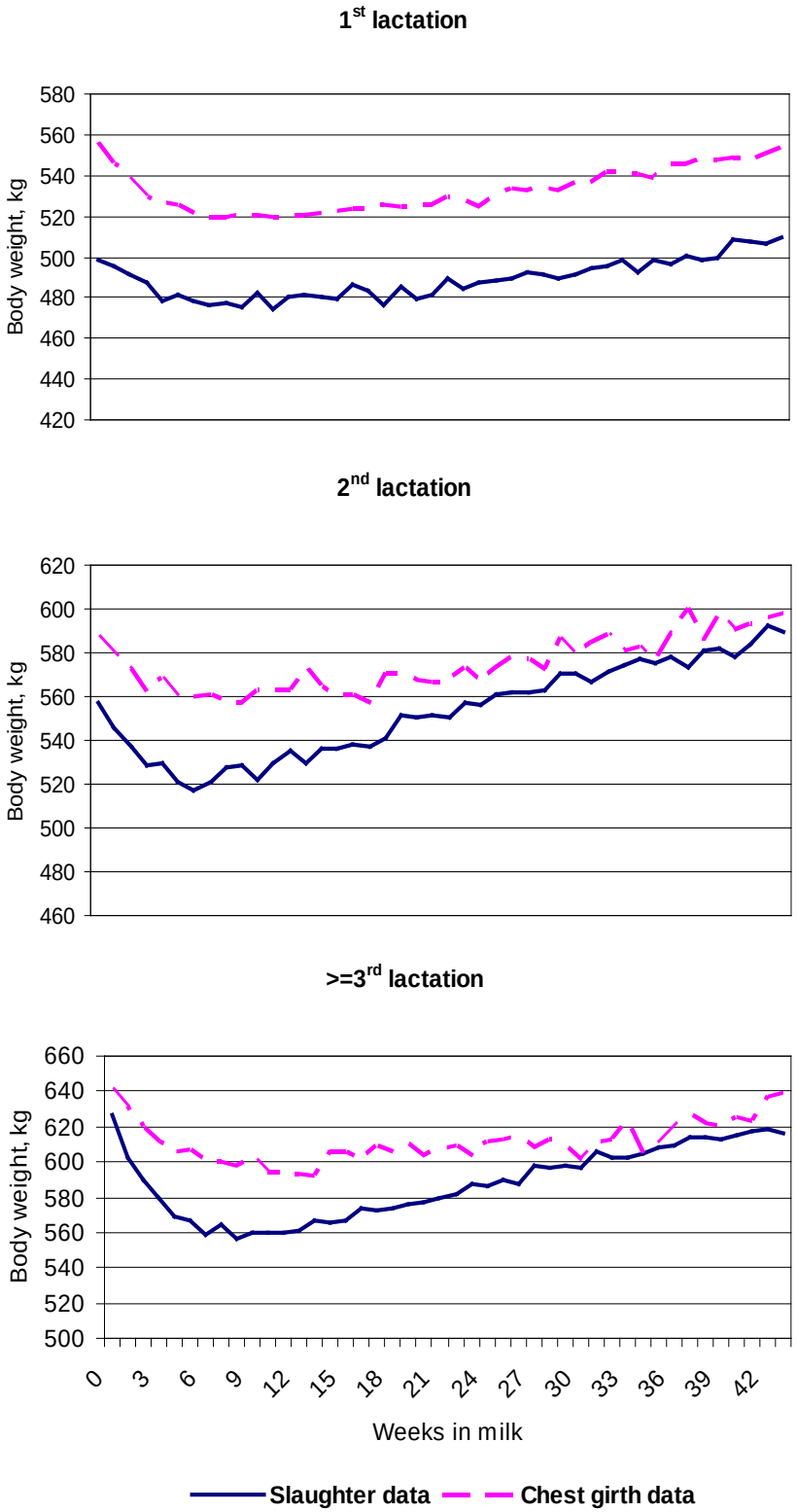

Figure 1 Body weight change (kg) during lactation estimated from slaughter weight and from chest girth measurement for 1st, 2nd, and >=3rd lactation cows.

cows are in bad condition. There is also a question concerning 1st lactation cows, whether slaughtered heifers are representative for the population of 1st lactating cows. Small heifers in poor condition have lower milk yield and thus might be slaughtered on the expense of larger heifers in better condition.

Our results indicate that, in particular for 1st lactation cows, the BW calculated from SW was under-estimated due to those cows that are alive. This was shown by the high difference between SW and CG estimates. This is also indicated by the high gap in BW estimated using the SW data between the dry off weight of 1st lactation cows (510 kg) and the initial weight at calving for 2nd lactation cows (560 kg). If the dry off period is 60 days then a daily weight gain of more than 800 g/dag is required, which is high even included an increased foetal growth.

Conclusions

In conclusion, BW estimated from SW data was under-estimated compared to estimates using CG data, in particular for cows in 1st lactation. To predict BW of cows based on SW data it is essential to use a slaughter percentage that gives the correct estimate not for slaughtered animals but for cows that are alive. For cows in 2nd and higher lactations there should be corrections for body condition, or different slaughter percentage in early lactating and late lactating cows should be used.

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Effect of abomasal phosphate infusion on inorganic phosphorus kinetics in dairy cows

K. Mogodiniyai Kasmaei[*] and K. Holtenius[†]
**MSc student in Animal Science, Swedish University of Agricultural Sciences, Uppsala, Sweden*
[†]Kungsängen Research Centre, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, SE-753 23 Uppsala, Sweden

Introduction

Phosphorous (P) is involved in various biological functions in the animal body with more known functions than any other mineral element (McDonald *et al.*, 2002). It plays important roles in energy metabolism pathways, DNA and RNA synthesis, bone formation and cell signaling (Hill *et al*., 2008). The importance of P in ruminants is even higher because rumen microbes have a high requirement for P (Van Soest, 1994). It has been pointed out that the P demand of rumen microbes could be greater than that of the host animal (Preston and Pfander, 1964; Kincaid and Rodehutscord, 2005). Salivary P is highly available to rumen microbes (Kebreab *et al*., 2005) and contributes with more P input into the rumen than dietary P does (McDonald *et al.*, 2002). Salivary P also plays an important part in control of rumen pH (McDonald *et al*., 2002). It appears that ruminants have developed an efficient P homeostatic mechanism so that both animal and microbial needs for P are met. Any intake of absorbable P above the requirement will eventually be excreted, mainly in the feces. However, the mechanisms regulating P homeostasis is not fully understood. Thus, the aim of this experiment was to investigate the effects of varying levels of phosphate infused into the abomasum on the P homeostasis in dairy cows.

Material and Methods

Three non-lactating non-pregnant rumen fistulated cows of the Swedish red and white breed with an average body weight of 682 kg were randomly assigned to an experiment with a 3×3 Latin square design. Cows were kept in a tie stall barn and were fed 4 kg of hay and 1.8 kg of concentrate every day at 8:00 and 16:00. The daily total P intake from the diet was 17.8 g. Cows had free access to water and salt lick. Each experimental period consisted of four days of continues infusion of monosodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$) solution with three different levels of P in each treatment. Treatments provided 0, 14.4 and 28.8 g of P/d in Treatment A, B and C, respectively. The infusion protocol is shown in Table 1.The solutions were prepared by dissolving calculated amounts of monosodium dihydrogen orthophosphate dihydrate in water.

Table 1 Concentration of phosphate solution and the rate of P infusion in treatment A (0 g/d infused P), B (14.4 g/d infused P) and C (28.8 g/d infused P)

Treatment	$NaH_2PO_4.2H_2O$ (g/l)	P concentration (g/l)	Phosphate solution (ml/day)	Infusion rate of P (g/d)
A	-	-	4800	Water only
B	15.11	3	4800	14.4
C	30.22	6	4800	28.8

The tubes used for infusion were placed in the abomasum through the rumen cannula. At the end of each period, the tubes were removed and the next period started after four days. One urine sample and one rumen liquid sample (at 12:00) were collected daily from each cow during the experimental periods. Collected urine and rumen liquid samples were frozen on a

daily basis. The blood samples were collected every day at 9:00 h each period. The blood samples were centrifuged for 15 minutes at 1800 x *g* and the extracted plasma was then frozen. The outflow of inorganic phosphorus (P_i) from the rumen and the rumen pool of P_i were calculated by means of fluid marker technique during the last infusion day. It was assumed that P_i was evenly distributed in the liquid phase. Cobalt-Lithium EDTA was used as fluid marker. Eight g of the marker dissolved in 350 ml of water was poured into the rumen via the cannula. Liquid samples were collected for eight hours on an hourly basis. They were then frozen after each sampling. The concentration of P_i in rumen liquid, urine and blood samples were measured by a colorimetric method on a spectrophotometer and cobalt (Co) concentration was measured by atomic absorption spectrophotometry. The rumen fluid volume and the outflow rate of the fluid fraction were determined by plotting the Co concentration against time assuming that the outflow followed first order kinetics. Statistical analysis of data was performed in Minitab 15 (Minitab Inc., State college, PA, USA) by using a general linear model procedure including treatment, period and animal. There were no significant interactions thus, they were excluded from the model.

Results and Discussion

The P requirement of the animals was covered by feed intake in this study according to NRC (2001). The diet and thus the P content was the same in all treatments and the P_i infusion gave rise to gastrointestinal levels well beyond animal' needs. Any change in P metabolism was a result of different P supplies. The P_i content of the rumen fluid and its outflow rose ($P<0.001$) as the amount of phosphate infusion increased (Table 2). There was no effect of treatment on the rumen fluid volume and outflow rate ($P>0.05$). It ranged from 76 to 100 (L) and from 5 to 5.7 (L/h), respectively. The result indicates that increased P_i outflow rate from the rumen was due to the elevated concentration of salivary P which is in accordance with conclusion of Challa and Braithwaite (1988). Although P supplied increased by approximately 100 % in treatment B and 200 % in treatment C, the rumen P_i concentration and content only increased by 21 % and 9 % in treatment B and 29 % and 23 % in treatment C respectively (Table 2). The results suggest that cows are able to efficiently adapt to markedly higher P intake without dramatic changes of the P_i content in the rumen fluid.

Table 2 Least square means and standard errors of means (SEM) of P_i outflow (mmol/h) from rumen, P_i concentration (mmol/l) and content (mmol) of the rumen in treatment A (0 g/d infused P), B (14.4 g/d infused P) and C (28.8 g/d infused P).

Treat	P_i outflow (mmol/h)	SEM	P_i concentration in rumen (mmol/l)	SEM	P_i content of rumen (mmol)	SEM
A	71	2.7	14	0.41	1232	45
B	91	2.7	17	0.41	1345	45
C	102	2.7	18	0.41	1518	45

Salivary P_i secretion (g/d) was calculated assuming that the contribution of ingested P from the diet to total outflow of P_i from the rumen was 15 (g/d) in each treatment. Furthermore, it was assumed that the net accretion of P was negligible in these adult non-lactating non-pregnant animals. Salivary P_i secretion (g/d) increased from treatment A to C (Table 3). It indicates that P_i absorption in the small intestine rose in response to increased P supply. A positive relation between P absorption and salivary P secretion was reported by Challa *et al*.

(1989). However, the absorption efficiency of P_i declined, as the amount of P_i entering proximal duodenum increased (Table 3). This indicates that P absorption in the small intestine was mediated by a carrier mechanism. A Na^+ and H^+ dependent transport system has previously been shown in small ruminants (Huber *et al*., 2002). According to the result of the current study, it appears that there was a gradual saturation of the intestinal transport of P_i. This could be regarded as a homeostatic response to increased P supply.

Table 3 Salivary P_i secretion, net absorption and absorption efficiency of P_i in treatment A (0 g/d infused P), B (14.4 g/d infused P) and C (28.8 g/d infused P).

Treatment	P_i outflow from rumen(g/d)	Salivary P_i secretion (g/d)	Total P_i entering proximal small intestine[a]	P_i net absorption[b] (g/d)	P_i absorption efficiency
A	53	38	53	38	0.71
B	67	52	82	52	0.64
C	76	61	105	61	0.58

[a] Total P_i entering proximal small intestine was calculated by adding infused P (g/d) to P_i outflow from rumen for each treatment; [b] P_i net absorption (g/d) was assumed to be the same as salivary P_i secretion for each treatment.

Concentrations of P_i in plasma and urine samples are presented in Figure 1. There was no effect of treatment on P_i concentration (mmol/l) of plasma (*P=0.28*) which is in contrast with results of Challa *et al*. (1989). This could be due to an immediate drainage of absorbed P_i into the saliva, hiding an increased P_i supply to the blood stream. The animals used in this experiment had a low requirement for P and the supply of P was well beyond their needs. It appears that in ruminants, the plasma P profile is not a good indicator for monitoring of P status (Pfeffer *et al*., 2005). There was also no effect of treatment on P_i concentration (mmol/l) in urine (*P=0.47*). In spite of an excessive P supply in this study, less than 1 % of absorbed P was excreted in urine. This shows that the contribution of urinary P excretion in P homeostasis was insignificant.

A relatively weak, but significant (*P<0.01*) relationship was observed between plasma and urine P_i concentrations (Figure 2). An exponential function (r^2= 0.47) fitted better to the data than a linear (r^2= 0.28) did. When plasma P_i concentration exceeded 3 mmol/l, urinary P excretion increased (Figure 2). This could be due to the renal threshold of plasma P concentration. Challa *et al*. (1989) reported a renal threshold of 2.3 (mmol/l) in growing calves.

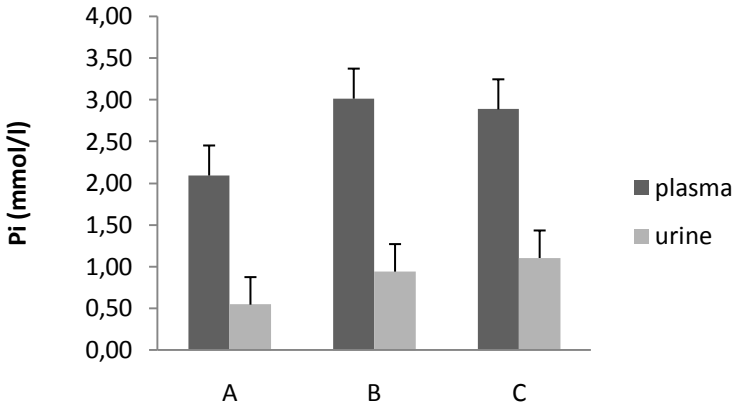

Figure 1 P_i concentration (mmol/l) of plasma and urine samples in treatment A (0 g/d infused P), B (14.4 g/d infused P) and C (28.8 g/d infused P). T-bars represent standard error of the mean (*n=3*).

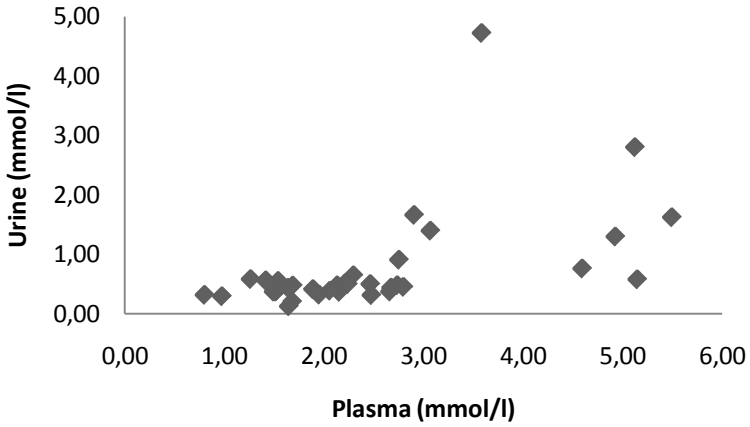

Figure 2 Relation between P_i concentration (mmol/l) in plasma and urine.

It is noteworthy to mention that there was an effect of cow and period on the P_i content of the rumen (*P<0.001*) and plasma P_i concentration (*P<0.05*). Individual cows (*P<0.001*) and period (*P<0.05*) also affected P_i concentration of the rumen. There was an effect of cow on P_i outflow rate from the rumen (*P<0.001*) and urinary P_i concentration (*P<0.05*). The impact of individual cows on the results could be explained by this fact that the number of animals was low and the effect of period could be due to the short interval between periods.

Conclusions

The net absorption of P_i and salivary P_i secretion increased with a reducing rate in response to the increased P supply as expected. The rumen P_i concentration and total amount showed only a marginal increase in relation to the excessive P supply. The results indicate that there are mechanisms in the small intestine that regulate P uptake. We suggest that these mechanisms are important in order to maintain P homeostasis when the P intake varies. The urinary P_i excretion was negligible but when the P_i concentration in plasma reached the renal threshold, urinary P_i excretion increased.

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Whole-crop maize for silage: Effects of maturity stage at harvest and feeding strategy on feed intake, chewing behaviour, diet selection and performance in growing bulls and ram lambs

K. Zaralis[1], C. Helander[1], E. Nadeau[1], S. Johansson[1], P. Nørgaard[2] and M. Murphy[3]
[1]Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, Sweden.
[2]Department of Basic Animal and Veterinary Sciences, Faculty of Life Sciences, University of Copenhagen, Frederiksberg C, Denmark
[3]Lantmännen Feeds, Uppsala, Sweden

Introduction

The whole-crop maize (WCM) cultivation has increased rapidly in Sweden and Denmark the recent years and is now occupying some 20 000 ha arable land in Sweden and at least 150 000 ha in Denmark. The increasing trend will most likely continue as new, earlier maturing, varieties are developed continuously. Maize has a high energy yield, which is advantageous in competition for acreages with small grains in flatlands and for limited acreages of land in forest-dominated areas. Limited research has been conducted on maize as a forage crop in the Nordic countries and feeding experiments have been conducted on dairy cows only (Hymøller et al., 2005). To our knowledge, no experiments have been conducted with feeding whole-crop maize silage (WCMS) to growing cattle and lambs in Scandinavia. In addition, growing cattle and lambs show different eating behaviours and they can differ in utilization of the ingested forages depending on maturity stages of the plants. However, comparisons between growing bulls and lambs in chewing behaviour, feed utilization and carcass quality characteristics when fed WCMS, are not currently available. It is therefore, a strong need for improvement of the scientific knowledge within the area of WCMS fed to large and small ruminants in Scandinavia. This paper describes two ongoing experiments that aim to investigate the effects of maturity stage at harvest and dietary inclusion of WCMS and their interactions on feed intake, chewing behaviour, diet selection, feed utilization and carcass quality in growing bulls and ram lambs and on economics of the producer.

Materials and Methods

The experiments started on December 2009 at the facilities of Götala Research Station of the Swedish University of Agricultural Sciences in Skara and are expected to be concluded by the end of June 2010. Experimental procedures have been approved by the Research Animal Ethics Committee (Swedish Animal Welfare Agency).

Whole-crop maize for silage.
Maize of the early maturing cultivar Avenir and grass forages (timothy, meadow fescue, perennial ryegrass) have been harvested for silage. The maize crop was harvested and chopped at two different stages of maturity; dough stage (15 September 2009 with 25% DM, 23% starch and 38% NDF) and dent stage (13 October 2009 with 34% DM, 36% starch and 38% NDF). At both occasions the WCM was treated with the chemical additive KOFASIL MAIZE® (Sodium-benzoate and Potassium-sorbate, Addcon Europe, GmbH, Bonn Germany) at a dosage of 2 litres/tonne of herbage and were pressed into round bales. The grass ley was harvested as a third cut in 2008, wilted to 35% DM, and ensiled with the acidic additive PROMYR® (formic acid, propionic acid and salts of organic acids, Perstorp Inc., Perstorp, Sweden) in a bunker silo.

Experiment I - Growing dairy bulls

Sixty-four growing dairy bulls (Swedish Holstein n=49; Swedish Red, n=15) were used. The animals were brought indoors approximately 10 weeks prior to the start of the experiment (week 50, 2009) in order to adapt to experimental conditions and diets. The maize silage inclusion rate was up to 75% of the forage portion when the experimental diets started to be fed. Prior to the start of the experiment, the bulls were assigned to 16 groups of 4 animals per group on the basis of their initial body weight (BW, week 47 2009) in order to form 8 groups of light and 8 groups of heavy bulls in total (Table 1). The 16 groups were then randomly allocated to 16 pens in the experimental shed. Two groups of light and two groups of heavy bulls were assigned randomly to one of the four feeding treatments (for details see below) resulting in a total of 16 bulls per treatment (Table 1).

Table 1 Group and treatment average body weight of the bulls at the time of allocation to the treatments

Treatment		*n*	Average group BW (kg) at allocation (n=4/group)				Treatment Average BW	SEM[1]
			Heavy 1	Heavy 2	Light 1	Light 2		
E100	100% Early Maize	16	421	423	362	363	392	9
E50	50% Early Maize 50% Grass	16	422	422	361	364	392	10
L100	100% Late Maize	16	423	421	362	360	391	10
L50	50% Late Maize 50% Grass	16	421	422	359	360	390	10

[1] SEM refers to the treatment average BW and is calculated on the basis of 16 animals.

Bulls in each treatment were offered a total mixed ration (TMR) that contained WCMS, harvested at dough or dent stage with or without inclusion of grass silage (Table 1).

Concentrates of the TMR diets consisted of rolled barley, dried distillers' grain and cold-pressed rapeseed cake and were included in the diets in a proportion of around 40% on a DM basis. The diets were formulated to an average daily gain of 1,5 kg and were balanced for NDF, starch, metabolizable energy and crude protein (Table 2). The animals are fed *ad libitum* on a pen level once daily at amounts corresponding to 105-110% of the average intake of the three previous days and all animals were supplemented with minerals and lime on top of their diet.

Measurements

The bulls were weighed once every two weeks throughout the experiment and were scored for body condition once every month. The average daily BW gain will be calculated. Amounts of offered TMR are recorded daily and refusals are weighed three times a week to allow calculation of intake and feed conversion ratio.

Chewing and ruminating behaviour was recorded by a closed circuit surveillance system (H.264 Network DVR, Övervakningsbutiken On Net GBG AB, Första Långgatan 6, 413 03 Göteborg) for 24h, replicated for four days on a replicated number of animals, in all

treatments. Video recording took place at two different stages of age of the animals (i.e. from middle of January to early February and 2-3 weeks prior to slaughter of the heavy animals). During the video recording periods diet selection will be estimated by analysis of the diets and refusals for contents of starch and NDF and by estimation of the particle size distribution of feed and refusals by using the Penn State particle separator (Kononoff et al., 2003).

Table 2 Ingredients and composition of experimental diets formulated for growing bulls of an average live weight gain of 1.5 kg/day

Item	Experimental Diets			
(kg DM)	E100	E50	L100	L50
Grass silage	-	3.8	-	3.8
Maize silage	7.6	3.8	7.6	3.8
Barley	2.8	4.6	2.4	4.1
Dried distillers grain	1.4	0.5	1.4	0.5
Cold pressed rapeseed cake	0.7	0.1	0.7	0.1
Mineral high	0.1	0.1	0.1	0.1
Total, kg DM	12.6	12.9	12.2	12.4
Forage proportion	0.6	0.6	0.6	0.6
Total DM fed, % of BW	2.4	2.4	2.3	2.4
Forage DM fed, % of BW	1.4	1.4	1.4	1.4
ME, MJ/kg DM	12	11.8	12.1	11.8
NDF, g/kg DM	314.5	326.8	307.8	326.8
NDF, % of BW	0.8	0.8	0.7	0.8
Starch, g/kg DM	262	279	365	322
CP, g/kg DM	123	127	124	128

Faecal samples are taken twice every day (morning - afternoon) from each pen during the video recording days. To evaluate the effects of treatments on rumen function, the faeces will be determined for consistency (Zaaijer & Noordhuizen, 2003), DM, number of undigested / partly digested kernels and number of long particles (>10 mm) by using a wet sieving technique (Nørgaard et al., 2007). Faecal particle size will be determined by a dry sieving technique followed by image analysis (Nørgaard et al., 2004). Contents of starch, NDF, lignin and acid-insoluble ash will be analysed in the composited faecal samples and digestibility will be calculated.

The animals will be slaughtered at an approximate weight of 630 kg and recordings for carcass weight, conformation, fatness and dressing percentage will be retrieved.

The economical conditions for whole-crop maize silage will be calculated for typical farms with finishing of dairy bulls and lamb production. Production costs for WCM and grass silages are calculated for the farms and these costs are used, together with experimental data for feed consumption and slaughter income for profitability calculations.

Experiment II - Growing ram lambs

Animals and experimental design.
Forty weaned ram lambs were randomly assigned to one of four dietary treatments. The lambs

were weaned at an age of 60.6±5.6 days and with an average live weight of 24.2±2.2 kg. They were kept in pens with straw bedding holding two lambs per pen and five pens per treatment. The dietary treatments were fed ad libitum once daily and the lambs were given free access to vitaminized minerals and water. The treatments were four TMR diets similar to those in experiment I (Table 1), but modified to 43% forage and 57% concentrate on DM basis. The four diets had similar contents of crude protein (CP), metabolizable energy (ME), neutral detergent fibre (NDF), starch and crude fat (CF) on DM basis (Table 3).

Table 3 Nutritive contents per kg DM of the diets in Experiment II

Item	ME	CP	Dig. CP	AAT	PBV	NDF	starch	CF	Ca	P
	MJ	g	g	g	g	g	g	g	g	g
1. E50	12.7	179	131	83	52	305	218	60	8.0	7.2
2. E100	12.7	173	118	84	49	314	208	61	5.5	7.2
3. L50	12.7	179	131	83	52	303	249	60	6.4	6.8
4. L100	12.8	173	119	84	49	301	282	61	5.2	7.2

Measurements

The lambs were adapted to the experimental diets for 14 days before the experimental recording started. The lambs were fed 115-120% of their *ad libitum* intake and feeds and refusals were weighed daily. The feeds were sampled once daily and the refusals three days per week. The animals were weighed weekly on the same week day, but twice weekly at start and end of the trial. At the start and the end of the trial the lambs were also body condition scored. The chewing behaviour was recorded using video cameras at mean body weight of 35 and 45 kg during 96 h, and offered feed, refusals and faeces samples were collected daily. Chewing behaviour, diet selection, feed, refusals and faeces samples with be analysed as described in experiment I and daily weight gain, feed conversion ratio and economics will be estimated from start of experiment to slaughter at 48-50 kg live weight.

Discussion

The experiments described above are still in progress and the authors are not in the position to present any results herein. Nevertheless, we would like to refer to some prospective contributions that this study can have towards a more sustainable agriculture in the Scandinavian countries.

The partial decoupling of the animal subsidies has to a major extent decreased the profitability of beef production in Sweden. Increased cereal prices make it even more economically difficult for the farmers and this trend will last for an extended time period. To handle these difficult situations, cost savings and production efficiencies are necessary. Maize cultivation and feeding is one way of saving costs as it gives high energy yields, only demands one harvest per year and potentially improves live weight gain and nitrogen utilisation as well as lowering methane emissions from the ruminants, since intensively raised bulls and ram lambs with short rearing periods result in lower methane emissions compared with long rearing periods. Intensively reared lambs slaughtered in spring are in demand from the slaughter industry. Feeding total mixed rations including new forages such as maize silage might increase the possibilities of a more profitable intensive beef and lamb production.

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The effect of diets containing increasing amounts of full fat sunflower seed meal on milk fat composition

M. Griinari[1], J. Westh Møller[2] and K. Sejrsen[2]
[1]*Department of Animal Nutrition & Management, Kungsängen Research Centre, Swedish University of Agricultural Sciences, S-753 23 Uppsala, Sweden*
[2]*Department of Animal Health and Bioscience, Faculty of Agricultural Sciences, Aarhus University, DK-8830 Tjele, Denmark*

Introduction

Over the past several years there has been a tremendous increase in interest to modify the composition of milk fat produced by dairy animals with dietary inputs. Typically the modifications involve improved nutritional and/or functional characteristics of high fat dairy products. More specifically, the targeted changes in milk fat composition include reduced ratio of saturated and unsaturated fatty acids, increased proportion of omega-3 fatty acids and increased level of conjugated linoleic acid, CLA (Givens and Shingfield 2004). Dietary inputs that provide the means to modify milk fat include oils and fatty acids in different forms. Processed oilseeds offer a practical solution to dietary oil supplementation.

Sunflower seeds contain 40-45% oil with typically 60-70% linoleic acid. Including oil in the dairy cow diet in the form of full fat seeds is considered to be less damaging to the rumen function than supplementation with free oil (Dewhurst et al. 2006). However, processing of the oil seed to allow digestion and release of the fatty acids is needed. Crushing the seeds with a roller mill is often sufficient.

The objective of this paper is to present an examination of changes in milk fatty acid composition in response to dietary supplementation of dairy cows with increasing amounts of crushed sunflower seeds. Effect of supplementation on milk production and composition is also examined. Furthermore, an estimate of the relative availability of sunflower seed fatty acids is produced based on the transfer efficiency of dietary fatty acids to milk fat.

Materials and methods

Animals

Twenty four lactating Holstein Friesian cows with daily milk yield of 25.3 ± 2.5 kg and 186 ± 20 days after calving were used in this experiment. The cows were blocked according to parity and days in milk and randomly assigned to four dietary treatments. Diets were fed for five weeks and the data were collected during the last week of the experiment.

Diets

The four treatments consisted of a total mixed ration (clover grass, barley, soybean meal, limestone and a mineral mix) supplemented with increasing amounts of crushed sunflower seeds (0, 51, 103 and 157 g/kg of diet DM; CTL, LoSF, MedSF and HiSF). The diets were balanced for crude protein and net energy by adjusting the levels of soybean meal and barley to increasing amounts of sunflower seed meal in the diet. These adjustments resulted in opposite changes in dietary fatty acid and starch concentrations (Table 1). The diets were fed once daily and the cows were allowed free access to feed and water.

Table 1 Diet ingredients and chemical composition.

	Treatment			
Item	CTL	LoSF	MedSF	HiSF
Ingredient, g/kg of DM:				
Sunflower seeds, crushed	-	51	103	157
Soybean meal	143	105	88	134
Barley	298	285	246	126
Clover grass	553	553	558	581
Minerals	2.8	2.8	1.7	-
Limestone	3.3	3.3	3.3	2.8
Chemical analysis, g/kg of DM:				
Crude protein	164	154	151	173
Crude fat	32	56	81	106
Added fatty acids	0	23	46	69
Total fatty acids	21	44	67	90
Oleic acid (C18:1)	2.2	8.1	14.2	20.5
Linoleic acid (C18:2)	8.3	22.6	37.1	51.9
α-linolenic acid (C18:3)	4.8	4.8	4.8	4.9
Starch	199	189	165	96
NDF	316	31.9	32.4	33.3
Chewing time (min/kg DM)	34.9	34.9	35.2	36.5
NE lactation (MJ/kg DM)	7.73	7.73	7.75	7.89
Fill factor (FFk)	6.75	6.75	6.75	6.75

Milk and fatty acids analysis

Pooled milk sample (am/pm milk) was obtained twice weekly and the samples obtained during wk 5 were analyzed for fatty acid composition. Milk samples were also analyzed for fat, protein and lactose content by infrared spectroscopy (MilkoScan^{TM} 4000, Foss Electric, Denmark). Analysis of milk fat fatty acid composition was performed as described by Tholstrup et al. (2006).

Results and Discussion

Increasing the proportion of crushed sunflower seeds in the diet did not influence feed intake and milk production at the lowest level of inclusion, but decreased feed intake as well as milk and protein yield at the intermediate and high level of sunflower seed supplementation (Table 2).

Table 2 Effect of increasing amounts of sunflower seeds on milk production.

	Treatment				
Item	CTL	LoSF	MedSF	HiSF	P
DMI, kg	17.9^{a}	17.5^{a}	14.4^{b}	14.7^{b}	0.006
Milk yield, kg	28.4^{a}	27.0^{a}	24.1^{b}	21.9^{b}	0.001
Fat, %	3.76	3.80	3.97	4.38	0.081
Protein, %	3.31	3.25	3.11	3.18	0.266
Fat yield, g	1051^{a}	1015^{ac}	826^{b}	951^{bc}	0.007
Protein yield, g	910^{a}	858^{a}	730^{b}	692^{b}	<0.001

Means with a row with different superscripts differ significantly ($p<0.05$).

Dietary supplementation with crushed sunflower seeds resulted in a marked shift in milk fat fatty acid composition: the proportion of fatty acids synthesized in the mammary gland (C4 to C14 and a portion of C16) was reduced and the proportion of fatty acids derived from the

diet (C18 fatty acids) was increased (Table 3). There was no change in milk fat linoleic acid (*cis*-9, *cis*-12 C18:2), the predominant fatty acid in sunflower seed oil while the most pronounced increase occurred in milk fat stearic (C18:0) and oleic acid (*cis*-9 C18:1). Stearic acid is an end product of ruminal biohydrogenation of unsaturated C18 fatty acids and milk fat oleic acid is mainly a Δ-9 desaturase product of stearic acid. Milk fat *trans*-18:1fatty acids also arise from rumen biohydrogenation and conjugated linoleic acid (CLA), analogous to milk fat oleic acid, is largely a Δ-9 desaturase product of *trans*-11 C18:1. Considering the amount of supplementary lipid and the predominance of linoleic acid in the supplement, increase in the milk fat content of *trans*-18:1 and CLA was less than expected. Overall, the changes in milk fat fatty acid composition suggest that the supply of sunflower seed fatty acids was hydrogenated in the rumen to a high degree. In addition, it appears that dietary supplementation with crushed sunflower seeds reduced the desaturase ratios in milk fat (Table 3). Observed decrease in desaturase ratios may indicate a decreased Δ-9 desaturase activity, which may contribute and explain, in part, the relatively modest CLA responses.

Table 3 The effect of increasing amounts of sunflower seeds on milk fatty acid composition.

Item	Treatment				P
	CTL	LoSF	MedSF	HiSF	
w-% of total fatty acids					
C4:0	4.31	4.65	4.15	4.05	0.276
C6:0	2.45^{a}	2.37^{a}	1.99^{b}	1.81^{b}	<0.001
C8:0	1.53^{a}	1.31^{b}	1.00^{c}	0.89^{c}	<0.001
C10:0	3.55^{a}	2.61^{b}	1.91^{c}	1.70^{c}	<0.001
C12:0	4.19^{a}	2.85^{b}	2.09^{c}	1.95^{c}	<0.001
C13:0	0.14^{a}	0.07^{b}	0.02^{c}	<0.01^{c}	<0.001
C14:0	12.50^{a}	10.33^{b}	8.62^{c}	7.90^{c}	<0.001
C14:1	1.14^{a}	0.72^{b}	0.58^{b}	0.64^{b}	<0.001
C15:0	1.40^{a}	1.06^{b}	0.97^{bc}	0.86^{c}	<0.001
C16:0	32.14^{a}	26.35^{b}	20.96^{c}	20.32^{c}	<0.001
C16:1	2.14^{a}	1.44^{b}	1.33^{b}	1.40^{b}	<0.001
C17:0	0.58^{a}	0.48^{b}	0.42^{c}	0.38^{d}	<0.001
C18:0	8.52^{a}	14.80^{b}	17.55^{c}	17.57^{c}	<0.001
trans-11 C18:1	1.04^{a}	1.98^{b}	3.06^{c}	3.21^{c}	<0.001
cis-9 C18:1	18.27^{a}	22.00^{a}	28.04^{b}	30.27^{b}	<0.001
C18:2 n-6	1.55	1.69	1.74	1.76	0.301
C18:3 n-3	0.47^{a}	0.37^{b}	0.26^{c}	0.24^{c}	<0.001
CLA	0.46^{a}	0.69^{a}	1.07^{b}	1.33^{b}	0.003
Desaturation ratios					
C14:1/C14:0	0.09	0.06	0.07	0.08	0,22
C16:1/C16:0	0.06	0.05	0.05	0.06	0,11
C18:1/C18:0	2.15^{a}	1.51^{c}	1.64^{b}	1.82^{b}	0,02
CLA /*trans*-11 C18:1	0.44	0.36	0.35	0.37	0,17

Means with a row with different superscripts differ significantly ($p<0.05$).

Conclusions

Supplementing dairy cow diets with increasing amounts of crushed sunflower seeds altered progressively the fatty acid composition of milk. The intermediate and high levels of supplementation reduced dry matter intake as well as milk and protein yield. In milk fat, the increase in stearic and oleic acids was the most pronounced with no increase in linoleic acid,

the predominant fatty acid in sunflower seed oil, indicating a high degree of biohydrogenation of unsaturated fatty acids in the rumen. Reduced supply of CLA precursor, *trans*-11 C18:1 and reduced Δ-9 desaturase activity could explain the relatively modest CLA responses in this study.

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Fibre digestion in different segments of the digestive tract of dairy cows fed grass silage based diets

S. Ahvenjärvi, A. Vanhatalo[1], T. Stefanski and P. Huhtanen[2]
MTT Agrifood Research Finland,31600 Jokioinen, Finland
[1]*Current address: University of Helsinki, Helsinki, Finland*
[2]*Current address: Swedish University of Agricultural Sciences, Umeå, Sweden*

Introduction

Studies on the nutrient metabolism in ruminants often rely on measurements of digesta flow entering the duodenum or omasal canal. In most cases nutrient flows are determined based on spot sampling of digesta and use of indigestible markers. Unrepresentative sampling and analytical problems associated with the use of markers may results in biologically improbable estimates of nutrient digestibility in the rumen and the intestines. Therefore, reasonable limits to nutrient digestibility in different segments of the digestive tract could be used as a guideline to critically evaluate the accuracy of flow measurements (Titgemeyer, 1997). Owing to limited capacity of the intestines relative to the forestomach the contribution of the rumen and reticulum to total tract fibre digestion in bovines is likely in excess of 80% (Paloheimo and Mäkelä, 1959; Titgemeyer, 1997). A number of studies that have assessed digesta flows entering the omasal canal have indicated that a major fraction of neutral detergent fibre (NDF) is digested in the rumen and reticulum of lactating dairy cows and only a small proportion is digested in the hindgut (Huhtanen et al., 2010). The objective of the current experiment was to determine the contribution of different segments of the digestive tract of dairy cows to total tract fibre digestibility using a slaughter technique.

Materials and Methods

Five lactating Finnish Ayrshire dairy cows equipped with rumen cannulas were offered grass silage and concentrates according to appetite allowing at least 5% for the refusals. Concentrate mixture consisted (on DM basis) of barley (0.39), molassed sugar beet pulp (0.39), soybean meal (0.20) and minerals (0.02). The proportion of concentrates in the diet varied between 0.27 and 0.54 (mean 0.37, standard deviation (SD) 0.117). The body weight of cows ranged from 548 to 729 kg (mean 645 kg, SD 76.8 kg) and their milk yield varied between 17.1 and 37.5 kg/d (mean 24.4 kg/d, SD 7.74 kg/d). Because the cows entered the experiment one or two at a time over three years the variation in digestibility between animals may be partly attributed to differences in experimental forages. Feed intake and milk yield were recorded daily over the entire experiment. Following an adjustment period of 10 d total collection of faeces was conducted on d 11 to 15 to determine the total tract digestibility of nutrients. On d 16 four hours after the morning meal the rumen contents were first evacuated and then the cows were slaughtered. The digestive tract was removed, the contents were evacuated and divided into five segments, 1) rumen and reticulum, 2) omasum, 3) abomasum, 4) small intestine and 5) caecum and colon. NDF digestibility in different segments was calculated from iNDF:pdNDF ratio in feed and digesta samples evacuated from these sites. These estimates tend to underestimate the digestibility in the segments where digesta was sampled from because particles leaving the segment, e.g. reticulo-rumen have higher iNDF:pdNDF ratio than those residing in the compartment (Ahvenjärvi et al. 2001).

Results and Discussion

Variation in dry matter (DM), organic matter (OM) and NDF intake between cows was large reflecting the wide range in milk yield (Table 1). On average OM and NDF digestibility were high but the variation between cows in NDF and potentially digestible NDF (pdNDF) digestibility was considerably larger than that in OM digestibility (Table 1).

Table 1 Intake and digestibility of chemical components

	Intake, kg/d				Digestibility		
Cow	DM	OM	NDF[1]	pdNDF[2]	OM	NDF	pdNDF
1	14.02	12.93	6.09	5.23	0.768	0.709	0.825
2	16.29	15.15	7.73	6.75	0.746	0.690	0.791
3	17.08	15.74	7.69	6.59	0.776	0.735	0.858
4	19.33	17.93	7.73	6.51	0.740	0.607	0.721
5	19.97	18.49	8.56	7.15	0.744	0.646	0.772
Mean	17.34	16.05	7.56	6.45	0.755	0.677	0.793
SD[3]	2.40	2.24	0.90	0.72	0.016	0.051	0.052

[1]Neutral detergent fibre.
[2]Potentially digestible neutral detergent fibre.
[3]Standard deviation between cows.

The mean retention time of indigestible NDF (iNDF) in the reticulo-rumen represented proportionally 0.72 of the total mean retention time in the digestive tract although with individual cows this varied between 0.67 and 0.76 (Table 2). The MRT in the omasum was similar to that observed for the small and large intestines. Part of the variation between cows in the MRT in a specific compartment may be explained by the fact that the pool sizes were not in a steady state and only a snapshot observation per animal could be determined using a slaughter technique.

Table 2 Retention time of indigestible neutral detergent fibre in different segments of the digestive tract of dairy cows

	Retention time in a compartment, h					
Cow	Reticulo-rumen	Omasum	Abomasum	Small intestine	Caecum and colon	Total
1	49.9	2.1	4.0	4.1	5.3	65.4
2	36.0	9.1	2.0	1.5	3.5	52.1
3	37.9	7.5	0.7	1.6	5.1	52.7
4	56.8	6.6	2.3	1.9	10.2	77.8
5	43.4	12.7	0.6	1.8	5.9	64.4
Mean	44.8	7.6	1.9	2.2	6.0	62.5
SD[1]	8.62	3.88	1.39	1.07	2.49	10.59

[1]Standard deviation between cows.

Based on the iNDF:pdNDF ratio of digesta particles sampled from the different segments the relative contribution of the reticulo-rumen, omasum and intestines to the total tract NDF digestibility was 0.78, 0.14, and 0.07 (Table 3). However, as suggested above these figures tend to underestimate the digestibility in the reticulo-rumen because digesta particles entering the omasal canal and duodenum have higher iNDF:pdNDF ratio than particles residing in that compartment (Ahvenjärvi et al., 2001). Assuming a similar rate of pdNDF digestion for all segments along the digestive tract the contribution of each compartment would be directly proportional to the pdNDF pool size. Pool sizes of pdNDF presented in Table 4 indicate that, assuming no digestion occurs in the abomasum and small intestine, the proportion of the

reticulo-rumen, omasum and caecum and colon of total NDF digestibility was 0.83 (SD 0.037), 0.10 (SD 0.046), and 0.08 (SD 0.019).

Table 3 Distribution of neutral detergent fibre (NDF) digestion between the segments within the digestive tract based on the ratio of indigestible and potentially digestible NDF in digesta sampled from the segment.

Cow	Reticulo-rumen	Omasum	Caecum and colon
1	0.790	0.050	0.160
2	0.694	0.219	0.087
3	0.718	0.172	0.110
4	0.896	0.115	-0.011
5	0.815	0.165	0.020
Mean	0.783	0.144	0.073
SD[1]	0.080	0.064	0.069

[1]Standard deviation between cows.

Table 4 Pool size of potentially digestible neutral detergent fibre in different segments of the digestive tract of dairy cows.

	Pool size of pdNDF, kg				
Cow	Reticulo-rumen	Omasum	Abomasum	Small intestine	Caecum and colon
1	3.78	0.14	0.21	0.43	0.36
2	4.57	0.71	0.12	0.15	0.28
3	3.99	0.49	0.04	0.14	0.33
4	5.45	0.49	0.18	0.18	0.68
5	4.79	0.92	0.04	0.17	0.44
Mean	4.52	0.55	0.12	0.21	0.42
SD[1]	0.66	0.29	0.08	0.12	0.16

[1]Standard deviation between cows.

Conclusions

The results from the current slaughter study indicate that in cows fed grass silage based diets over 90% of fibre digestion occurred in the forestomach.

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Effect of incremental amounts of docosahexaenoic acid enriched marine oil on enteric methane production in growing cattle fed grass silage based diets

T. Stefański, S. Ahvenjärvi, P. Kairenius and K. J. Shingfield
Animal Production Research, MTT Agrifood Research Finland, FI 31600, Jokioinen, Finland

Introduction

Ruminant livestock systems are a significant source of methane and nitrous oxide into the environment that contribute to global warming. Numerous countries have agreed in principle to decrease greenhouse gas emissions (GHG), targets that have to be met within the context of expected increases in global foods production. In order for the livestock industry to remain viable, there is an urgent need to develop strategies to mitigate GHG emissions from ruminant milk and meat production systems. Methane, nitrous oxide and carbon dioxide are the main GHG. Enteric methane production that accounts for between 2 and 12% of gross energy intake (Johnson and Johnson. 1995) is considered to be the most important GHG released into the environment from ruminant livestock (Ogino et al., 2007). Several approaches for decreasing ruminal methane production have been examined including vaccination, defaunation, use of probiotic treatments and alterations in diet composition (Martin et al., 2010). Inclusion of oils and oilseeds in the diet typically depress enteric ruminal methanogenesis (Beauchemin et al., 2008; Eugène et al., 2008; Martin et al., 2010), effects thought to be related to the anti-protozoal properties of 12:0 and 14:0 fatty acids and inhibitory effects of unsaturated≤ 18 carbon fatty acids on the growth of rumen methanogens. Several recent studies have demonstrated the potential of plant oils rich in polyunsaturated fatty acids to lower methane emissions from cattle in vivo (Martin et al., 2008; Beauchemin et al., 2009). Furthermore, fish oil and marine lipids containing 20:5n-3 and 22:6n-3 fatty acids are known to induce potent inhibitory effects on methanogenesis in vitro (Dong et al., 1997; Fievez et al, 2003; 2007), but evidence on the efficacy of long chain n-3 fatty acids on enteric methane production in vivo is limited (Woodward et al., 2006). In the current experiment the effect of incremental amounts of fractionated marine oil enriched in 22:6n-3 (docosahexaenoic acid) on methane production and ruminal methanogen populations in growing steers fed grass silage based diets was examined.

Materials and Methods

Four Aberdeen Angus steers of mean ± SD initial live weight 621.8 ± 39.8 kg fitted with rumen cannula and and a rigid T-piece cannula located within 50 mm of the pylorus were used in a 4 x 4 Latin square design with 28 d experimental periods. Steers were housed in a dedicated metabolism unit and offered daily rations as equal meals at 06.00 and 18.00 h. Steers were offered total mixed rations based on restrictively fermented grass silage and cereal based concentrates (forage: concentrate ratio 60:40 on a dry matter basis) fed at a rate of 85 g DM/kg metabolic live-weight/d equivalent to 95% of ad libitum intake measured immediately before the start of the experiment. Experimental treatments comprised 0, 21.4, 42.9 and 85.7 g/d of a fractionated fish oil (Aker BioMarine, Oslo, Norway) containing 70 g of 22:6n-3/100 g total fatty acids and designed to supply 0, 15, 30 and 60 g/d of 22:6n-3, respectively. Oil supplements were offered as two equal amounts/d by mixing with 0.5 kg of concentrate components immediately before feeding the total mixed ration. Supplements of marine oil were excluded from the diet for the last 3d of each experimental period to minimize possible treatment carry over effects. Samples of the gas produced in the rumen were collected on d 23-25 of each period. Daily gas production was determined by the tracer gas technique using permeation tubes that release the SF_6 tracer gas at a steady rate into the

rumen. During the collection period, gases were continuously collected via the rumen cannula using plastic tubing (circa 2 L/d) and sub-sampled into evacuated 10 ml glass tubes equipped with a rubber stopper and submitted for the analysis of CH_4, CO_2 and SF_6. Gas samples were analysed using a gas chromatograph (HP 6890 Series, GC System, Hewlett Packard, USA) equipped with flame ionization and electron capture detectors and a nickel catalyst for converting CO_2 to CH_4 according to Kanerva et al. (2007).

Results and Discussion

Preliminary results from this experiment indicated no significant effects of fractionated marine oil enriched in 22:6n-3 on oven dry matter intake or methane production in growing steers fed grass silage based diets (Table 1). Previous studies have shown that inclusion of incremental amounts of fish oil supplying up to 24 g/d of 22:6 n-3 has no effect on nutrient intake or total tract neutral detergent fibre digestibility coefficients in growing cattle fed grass silage or red clover silage (Lee et al., 2008) or total mixed rations based on maize silage (Shingfield et al., 2010).

Table 1 Effect of incremental amounts of fractionated marine oil enriched in 22:6n-3 on dry matter intake and gas production in growing steers fed grass silage based diets

	Intake of 22.6n-3 (g/d)				SEM	P-value[1]	
	0	15	30	60		Lin	Quad
DMI kg/d	9.46	9.46	9.46	9.45	0.013	0.45	0.77
CH_4 L/d	294	346	316	298	30.8	0.80	0.39
CO_2 L/d	929	1142	1074	1015	134	0.86	0.38
CH_4 L/kg DMI	31.2	36.6	33.5	31.5	3.27	0.79	0.38
CO_2 L/kg DMI	99.2	122	115	108	14.4	0.89	0.36

[1] Significance of linear (Lin) and quadratic (Quad) components of the response to fractionated marine oil in the diet; DM, Dry matter; DMI, Dry matter intake.

Incubations of fish oil or marine algae containing 20:5n-3 and 22:6n-3 fatty acids with mixed rumen bacteria (Dong et al., 1997; Fievez et al., 2003; 2007) has been reported to decrease methane production in vitro up to a maximal inhibition of 80%. Plant oils rich in polyunsaturated fatty acids are known to lower enteric methane production in cattle (Martin et al., 2008; Beauchemin et al., 2009), suggesting that the highly unsaturated fatty acids in fish oil, including 20:5n-3, 22:5n-3 and 22:6 may inhibit the growth and/or metabolic activity of methanogens in the rumen. Data from this experiment provided no support that 22:6 n-3 in the diet alters methane production in growing cattle fed grass silage based diets, with the implication that other fatty acids in fish oil and marine algae may be responsible for the effects in vitro. Furthermore, there is no evidence to indicate that 20:5n-3 or 22:5n-3 in isolation decrease ruminal methane production in vivo, whilst 14:0 is also relatively abundant in fish oil and marine algae. Studies in lactating cows have shown that supplementing the diet with 14:0 decreases methane production (Dohme et al., 2004; Odongo et al., 2007) suggesting that the possible effects of marine oils on methanogenesis in ruminants are related to the concentrations of medium chain saturated fatty acids in these lipid supplements.

Conclusions

Incremental amounts of fractionated marine oil enriched in 22:6n-3 had no effect on dry matter intake or enteric methane production in growing cattle fed grass silage based diets.

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