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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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Foreword

The organizing committee of the 1st Nordic Feed Science Conference 2010 was very pleased with the large number of participants and the overwhelmingly positive response to the conference. The original idea was to arrange similar conferences on a biannual basis, but we have now changed our minds and decided to arrange it annually.

It has become apparent that conflicts with other conferences will occur every year. Therefore, the NFSC will have to adapt its theme, not to overlap with these conferences. This year NJF arranges its 24th Congress in Uppsala and had planned a parallel session on feeds. The NJF organizing committee graciously accepted our suggestion of a joint meeting something which solved the problems of two conferences in the same month. What also occurs this year is that Norway arranges the EAAP meeting August 29 to September 2. Next year, Finland hosts the International Silage Conference July 2 – 4 and 2012, we clearly have to modify our program to exclude focus on forage conservation.

The 2011 NFSC has two sessions which differ from the normal oral and poster presentations. The first one takes place on Wednesday night after the NFSC dinner. It focuses on equine research capacities of the Nordic countries. The idea of this session was initiated by the very encouraging signals of increased cooperation among Nordic countries. Leading equine scientists have expressed great interest in joining forces and also to share each other's research facilities to a greater extent in the future. The second session takes place on Thursday before lunch. The topic is "Experimental design for production studies on dairy cows". The intention of this session is to share information and ideas on design and statistical evaluations of dairy cow studies. We have hopes that statistically oriented sessions will be a permanent part of future conferences.

Uppsala 2011-05-30

Peter Udén

Contents

Methods and laboratory procedures

Ruminant nutrition

Comparison of *in vitro* and *in situ* methods in evaluation of forage *in vivo* organic matter digestibility in ruminants

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Introduction

Traditionally, feeding value of diets has been determined from tabulated digestibility coefficients and proximate analysis. The digestibility coefficients have been determined in trials with sheep fed at maintenance level of intake. This would be the most accurate reference method for further development of the rapid and cost-effective use of near infrared reflectance spectroscopy (NIRS) in commercial analysis of forage farm samples. However, more easily conducted laboratory methods have been developed for routine determination of forage digestibility. Different *in vitro* methods, based on rumen inoculums or enzymes, related to *in vivo* organic matter digestibility (OMD) by general or forage specific equations have been applied as a basis for NIRS calibrations in the Nordic countries (Huhtanen et al., 2006; Åkerlind et al., 2011). Recently, an accurate association between NIRS and indigestible neutral detergent fiber (iNDF) in grass silage was reported by Nousiainen et al. (2004). The iNDF fraction of primary and regrown grass, conserved as silage, were equally well related to *in vivo* OMD, while separate equations performed better for pepsin-cellulase solubility (Huhtanen et al., 2006). The objective of this study was to compare the application of different *in vitro* and *in situ* methods in empirical and mechanistic predictions of *in vivo* OMD for a wide variety of forages.

Materials and Methods

Samples from a total of 50 forages, which had been used earlier in digestibility trials with sheep, were collected from Bonn University in Germany, and from MTT Agrifood Research and Helsinki University in Finland. The sample set comprised silages of alfalfa (n=3), corn (n=9), corn stover (n=2), grass (n=11), whole crops of wheat and barley (n=8), red clover (n=7), and grass hays (n=5) and wheat straws (n=5).

Concentrations of iNDF in all forage samples were determined following a 288-h *in situ* incubation in the rumen using 3 cows. Samples of 2 g were weighed into pre-weighed polyester bags with pore size of 12 µm and a pore area equal to 6% of the total surface (Saatifil PES 12/6; Saatitech S.p.A., Veniano, Como, Italy). Residues were analyzed for NDF including sodium sulfite and iNDF was expressed exclusive of residual ash. Isolated forage NDF of all samples was subjected to *in vitro* incubations with automatically recorded gas volumes. About 500 mg of sample were incubated in 60 mL of buffered rumen fluid for 72 h. Incubations were conducted at 39°C and recordings of each sample were repeated in 3 consecutive runs. *In vitro* pepsin-cellulase organic matter solubility (OMS) of the forages was determined in a 2-step gravimetric digestion method. Initially, samples were subjected to a pre-treatment with pepsin-HCl (1000 mL 0.5 M HCl and 2 g pepsin; Merck No. 7190, 2000 FIP U/g) for 24 h at 40°C, further incubated for 48 h at 40°C using crystalline cellulase from *Trichoderma viride* (3.3 g and 11.7 U/g; Onozuka R-10, Yakult Pharmaceutical Ind. Co. Ltd., Japan) and the insoluble residue was determined exclusive of residual ash. The *in vitro* methods based on the use of rumen inoculum were conducted at the Department of Animal Science of Helsinki University and at the Swedish University of Agricultural Sciences at

Kungsängen Research Center. The 2-step *in vitro* procedure for determination of *in vitro* OMD at Helsinki University was conducted according to Tilley and Terry (1963). Samples were incubated using rumen fluid mixed with buffer for 48 h at 38°C. The second step of digestion was performed by adding 50 mL of a pepsin solution made from 2 g pepsin (Merck No. 7190, 2000 FIP U/g) dissolved in 1000 mL 0.5 M HCl; samples were kept additional 48 h at 38°C. The incubation residues were dried overnight at 103°C and thereafter combusted at 600°C for 16 h. At Kungsängen Research Center, *in vitro* OMD was determined by incubation in rumen fluid, as described by Lindgren (1979). The dried forage samples were incubated in 49 mL buffer and 1 mL of rumen fluid at 38°C for 96 h. Incubation residues were dried at 105°C for 3 h followed by ashing at 500°C for 40 min to determine rumen fluid organic matter solubility (VOS).

Digestibility of organic matter from the prolonged *in situ* incubations and from analyzed OMS values was calculated according to the general as well as the forage specific empirical equations reported by Huhtanen et al. (2006). Specific equations were used to predict OMD from iNDF (OMD_{iNDF}) and OMS (OMD_{OMS}) concentrations in hay and grass (combined primary and secondary growth or primary growth alone), legume, and whole crop silages, for other forages the general forage equation of Huhtanen et al. (2006) was used. Indigestible neutral detergent fiber was also used in a mechanistic model to estimate a first-order degradation rate (k_d) of neutral detergent fiber (NDF) from gas profile recordings to predict OMD (OMD_{GP}). A 2-pool Gompertz model was fitted to the cumulative gas production profile of each sample and the parameter estimates were then used in a dynamic mechanistic rumen model to determine digestibility of potentially digestible NDF (pdNDF) as described by Huhtanen et al. (2008). The equation of Allen and Mertens (1988) was solved for k_d using known values of pdNDF digestibility and a rumen residence time of 50 h distributed between the non-escapable and escapable pools in a ratio of 0.4:0.6. The OMD_{GP} was calculated according to Eq. 1:

$$OMD_{GP}\ (g/kg) = ((pdNDF \times pdNDFD) + dNDS) / (1000 - Ash), \quad [1]$$

where, pdNDF (g/kg of dry matter (DM)) = NDF – iNDF, pdNDFD (kg/kg) = digestibility of pdNDF, and dNDS (g/kg) = digestible neutral detergent soluble (NDS) part = (1000 – NDF – Ash) × 0.963 – 92. A general forage equation was applied in calculations of digestible NDS according to Huhtanen et al. (2006). Two empirical relationships according to Lindgren (1983), which only distinguished between more or less than 50% legumes in the silage, were used to calculate rumen fluid OMD from VOS (OMD_{VOS}). Predictions of *in vivo* OMD from *in vitro* determined OMD by the modified method of Tilley and Terry (1963) (OMD_{TT}) was determined from two equations (Møller et al., 1989; Søegaard et al., 2001), which only separated corn silage from other forages in the calculations.

The GLM procedure of SAS (2002-2003) was used to produce linear regressions to predict *in vivo* OMD from the different *in situ* and *in vitro* derived methods used as estimators of OMD. Deviating properties of forage types were investigated from leverage and influence by the diagnostics $DFFITS_i$ and $DFBETAS_{j,\,i}$, where i = 1, 2, …, 50 and j, i denotes the jth regression coefficient in the regression equation (= 0 or 1) estimated without observation i, where i = 1, 2, …, 50, respectively. Cut off values suggesting that an observation warrants examination were set at $|DFFITS_i| > 2\sqrt{(p/n)}$ and $|DFBETAS_{j,\,i}| > 2/\sqrt{n}$, where p = number of model parameters, and n = total number of observations. Prediction equations were further evaluated by analysis of residuals. Predicted values were centered to a mean value of zero by subtracting the mean of predicted values from each prediction within method in combination

with general or forage specific prediction equations. The slope and intercept estimates in the regression were orthogonal and could thereby be assessed as independent. Residual values were calculated as observed *in vivo* OMD minus predicted OMD. Prediction equations were evaluated by regressing residual values on the centered predicted values. Mean biases were assessed by using the intercepts of the regression equations, and the slopes of the regression equations were used to determine the presence of linear biases. Root mean square error of prediction (RMSEP) was calculated as RMSEP = $\sqrt{[\Sigma (\textit{In vivo}\ \text{OMD} - \text{Predicted OMD})^2 / n]}$.

Results and Discussion

The forages covered a wide range in chemical composition and *in vivo* OMD (Table 1). Four of the 5 wheat straws were treated with either NH_3 (3) or NaOH (1), which contributed to high standard deviations of the concentrations of CP (62±28.8 g/kg of DM) and iNDF (166±62.1 g/kg of DM) within forage type. One alfalfa sample was not preserved and instead fed as fresh herbage. This alfalfa was lower in DM (165 versus 369 and 421 g/kg) and higher in CP (258 versus 184 and 188 g/kg of DM) compared to the ensiled alfalfa.

Table 1 Chemical composition and *in vivo* organic matter digestibility (OMD) of all forages.

Parameter	Mean	SD[a]	Minimum	Maximum
Dry matter (DM), g/kg	414	233.6	165	914
Organic matter, g/kg of DM	901	35.7	790	949
Crude protein, g/kg of DM	139	62.0	27	261
Neutral detergent fiber (NDF), g/kg of DM	498	131.1	281	795
Indigestible NDF, g/kg of DM	121	48.8	52	264
In vivo OMD, g/kg	677	91.0	386	808

[a]Standard deviation.

The predictive ability and source of bias of the different *in situ* and *in vitro* based predictions of *in vivo* OMD were evaluated using residual analysis (Table 2). Predictions of OMD based on OMS values, using either a general equation or the forage specific equations, overestimated *in vivo* OMD ($P \leq 0.05$; Table 2). Predictions by OMD_{TT} and OMD_{GP} displayed a positive linear bias ($P < 0.01$; Table 2), which indicated an overestimation of *in vivo* digestibility of OM at low values that changed to an underestimation at higher predicted OMD. Root mean square error of prediction was smallest for OMD_{OMS} estimated by forage specific equations and highest for OMD_{iNDF} by the general forage equation (Table 2). Samples of straw (3 out of 5) and alfalfa (all) appeared as leverage points and were influential observations in predictions of OMD based on iNDF using the general forage equation (results not presented). Further, 3 and 4 of the 5 straw samples were potential outliers in predictions of OMD based on VOS and OMS using the general forage equation, respectively (results not presented). Regenerating the residual analysis of predictions of *in vivo* OMD by OMD_{iNDF} (general equation), VOS and OMD_{OMS} (general equation) omitting the influential forage types decreased RMSEP to 26.9, 29.2 and 36.5, respectively. A mean bias of -0.024 was significant ($P < 0.001$) for the predictions based on OMD_{OMS} (general equation) despite omitting the straw samples. Further, the small linear bias of 0.17 in the residual analysis of predictions of *in vivo* OMD by OMD_{iNDF} (general equation) became significant ($P = 0.03$) in the regression when straw and alfalfa samples were not included.

Table 2 Assessment of sources of bias by regression analysis of the residual analysis of different *in situ* and *in vitro* based predictions of *in vivo* organic matter digestibility.

Prediction method[a]	Mean bias	SE[b]	*P*-value	Linear bias	SE	*P*-value	RMSEP[c]
OMD_{iNDF1}	-1.92	7.34	0.80	0.17	0.115	0.16	52.0
OMD_{iNDF2}	-5.30	6.77	0.44	0.21	0.106	0.06	49.0
OMD_{GP}	3.14	5.48	0.57	0.26	0.084	<0.01	41.6
OMD_{OMS1}	-21.5	4.54	<0.001	-0.04	0.052	0.47	38.2
OMD_{OMS2}	-9.86	4.85	0.05	-0.01	0.058	0.89	35.1
OMD_{VOS}	-9.37	5.63	0.10	0.09	0.076	0.23	40.7
OMD_{TT}	4.30	5.29	0.42	0.22	0.079	<0.01	39.9

[a]Organic matter digestibility (OMD) determined using indigestible neutral detergent fiber concentration in a general empirical equation (OMD_{iNDF1}) or in forage specific equations (OMD_{iNDF2}); OMD determined using *in vitro* gas production derived data (OMD_{GP}), from pepsin-cellulase solubility in a general empirical equation (OMD_{OMS1}) or in forage specific equations (OMD_{OMS2}); OMD determined from rumen fluid OM solubility in forage specific equations (OMD_{VOS}); OMD determined from forage specific equations related to the 2-step *in vitro* incubation by Tilley and Terry (1963) (OMD_{TT}). [b]Standard error. [c]Root mean square error of prediction.

Variations in forage OMD have not been satisfactorily predicted from feed chemical composition (Van Soest, 1994; Nousiainen et al., 2004; Huhtanen et al., 2006). For this reason different *in situ* and *in vitro* methods have been developed and more or less successfully related to *in vivo* data on OMD. True digestibility of NDS is nearly complete and with small variation mostly caused by random effects. Consequently, an *in situ* or *in vitro* method with accurate predictive ability should reveal and correlate to differences in forage NDF quality. The empirical relationship between the iNDF concentration and OMD is based on the assumptions that iNDF and NDS are uniform nutritional entities and that the digestibility of pdNDF is strongly correlated with the concentration of forage iNDF (Huhtanen et al., 2006). However, samples of straw and alfalfa were pointed out as outlier forage types in predictions of OMD from a general empirical forage equation based on iNDF. Separating the effect of NDF quality and rate of fiber degradation in a more mechanistic approach did not improve the predictions compared to excluding influential forage types in the empirical relationship between iNDF and OMD. Among the *in vitro* methods OMD_{VOS} generated predictions with smallest prediction error and without any bias. Likely, this method was less sensitive to fluctuations in rumen fluid (small amount used) and the prolonged *in vitro* incubation more correlated to forage iNDF concentration.

Conclusions

Indigestible NDF performed only slightly better than rumen fluid organic matter solubility in predictions of *in vivo* OMD when outlier forage types (alfalfa and straw) were not included in the predictions. To account for forage specific equations for outlier forage types would improve overall predictions of forage *in vivo* OMD for both methods.

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A ring-test of a wireless in vitro gas production system

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Introduction

The use of *in vitro* cumulative gas production (GP) as an evaluation of digestibility of feed products has been a focus of interest in the last decade. It allows assessment of feed degradability with a minimal use of surgically prepared animals and allows screening a large number of samples compared to *in vivo* studies. Early methods were based on regular manual measurement of produced gas as shown by translocation of the plunger in syringes, which is very time and labor consuming and gives limited data on fermentation kinetics. Alternatively, the increase in pressure during incubation in closed vessels of a known volume can be measured. However, accumulated gasses in the vessels may affect the fermentation processes (Rymer et al., 2005). Fully automatic systems have therefore been developed which measure pressure at much more frequent intervals and keep the pressure at an acceptable low level by automatic release of gas at a pre-set pressure level (Ankom, 2011). This increases the information about GP kinetics and raises a need for more thorough mathematical descriptions of the GP curves. The aim of this study was to assess, by mean of a ring test, whether the ANKOM^{RF} (Ankom Technology, Macedon, NY, USA) GP system produces repeatable and reproducible results.

Materials and methods

Four laboratories, located in four different countries participated in the ring test: Denmark (DK), Italy (IT), Spain (SP) and Wales (WA). All participating countries used the wireless ANKOM^{RF} Gas Production System (ANKOM, 2011) for measurement of *in vitro* GP. Three types of substrates were used: hay and barley straw were milled to pass a 1.0-mm screen in a Cylotec 1093 sample mill (Foss Analytical, Hilleroed, Denmark). Maize starch was a commercially available purified starch product (Maizena) for human consumption. All substrates were shipped from DK to all participating laboratories.

The types of donor animals varied: DK used 2 Jersey heifers; IT used 2 dry Holstein cows; SP used 2 Segureña sheep and WA used 3 dry Holstein cows. Rumen fluid was collected before morning feeding for SP and WA, 1 hour after morning feeding for DK and 2 hours after morning feeding for IT. Time from rumen fluid collection to addition to the buffer was 45 min for DK, 30 min for IT, 10 min for SP and 1 to 2 hours for WA. Upon arrival at the laboratory, and before addition to the buffer, the rumen fluid was sieved through cheese cloth.

DK defined the standard experimental protocol. The buffer was prepared as described by Menke et al. (1988). In each country, 20 modules (250 ml Duran® bottles) were prepared: five modules per substrate and five blank modules. Each module contained 0.5 g of substrates and 60 ml of mixed buffer and inoculum (ratio 2:1) and was incubated at 39.5°C for 72 hours. Gas was automatically released when the pressure inside the modules was 6.9 kPa (13.8 kPa in SP) above ambient pressure and the cumulative GP was automatically registered at 5-minute intervals. Ten modules were omitted from data analysis: two due to battery failure (from WA), and eight due to leak of gas during incubation (four from IT and four from SP). For hay, the number of modules available was 5 for each country; for maize, 5 for DK and WA, 4 for SP and 3 for IT; and for straw, 5 for DK and IT, and 4 for WA and SP.

Correction for blank modules was performed within each country. Thereafter, the recorded cumulative gas pressures were converted into volume of gas produced, in ml /g DM as $VOL_{kct} = P_{kt} \times C/(P_{0c} \times SW_k)$ where VOL_{kct} is the volume of gas produced for the module k of the laboratory of the country c at time t, P_{kt} is the pressure in PSI, observed in the module k at time t, C is a constant determining the headspace volume (211 ml), P_{0c} is the average atmospheric pressure recorded from 0 to 72 hours for the country c, and SW_k is the sample weight of the feed (g DM) in the module k.

Curve fitting was performed using the software R (R Development Core Team, 2009), using the *drc* package (Ritz and Streibig, 2005). GP profiles at time t (Y_t) were fitted using two models. The first model (M1) is described as $Y_t = a + b(1 - e^{-ct})$, where Y_t is the total GP at time t (ml/g DM incubated), a is the intercept and $a+b$ is the asymptotic gas production (ml/g DM) and c is the constant determining the steepness of the curve. This corresponds to the model for feed degradation as described by Ørskov and McDonald (1979). The second model (M2) is described as $Y_t = b(1 - e^{-c(t-L)})$, where Y_t is the total gas produced at time t (in ml/g DM incubated), L is the lag time (in hours) before the GP starts, b is the asymptotic gas production (ml/g DM) and c is the constant determining the steepness of the curve. This model corresponds to a simple exponential model that includes a lag time.

For each module, the best fitting model was selected as being the one with the smallest Akaike Information Criterion. Thereafter, for each substrate, the model that fitted best the majority of the modules was selected: the (M1) model for hay and straw and the (M2) model for maize.

Two general parameters, as widely used in published literature, were calculated for all substrates: A1: asymptotic GP (in ml/g DM), computed as $a+b$ from (M1), and b from (M2); H1: time where half A1 is produced (in hours), computed as $ln(2)/c$ from (M1) and $(ln(2)/c) + L$ from (M2). These model parameters can give unrealistic results and therefore a more detailed analysis of the GP profiles was performed using a set of predicted values based on fitted curves: PV_6, PV_{12}, PV_{24}, PV_{48} are predicted values at 6, 12, 24, and 48 hours; t48½: time to reach half the maximum predicted value in interval 0-48h; GP_{MR}: rate of maximum predicted GP (in ml/hour) and T_{MR}: time (in hours) where predicted maximum rate occurs. 48 hours is chosen as a realistic time interval in which the asymptotic production should be reached.

For each substrate, the general and predicted parameters describing the GP profiles were compared between countries, using one-way analysis of variance as $y_{kc} = \alpha_c + \varepsilon_{kc}$, where y_{kc} is the value for module k from laboratory of country c; α_c is the effect of country, and ε_{kc} is the error term, assumed to have a normal distribution with mean zero and variance σ^2. Thereafter, pairwise comparisons were performed using the software R, using the function

'glht' from the *multcomp* package (Hothorn *et al.*, 2008). The three substrates were analysed independently.

The accuracy of measurements was assessed for repeatability and reproducibility (ISO 5725-2, 1994). Estimation of the variance components were obtained by fitting the following mixed model, using the function lmer from the *lme4* package (Bates and Maechler, 2009): $y_{ick} = S_i + \alpha_c + \varepsilon_{ick}$, where y_{ick} is the value for module k from laboratory of country c using the substrate i; S_i is the substrate (fixed) effect, α_c is the effect of the random factor country c and ε_{ick} is the error term, assumed to have a normal distribution with mean zero and variance σ_{eR}^2. The restricted maximum likelihood (REML) was used for estimating the standard deviation of the factor country (eC) and the residual standard errors (eR). Repeatability (RT) refers to within country variation and was computed as $RT = 2\sqrt{2\sigma_{eR}^2}$. Reproducibility (RP), which refers to variation among countries, were thereafter computed as $RP = 2\sqrt{2(\sigma_{eC}^2 + \sigma_{eR}^2)}$.

Results and discussion

In SP, blank values stayed positive the entire period. In DK, blank values became negative after the first 36 hours of incubation, whereas values observed both in WA and IT became negative within the first 12 hours of incubation. The maximum negative accumulation, as observed in IT, corresponded to 0.21 kPa/hour. This is in accordance with the negative accumulation due to CO_2 permeability reported by ANKOM (2011), which can range from 0.14-0.21 kPa/hour.

Results for comparison of GP profiles per feed type are shown in Table 1. The values for the general parameters (asymptotic GP A1 and half time H1) for hay from WA were much larger than for the other countries. This is because two modules for hay from WA had very high asymptotic GP (A1): 335 and 476 ml/g DM as compared to the other three, ranging from 176 to 236 ml/g DM observed for all other modules. These very high model estimates were not observed using predicted values. For DK, the time for maximum rate was higher ($P<0.001$) than for the other countries: 4.9 hours as compared to 0.5 to 1.15 hours. Using predicted values, DK and SP hay samples showed no difference, but were higher ranked ($P<0.01$) than IT and WA. Therefore, use of predicted values as well as the general model parameters is suggested.

For maize, results from the two general parameters showed large variations in the GP profile results. IT and WA were not different in the asymptotic GP (A1) and IT and SP showed no difference in time to reach half the asymptotic production (H1). Using predicted values, a better homogeneity of results was observed (PV_6, PV_{24}, PV_{48}). While the GP profile of the SP modules had a lag time of 0.5 hour, the lag time of the other countries ranged from 5.7 to 8.3 hours.

Results of the general parameters for GP profile of the substrate straw indicated large variations between countries. A1 and H1 were not different between IT and SP. The results of the predicted values showed the same trend as observed for hay: values for DK and SP were ranked higher ($P<0.05$) than IT and WA. The maximum rate of GP was not different between DK and SP and the time of maximum rate of GP was not different between DK and IT.

Table 1 Results of gas production profiles for hay, maize and straw. Different superscript letters in each column indicate significant differences (P<0.05).

	General Parameters		Predicted Parameters						
	A1	**H1**	**GP_{MR}**	**t_{MR}**	**PV_6**	**PV_{12}**	**PV_{24}**	**PV_{48}**	**t48½**
					Hay				
DK	190.9 ^{a,b}	13.7	6.9	4.9 ^{a}	48.9 ^{a}	86.8 ^{a}	140.5 ^{a}	181.6 ^{a}	12.7 ^{a}
IT	181.1 ^{a}	18.1	8.3	0.7 ^{b}	36.9 ^{b}	66.9 ^{b}	108.3 ^{b}	150.1 ^{b}	13.3 ^{a}
SP	203.7 ^{a,b}	17.3	6.8	1.15 ^{b}	52.3 ^{a}	84.8 ^{a}	131.3 ^{a}	177.5 ^{a}	12.8 ^{a}
WA	294.2 ^{b}	66.9	7.8	0.5 ^{b}	37.4 ^{b}	60.9 ^{b}	96.8 ^{b}	144.5 ^{b}	15.3 ^{b}
					Maize				
DK	299.6 ^{a}	9.4 ^{a}	50.5 ^{a}	6.2 ^{a}	16.4 ^{a}	208.2 ^{a}	290.0 ^{a}	299.5	9.3 ^{a}
IT	309.8 ^{a,b}	13.2 ^{b}	32.2 ^{b}	7.3 ^{b}	0.0 ^{a}	132.3 ^{b}	261.8 ^{b}	306.3	13.1 ^{b}
SP	337.9 ^{c}	11.8 ^{b}	20.4 ^{c}	1.0 ^{c}	98.2 ^{b}	172.3 ^{c}	258.1 ^{b}	318.7	10.8 ^{c}
WA	324.8 ^{b,c}	16.3 ^{c}	27.1 ^{d}	8.8 ^{d}	0.0 ^{a}	89.1 ^{d}	242.0 ^{b}	314.5	15.9 ^{d}
					Straw				
DK	190.1 ^{a}	13.6 ^{a}	7.3 ^{a}	9.5 ^{a}	29.0 ^{a}	71.6 ^{a}	140.7 ^{a}	185.9 ^{a}	15.1 ^{a}
IT	170.8 ^{b}	15.5 ^{b}	5.9 ^{b}	9.8 ^{a}	17.8 ^{b}	52.5 ^{b}	112.1 ^{b}	161.8 ^{b}	17.1 ^{b}
SP	213.4 ^{c}	15.9 ^{b}	7.0 ^{a}	5.4 ^{b}	38.6 ^{c}	78.5 ^{a}	139.4 ^{a}	194.9 ^{a}	15.2 ^{a,b}
WA	155.9 ^{d}	22.7 ^{c}	3.7 ^{c}	19.7 ^{c}	11.6 ^{b}	28.2 ^{c}	70.8 ^{c}	134.0 ^{c}	22.9 ^{c}

A1: asymptotic GP (ml/g DM); H1: time where half A1 is produced (h); GP_{MR}: rate of maximum predicted GP (ml/h) and T_{MR}: time (h) of predicted maximum rate; PV: predicted values at 6, 12, 24, and 48 hours (ml/g DM); t48½: time to reach half the maximum predicted value in 0-48h.

Table 2 Mean (± SD) of the parameters and predictive values for each substrate and in total, and results of repeatability (RT) and reproducibility (RP)

	General Parameters		Predicted Parameters						
	A1	**H1**	**GP_{MR}**	**t_{MR}**	**PV_6**	**PV_{12}**	**PV_{24}**	**PV_{48}**	**t48½**
Hay	218±70	29±36	7±2	2±2	44±8	75±13	119±20	163±19	14±1
Maize	318±18	13±3	33±12	6±3	28±42	151±50	263±21	310±12	12±3
Straw	182±23	17±4	6±1	11±5	24±11	58±20	117±29	170±25	17±3
Mean	**237±72**	**20±23**	**15±14**	**6±5**	**32±26**	**93±50**	**163±71**	**210±70**	**14±3**
RT	*122(46)**	*57(6)**	*16*	*8*	*49*	*50*	*34*	*43*	*4*
RP	*130(57)**	*63(14)**	*20*	*11*	*75*	*94*	*72*	*58*	*8*

A1: asymptotic GP (ml/g DM); H1: time where half A1 is produced (h); GP_{MR}: rate of maximum predicted GP (ml/h) and T_{MR}: time (h) of predicted maximum rate; PV: predicted values at 6, 12, 24, and 48 hours (ml/g DM); t48½: time to reach half the maximum predicted value in 0-48h.
*Exclusion of two modules from WA (see text)

As seen in Table 2, repeatability (RT) values showed variable results. The very high RT results for A1 and H1 were due to the 2 modules for hay from WA, which had very high

values. Excluding these gave a RT of 46 and 6 for A1 and H1 and an RP of 57 and 14 for A1 and H1 (values in parentheses in Table 2). Differences in RP may be explained by species and feeding of the donor animals, as well as conditions of inoculums collection and treatment. For instance, the use of different types of cheese cloth may have had an effect on the retained particle size and microbial population in the sieved fluid, and influenced feed degradation kinetics and gas accumulation.

Conclusion

The similarity of ranking between countries with the ANKOM^{RF} System using this analytical approach shows promise. While general model parameters alone may give unrealistic results, these can still be used to calculate predicted values at realistic time intervals. When unrealistic results are obtained from general model parameters, predicted results in a relevant time interval should be considered. Repeatability and reproducibility were acceptable using this approach. Nonetheless, the results from this ring-test suggest the need for more standardization.

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Urine excretion relative to K intake in Swedish Red cattle

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Introduction

Urinary volumes excreted by cattle affect urea concentrations and hence ammonia volatization from stables, storage facilities, pastures and arable land at manure spreading (Monteny et al., 2002). The vast amount of literature data suggests that dietary potassium (K) concentration is a main determinant of urinary volume (Bannink et al., 1999; Nennich et al., 2006; Kume et al., 2008; Weiss et al., 2009). For Swedish Red cattle, reports on urine output together with details on both intake and excretion of minerals has been published for one experiment with lactating cows (Gustafson, 2001) and one with growing steers (Gustafson and Olsson, 2004). The lactating cow experiment compared hay and silage from similar grass/clover swards in rations with forage proportions of either 0.3 or 0.5 and reported the highest urine volume when forage was provided as silage. The steer experiment included incremental forage proportions, provided as silage and displayed increasing urine volume with increasing forage proportion.The objective of this paper was to utilize data on urine excretion from N balance experiments performed at Kungsängen Research Centre to investigate the relationship between K intake and urine volume for Swedish Red cattle fed rations mainly based upon domestic ingredients, with typical mineral contents.

Materials and Methods

Data from eight change-over experiments performed at Kungsängen Research Centre was utilized (Table 1). Each experiment included 6-8 animals. Total urine collection was performed in all experiments, except for G and H, where urine output was based upon 17 spot samples per animal and period together with volume estimates based on creatinine concentrations and an assumed daily creatinine excretion according to Chizzotti et al. (2008). The equation for lactating cows from Chizzotti et al. (2008; daily creatinine excretion = 24.1 mg/kg BW) corresponded well with results for experiments A-D, suggesting similar effects of experimental site and hereby justifying the choice of equations. Weight-based urine recordings (A, C, D) were assumed to have density 1.00. Feed sample K concentration was analyzed by inductively coupled plasma-atomic emission spectroscopy (JY 50P, Instruments S.A., Division Jobin Yvon, Longgjumeau, France for experiments E and F; Spectro flame, SPECTRO Analytical Instruments, Kleve, Germany for the other experiments) after digestion with nitric acid.

Treatment means of daily urine volumes were regressed against K intake by simple linear regressions in procedures REG and GLM of SAS 9.1 (SAS Institute Inc., Cary, NC, USA) and by the mixed model approach outlined by St-Pierre (2001) with random intercept and slope for individual experiments under the assumption of unstructured covariance, i. e. intercept and slope have different variances but there is a covariance between them.

Table 1 Change-over experiments used for evaluating effects of K intake on cattle urinary excretion. Arranged by descending urine output.

Exp.	Animals	Treatments	Diets	DM intake, kg/d[1]	Reference
A	Lactating cows	Mixed silage with birdsfoot trefoil or white clover, Yr 1	2	19.4	Eriksson et al., 2008
B	Lactating cows	Fodder beets, potatoes and barley as concentrates	3	21.6	Eriksson et al., 2004
C	Lactating cows	Lupins or pea as protein concentrate	2	19.1	Eriksson, 2010
D	Lactating cows	Mixed silage with birdsfoot trefoil or white clover, Yr 2	2	20.2	Eriksson et al., 2008
E	Lactating cows	Forage as hay or silage, comprising 0.3 or 0.5 of total ration DM	4	17.3	Gustafson, 2001
F	Steers	Silage proportion 0.15-1.00 of total ration DM	4	8.9	Gustafson and Olsson, 2004
G	Dry cows	Forage harvested from semi-natural pastures	3	6.8	Pelve, 2010
H	Heifers	Forage harvested from semi-natural pastures	3	5.6	Pelve, 2010

[1]Experimental mean, largest relative treatment mean range within experiment (highest divided by lowest) 1.07

Results and Discussion

Simple linear regression and the mixed model yielded slopes of 0.056 and 0.053 L urine/g K intake, respectively, with significant intecepts in both cases (Fig. 1). For both models, K intake alone explained >0.95 of the variation in urine volume on this limited data set. The mixed model approach is intended to give a more correct description of the true relationship between variables than a simple linear regression across experiments, where the grouping of results within experiment, at least theoretically, could result in a regression line with opposite sign compared to the outcome of any of the individual experiments (St-Pierre, 2001). A visual inspection of the simple regression graph suggests a positive slope also within each experiment for the actual data, with curvilinear appearance in experiments E and F. Fig. 1 also suggests that the treatment differences in experiment E (Gustafson, 2001) were not caused by the conservation method but rather by differences in K intake.

The adjustments of data that the mixed model created (Fig. 1,) by allowing a random intercept and slope within experiment, was in most cases a level shift that centered the

observations of each experiment around the common regression line. The different levels could have been due to true biological differences between experiments as well as to the methods for measuring urine volume. All experiments were performed at the same research facility over a period of about 10 years including some variations in the methodology. Experiments B and F involved total collection and constant monitoring of the animals; experiments A, C, D and E used total collection but less extensive monitoring and in experiments G and H spot sampling and estimation of urine volume from creatinine concentration were used. The possible error associated with total collection is underestimation of urine volume as a result of collection losses, whereas volume estimation from spot sampling could result in both over- and underestimation.

The slopes of 0.056 and 0.053 L urine/g K in Fig.1 are close to the slope 0.058 that Bannink et al. (1999) reported for K in a bivariate model, where intakes of Na and K explained urine volume. If K was the sole regulator of urine volume with a strictly linear relationship, then the inverse of the slope should simply correspond to urinary K concentration after correcting for intake K not allocated to urine. The slope 0.053 L urine/g K intake would then equal a urinary K concentration of 17.7 g/L if K digestibility coefficients from Bannink et al. (1999) are applied. However, urinary K concentration is not a constant, as other elements and compounds also have to be cleared from the blood, which affects urine volume and the dilution of urinary K at low K intakes. Gustafson (2001) reported urinary K concentrations of 7-18 g/L for individual cows, with a mean of 12 g/L. Kume et al. (2008) found an exponential relationship between urinary K concentration and urinary K excretion, where K concentration increased rapidly from 5 to 13 g/L, with increased urinary K excretion and then leveled off. With such a relationship, K intake would regulate urine volume linearly whenever K intake is sufficient to approach the asymptotic urinary concentration of 13 g K/L.

However, at low K intakes, it is reasonable that both Na and N intakes (Bannink et al., 1999; Nennich et al., 2006; Kume et al., 2008; Weiss et al., 2009) would be the driving forces for urine volume. The slope for urinary volume against K intake would in that case be smaller than when the K intake is so high that the asymptotic value of urinary K concentration is reached. Measurements on urinary K concentration were only avaliable for experiments E and F in the actual dataset. These values together with estimates from K intake and assumed allocation to faeces and milk (Bannink et al., 1999) for the rest of the dataset suggests urinary K concentrations relatively close to the asymptote (11.8 g/L; SD = 1.2) for most of the treatment means. Exceptions are experiment F, where incremental silage proportion to steers increased urinary K concentration from 7 to 11 g/L and the diet lowest in K of experiment G and H, respectively, which both gave estimates of 8 g K/L urine.

The actual dataset displayed treatment means of urinary excretion for lactating cows ranging from 13 to 30 L/d, with DM intakes of 17-22 kg/d. In the current analyses of Swedish farm samples from grass-clover forages collected during 2010 (Personal communication M. Åkerlind, Swedish Dairy Association), mean K concentration were 20.5 g/kg DM, with a standard deviation of 4.7 g/kg DM. Inserting these values into the regression equation from the mixed model in Fig. 1 to a ration with 12 kg DM grass-clover forage and 8 kg DM concentrates (assumed concentrate K concentration 10 g/kg DM (Gustafson, 2001) yields a mean estimate of 20 L urine/d, with 14 L/d for the 5% percentile and 26 L/d for the 95% percentile.

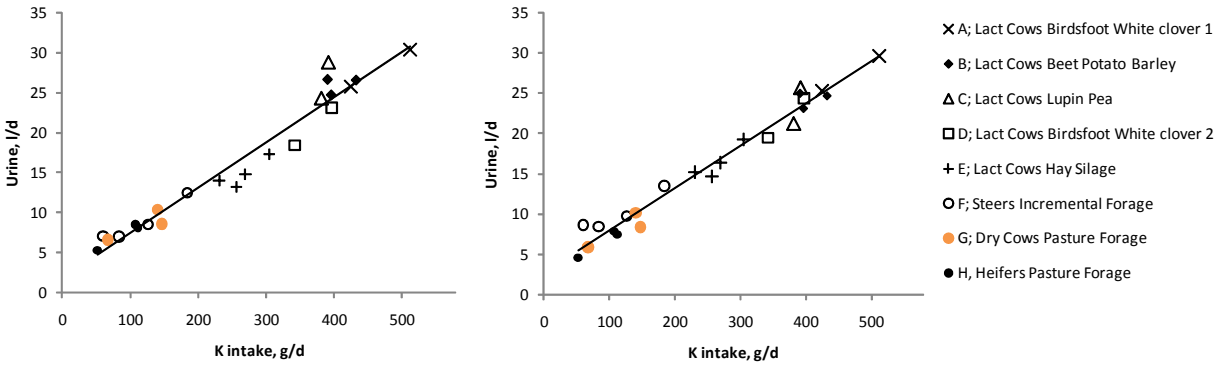

Figure 1 Plots of urine excretion (L/d) vs K intake for experiments A-H. Left: simple linear regression (y = 1.9 + 0.056 x; R^2 = 0.956; RMSE = 1.8). Right: Adjusted y:s from mixed model with random intercept and slope for individual experiments (y = 2.7 + 0.053 x; R^2 = 0.974; RMSE = 1.3)

Conclusion

The limited data analyzed here suggests that urinary volume is mainly regulated by K intake in Swedish Red cattle fed rations based on grass-legume forages. The results also suggest that urine volume on a group level may be predicted from K intake. The range of K concentrations in farm samples of grass-clover forage corresponds to differences of urine volume by a factor 1.9 for dairy rations.

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Prediction of rumen forage and concentrate residence times using data from marker excretion studies

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Introduction

Mechanistic or semi-mechanistic feed evaluation models need forage and concentrate residence times to estimate digestibility of potentially digestible neutral detergent fibre, starch and protein fractions in the rumen of cattle. Passage rates have most often been measured using rare earths or Cr-mordanted fibre as markers and estimated from the descending phase of the marker excretion curves. The prediction equations of passage rate currently used in calculations of ruminal digestibility of carbohydrate and protein fractions are influenced by differences between markers, marker preparations, kinetic models and sampling site. In addition, there is variation in passage rate due to animal and dietary characteristics. The objective of this study was to generate biologically valid predictions of forage and concentrate rumen residence time primarily using marker kinetics data from the literature.

Materials and Methods

A database was constructed from experiments with cattle where the experimental objective was to study dietary effects on passage kinetics of different feeds from marker excretion curves. A total of 68 studies comprising 370 dietary treatment means were added to the database. Forty one (n=240) of these were conducted with dairy cows and 27 (n=130) with growing cattle. Diets consisted of forages; fresh or grazed grass, grass hay or silage, legumes, whole crops including maize, and mixtures of lucerne and maize silage. Diets were fed either with or without concentrate supplements at different levels of feeding. Feeds were either labeled with Yb or other rare earths (n=277), or mordanted with Cr (n=93). Ytterbium and Cr were used as digesta kinetic markers for the same feed in four studies comprising 31 treatment means. When rare earths and Cr were applied to different feeds in the same study, they were counted as different treatment means. Rare earths were applied to the feed either by soaking the material in marker solution followed by rinsing to remove unbound or loosely bound marker, or simply sprayed on the feed. Mordanting of fiber was conducted according to Udén et al. (1980). Marked feed were fed in separate meals, but most often directly dosed in the rumen when cannulated animals were used in the experiment. One hundred five and 312 treatment means estimated retention time of concentrates and forages, respectively. Retention times of both forage and concentrate were estimated with the same marker within experiment in 11 studies comprising 60 pairwise comparisons. Rumen content, mixed feed or total mixed rations that were applied with marker were classified as concentrate when diet proportion of concentrate in the diet was larger than 0.5, else as forages. Pairwise comparison of retention times estimated from duodenal and fecal marker excretion curves of the same labeled or mordanted feed within study was made from 13 studies comprising 56 comparisons; with 40 and 16 between forages and concentrate feeds, respectively.

The prerequisite for an experiment to be included in the analysis was that compartmental mean retention time (CMRT) or TMRT (CMRT + transit time (TT)) was estimated from

either duodenal or fecal marker excretion curves. In the experiments included in this database, CMRT and TMRT were calculated from one- or two-compartmental models without or with different degrees of time-dependency. Information of model/models used in each study and degree of time-dependency behind all or individual treatment means within study were not available for the complete database. Therefore, it was not possible to examine the need for a statistical correction of the effect of kinetic model on retention time. A further prerequisite for inclusion of an experiment was that production parameters (forage and total dry matter intake (DMI), and body weight (BW)) and forage, concentrate and diet concentration of neutral detergent fiber (NDF) was determined or estimated. If concentrate chemical composition was not completely reported, default feed table values from National Research Council (NRC; 2001) were used. When forage NDF concentration was not reported the value was estimated from diet and concentrate NDF concentrations, and proportion of concentrate in diet dry matter (DM).

The relationship between CMRT estimated from duodenal sampling ($CMRT_{Duo}$) and CMRT or TMRT estimated from fecal sampling ($CMRT_{Fec}$ or $TMRT_{Fec}$) were analyzed using the mixed model procedure of SAS. The model was $Y = B_0 + B_1X_{1ij} + b_0 + b_1X_{1ij} + B_2X_{2ij} + \ldots + B_nX_{nij} + e_{ij}$, where B_0, B_1X_{1ij}, B_2X_{2ij} . . . X_{nij} are the fixed effects and b_0, b_1, and e_{ij} are the random experiment effects (intercept, slope and error), where i = 1 . . . n studies and j = 1 . . . n_i values. The effect of marker (Yb *vs*. Cr) was estimated using the sub data set comprising 4 studies. Concentrate CMRT was also related to forage CMRT from univariate mixed model regression of a data sub set. The relationships between TT and TMRT were also examined for data collected from duodenal and fecal sampling. In the univariate regressions either the intercept or both intercept and slope were treated as random factors.

Multivariate mixed model regression was further used to analyze the relationship between $CMRT_{Duo}$ and independent variables/extrinsic animal and diet characteristics using all treatment means (n=370) in the database. The best-fit model was chosen based on the lowest root mean square error (RMSE). Intercept and slope were both treated as random factors in the regressions.

Results and Discussion

Mean and ranges of experimental animal and diet characteristics are given in Table 1.

Proportions of $CMRT_{Duo}$ and $CMRT_{Fec}$ to TMRT from fecal sampling were 72 and 79%, respectively. These proportions were consistent with ranges of the proportions of the retention time in reticulorumen and forestomachs (including the omasum) of TMRT of 74-75% and 78-85%, respectively, determined in 3 slaughter studies (Huhtanen and Ahvenjärvi, 2008). Relationships between $CMRT_{Duo}$ and $CMRT_{Fec}$, and $CMRT_{Duo}$ and $TMRT_{Fec}$ are given in Eq 1 and 2. Intercept and slope were not adjusted for feed as class variable (forage or concentrate) since the fit of the model was not improved (i.e. smaller RMSE) compared to the common relationships.

$CMRT_{Duo}$ (h) = -0.413 + 0.936 × $CMRT_{Fec}$ (h) (RMSE=1.82 h) [1]

$CMRT_{Duo}$ (h) = -7.48 + 0.883 × $TMRT_{Fec}$ (h) (RMSE=1.99 h) [2]

Table 1 Mean and range of experimental animal and diet characteristics.

	n	Mean	SD	Minimum	Maximum
Intake (g/kg of BW)					
Dry matter (DM)	370	26.7	7.80	8.4	42.2
Neutral detergent fiber	370	10.8	3.27	2.4	21.5
Concentrate proportion (DM basis)	370	0.35	0.227	0	0.96
Milk production (kg)	227	22	11.9	0	46
Body weight (kg)	370	528	156.3	115	747
Diet composition (g/kg of DM)					
Organic matter	255	913	27.2	812	953
Neutral detergent fiber	370	423	124.7	112	721
Crude protein	355	149	43.1	19	306
Compartmental mean retention time (h)					
Forage	262	40.3	9.14	21.6	70.1
Concentrate	88	26.8	7.10	15.7	49.6
Total mean retention time (h)					
Forage	249	52.2	10.90	31.0	96.2
Concentrate	82	37.6	9.19	24.6	66.1

The univariate regression of CMRT estimated from Cr-mordanted feed particles ($CMRT_{Cr}$) on CMRT derived from excretion curves of feed labelled with Yb ($CMRT_{Yb}$) was (Eq. 3):

$CMRT_{Cr}$ (h) = -4.20 + 1.29 × $CMRT_{Yb}$ (h) (RMSE=6.22 h) [3]

The relationship between concentrate and forage CMRT estimated from a data sub set is presented in Eq. 4. The intercept in Eq. 4 was not different from zero (P=0.59).

CMRT concentrate (h) = 2.17 + 0.59 × CMRT forage (h) (RMSE=2.43h) [4]

The relationships between TT and TMRT derived from models of duodenal (Eq. 5) respectively fecal (Eq. 6) marker excretion curves of the sub data set were:

TT_{Duo} = 2.24 + 0.01 × $TMRT_{Duo}$ (RMSE=0.83 h) [5]

TT_{Fec} = 7.03 + 0.07 × $TMRT_{Fec}$ (RMSE=1.64 h) [6]

Intercept and slope were not significant (P≥0.09) in Eq. 5, but both were significant in Eq. 6 (P≤0.03).

A correlation between TT and TMRT could indicate that the different mathematical models influence the estimate of TT. However, the positive correlation in Eq. 6 indicates increased transit time with increased TMRT, which is consistent with reduced transit time with increased intake (Huhtanen and Kukkonen, 1995).

The effect of diet and animal characteristics on $CMRT_{Duo}$ in univariate and multivariate regressions estimated from mixed model regression analysis is presented separately for forages and concentrates in Table 2 and 3. Type of marker (rare earths or Cr) was significant as class variable in equations for forages ($P<0.001$) and gave best fit of the models as an adjustment of the intercept. Either intercept or slope was non-significant ($P\geq0.10$) when marker type was used as an adjustment of both. Marker was not significant ($P\geq0.61$) as class variable in mixed model regression analysis of $CMRT_{Duo}$ of concentrates. The bivariate model including DM intake and concentrate proportion on DM basis gave lowest RMSE in regressions of $CMRT_{Duo}$ of both forages and concentrates (Table 2 and 3).

Table 2 Effect of diet and animal characteristics on pre-duodenal compartmental retention time of forages estimated by mixed model regression analysis ($Y = A + BX_1 + CX_2 + DX_3$)[a].

Marker[b]	X_1	X_2	A[c]	B	P-value	C	P-value	RMSE
1	DMI		57.5	-0.599	<0.001			3.24
2	DMI		51.9	-0.599	<0.001			3.24
1	NDFI		50.5	-0.872	<0.001			3.27
2	NDFI		45.9	-0.872	<0.001			3.27
1	DMI	CProp	57.0	-0.719	<0.001	11.6	<0.001	3.07
2	DMI	CProp	51.7	-0.719	<0.001	11.6	<0.001	3.07

[a]DMI=dry matter intake (g/kg of body weight(BW); NDFI=intake of neutral detergent fiber (NDF; g/kg of BW); CProp=concentrate proportion on DM basis.[b]Marker 1=Cr; Marker 2=Yb and other rare earths. [c]A: P<0.001.

Table 3 Effect of diet and animal characteristics on pre-duodenal compartmental retention time of concentrates estimated by mixed model regression analysis ($Y = A + BX_1 + CX_2 + DX_3$)[a].

X_1	X_2	A[b]	B	P-value	C	P-value	RMSE
DMI		34.9	-0.374	<0.001			2.18
NDFI		33.4	-0.950	<0.01			1.96
DMI	CProp	26.4	-0.290	0.02	12.4	<0.001	1.90

[a]DMI=dry matter intake (g/kg of body weight(BW); NDFI=intake of neutral detergent fiber (NDF; g/kg of BW); CProp=concentrate proportion on DM basis.[b]A: P<0.001.

The best univariate mixed regression equation generated by Krizsan et al. (2010) from a meta-analysis of passage rate (k_p) estimated by rumen evacuation with cattle (based on NDF intake) was used to predict pre-duodenal CMRT of both forages and concentrates. Predicted CMRT ($CMRT_{Pred}$) was estimated as the inverse of k_p. Average $CMRT_{Pred}$ was 39.2 and gave a comparable estimate to the average $CMRT_{Duo}$ of 38.7 from this data base. Regression of $CMRT_{Duo}$ on $CMRT_{Pred}$ with mixed model regression is given in Eq. 7. The intercept of Eq. 7 was not significantly different from zero (P=0.61).

$$CMRT_{Duo} = -3.33 + 1.10 \times CMRT_{Pred} \quad \text{(RMSE=6.31 h)} \quad [7]$$

Conclusions

There was a close relationship between retention time estimated from duodenal or fecal sampling and the data indicated that proportionally about 90% was pre-duodenal. Correcting estimates of retention time derived from duodenal and fecal marker excretion curves for sampling site and marker type gave estimates of pre-duodenal compartmental retention time that were comparable with predictions derived from an equation developed based on rumen evacuation data. Pre-duodenal retention time was longer when estimated with Cr-mordanted feeds compared with rare earths. Further, bivariate prediction equations based on DM intake performed slightly better than univariate prediction equations based on intake of NDF for both forages and concentrates. Increases in DM intake predicted decreased pre-duodenal compartmental retention time, which was counteracted with an increased proportion of concentrate in diet DM.

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Development of *in vitro* method for determination of methane production kinetics

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Introduction

Methane is a potent greenhouse gas with strong infrared absorbance contributing about 20% to anthropogenic "radiative forcing" and is responsible for one third of all global warming over the last 250 years (Thorpe, 2009). Methane production from the agriculture sector in European countries has been estimated to be mostly produced by enteric fermentation (two-thirds, 80 million tons per year) while one-third originates from livestock manure (Moss *et al.,* 2000). Methane is an end-product of microbial fermentation of carbohydrates in the digestive tract of ruminant animals along with short chain fatty acids such as acetate, propionate and butyrate as well as CO_2. There are several methods for measuring methane production from fermentation of feedstuffs in the rumen fluid. *In vivo* techniques are laborious, expensive and difficult to standardize (Cone *et al.,* 1996). *In vitro* cumulative gas production technique has been used to predict fermentation of ruminant feedstuffs. The feedstuff incubated will be degraded to either gas, microbial mass or volatile fatty acids (Rymer *et al.,* 2005).

Therefore, the objective of this experiment was to determine the suitable sample size for *in vitro* studies, and to estimate the kinetic parameters of CH_4 production at different substrate levels.

Materials and Methods

Five replicate samples of 300, 600, 900 and 1200 mg of low quality timothy hay were weighed into serum bottles that were filled with 60 ml buffered rumen fluid and incubated at 39°C. The gas production was conducted by a fully automated system (Cone *et al*., 1996); recordings were done every 12 min and corrected to the normal air pressure (1013.5 kPa). Gas samples were drawn from each bottle by a gas tight syringe (Hamilton, Bonaduz, Switzerland) at 4, 8, 24, 32 and 48 hr through the Suba-Seal rubber septa. Methane was determined by injecting 0.2 ml of gas into a Star 3400 (CX series) gas chromatograph (Varian Chromatography, USA) equipped with a thermal conductivity detector (TCD). One ml was collected for VFA determination from each bottle at 24 and 48 hr of incubation. After 48 hr of incubation, the bottles were put on ice in order to terminate the fermentation. Methane data (concentrations at each time point) was subjected to a logarithmic model to estimate methane concentrations at each time point (Figure 1). Methane and total gas data were fitted to a two-pool Gompertz model to estimate kinetic parameters. Gas volumes in each pool were calculated per g dry matter (DM). Methane production was predicted according to VFA stoichiometry equations, the equation used was as below:

$$\text{Predicted } CH_4 = 22.3 \times (0.5AA - 0.25PA + 0.5BA + 0 \times iV - 0.25V)$$

AA, PA, BA, iVA and VA are the production of acetate, propionate, butyrate, iso-valerate and valerate (mmol) corrected for blanks.

The kinetic parameters were then subjected to a mechanistic rumen model described by Huhtanen et al. (2008) to estimate first order production rate and total production of methane. Data for VFA were subjected to the GLM procedure of SAS using the following model:

$Y_{ij} = SL_i + e_{ij}$, where SL_i is substrate level (i = 4) and e_{ij} is random error term. The sum of squares of SL effect was further partitioned into linear, quadratic and cubic effects of the substrate level using orthogonal polynomial contrasts.

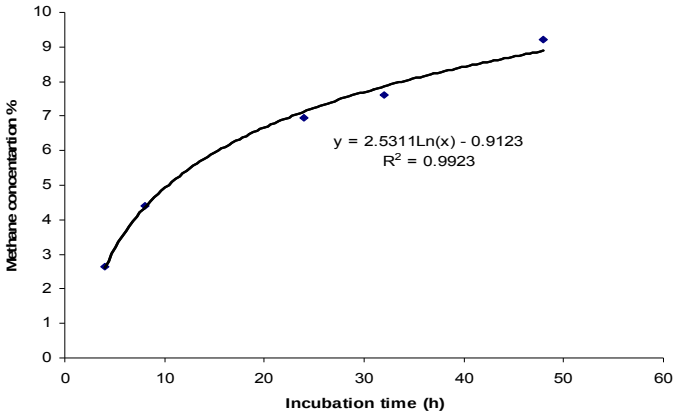

Figure 1 Logaritmic model used for methane calculations

Results and Discussion

The composition of the hay was (g/kg): DM 956, (kg DM): NDF 570 and ash 35.4. The results showed that methane production per gram of DM sample decreased as the sample size increased from 300 to 1200 mg, the reduction in methane production could be explained as the change in partioning carbon between VFA and microbial cells (Table 1). Assuming a gross energy (GE) concentration of 18.5 MJ/kg DM, the predicted CH_4 production varied from 8.0% (300 mg) to 6.1% (1200 mg) of GE. These values are close to observed *in vivo* values at the maintenance (300) level of intake in sheep and production (1200) levels of intake in dairy cows (Yan *et al*., 2000)

Table 1 The effects of increasing sample size on methane and total gas production (ml/g DM) after 24 and 48hr of incubation

		Substrate level (mg)					Contrast[a]	
		300	600	900	1200	SEM[b]	L	Q
Methane								
	Asymptotic CH_4	45.6	40.9	39.7	36.3	0.98	<0.01	0.48
	Rate (/h)	0.052	0.046	0.046	0.045	0.001	0.001	0.10
	Predicted CH_4	36.9	32.0	31.1	28.2	0.74	<0.01	0.20
Total gas								
	Asymptotic gas	241	223	223	212	4.26	0.0002	0.42
	Rate (/h)	0.072	0.061	0.061	0.059	0.002	0.002	0.10
	Predicted gas	209	187	188	177	3.31	<0.01	0.11

[a] L=linear effect of substrate level, Q=quadratic effect of substrate level
[b] For 600 and 900 mg substrate level SEM, is 1.1 times higher (at 24 hr)
For 300 and 600 mg substrate level SEM, is 1.1 times higher (at 48 hr)

The first-order production rate was constant except for the 300 level substrate size (Table 2). Total volatile fatty acid production (VFA) decreased with increasing sample size (Table 2). This suggests that more carbon was partitioned towards microbial cells with increased

substrate level, since the changes in true OM digestibility were minimal. Molar proportions of acetate decreased and the propionate increased as the sample size increased from 300 to 1200 mg (Table 2).

Table 2 The effect of sample size on VFA production and molar proportions after 24 and 48hr of incubation

	Substrate level (mg)					Contrast[a]	
Time (h)	300	600	900	1200	SEM[b]	L	Q
24							
Total VFA (mmol/g DM)	2.86	2.89	2.72	2.53	0.11	0.03	0.36
Molar proportion (mmol/mol)							
Acetate	636	614	591	584	7.72	0.00	0.35
Propionate	273	267	281	276	5.38	0.37	0.94
Butyrate	96	98.4	99.7	108	3.39	0.02	0.35
i-valerate	0	0	0	0	2.81	0.69	0.32
Valerate	0	28.6	30.7	33.5	9.43	0.01	0.16
48							
Total VFA (mmol/g DM)	4.41	4.25	3.96	3.82	0.11	0.00	0.96
Molar proportion (mmol/mol)							
Acetate	620	612	604	590	2.41	<0.001	0.26
Propionate	248	258	262	266	3.12	0.00	0.34
Butyrate	90.6	93.0	95.2	103	1.48	<0.001	0.09
i-valerate	5.13	8.35	6.18	4.37	1.78	0.60	0.20
Valerate	35.4	27.2	31.5	35.1	2.65	0.78	0.05

[a] L=linear effect of substrate level, Q=quadratic effect of substrate level
[b] At 24 hr SEM is 1.1 times higher for 600 and 900 mg substrate level
At 48 hr SEM is 1.1 times higher for 300 and 600 mg substrate level

Production of CH_4 was also calculated stoichiometrically, using VFA production at 24 and 48 h. There was a close relationship (R^2=0.98) between predicted and observed values (Figure 2) with 0.0 intercept and a slope close to 1.0, indicating small mean and linear biases.

Conclusion

It is concluded that increasing sample size did not change the kinetic rate of methane production and it is possible to use a substrate level of 1000 mg in the present system. This is of importance in order to decrease the depletion of substrate in the *in vitro* gas system. A high correlation was achieved between methane measured and methane predicted stoichiometrically. Realistic values of predicted CH_4 production suggest that automated *in vitro* gas production system combined with modeling approaches is a useful tool for evaluating the effects of diet composition and feed additives on CH_4 production.

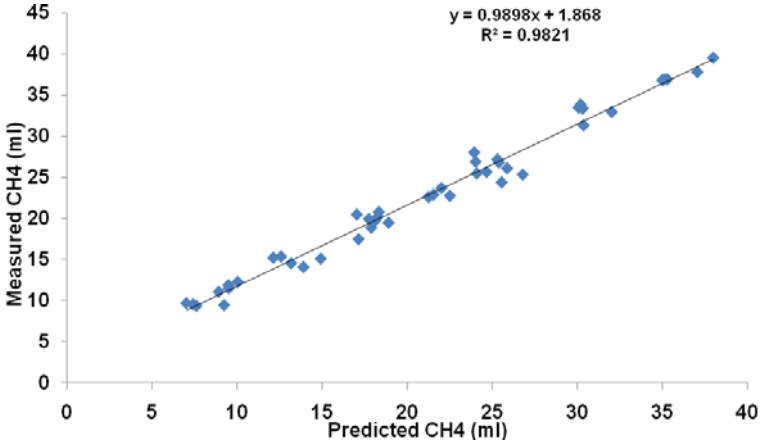

Figure 2 Predicted *vs*. Observed values of methane production (ml) for the *in vitro* measurements.

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In vitro estimation of rate and extent of ruminal digestion of cereal feed fractions

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Introduction

Cereal grains are important components in diets for high producing dairy cows and fast growing beef cattle. The most important feed fraction in cereals is starch, which constitutes the major portion (70-80%) of cereal grains. In ruminant nutrition different starch sources are characterized by their physical and chemical properties and might be ranked as highly and poorly degrading starch after their extent and rate of degradation in rumen (Nocek and Tamminga, 1991). It is hypothesized that rumen fermentation rate of cereal starch varies considerably and that this might affect fibre digestibility, forage intake, and animal performance (Nocek and Tamminga, 1991; Mills et al., 1999). It would, therefore, be useful to reliably determine the differences in rumen fermentation characteristics of cereals. Part of the variation can be attributed to the methods used for evaluation (Stensig et al., 1998b; Huhtanen and Sveinbjörnsson, 2006). The in sacco technique has been used extensively in the recent past (Stensig et al., 1998a; Larsen et al., 2009) and is still an important analytical method in modern feed evaluation systems (Volden et al., 2011) even though the method has several limitations. Particle loss and microbial contaminations during incubations are associated with this technique, consequentially, leading to over- or under-predictions (Huhtanen and Sveinbjörnsson, 2006). The *in vitro* gas production (GP) technique, on the other hand, has some advantages over the in sacco method. The GP technique is characterized with negligible primary and secondary particle-losses (Huhtanen and Sveinbjörnsson, 2006), minimizing the risks of over- or under-predictions. In addition, it can be automated to generate and record a large number of data points and can be potentially used to study the fermentation characteristics of soluble and insoluble fractions of forages (Hetta et al., 2004).

The objective of this experiment was to study the rumen degradation characteristics of fibre and non fibre fractions of a range of cereals by use of *in vitro* gas production technique.

Materials and Methods

Description of feeds and isolation of fibre

This study included nine cereal feeds with a range in starch concentration from 389 (rolled oats) to 712 g starch/kg DM (ground maize) with known *in vivo* ruminal degradation characteristics. The cereals came from two different experiments conducted at Research Centre Foulum, University of Aarhus, Denmark (Exp. 1) and the Forage research centre, Swedish University of Agricultural Sciences, Umeå, Sweden (Exp. 2) as shown in Table 1.

Feed samples were dried at 60^{o}C and milled through 2-mm screen (cutter mill; SM 2000, Retsch GmbH, HAAN, Germany). The sub-samples of whole feeds (WF) were used to prepare neutral detergent fibre (NDF) fractions (NDFF) by extraction of WF with some modifications to the original method of Van Soest et al. (1991). In brief, accurately weighed 4 g feed sample were put into nylon bags (38 µm and 28% effective surface area) and boiled

in neutral detergent solution (1 g feed in 100 mL neutral detergent solution) for one hour at 95°C with double amounts of heat stable α-amylase. The heat stable α-amylase was first added at the start of boiling (2 mL/L of neutral detergent solution) and then at first hot water rinsing (1 mL/L hot water). After boiling, the bags were washed with hot water (70°C) to remove all neutral detergent solution. The bag residues were then transferred to glass filter crucibles and rinsed three times with acetone (30-50 mL/bag residue per rinsing). The residues were dried at 105°C for 16 h and weighed to determine NDF concentration. The WF and NDFF were also double-checked for NDF concentration according to the method as described by (Chai and Uden, 1998).

Table 1 Chemical compositions and apparent ruminal starch digestibilities of feeds.

	Experiment 1[a]							Experiment 2[b]	
Feed type	Barley R	Barley RG	Maize G	Oats R	Smooth peas G	Wheat R	Wheat RG	Barley 371	Maize 371
g/kg DM									
Ash	19	20	17	20	29	16	18	57	55
CP	101	114	96	111	221	108	119	114	123
NDF	170	158	98	271	92	127	92	155	183
Starch	651	562	712	389	429	608	598	514	491
Digestibility[c]	882	881	746	857	654	882	897	-	-

[a] From Larsen et al. (2009).
[b] Data obtained from the experiment conducted at Forage Research Centre, Swedish University of Agricultural Sciences, Sweden.
[c] *In vivo* apparent ruminal starch digestibilities reported as g/kg starch intake, from Larsen et al. (2009).
R=rolled, RG=rolled and gelatinized, G=ground, 371=pelletized compound feed.

In vitro GP recordings

In vitro degradation characteristics of OM and NDF for WF and NDFF were determined by use of an automated gas production (GP) and recording system (Cone et al. 1996). The analyses were carried out using 0.5 g samples (2-mm screen) in duplicate in two consecutive series of runs (four replicates in total for each WF and its respective NDFF). The samples were incubated in 250-ml serum bottles (Schott, Mainz, Germany), already flushed with CO_2, containing 60 mL buffered rumen fluid for 72 h at 39°C. The bottles were placed in a water bath and shaken continuously (40 rpm). The bottles were connected to an automated recording and measuring system as described by Cone et al. (1996). After 72 h, the incubations were terminated by putting the bottles on ice. The residues remaining after 72 h fermentation were filtered in glass filter crucibles and analysed for NDF to determine NDF digestibility. True OM digestibility (true OMD) was calculated as ((OM-NDF residue)/OM). Ruminal neutral detergent soluble (NDS) digestibility was calculated using gas production rates for NDS fraction and a two-compartment rumen model (0.20 non-escapable pool: 0.80 escapable pool) assuming 24 h retention time in the rumen.

Rumen fluid was collected from two rumen cannulated lactating dairy cows fed a forage-based diet supplemented with whole crop barley silage. The fluid was collected 2 h post-feeding in the morning and transported from dairy barn to the gas *in vitro* laboratory into CO_2 flushed insulated flasks. The fluid, after collection, was handled according to the instructions as described by Cone et al. (1996). In brief, the fluid was filtered through a double layer of

cheese cloth and mixed with buffered mineral solution at a volume ratio of 1 mineral: 2 rumen fluid, and was kept at 39^{o}C under constant CO_2 flushing.

The DM and ash contents of the WF and NDFF were determined at 60^{o}C for 48 h and at 525^{o}C for 6 h (method 923.03; AOAC, 1990), respectively.

Curve fitting and model

The recorded gas volumes were adjusted to the normal air pressure. The corrected GP data were averaged for all four replicates for each WF, its respective NDF and NDS fractions. The GP profile for the NDS fraction was estimated by subtracting the GP of the NDFF from the GP of the intact feeds (cereal). The GP from the NDFF was adjusted in volume at each recording by its fractional contribution on OM basis. The GP profiles (ml gas/g OM) were fitted to a first order kinetic model without intercept:

$GP^t=A\ (1-\exp(^{-bt}))$

Table Curve 2D (ver. 5.0® SPSS INC.) was used for fitting the model, where GP^t=gas production at time t, A=asymptotic commulative gas volume, and b^t=fractional gas production rate.

The mean values for digestibilities, cumulative gas volumes and fitted degradation parameters for each feed, its respective NDF and NDS fractions were calculated by general linear model procedure of MINITAB 16.1.1.0

Results and Discussion

The mean chemical composition and apparent ruminal starch digestibilities are presented in Table1. The NDF and crude protein contents of feeds varied from 92 to 271 g/kg DM and from 96 to 221 g/kg DM, respectively.

The average values for digestibilities, cumulative gas volumes and fitted degradation parameters for each feed, its respective NDF and NDS fractions are shown in Table 2. True OM and NDF digestibilities varied among whole feeds being the highest for smooth peas (999 g/kg OM and 999 g/kg NDF, respectively) and the lowest for oats (804 g/kg OM and 363 g/kg NDF, respectively). The cumulative gas volumes for 72 h incubations and fitted gas production rates (which corresponded degradation rates) varied from 373 to 480 mL gas/g OM and from 0.092 to 0.157/h, respectively. Among all WF, only maize samples showed degradation rates less than/close to 0.10/h. The mean estimated degradation rates for barley, maize and wheat were lower than those reported by Lanzas et al. (2007). In their study, they used a discrete lag for gas production rate calculations. The degradation rates were also lower than those reported in NorFor Feed Tables (Volden et al., 2011) for grain. However, the mean degradation rates of maize in our study were in agreement with those reported by Deaville et al. (2001).

The cereal NDF digestibilities and their respective cumulative gas production rates varied from 410 to 985 g/kg NDF and from 0.033 to 0.082/h, respectively. Calculated values of ruminal NDS digestibilities and their respective cumulative gas production rates were in the range from 762 to 880 g/kg NDS and from 0.097 to 0.180/h, respectively. Ruminal NDS digestibilities reported in our study are in agreement with the *in vivo* data except for smooth peas.

Table 2 Gas production kinetics of whole feed (WF), the neutral detergent fibre (NDF) and the neutral detergent soluble (NDS) fractions. The values are presented as means of four replicates.

	Experiment 1[a]							Experiment 2[b]	
Feed type	Barley R	Barley RG	Maize G	Oats R	Smooth peas G	Wheat R	Wheat RG	Barley 371	Maize 371
WF									
True OMD	958	955	977	804	999	991	995	941	956
NDFD	780	729	787	363	999	937	945	648	780
GP	480	446	468	414	373	468	442	469	473
Fitted degradation parameters									
Asymptotic GP	468	425	464	408	356	457	426	459	465
Rates /h	0.136	0.153	0.092	0.129	0.157	0.126	0.142	0.109	0.098
NDF fraction									
NDFD	712	715	883	410	985	789	788	700	734
GP	322	345	401	238	383	417	364	338	349
Fitted degradation parameters									
Asymptotic GP	322	348	423	262	380	432	362	332	358
Rates /h	0.062	0.058	0.050	0.033	0.065	0.061	0.063	0.082	0.056
NDS fraction									
NDSD	849	880	762	855	876	840	857	788	786
GP	424	390	428	348	337	414	408	434	406
Fitted degradation parameters									
Asymptotic GP	416	372	424	352	321	404	394	459	398
Rates /h	0.149	0.180	0.097	0.154	0.176	0.141	0.151	0.109	0.108

[a] From Larsen et al. (2009).

[b] Data obtained from the experiment conducted at Forage Research Centre, Swedish University of Agricultural Sciences, Sweden.

OMD=organic matter digestibility (g/kg OM), NDFD=NDF digestibility (g/kg NDF), GP=gas production at 72 h (mL gas/g OM), Asymptotic GP=Asymptotic gas production (mL gas/g OM), NDSD=NDS digestibility was calculated using degradation rates for NDS fraction and assuming 24 h retention time using a two-compartment rumen model.

For abbreviations see Table 1

Conclusions

The results of present study indicated that the differences in the degradation characteristics of cereals, measured with the GP technique, are smaller than those which are generally assumed in the literature (e.g. NorFor Feed Tables; Volden et al., 2011). It is also obvious that predicted NDS digestibility based on GP data corresponded better to *in vivo* data than estimates reported in NorFor Feed Tables (Volden et al., 2011).

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Magnesium chloride in dry cow silage to prevent parturient hypocalcemia in dairy cows

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Introduction

The calcium homeostasis in the cow is carefully governed by hormones, with parathyroid hormone being the most important. Still, parturient clinical hypocalcemia, often manifested as milk fever, is the second most treated disease among dairy cows in Sweden (Swedish Dairy Association, 2010). During the dry period the mechanisms regulating calcium homeostasis are relatively inactive due to the low need for calcium (Ramberg *et al*., 1970). At the beginning of lactation, the cow is then momentarily unable to adequately replace the amount of calcium lost through milk, and generally all cows therefore experience a transient decrease in plasma calcium level. In response to the reduced plasma calcium levels the calcium mobilizing mechanisms are activated. In some cows, this activation is delayed, resulting in development of clinical hypocalcemia. The activity and response of the calcium mobilizing mechanisms is affected by the acid-base status of the cow. Metabolic alkalosis, which is the common state of an herbivore, blunts the responses to hypocalcemia and may increase the risk of developing milk fever (Seifi *et al*., 2004). Intake of easily absorbed anions, e.g. chloride and sulphate, shifts the cow towards metabolic acidosis according to the theory of strong ions (Stewart, 1983). The dietary cation-anion difference (**DCAD**) is a way to measure the impact of the feed on the acid-base status in the animal. Diets with low DCAD, i.e. high in anions, has been used as a means to decrease the incidence of milk fever (Rérat *et al*., 2009). However, dry cows are usually offered grass silage-based diets that are high in potassium, which increases the DCAD. High amounts of anions must then be added to decrease the DCAD, e.g. in the form of anionic salts. However, anionic salts are known to have a low acceptance and are therefore difficult to distribute separately. Mixing the salts in e.g. a total mixed ration has successfully been tested, but most dry cow diets consists mainly of roughage and total mixed rations are not used to a large extent. A sufficient intake of magnesium during the dry period is also of importance for the ability of the cow to restore calcium homeostasis at calving. Intake of magnesium chloride prior to calving would, thus, prevent parturient hypocalcemia through two mechanisms. The aim of this experiment was to produce a silage designed for dry cows with magnesium chloride added at ensiling and evaluate the effects on feed intake and periparturient calcium homeostasis in dairy cows and heifers.

Materials and Methods

Twelve cows (parity 1 to 3) and 12 pregnant heifers were used in this experiment. The heifers were included to better evaluate the palatability of the feed, because they could be assumed to be more sensitive to the magnesium chloride addition also at restricted feeding, due to their lower intake capacity. All animals were non-lactating and in their last month of pregnancy when started on treatment diets. The animals were blocked according to parity and expected calving date and two animals in each block were randomly assigned to each treatment. The treatments consisted of grass silage (control), grass silage with 7 g magnesium chloride/kg DM added at ensiling ($MgCl_2$) and grass silage with 23g magnesium oxide added at feeding (MgO). Cows and heifers on all treatments were housed individually and fed 8 kg DM of the silage and additionally 1.8 kg DM of concentrates during the last three weeks of gestation.

After calving, all cows were fed a grass and clover silage *ad libitum* and increasing amounts of concentrates. Plasma was sampled twice weekly until calving and 6, 12, and 24 h, 2, 4, and 7 days after calving. Urine was sampled by manual stimulation twice weekly until calving and 24 h, 2, 4, and 7 days after calving. The urine pH was measured immediately after sampling. Calcium was analyzed in plasma and urine using a colorimetric method with O-cresophtalein. Concentration of creatinine in urine was analyzed using Jaffees reaction with picric acid. Daily excretion of urine was estimated using a creatinine output of 29 mg/kg body weight per day (Valadares *et al*., 1999). One of the control cows was treated with intravenous calcium after calving and was excluded from the experiment. Statistical analyses of the difference among treatments were made using procedure MIXED in SAS 9.1 (SAS Institute Inc., Cary, NC, USA). The time before calving was combined into three-day periods after the cow had been on the treatment diet for one week. Treatment, day and block were included as fixed factors and the interaction between treatment and day was included if $P < 0.05$. Cow was included as a random effect and the covariance among samples taken over time within each cow was modeled with a spatial power covariance structure. The correlation between calcium concentration in the urine and urinary pH was analyzed using procedure CORR in SAS 9.1 (SAS Institute Inc., Cary, NC, USA). Time series data are presented as least squares means and the standard error of the mean in figures, whereas probabilities for treatment effects are presented in the text . Statistical significant differences were considered when $P < 0.05$.

Results and Discussion

All cows and heifers consumed the entire ration prepartum. Animals on all treatments experienced decreased plasma calcium on the first two days after calving, but there were no effects of treatment on plasma calcium ($P = 0.45$, Figure 1). This may be explained by the low parity of the animals in the present experiment. Cows in higher parities are more susceptible to hypocalcemia, but since this experiment contained a large fraction of heifers and most of the cows were in their second gestation, the susceptibility for hypocalcemia was probably low, and this may explain the lack of treatment effect.

Urinary pH was not different among treatments ($P = 0.12$, Figure 2). The reason for this may be that the difference in DCAD among the diets was too small, 125 meq/kg DM calculated as $(Na + K) – (Cl + S)$, and that the $MgCl_2$ diet was markedly above 0 meq/kg DM. For an efficient reduction in urinary pH, Thilsing-Hansen *et al*. (2002) suggests that the DCAD should be reduced to below 0.

Urinary excretion of calcium was higher in cows receiving the $MgCl_2$ diet compared to control cows ($P = 0.04$, Figure 3). An increased excretion of calcium in urine when diets with low DCAD are fed has been seen in other studies (Rerát *et al*., 2009) and has been suggested to be the result of an attempt to buffer the metabolic acidosis by exchange of hydrogen ions and release of calcium from bone to the extracellular fluids. The extra calcium is then excreted in the urine to maintain normocalcemia (Lehmann *et al*., 2003). In the present study, there was a negative correlation ($r = -0.26$, $P = 0.002$) between the urinary pH and the concentration of calcium in the urine, which could support the theory of increased excretion of calcium as a way to buffer when the cow was shifted towards acidosis. However, in this experiment no significant effects of treatments on urinary pH could be seen. The increased excretion of calcium in urine may indicate that the calcium was more available in cows fed the $MgCl_2$ diet and that they therefore had a higher capacity to maintain plasma calcium after calving.

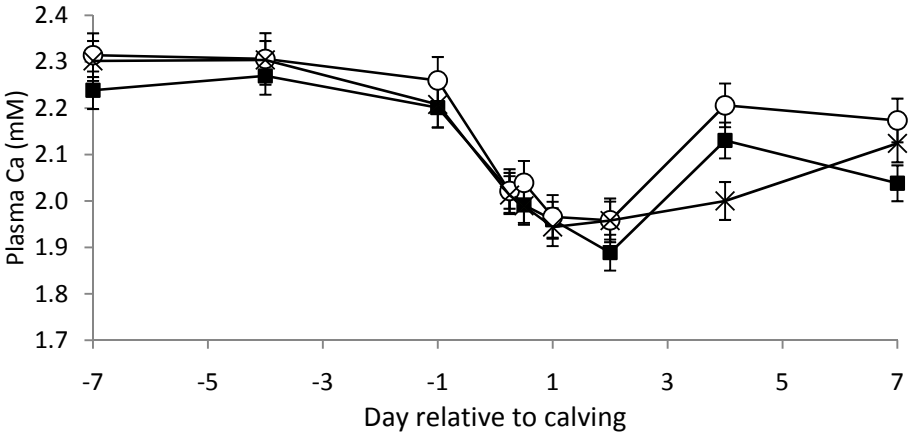

Figure 1. Plasma calcium (mM) around calving in cows fed the control diet (■), the MgO diet (○) and the $MgCl_2$ diet (×). Data are shown as least squares means ± standard error of the mean.

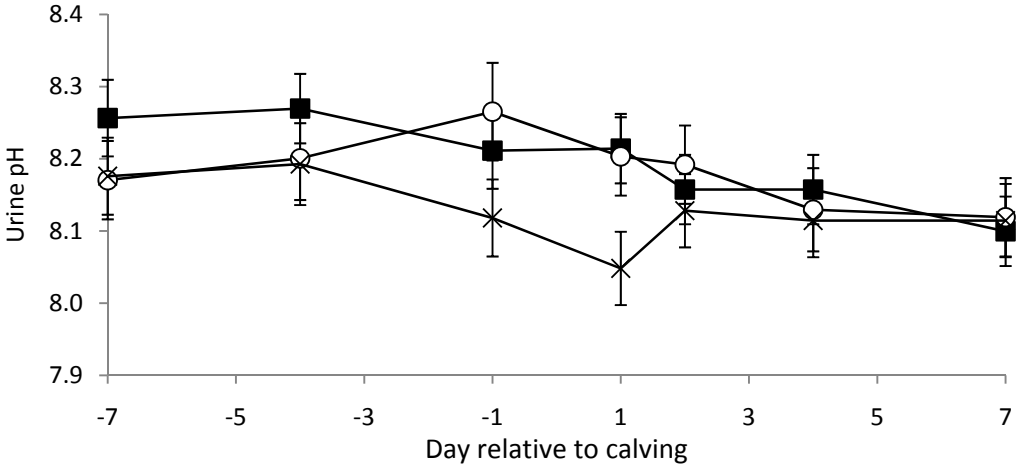

Figure 2. Urine pH around calving in cows fed the control diet (■), the MgO diet (○) and the $MgCl_2$ diet (×). Data are shown as least squares means ± standard error of the mean.

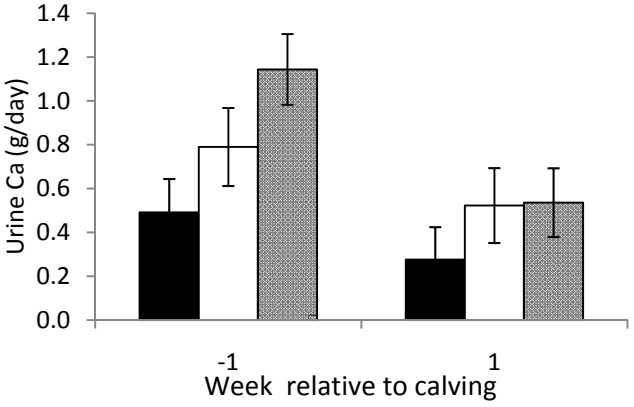

Figure 3. Urine excretion of calcium (g/day) around calving in cows fed the control diet (■), the MgO diet (□) and the $MgCl_2$ diet (■). Data are shown as least squares means ± standard error of the mean.

Conclusions

Inclusion of 7 g magnesium chloride/kg DM at ensiling had no detrimental effects on feed intake by dairy cows and heifers fed restrictedly. No effect of the added chloride ions could be seen on urinary pH or plasma calcium at calving, although the cows fed silage with magnesium chloride excreted more than twice the amount of calcium through urine compared to control cows, which may indicate that the calcium was more available in those cows, resulting from calcium release from bone tissue. This may be beneficial for cows that are in risk of developing milk fever, but more studies are needed to establish if this is the case. Addition of magnesium oxide had no effect on plasma calcium, urinary pH or calcium excretion in urine.

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Locally produced protein feeds for dairy bull calves

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Introduction

Some cases have shown that feeds grown on the own farm contributes less to environmental problems than imported feeds, for example due to less energy needed for transportation (Flysjö et al., 2008; Wallman et al., 2010). A ration with home-grown protein feeds and silage from nitrogen fixating legumes would be the best alternative for the environment when emissions from excessive fertilization are restricted. Soya bean meal is widely used in the world as a protein feed of good nutritional quality but the ethics around the use are often questioned because production of soya beans e.g. contributes to the cutting of rainforests and contamination of water. Calves need high concentrations of protein in their feed for proper growth. In addition, the protein quality must be high with sufficient amounts of rumen undegradable protein since the proteins synthesized by the rumen microorganisms will not be enough (Phillips, 2010). The purpose of this study was to compare dry matter intake (DMI), live weight gain (LWG), feed efficiency (FE), rumen function and profitability in calves fed protein feeds produced in Sweden vs. soya bean meal.

Materials and Methods

The experiment was carried out at Götala Beef and Sheep Research Station, Swedish University of Agricultural Sciences (SLU), Skara. Dairy bull calves (Swedish Red and Swedish Holstein) were used in a completely randomized design. The protein feeds studied were rolled peas (P) and rolled field beans (FB) in year 1, Swedish grown rolled soya beans (SSB) and dried distiller's grains with solubles (DDGS) in year 2, which were compared to imported soya bean meal (SBM) both years. The DMI, FE and faecal traits were recorded at a pen level while LWG was recorded on the individual bulls. In both years, there were seven animals in each pen and four pens per treatment. The calves averaged 90 and 93 kg in live weight at the start of year 1 and 2, respectively, whereas at the end of the periods, the calves had reached an average weight of 245 and 271 kg. Calves were weighed once every 14 days during the experimental period and average daily LWG of the calves was calculated.

Total mixed rations (TMR) consisting of a grass/clover silage, rolled barley and vitaminised minerals, together with either P (0.57), FB (0.37), SSB (0.44), DDGS (0.63) or SBM (0.28), was daily fed to the calves (mean kg DM, respectively). The silage consisted of approximately 90% grass and 10% clover. The P, FB and SBM were purchased from Lantmännen Feeds, Sweden. The DDGS was manufactured and sponsored by Agroetanol (Lantmännen AB, Sweden) and the SSB was grown in south of Sweden. Feed was offered *ad libitum* and feeding was done once a day. The diets were balanced to be isonitrogenic and isoenergetic. Diets were rebalanced four times according to changed nutrient requirements during growth (Spörndly et al., 2003). To fulfill the recommended protein requirements of calves given P, FB, DDGS and SSB, cold-pressed rapeseed cake (CRC; 0.23 and 0.13 kg DM year 1 and 2, respectively) was included in the diet until the calves reached an average live weight of 175 kg. Nutrient composition in DM of the silage (10.9 MJ ME, 156 g CP, 497 g NDF in year 1 and 11.6 MJ ME, 152 g CP, 534 g NDF in year 2) and concentrates (Table 1) were analysed by conventional methods. The DDGS contained 21% ADIN of total N.

Table 1 Chemical composition *of peas (P), field beans (FB) and cold-pressed rapeseed cake in years 1 and 2 (CRC1 and CSC2), soya bean meal in years 1 and 2 (SBM1 and SBM2), dried distiller's grains with solubles (DDGS) and Swedish rolled soya bean (SSB) used in the experiment, means and SD, shown as g kg^{-1} DM if nothing else is stated.*

	Year 1		Year 2					
	P	FB	CRC1	SBM1	DDGS	SSB	CRC2	SBM2
	n=5	n=5	n=3	n=5	n=5	n=4	n=5	n=5
DM, %	89 (0.1)	87 (0.3)	89 (0.2)	87 (0.7)	90 (0.5)	82 (7)	92 (1)	87 (1)
ME, MJ kg^{-1} DM	14.0 (0.1)	13.8 (0.3)	15.1 (0,1)	14.7 (0.1)	13.7 (0.1)	15.6 (0.8)	17.4 (1.3)	14.7 (0.2)
CP	211 (11)	281 (18)	348 (5)	528 (11)	349 (5)	400 (18)	296 (28)	528 (6)
C. fat	21 (1.3)	20 (2.6)	150 (3)	24 (2)	68 (1)	158 (72)	253 (53)	31 (2)
Ash	30 (1)	37 (1)	63 (1)	65 (1)	49 (8)	54 (2)	57 (3)	62 (3)
NDF	99 (25)	126 (48)	268 (3)	126 (47)	335 (24)	138 (16)	276 (30)	115 (9)
Starch	581 (9)	498 (22)	253	163	25 (35)	63 (49)	20 (27)	38 (53)
AAT[1]	99 (1)	98 (3)	94 (1)	169 (2)	110 (12)	108 (27)	81 (6)	193 (56)
PBV[2]	52 (11)	123 (19)	205 (4)	278 (9)	173 (28)	239 (55)	174 (19)	243 (75)

[1]*Amino acids absorbed in the small intestine (Spörndly, 2003),* [2]*Protein balance in the rumen (Spörndly, 2003),* [3]*According to NORFOR feed table*

One fresh faecal sample was collected from each pen on two consecutive days at four times during each year. When collecting the faeces the consistency was determined visually on a scale from 1 – 5 where 1 means very runny and 5 dry and hard. The two daily samples from the same time and pen were pooled and their DM was determined. To determine the content of whole and partial grains and long particles (> 10 mm) in the faecal matter, the pooled samples of faeces were weighed into a sieve with a pore size of 2.36 mm. After rinsing, the number of particles > 10 mm and whole and partial grains were recorded while they were collected and put into separate dishes, which were weighed after drying.

Statistical analyses were done separately for each year. To compare the effects of the three protein feeds per year two different procedures in SAS (2003) were used. Analyses of DMI and FE were done on a pen level with the GLM procedure whereas the MIXED procedure was used for analyses of faecal traits on a pen level, with pen nested within treatment, and LWG on an individual level, with individual nested within pen. Results with a *P*-value lower than 0.05 were considered as significant and results with *P*-values between 0.05 and 0.10 were considered as tendencies to significance.

The profitability was calculated as value of calf growth less cost of feeds consumed at 2010 price level in Southern Sweden. In sensitivity analyses different prices were used. It was supposed that differences in calf weights at the end of the experiments would remain until slaughter as young bulls at 18 months of age. Thus, the value of kg calf growth was calculated as carcass price multiplied by dressing percentage. Climate impact was calculated as consumption of each feed used multiplied by emissions of carbon dioxide equivalents for producing 1 kg of respective feed (Berglund et al., 2009). Possible differences of emissions from enteric fermentation and manure were omitted.

Results and Discussion

In year 1, no differences in daily DMI or LWG were found between the diets (Table 2). There was, however, a tendency for a higher intake of ME for the calves fed peas than for the other calves, but the FE did not differ between treatments.

Table 2 Average daily intake, average *daily live weight gain and feed efficiency of bull calves fed diets containing either rolled peas (P), rolled field beans (FB) or soya bean meal (SBM) during year 1, means and standard error of the means (SEM).*

	P	FB	SBM	SEM	*P* - value[1]
Intake of dry matter (kg day-1)	4,66	4,38	4,41	0.091	NS
Intake of dry matter (% of live weight)	2.91	2.93	2.79	0.059	NS
Intake of NDF (kg day^{-1})	1.65	1.62	1.64	0.033	NS
Intake of NDF (% of live weight)	1.00	1.05	1.01	0.022	NS
Intake of metabolisable energy (MJ day^{-1})	57.1	53.2	53.3	1.11	0.054
Intake of crude protein (g day^{-1})	760	710	741	17.3	NS
Live weight gain (kg day^{1})	1.16	1.08	1.12	0.029	NS
Feed efficiency (g gain MJ^{-1} ME)	20.1	20.2	21.0	0.27	NS

[1]NS = not significant, Values that have a tendency to differ ($0.05 < P < 0.10$) have their *P*-values written in the table

In year 2, feeding DDGS resulted in the highest LWG due to higher intakes of ME, CP and also to a strong tendency for higher DM intake than calves fed SBM, but FE was similar in all treatments (Table 3). A higher DMI when feeding wheat DDGS has been observed by others (Gibb et al., 2008; Walter et al., 2010), but in those studies the DDGS was used as a replacement for barley. Also, the studies were performed on older animals fed rations containing 85% concentrates and up to 40% DDGS in the diets, which is very different from the conditions in the present experiment. The effect of DDGS on FE differs between studies. Gibb et al. (2008) observed that the FE was impaired when feeding DDGS compared to the 85% barley control diet while Walter et al. (2010) did not find any difference. However, the feed rations contained more CP, on average, in the study by Gibb et al. (2008). The diets in these two trials were not balanced to be isonitrogenic as was the case in the present experiment. Also, the nutrient content in DDGS varies due to use of grain and processing. In the present study all DDGS came from the same batch.

As calves fed DDGS had higher intakes of ME and CP, of which more was rumen degradable, than SBM calves it is possible that the rumen microbes of the DDGS calves produced more microbial protein that could be enzymatically degraded and absorbed in the small intestine. This might also be the reason for the similar LWGs between calves fed P, FB, SSB and SBM. Also, the higher intake in calves fed DDGS resulted in similar daily intakes of AAT between the SBM and DDGS fed calves (data not shown). Feeding energy and protein at the same time has been identified as a way to optimize the protein utilisation (Børsting et al., 2003). In the present study, TMR feeding was used and thus energy and protein were offered simultaneously, which improved the utilisation of rumen degradable protein for synthesis of microbial protein. The high ADIN concentration of DDGS should decrease the availability of important amino acids needed for optimizing LWGs of the calves fed the DDGS diet. However, this was apparently not the case in this study and leads us to discuss the possibilities that part of the ADIN in DDGS is digested and can be used by the calves as supported by results on sheep by Nakamuura et al. (1994).

Table 3 A*verage daily intake, average daily live weight gain and feed efficiency of bull calves fed diets containing either dried distiller's grains with solubles (DDGS), Swedish rolled soya bean (SSB) or soya bean meal (SBM) during year 2, means and standard error of the means (SEM).*

	DDGS	SSB	SBM	SEM	*P* - value[1]
Intake of dry matter (kg day^{-1})	4.81	4.50	4.45	0.098	0.058
Intake of dry matter (% of live weight)	2.72	2.61	2.69	0.036	NS
Intake of NDF (kg day^{-1})	1.78^{a}	1.57^{b}	1.66^{b}	0.035	**
Intake of NDF (% of live weight)	1.01^{a}	0,91 ^{b}	1.00^{a}	0.013	***
Intake of metabolisable energy (MJ day^{-1})	60.9^{a}	57.7^{ab}	55.5^{b}	1.24	*
Intake of crude protein (g day^{-1})	811^{a}	750^{b}	725^{b}	16.8	*
Live weight gain (kg day^{1})	1.34^{a}	1.25^{b}	1.21^{b}	0.027	**
Feed efficiency (g gain MJ^{-1} ME)	22.2	21.7	22.0	0.21	NS

[1]* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant, Values that have a tendency to differ ($0.05 < P < 0.10$) have their *P*-values written in the table, Values on the same row that are significantly different ($P < 0.05$) have different superscripts (a, b)

There were no differences in faecal traits found between treatments in year 1. In year 2, the faecal traits differed little or not at all between the treatments except for the consistency, DM content and number of grains. The firmer consistency of the faeces from calves fed DDGS (and SSB) compared to calves fed SBM can partly be explained by a higher NDF intake, at least by the DDGS calves. In this experiment, differences in consistency were accompanied by differences in faecal DM content, which has been observed before (Hessle et al., 2008). A lose or runny consistency can indicate a disturbed rumen function with too high passage rates of the feed and therefore the rather firm consistency observed in all treatments in the present experiment indicated that the rumen functioned normally. There were no differences between treatments in the number and dry weight of long particles in faeces. This indicates that all calves were fed diets that resulted in similar retention times of the digested feed in the rumen before the feed was passed through the intestinal tract. Number of grains in faeces differed between the treatments, but there is no clear explanation for this. In general, no obvious advantage or disadvantage of the treatments on faecal traits and, hence, rumen function could be observed.

Highest profitability per calf in the basic price situation had P year 1 and DDGS year 2. However, relatively small increases in cost of pea production or decreases in SBM-price would make SBM most profitable year 1. DDGS was most profitable year 2 at any probable price situation. By using P or DDGS instead of SBM, greenhouse gas emissions from production of feed consumed during the calf period would decrease by c. 30 kg carbon dioxide equivalents per calf. This corresponds to c. 0.1 kg per kg carcass weight of young bulls or nearly 1 % of the total green house gas emissions from the bulls during the rearing.

Conclusions

Swedish grown protein feeds can replace imported SBM with maintained or, regarding DDGS, even improved performance in dairy calves. All diets resulted in good rumen function and diets with peas and DDGS resulted in the best profitability and lowest emission of greenhouse gases.

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Plant development, agronomic performance and nutritive value depending on hybrid, maturity and site in forage maize grown at high latitudes.

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Introduction

Access to new maize hybrids have increased forage maize cultivation in Scandinavia. Production of good quality forage maize is uncertain at higher latitudes (55° - 60° N) in Sweden, as no maize breeding occurs in the region and the producers are dependent on the hybrids and the FAO maturity ranking provided by the breeders in continental Europe for the choice of hybrids. Forage maize grown on high latitudes may be harvested immature because of average cooler temperatures and early frost (Darby and Lauer, 2002). Feeding immature forage maize with high moisture and low starch concentration could lead to reduced feed intake and can negatively affect animal performance (Bryant et al., 1965). The concentration of starch and quality of fiber in forage maize at harvest is a major factor for describing forage quality (Jensen et al., 2005).

Maize hybrids grown at higher latitudes have been shown to require more accumulated thermal units for plant development and maturation compared to more southern locations (Smith et al., 1982). Easson et al., (2003) reported significant variations in thermal units required for developmental stages in forage maize affected by hybrid, season and geographic location.

The aim of this experiment was to study the effects of site, maturity and hybrid on plant development, agronomic performance and nutritive characteristics in forage maize grown at higher latitudes.

Material and Methods

This experiment was conducted during the growing seasons of 2008 and 2009 at three experimental sites, Kristianstad (56°04´13"N; 14°19´11"E), Skara (58°23'15"N; 13°29'03"E) and Västerås (59°36'47"N; 16°38'07"E) in Sweden. The soils in Kristianstad and Skara were sandy clays and in Västerås it was a clay loam. Soil pH ranged from 6.2 to 6.8. Fertilizers were applied in accordance with cropping history, soil type and fertility. The experimental design was based on two replicates of randomized complete blocks at each site. Three commonly used forage maize hybrids Avenir (FAO maturity ranking 180; Syngenta Seeds, Switzerland), Isberi (FAO maturity ranking 190; Caussade Semences, France) and Burli (FAO maturity ranking 210; Caussade Semences, France) were used in the study. Seeds were sown at the density of 72000 seeds ha^{-1} in four rows with spacing of 75 cm in 12 x 3 m plots. In 2008, Avenir was harvested at four occasions and Isberi and Burli were harvested at three occasions with increasing maturity. In 2009, all the hybrids were harvested at four occasions with increasing maturity. The harvest occasions represented approx. development stages R1-R5 (OMAFRA, 2009) for Kristianstad and stages R1-R3 for the other sites.

Plant development was observed from tasseling to physiological maturity by visiting plots frequently (5 to 7 times per site and season) until the last harvest. At each visit the proportion of the plants in each stage of development were estimated for each plot. Plants were assumed to have reached a specific growth stage when 0.5 of the plants had attained it. Harvested plant samples were analyzed for concentration of crude protein (CP) (AOAC, 1984; method 7.015) neutral detergent fibre (NDF) with addition of sulphite and amylase (Chai & Udén, 1998), water-soluble carbohydrates (WSC) (Larsson & Bengtsson, 1983) and starch (Larsson & Bengtsson, 1983). Indigestible fibre (iNDF) was determined using near infrared spectroscopy (NIRS) and the concentration of net energy lactation at an intake of 20 kg DM (NEL20) was determined according to Nordic feed evaluation system Norfor^{TM} (Volden, 2011). The potential degradability of the fibre (pNDFD) was calculated as (NDF-iNDF)/NDF. Organic matter digestibility *in vivo* was estimated from *in vitro* OM degradability (Lindgren, 1979). The accumulation of crop heat units (CHU) for each site was calculated as described by Kwabiah et al. (2003).

Statistical analysis was performed with the GLM procedure of ANOVA, utilizing the software Minitab (v. 16, Minitab Inc., State College, PA, USA) with block and year treated as random factors. Significance was declared at $P \leq 0.05$. The requirements of CHU to reach plant development stages from R1-R5 are presented as least square means within development stage for hybrids and sites, respectively. The model for DM yield and nutritive values included linear and quadratic effects of DM concentration at harvest and the results are presented as least square means within sites and hybrids across all harvest occasions. For the quadratic effects found, DM concentrations at maximum or minimum of response variables were also calculated.

Results and Discussions

All the hybrids (Table 1) required different amount of accumulated CHU to reach observed stages of development, which is consistent with the findings of Millner, (2005). The earliest maturing hybrid Avenir needed least CHU to reach each stage of plant development except for R2 (blister) when it was comparable with Isberi. And late maturing hybrid Burli needed most CHU to reach each stage accept for R4 (dough) when it was comparable with Isberi. The hybrids in Skara needed least CHU to reach R1 followed by Kristianstad and Västerås. To reach R2 and R3 the maize in Skara and Kristianstad had the same requirements but the maize in Västerås needed about 450 CHU more to reach the same plant development stage. This can be due to longer vegetative period for hybrids in Västerås due to which plants arrived late in R1 stage consuming more CHU. Increasing thermal unit accumulation for a certain stage of development at higher latitudes is in agreement with the results of Smith et al. (1982). Kristianstad was the only site where all the hybrids reached physiological maturity (R5) during both years, which is the recommended stage for ensiling forage (Cummins, 1970).

Early maturing hybrids (Table 2) gave lower DM yield as compared to late maturing hybrids at all sites, which is in line with the conclusions of Millner et al. (2005). Maize hybrids in Kristianstad were significantly different for starch, OMD, NDF, WSC and NEL20. In Skara hybrids were different for OMD, starch and NDF concentration. In Västerås hybrids were different only for WSC. The Table 2 also indicates DM concentration for the minimum or maximum responses to maturity for the quadratic functions.

Table1 Least Square means (LSM) for both years of requirement of crop heat units (CHU) for three maize hybrids (n=12) to reach stages of development R1- R5 at three sites (n=12).

	Maize Hybrids			Sites				P≤	
Stages	Avenir	Isberi	Burli	Kristianstad	Skara	Västerås	SEM	Hybrid	Site
R1[1]	1287[c]	1415[b]	1509[a]	1310[b]	1262[c]	1640[a]	7.70	0.01	0.01
R2	1620[b]	1627[b]	1720[a]	1496[b]	1510[b]	1961[a]	10.88	0.01	0.01
R3	1854[c]	1915[b]	1993[a]	1792[b]	1773[b]	2198[a]	11.88	0.01	0.01
R4	2029[b]	2243[a]	2280[a]	2184	-[2]	-	29.80	0.01	-
R5	2352[c]	2503[b]	2565[a]	2473	-	-	11.72	0.01	-

[a-c] Means for hybrids and Sites followed by different superscripts in the same row are different (P≤0.01).

[1]R1, silking; R2, blister; R3, milk; R4, dough; R5, physiological maturity.

[2]Hybrids did not reach R4 and R5 in Skara and Västerås during both years.

Conclusions

In conclusion, plant development of maize hybrids required higher accumulation of thermal units with increasing latitudes. Hybrids were unable to achieve physiological maturity at higher latitudes; this represents the limitations for forage maize production in Sweden. Forage maize hybrids with equal DM concentration can have differences in nutritive values. Early maturing hybrids gave relatively lower DM yields but with higher nutritive value compared to later maturing. Increased maturity causes linear and quadratic responses in agronomic performance and nutritive characteristics that must be considered in risk management, selection of hybrids, crop and harvest management.

Table 2 Dry matter yield and nutritive value (g/kg DM) for three maize hybrids (Avenir n=16, Isberi and Burli n=14) at three sites. Results are least square means at a global DM concentration for both years with 3-4 harvest occasions per year, representing approx. developmental stages R1-R5 for Kristianstad and stages R1-R3 for the other sites, linear (L) and quadratic (Q) effects to increasing DM concentration are shown together with the DM concentration when quadratic effects reached maximum or minimum.

	Maize hybrids				P≤			DM at Q[1]	
Item	Avenir	Isberi	Burli	SEM	Hybrid	L	Q	Max	Min
Kristianstad (Global DM: 294 g/kg)									
Kg DM/ha	13999[b]	17522[a]	18310[a]	443	0.01	0.01	0.01	350	
Starch	310[a]	258[b]	234[b]	13	0.01	0.01	0.01	360	
OMD	732[a]	709[b]	692[c]	4	0.01	0.01	0.02	350	
Crude protein	82[a]	81[a]	84[a]	1.70	0.31	0.01	0.01		410
NDF	384[b]	404[ab]	414[a]	9.9	0.05	0.01	0.01		390
WSC	33[c]	51[b]	72[a]	5.1	0.01	0.01	0.01		360
NEL20 (MJ/kg DM)	5.9[a]	5.7[b]	5.6[b]	0.05	0.01	0.01	0.01	340	
Skara (Global DM: 252 g/kg)									
DM yield (kg/ha)	9451[b]	11550[a]	11446[a]	560	0.01	0.01	0.01	340	
Starch	204[a]	231[a]	191[a]	18.1	0.03	0.01	0.01	274	
OMD	705[a]	698[a]	673[b]	6.5	0.03	0.01	0.01	254	
Crude protein	93[a]	90[a]	90[a]	2.5	0.58	0.01	0.01		320
NDF	471[a]	457[a]	493[a]	14.2	0.01	0.01	0.01		270
WSC	49[a]	42[a]	48[a]	10.2	0.86	0.01	0.02		309
NEL20 (MJ/kg DM)	5.5[a]	5.4[ab]	5.3[b]	0.10	0.11	0.01	0.01	270	
Västerås (Global DM: 205 g/kg)									
DM yield (kg/ha)	8085[b]	9336[a]	9703[a]	370	0.01	0.01	-	-	
Starch	79[a]	80[a]	44[a]	14.2	0.06	0.03	0.01		170
OMD	687[a]	683[a]	674[a]	8	0.45	0.77	-	-	
Crude protein	82[a]	77[a]	74[a]	2.4	0.11	0.01	-	-	
NDF	559[a]	542[a]	543[a]	9.9	0.23	0.04	0.046	-	210
WSC	117[b]	127[b]	175[a]	10.4	0.01	0.01	0.01	210	
NEL20 (MJ/kg DM)	5.4[a]	5.3[a]	5.3[a]	0.08	0.70	0.41	-	-	

[1]DM concentration when quadratic response to harvest DM reached maximum or minimum.

[a-c] Means followed by different superscripts in the same row are different (P≤0.05), SEM: standard error of mean; OMD: organic matter digestibility; NDF: neutral detergent fiber; WSC, water soluble carbohydrates; NEL20: net energy of lactation at 20 kg DM/ day.

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Methane production in bull calves fed rations based on grain or different levels of high-moisture corn kernel silage

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Introduction

Estimation of methane production from bull calves is based on ICCP standard methods in Denmark. The loss of methane is fixed to 4% of the gross energy (Mikkelsen et al. 2005). However, the methane production is affected by for instance forage:concentrate ratio, feeding level (Blaxter and Wainman, 1964), and presence of protozoa or not (Whitelaw et al., 1984). Loss of methane in percent of gross energy varies from 2% (Mwenya et al., 2005) to 12% (Whitelaw et al., 1984) in the literature. The objective of this study was to measure the methane production in 300 kg bull calves fed three different diets: Pelleted concentrate based on mature grain (mainly barley, wheat, triticale) and oil seed meal (rape and soya) supplemented with straw (CONC) or two total mixed rations (TMR) with either 25% (M25) or 50% (M50) of dry matter from high-moisture corn kernel silage.

Material and methods

Twelve Danish Friesian bull calves were allocated to the three diets (4 per treatment) from 130 kg body weight. The twelve bull calves used this experiment were a part of a production experiment with different high-energy corn silage products to bull calves. The pelleted concentrate was bought at a commercial feed company. The two other rations were mixed daily and the composition is given in table 1. Only chemical analyses for the M25 and M50 were available and given in table 2. The gross energy content of the diets were calculated according to Norfor feed evaluation system (Volden and Nielsen, 2011). During methane measurements individual feed intake was measured. The diets were fed ad libitum except barley straw which was limited to 0.5 kg. The calves were submitted to four days in an open circuit respiration unit with measurement of production of methane, hydrogen and carbon dioxide and consumption of oxygen (Columbus instruments, Ohio, USA). Heat production was calculated according to Brouwer (1965). Data was analyzed with PROC GLM in SAS® with diet as main effect. Results are presented as least squares means (LSmeans) and mean square error (MSE) was given as measurement of variation. Pair wise comparison of LSmeans was made by the PDIFF option in SAS. Difference was considered significant if $P<0.05$.

Results and discussion

Both dry matter intake (DMI) and intake of gross energy (GE) were lower on M50 ($P<0.05$). The intake of net energy was higher on CONC ($P=0.001$) then M25 and M50. The data from the production experiment are not yet calculated but life weight gain seemed be the same between the treatments (Kirstine Jørgensen, Personal communication). One possible explanation of the difference in feed intake is that calves on the two TMR diets have selected in the diet. Visual inspection of the feed residues during the methane experiment support this

hypothesis. DMI a week before and during methane measurements was the same (Data not shown).

Table 1 Feed composition of the three diets.

	M25	M50		CONC
	[g/kg DM]			[g/kg feed]
Spring barley	312		Grain[a]	559
High-moisture corn silage	352	613	Oil seed meal[b]	134
Rape seed meal	95	97	Peas	100
Soya bean meal	95	97	By-products[c]	144
Sugar pulp dried	68	97	Art. dried grass	30
Barley straw, spring	54	70	Fat, palm	10
Calciumcarbonat	14	14	Calcium carbonat	17
Minerals[e]	11	11	Minerals[d]	2
			Sodium chloride	4

CONC: Commercial pelleted concentrate with different cereals supplement with straw. M25 and M50 total mixed ration with respectively 25% and 50% of the dry matter from high-moisture corn.
[a]Barley 41%, Wheat 27 %, Triticale 21%, Oat 5% and Corn 5%; [b]Rape seed meal 78%, soya bean meal, decorticated 22%; [c]Sugar beet pulp, dried 69%, citrus pulp, dried 21%, molassed sugar beet10%; [d]Vitamins and minerals per kg feed: $CuSO_4$ 5 mg, ZnO 240 mg, $FeSO_4$ 50 mg, $CoSO_4$ 0.1 mg, CaI_2 0.27 mg, MnO_2 40 mg, Na_2SeO_3 0.4 mg, Vitamin A 10000 I.E., Alfa-tocoferol 45 mg, B1 vitamin 5 mg and D3 vitamin 1000 I.E; [e]Vitamins and minerals per kg dry matte in supplement: Calcium145 g, Magnesium 85 g, Sodium 100 g, Chlorine 147 g, Sulfur 40 g, Manganese 4000 mg, Zinc 4500 mg, Copper 1500 mg, Cobalt 25 mg, Selenium 50 mg, Iodine225 mg, Vitamin A 900000 I.E., Vitamin E 6000 mg and Vitamin D 190000 I.E.

Table 2 Chemical composition of the maize kernel diets, concentrate and barley straw

	M25	M50	CONC	Barley straw, spring
Dry Matter [g/kg]	768	689	867	850[b]
Ash [g/kg DM]	34	58	61[a]	60[b]
Crude protein [g/kg DM]	169	165	164[a]	51[b]
Crude fat [g/kg DM]	31	33	41[a]	13[b]
Crude fibre [g/kg DM]	78	82	84[a]	438[b]
Starch [g/kg DM]	401	394		
NDF [g/kg DM]	178	169		771[b]
Gross energy [MJ/kg DM][c]	18.9	19.0	18.9	17.9
Net energy lactation[c] [MJ/kg DM]	7.60	7.83	9.15[a]	2.45[b]

CONC, M25 and M50: See Table 1.
[a]Declared composition given by the feed company; [b]Chemical composition given in Norfor feed table (accessed April 2011); [c]Calculated according to the Norfor feed evaluation system (Volden and Nielsen, 2011).

Daily methane production was not different between treatments (P>0.05). Related to DMI the daily methane production was14 L CH_4/kg DM on CONC which was lower (P=0.03) than 24.1 and 24.7 L CH_4/kg DM on M25 and M50, respectively. Also the loss of GE as methane was lower on CONC (P=0.04). One animal on diet M25 excreted 35.9 l CH_4/kg DM. If this calf was omitted from the dataset the mean methane excretion was 20.7 l CH4/kg DM for M25. Plots of methane production per kg DM in relation to content of high-moisture corn kernel silage indicate that there could be a linear or a curvilinear relationship. All three diets are characterized by high contents of starch, which increase propionate production and reduce methane excretion. The difference between CONC and two others diets are possible related to type of cereal, maturity state of cereal and physical form. Compared with starch from wheat, barley and oat, more starch from corn escape rumen fermentation (Ørskov, 1986). This would lead to change in proportion of volatile fatty acids with lower ratio of propionate and higher ratio of acetate. This could lead to higher methane production. However starch from high-moisture corn has a rumen digestibility of 100% (Cooper et al., 2002). Studies by Szasz et al. (2007) have shown that increasing dry matter content in corn kernel silage decrease the degradability in the rumen. The moisture content of corn kernel silage in this study was 45%. From studies by Cooper et al. (2002) and Szasz et al. (2007) this would imply that rumen starch digestibility should be high. Therefore differences in degradation pattern of cereal starch in the rumen can probably not explain the differences in methane production.

Table 3 Body weight, intake of dry matter and gross energy, methane and hydrogen excretion and heat production.

	CONC	M25	M50	MSE	P-value (Diet)
Body weight [kg]	291	300	290	10.8	0.76
Dry matter intake [kg/day]	6.8[a]	6.4[a]	5.5[b]	0.29	0.03
Gross energy intake [MJ/day]	129[a]	121[a]	105[b]	5.6	0.04
Net energy lactation [MJ/day]	62.6[a]	48.7[b]	43.3[b]	4.5	0.001
Methane [l/day]	101	160	134	17.7	0.11
Methane [l/kg DM]	14.1[b]	24.4[a]	24.7[a]	2.6	0.03
Methane [% of GE]	3.1[b]	5.2[a]	5.1[a]	0.5	0.04
CO_2/CH_4	33.0[a]	22.3[b]	23.5[b]	2.0	0.01
Hydrogen [l/day]	1.4	3.5	0.5	1.1	0.23
Hydrogen [ml/ kg DM]	192	550	91	179	0.22
Heat production [MJ/day]	69	69	66	2.5	0.60

CONC, M25 and M50: See Table 1. a,b superscript with different letters within row are significantly different (P<0.05).

The loss of methane from GE on CONC is comparable with other studies with high feeding level and concentrate level (Mwenya et al., 2005; Blaxter and Wainman, 1964). The higher methane production on M25 and M50 cannot be explained at present.

The key in the regulation of methane production is hydrogen. Hydrogen is produced during fermentation of carbohydrates to acetate and butyrate whereas propionate is a hydrogen sink. The hydrogen production was small and no differences were found between diets (Table 3). In figure 1 the hydrogen production in ml/minute is given for all animals in the measured period. The emission of hydrogen varies during the day and there can be high variation from

day to day for the individual animals. Neither level nor variation in hydrogen emission can be explained and further studies are needed.

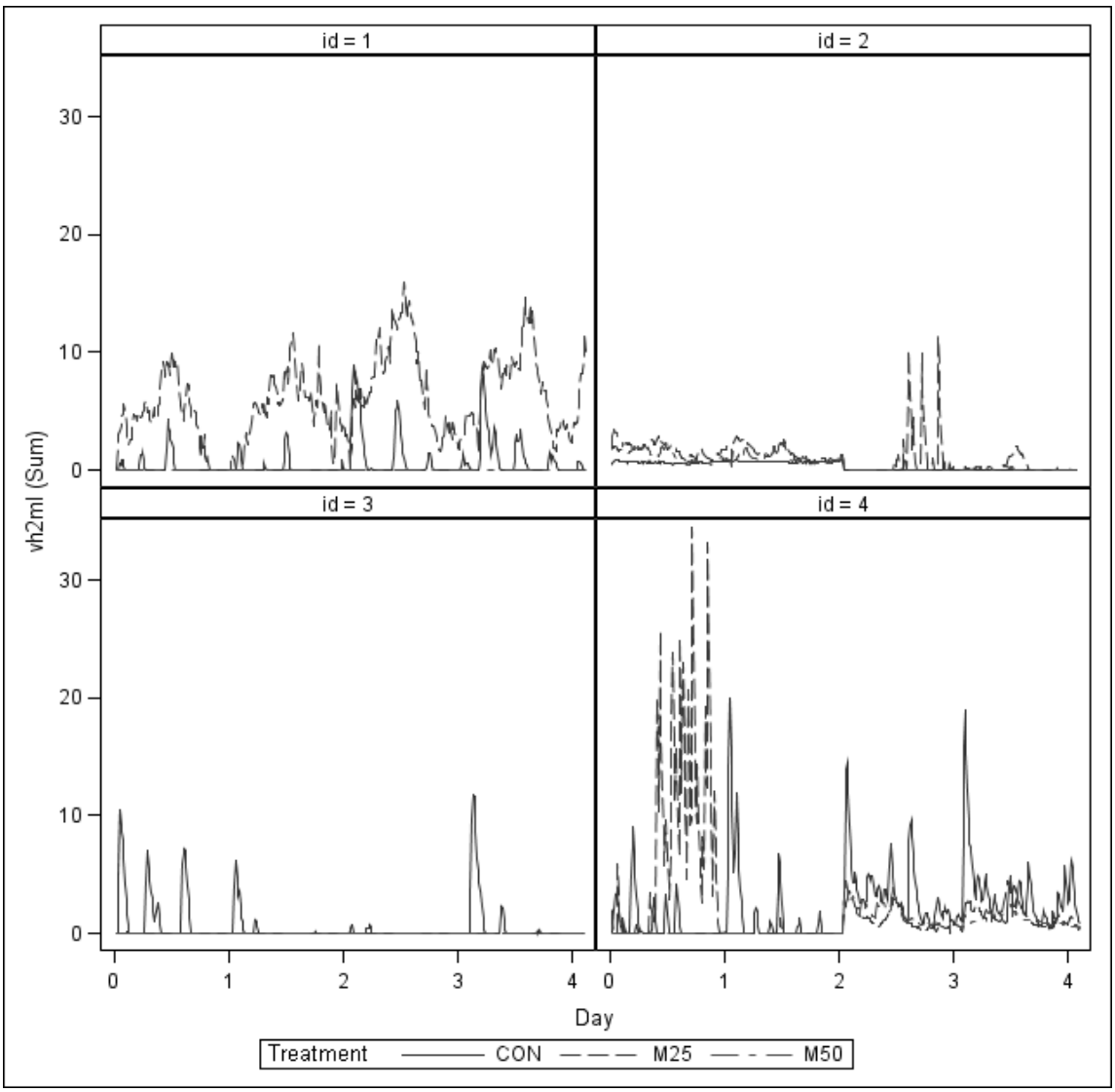

Figure 1 Emission of hydrogen is given in ml per minute for each bull calves. CONC: Commercial pelleted concentrate with different cereals supplement with straw. M25 and M50 total mixed ration with respectively 25% and 50% of the dry matter from high-moisture corn.

The calf with the high emissions of methane indicates that there can be considerable variation between individuals. It has not been possible to explain the high methane production for this calf. It is expected that the pH in rumen of steers feed high concentrate diets is low, and the number protozoa is probably zero. Cattle without protozoa are expected to have lower methane emission than cattle with protozoa (Whitelaw et al., 1984 and Schönhusen et al., 2003). Franzolin and Dehority (1996) have shown that even after prolonged feeding with high concentrate diets some steers had protozoa in the rumen. Such variation in rumen fauna might explain some of the observed emission for this particular calf.

Heat production did not differ between animals (P=0.60) although bull calves on M50 had lower feed intake.

Conclusions

Exchange of the pelleted concentrate with high-moisture corn silage increased methane production in 300 kg bull calves.

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Relationships between milk urea and production traits in dairy herds in Latvia

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Introduction

Normal bovine milk contains 30 to 35 g of protein kg^{-1}. Milk total proteins are composed of casein, whey proteins and non-protein nitrogen (DePeters and Cant, 1992). Caseins constitute 76 to 86% of the total milk protein. Whey proteins represent 14 to 24% of milk proteins and are in solution in the serum phase of the milk (Hui, 1993).

Urea is a normal constituent of milk and comprises part of the non protein nitrogen fraction. Although opinions do vary to some extent, milk urea levels between 20 and 30 mg dL^{-1} are generally considered as normal for cow's milk. Urea accounts for roughly 50% of the non-protein nitrogen fraction in herd bulk milk of dairy cows, although this may vary from 35 to 65%. For milk from individual cows, this variation may be even larger (Bijgaart, 2003).

In different countries milk urea is used to monitor the efficiency of protein utilization in dairy herds (Godden et al., 2001). The most important of all milk components for the manufacture of cheese and curd are milk proteins. In Latvia, the milk payment system is based on total protein and milk amount. Therefore, Latvian breeding programmes will be focusing on high milk yield with high protein concentration.

The objective of this study was to evaluate the milk urea content in relation to milk yield and milk content in four different farms.

Materials and Methods

In the present study individual cow milk samples (n=1522) were collected monthly from 2008 August to 2009 February from four dairy farms (Farm A-D). The dairy herds represented three breeds Holstein Black and White (HB), Latvian Brown (LB) and cross breeds XP (cross breed from HB and LB). Average lactation number for cows in farms was 2.04. The herds were under official performance and pedigree recording. Monthly control milk samples were analyzed for total protein, casein, fat, lactose, urea content and somatic cells count. All of these parameters were analyzed in accredited milk quality laboratory SIA 'Piensaimnieku Laboratorija' with FOSS instrument CombiFoss FC. Somatic cells counts were transformed to SCS (Somatic Cell Score) with formula (Schutz, 1994). Data regarding breed of cows and date of milk analysis were available from monthly records of the herds from Agricultural Data Centre program. Lactations were arranged in three groups according to lactation number (1^{st}, 2^{nd} and $\geq 3^{rd}$). Lactation stages (days in milk; DIM) were also arranged in three groups, DIM1 (1-100), DIM2 (101-200) and DIM3 (>200).

The statistical analyses were performed with the SPSS program package and Microsoft Excel for Windows. To determine the effects of farm, breed, month of recording, lactation and lactation stage on a milk component and milk yield, these factors were tested as fixed in a General Linear Model using SPSS.

Results and Discussion

The evaluation of effects of breed, months of recording, lactation no and DIM on milk composition is shown in Table 1 and milk composition for the individual farms in Table 2.

Table 1 The influence of researched factors on milk compostion

Traits	Farms	Breeds	Month	Lactation	Lactation stage
Milk yield, kg	***	***	***	***	***
Protein content, %	***	***	***	***	**
Casein content, %	***	**	***	**	***
Fat content, %	**	n.s.	***	n.s.	***
Lactose content, %	***	n.s.	**	***	***
Urea content, mgdL^{-1}	***	*	***	***	n.s.
SCS	***	***	***	***	***

*p<0.05, **p<0.01, ***p<0.001, n.s. not significant

Table 2 Average milk yield and quality traits during the study in different farms (LS-Means±S.E.).

Traits	Farm			
	A (n=791)	B (n=478)	C (n=149)	D (n=104)
Milk yield, kg	20.9±0.22^{a}	23.7±0.26^{b}	23.7±0.32^{b}	16.1±0.24^{c}
Protein content, %	3.60±0.016^{a}	3.46±0.014^{b}	3.37±0.021^{c}	3.57±0.029^{a,b}
Casein content, %	2.75±0.011^{a}	2.67±0.010^{b}	2.58±0.014^{c}	2.71±0.020^{a,b}
Fat content, %	4.45±0.042	4.41±0.025	4.38±0.030^{a}	4.56±0.038^{b}
Lactose content, %	4.67±0.012^{a}	4.84±0.006^{b}	4.76±0.008^{c}	4.79±0.012^{b,c}
Urea content, mg dL^{-1}	17.1±0.33^{a}	21.1±0.22^{b}	17.4±0.27^{a}	27.3±0.39^{c}
SCS	2.32±0.059^{a}	2.75±0.052^{b}	2.58±0.100^{a,b}	1.71±0.105^{c}

$^{a; b; c}$ milk productivity and quality traits by different superscripts are significantly different between farms (p<0.05)

In the study urea content and all milk productivity traits were significantly influenced (p<0.001; p<0.01) by farm and month of the year. Breed and lactation number did not influence milk fat content. Breed did not influence lactose content, but had a significant influence (p<0.001) on milk yield, protein and casein content, SCS and urea content (p<0.05). Lactation stage significantly influenced all milk productivity traits except the urea content. The factors with significant influences were analyzed separately.

Milk yields differed among farms and ranged from 23.7 to 16.1 kg/d. The lowest milk yield (16.1 kg) and highest urea content (27.3 mg dL^{-1}) was found in farm D with LB breed cows and where cows were managed in one feeding group. Likewise significant differences were found in milk constituents and milk quality among farms. Farm A had the highest protein and casein content (3.60 % and 2.75 %). At farm B, the highest lactose content and SCS were found. The European Union requires that milk used for dairy products sold in its territory have SCC levels below 400,000 cells mL^{-1} and SCS in this study farms was below this level.

Milk protein (3.53 %) and fat (4.44 %) contents were higher for all farms than the average for Latvia milk recordings in 2009 year (3.36 and 4.38 %, respectively). Urea content varied from 17.1 to 27.3 mg dL^{-1}. The average urea content in Farm D was significantly higher (27.3 mg dL^{-1}) than in the others farms, indicating problems with the feeding balance and management. The average milk yield and quality traits were significantly different among breeds (Table 3).

Table 3 Average milk y and quality traits with different breeds (LS-Means±S.E.).

Traits	Breed		
	LB (n=920)	HM (n=415)	XP (n=187)
Milk yield, kg	20.5±0.23[a]	24.0±0.34[b]	22.9±0.51[b]
Protein content, %	3.59±0.015[a]	3.42±0.023[b]	3.44±0.034[b]
Casein content, %	2.75±0.011[a]	2.63±0.016[b]	2.66±0.024[b]
Fat content, %	4.51±0.033[a]	4.33±0.049[b]	4.37±0.073[a,b]
Lactose content, %	4.70±0.009[a]	4.77±0.014[b]	4.88±0.020[c]
Urea content, mgdL^{-1}	18.4±0.28[a]	19.6±0.42[b]	21.3±0.63[c]
SCS	2.34±0.058[a]	2.51±0.086[a,b]	2.76±0.128[b]

[a; b; c] milk productivity and quality traits by different superscripts are significantly different between breeds ($p<0.05$)

The average urea and protein content and milk yield differed significantly between months (Table 1 and Figure 1). Both protein and urea content was highest in September. In December, the urea content peaked again but at this time the protein content was at one of its lowest levels. The protein and urea content and milk yield decreased in October coinciding with the end of the pasture season. The results of this study are consistent with a previous study that reported milk urea and protein varied by month of recording and with the highest urea content in September (Godden et al., 2001). The lowest milk yield was in February (17.9 kg) and the average milk protein and urea content was 3.58 % and 17.2 mg dL^{-1}. The coefficient of variation was 32.8% for milk yield and 45.3% for urea, indicating high variability.

The average milk yield, protein, fat and casein content, and SCS of cow milk were significantly lower in the first lactation than in the second lactation, but milk yield, casein and urea content did not differ from third lactation (Table 4).

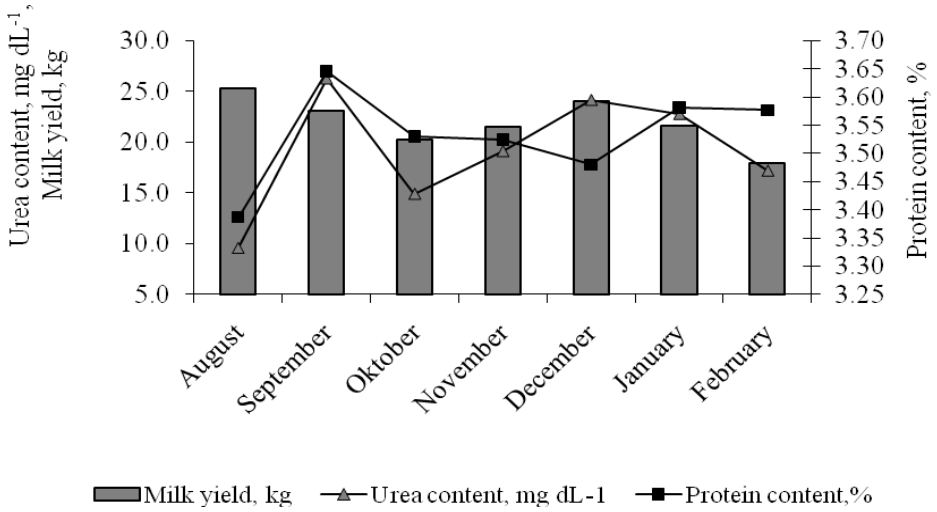

Figure 1 Urea and protein content and milk yield by month of recording.

Table 4 Average milk productivity and quality traits in different lactation (LS-Means±S.E.).

Traits	Lactation		
	1 (n=708)	2 (n=405)	3 (n=409)
Milk yield, kg	21.4±0.27[a]	22.5±0.36[b]	21.7±0.35[a,b]
Protein content, %	3.47±0.018[a]	3.60±0.023[b]	3.56±0.023[b]
Casein content, %	2.68±0.012[a]	2.75±0.016[b]	2.71±0.016[a,b]
Fat content, %	4.38±0.038	4.50±0.050	4.50±0.049
Lactose content, %	4.79±0.011[a]	4.72±0.014[b]	4.70±0.014[b]
Urea content, mgdL^{-1}	18.8±0.32[a]	20.2±0.43[b]	18.5±0.43[a]
SCS	2.17±0.065[a]	2.45±0.086[b]	2.88±0.085[c]

[a; b; c] milk productivity and quality traits by different superscripts are significantly different between lactation ($p<0.05$)

Stage of lactation (Table 5) significantly influenced average protein, fat, casein and lactose content and SCS in milk. The lowest average protein and casein content was seen in DIM1 (3.23 and 2.51%), and the highest (3.75 and 2.85%) in DIM3. The influence of the lactation stage on the milk composition has also been observed previously by Ng-Kwai-Hang et al. (1984) and Jonkus et al. (2004). The lowest levels of fat and protein content were observed together with the highest milk yield in the 2nd to 12th lactation week. At the fourth to fifth lactation month, these parameters in milk started to increase and the highest contents were found at the end of the lactation.

In the present study the milk urea content was positively correlated (r=0.053) with milk yield and also positively correlated (r=0.147) with lactose content. A weak, but significant ($p<0.05$), negative correlation was found between milk urea content and SCS.

Table 5 Average milk yield and quality traits in different lactation stage (LS-Means±S.E.).

Traits	Lactation stage		
	1 (n=387)	2 (n=493)	3 (n=642)
Milk yield, kg	27.1±0.31[a]	22.4±0.28[b]	18.0±0.24[c]
Protein content, %	3.23±0.022[a]	3.48±0.019[b]	3.75±0.017[c]
Casein content, %	2.51±0.015[a]	2.67±0.013[b]	2.85±0.012[c]
Fat content, %	4.19±0.050[a]	4.41±0.044[b]	4.62±0.039[c]
Lactose content, %	4.81±0.014[a]	4.75±0.012[b]	4.68±0.011[c]
Urea content, $mgdL^{-1}$	18.6±0.44	19.6±0.39	19.0±0.34
SCS	1.92±0.087[a]	2.35±0.077[b]	2.82±0.068[c]

[a; b; c] milk productivity and quality traits by different superscripts are significantly different lactation stage ($p<0.05$)

Conclusions

This study supports earlier studies showing that milk protein, urea, casein, fat and lactose content statistically varies between different Latvian farms. The protein and urea content in milk were significantly ($p<0.05$) influenced by the dairy cows breed, month of recording and lactation. The highest milk yield had HM breed cows and highest urea content had XP breed cows. The milk yield was highest in the second lactation. The milk urea and quality traits in dairy herds in Latvia were significantly different by month of recording. The influence of the stage of lactation on the milk content was significantly to all productivity traits but the stage of lactation had no significant influence on the milk urea content. It was found very low positive but significant ($p<0.05$) correlations between urea content and milk protein, casein, fat and lactose content and with milk yield.

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Estimating milk yield response to increasing feed intake in dairy cows – evaluation of cow level versus group level approaches

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Introduction

A model for economical optimization of the feeding level for dairy cows in Scandinavia is to be developed and evaluated in an ongoing PhD project. The aim of the project is to develop and evaluate a model (a decision tool) which can offer an economical optimization of the feed ration and in particular the energy level in a specific herd. The model for optimal feeding level should be operational together with new mechanistic physiological models for ration composition. Estimation of valid production functions for expected milk yield response to increasing feeding level is essential. It is expected that the new optimization model will improve the economy of dairy production compared to other currently available tools for planning feed ration composition.

Formulation of feed rations and feeding level (energy fed per cow per day) is of great importance for the milk production in dairy herds. Feed costs typically constitute 80% of the variable costs in Denmark (Thøgersen and Laursen, 2009). Thereby feed is the single factor of greatest importance to the economy in the dairy herd. The increasing focus on dairy herds being more economical efficient as well as the more frequent fluctuations in feed and milk prices make an economical approach to the feed planning, including the feeding level quite necessary. To improve formulation of feed rations nutritionally and improve milk production new mechanistic physiological models have been developed in recent years. NorFor Plan is such a model and is implemented in Scandinavia and Iceland (Volden, 2011). NorFor Plan is used for feed ration composition and nutrient supply in relation to the needs of the cow for a given production, but this feed ration will seldom be the economically optimal although the model can minimize feed costs. This is due to a decline in excess profit as the utilization of feed energy for production decreases with increasing feeding level (Østergaard, 1979). There is currently no mechanistic physiological feed planning systems available, where determination of feeding level is economically optimized because we are lacking knowledge of production functions applicable to a contemporary level of production of modern high yielding dairy cows (Østergaard et al., 2009).

The first part of the project involves estimation of milk yield response to increasing energy intake by doing a meta-analysis of data from cows fed at different feeding levels within the experiments and where concentrate levels or concentrate to roughage ratio are independent of milk yield.

Data material in project

The data material to be used in modeling the response curves are derived from 13 production experiments originating from Norway, Sweden and Denmark from 1998-2010. The experiments, mostly being block experiments, lasted from eight weeks to six years with varying levels of feed intake (from 13.4 to 24.3 kg dry matter) and roughage fed ad lib. Data from experiments with grazing periods are discarded, due to estimated feed intake. Cows in

experiments were from first to seventh lactation cows with proportions of primiparous to multiparous varying from 14:86 to 75:25.

To be able to take into account the effect of parity and stage of lactation the data material is grouped. The grouping is done according to parity in two levels as primiparous or multiparous. Also three levels are used according to stage of lactation. These are Early, Mid and Late being 0-105, 106-203 and 204-301 days in milk, respectively.

Pilot study on individual vs. group level data

It has been questioned which observational unit should be used in this meta-analysis as opposite to most meta-analyses there is access to original individual cow data for all the experiments. Therefore, a pilot study was conducted to evaluate the consequences of assigning observational units to individual cows versus groups of primi- and multiparous cows within each treatment group.

Materials and Methods

In the pilot study data from two block experiments (Kristensen et al., 2003) at Research Centre Foulum, Aarhus University, Denmark were used. The experiments each lasted from 3 to 15 weeks after parturition and included 63 Danish Holstein cows, which were fed TMR rations. There were nine treatments consisting of one of three roughages (grass-clover silage, whole-crop barley and NH3-treated straw) each combined with low, medium or high levels of concentrates giving nine combinations of energy intake and quality of roughage.

The dataset on individual level has 126 observations (one per cow) coming from the two experiments. The dataset on group level has 36 observations coming from the two experiments, with nine treatments each and two levels of parity.

To compare regression estimates from the individual data with the group data the same models are used on both datasets. The response variable was milk production (kg ECM) and the explanatory variables were feed intake in feed units (continuous), parity (categorical with two levels) and experiment (categorical with two levels). The order of the models was first to use a simple linear regression analysis with all three variables and all interactions (model 1 and 2). Graphs showing plots of data from individual and group level datasets with simple regression lines is shown in figure 1. Then linear regression without interaction of experiment (model 3 and 4) and afterwards linear mixed-effect analysis with experiment as a random factor were done (model 5 and 6). Finally for the dataset on group level a linear mixed-effects model with experiment as a random factor and the data being weighted by the number of observations behind each group mean.

The ‘lm’ and ‘lme’ statement of R (2011), version 2.12.2 was used for the linear and linear mixed-effects models to calculate estimates for the linear regression coefficients.

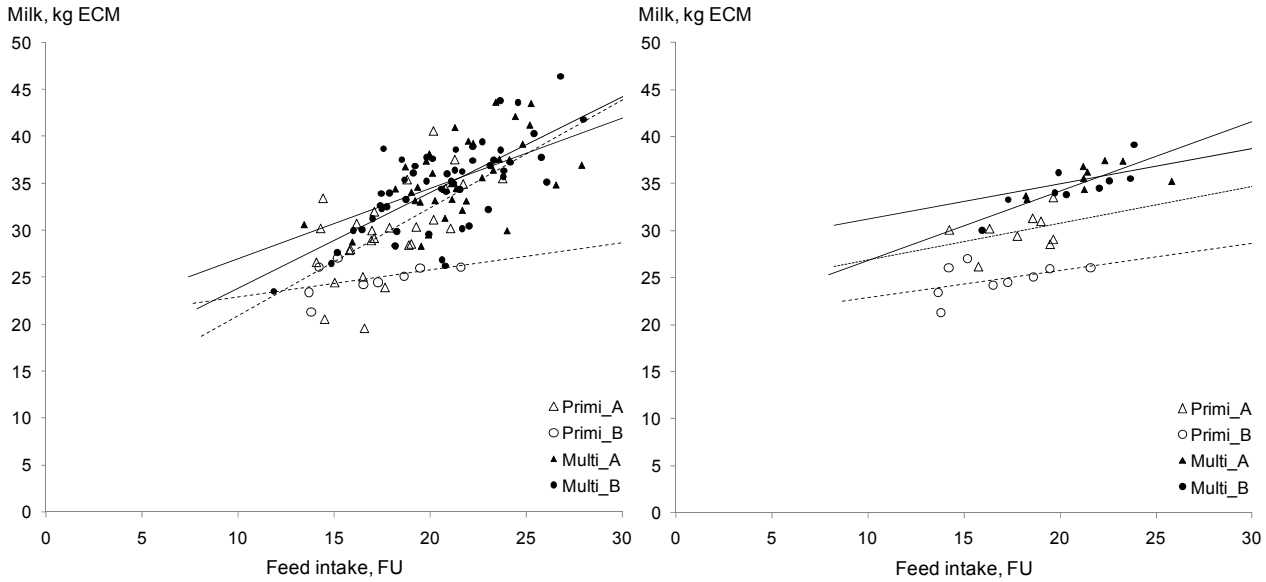

Figure 1 Graphs showing milk production data based on either individual level (n=126) or on group level (n=36) from two experiments (A and B) with cows as primiparous (Primi) or multiparous (Multi). Regression lines are by simple linear analysis.

Results and Discussion

There were large differences in the estimates for milk production whether based on data from individual level or group level (Table 1). The simple linear regression models (model 1– 4) estimated slopes from 0.29 to 1.15 for individual data and from 0.28 to 0.74 for group data. The mixed-effects models with experiments as a random factor (model 5-6) estimated slopes of 1.03 for primiparous cows and 0.91 for multiparous cows. The mixed-effects model also including weighting by number of observations in each group (model 7) estimated a slope of 0.52 for primiparous cows and 0.60 for multiparous cows.

Conclusions

The pilot study on regression analysis of data based on data of either individual level or group level showed large differences in estimates for milk production response curve. With data from two production experiments based on individual level the estimates of ECM per feed unit increase is roughly about twice the size as the estimate based on group level data. The result stresses the need for caution when recalculating data from various experiments for use in a meta-analysis.

Table 1 Estimates (slope) from regression models on milk production according to data on individual level vs. group level and further models with the variable 'experiment' as random and a model also including weighting of data by the number of observations behind each group mean. Data are from two experiments (A and B).

		- - - - - - Linear models - - - - - - - -				**- - - Mixed models - - -**		
Model characteristic		1	2	3	4	5	6	7
Data level		Indiv.	Group	Indiv.	Group	Indiv.	Group	Group
Exp. as random		-	-	-	-	+	+	+
Weighted (n obs.)		-	-	-	-	-	-	+
Parity	**Exp.**	**Slope (ECM/FU)**						
Primi	A	1.15	0.39	1.01	0.44	1.03	0.44	0.52
Primi	B	0.29	0.28					
Multi	A	0.75	0.38	0.91	0.51	0.91	0.51	0.60
Multi	B	1.02	0.74					

Model 1 + 2: ECM ~ FU * PARITY * EXP

Model 3 + 4: ECM ~ FU * PARITY + EXP

Model 5 + 6: ECM ~ FU * PARITY, random=EXP

Model 7: ECM ~ FU * PARITY; random=EXP, weights=n.obs.

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LYPSIKKI – a novel approach for the formulation of dairy cow rations

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Introduction

The dairy cow ration formulation and economical optimization is facing several challenges within the current EU market. Firstly, the prices of feed components (both grain and protein feeds) and raw milk are fluctuating according to existing market condition. Traditionally ration formulation systems have been based on static feed values and standard feeding recommendations. However, with variable feed costs and milk prices this approach seldom maximizes the margin over feed cost, i.e. the optimal solution may be higher or lower feeding intensity than the average recommendations. Milk quota system can further complicate the situation. Secondly, metabolisable energy (ME) and protein (AAT) intake may not completely explain the variation in responses to the changes in feed intake and diet composition. In addition to ME and AAT intake dietary characteristics like fat concentration, components that increase glucose supply and carbohydrate composition may influence both milk output and composition. Consequently, an ideal ration formulation system (1) includes an intake model that takes in account both dietary and animal characteristics, (2) calculates the true nutrient intake with varying feeding level and diet composition and (3) predicts realistic production responses according to ME and AAT intake and diet composition. The objective of this project was to develop and validate a ration formulation system that optimizes margin over feed cost (per cow or liter of milk) in addition to traditional minimum diet cost solution. The model is based on the Finnish feed evaluation systems (MTT 2010) for energy (ME) and metabolisable protein (AAT). The dry matter (DM) intake (DMI) model used in this study is based on relative total diet intake index (Huhtanen et al. 2008) and integration of the dietary and animals characteristics (Huhtanen et al. 2011) to obtain absolute DMI estimates. ME intake is discounted for feeding level and associative diet composition effects (Huhtanen et al. 2009). The basic model construction of LYPSIKKI is described in Nousiainen et al. (2011). In this paper the further model development, validation and prerequisites of use are presented and discussed.

Materials and Methods

The model named Lypsikki is a whole farm nutrient management model, which includes a dairy cow ration formulation tool (Nousiainen et al., 2011). Ration balancing is made using the mean daily milk yield and composition for the whole lactation that can be justified, because the effects of milk production and composition on nutrient requirements are linear (Mäntysaari et al., 2003, 2005). The feeding of dairy cows was balanced on a daily basis to meet the requirements of ME, AAT, Ca, P, Na and Mg calculated according to MTT system (MTT, 2010). The concentrations of ME (MJ/kg DM) and AAT (g/kg DM) in forages were calculated from digestible organic matter (D-value, g/kg DM) and CP (g/kg DM) concentrations. The ME and AAT values for concentrate feeds were calculated using analyzed chemical composition and tabulated digestibility and degradability coefficients (MTT, 2011). The total ME requirement of the cows was calculated as follows:

$$\text{ME requirement (MJ/d)} = 0.515 \times LW^{0.75} + 5.15 \times ECM \quad [1]$$

where LW = live weight (kg) and ECM = energy corrected milk yield (kg/d). Calculated ME intakes based on ME concentration at maintenance level of feeding in sheep were discounted for the feeding level and associative effects (dietary concentrations of CP and ME at maintenance) to correspond "true" ME intake at actual intake level. The equation based on more than 500 diets was derived from Huhtanen et al. (2009). The requirement of AAT was calculated as follows:

$$\text{AAT (g/d)} = 1.8 \times LW^{0.75} + 14 \times \text{TDMI} + (1.46 - 0.0017 \times \text{ECM}) \times \text{PY} \quad [2]$$

where TDMI = total DM intake (kg/d) and PY = milk protein yield (g/d). The optimal diet composition (least feed cost or maximal margin over feed cost) were formulated fulfilling the nutrient requirements within the limits of intake potential. The DM intake potential (DMI_{max}) was calculated according to Huhtanen et al. (2011) using the following equation:

$$DMI_{max}\text{ (kg/d)} = -2.9 + 0.0148 \times \text{LW (kg)} + 0.258 \times \text{sECM (kg/d)} + 0.09 \times \text{TDMI index} - 0.0175 \times \text{DIM} - 5.85 \times \text{EXP}(-0.03 \times \text{DIM}) \quad [3]$$

where TDMI index describes the relative intake potential of the diet, sECM the standardized ECM yield and DIM days in milk (d). The factor sECM describes the genetic potential and permanent management level of the dairy herd and is determined within herd before ration formulation from observed milk yield and diet characteristics as follows:

$$\text{sECM (kg/d)} = \text{ECM (kg/d)} + (100 - \text{TDMI index}) \times 0.131 + [90 - \text{AAT (g/kg DM)}] \times 0.142 + -0.0481 \times (150 - \text{DIM}) + 6.96 \times [\text{EXP}(-0.07 \times \text{DIM})] \quad [4]$$

The animal (sECM, LW) and dietary (TDMI index, AAT) characteristics are independent constraints of DMI.

The optimization of rations was made by using the Solver tool (Fylstra, 1998) in Microsoft® Excel that employs the Generalized Reduced Gradient non-linear optimization code (Lasdon, 1978). The diets were optimized to meet ME, AAT and mineral (Ca, P, Na and Mg) requirements within the constraints of intake potential, a healthy diet (minimum forage NDF) and maximum non-fibre carbohydrates (NFC) in diet DM. It is also possible to set constraints for excess N and P output per kg milk produced. The feeding cost of on-farm produced forage is a sum of the total cost (fixed plus variable) until the minimum amount of forage is fulfilled (8 kg DM /d) and variable cost for forages fed above minimum requirement. A value of 33 eurocents/kg DM was assumed for the fixed cost of forage (this value does not influence optimal ration composition). A variable cost of 8 eurocents per kg DM was used on farm produced silages, but zero value can be used for forages in silos to optimize the utilisation of forages in the storage. For concentrate ingredients, market prices were used in optimization. The performance of the cows fed optimized rations was predicted from calculated nutrient supply using empirical polynomial regression equations derived from a large data set from milk production trials with cows fed predominantly grass silage-based diets. Mixed model regression analysis was used to remove the study effect. The data from studies with average daily milk yield above 26 kg/d (1098 treatment means in 245 studies) were used in the model tool to correspond better the current production levels. The equations for milk, ECM and protein (N × 6.38) are presented in Table 1. The fat yield is then

calculated assuming a constant lactose concentration (48 g/kg). For estimation of milk prise the milk concentrations of fat and protein are derived as component yield/milk yield.

Results and Discussion

Prediction models of milk, ECM and protein yield derived from the whole data set are shown in Table 1. In Lypsikki only the data from studies with mean milk yield above 26 kg/d were used. In addition to ME and AAT intake the concentrations non-fibre (NFC) carbohydrates and concentrate fat influenced production in a quadratic manner. Feed AAT rather than total AAT were used in the models, since microbial AAT is almost completely correlated to ME. Adjusted root mean squared errors were only marginally greater than random variation in production trials with dairy cows assuming 5% residual coefficient of variation and 10 cows per treatment.

Table 1 Production response equations for milk, ECM (kg/d) and protein (g/d) yields derived from milk production trials[b] with cows fed predominantly grass silage-based diets and using mixed model regression analysis to remove the study effect.

Item	Milk	ECM	Protein
Intercept	5.30	1.84	122
Production ME intake (MJ/d)	0.140	0.175	3.25
Production ME intake $(MJ/d)^2$	-0.00018	-0.00023	
Feed AAT (g/kg DM)	0.109	0.071	
Feed AAT intake (kg/d)			513
Feed AAT intake (kg/d)			-242
Non-fibre carbohydrates (kg/kg)	19.3	28.3	521
Non-fibre $carbohydrates^2$ (kg/kg)	-32.5	-51.7	-702
Concentrate crude fat (g/kg DM)	0.0913	0.0473	1.21
Concentrate $crude\ fat^2$ (g/kg DM)	-0.00105	-0.00067	-0.0176
DIM (d)	-0.0224	-0.0146	-0.434
Adjusted root mean square error [a]	0.424	0.497	15.2

[a] Adjusted for random study effect; [b] n=1098 from 245 studies.

All production models showed diminishing returns to increased supply of ME available for production (ME intake – ME for maintenance) and Feed AAT. In practical ration formulation it is important to determine the current ME (and AAT) status of the herd. The principle is demonstrated in Figure 1. For example, if the cows are producing more than expected from their ME intake (point A), i.e. they are in a negative ME balance, increasing ME intake (ΔME) to point B would produce a greater production response than the corresponding ΔME from B to C.

When the diet is optimized for maximum income over feed cost, dietary ingredients that produce a more optimal dietary fat and NFC concentrations are preferred at the same ME and AAT price.

An example of formulation the ration according to minimum diet cost and maximum margin over

feed cost is shown in Table 2. With feed and milk prices used the margin over feed cost feed cost clearly increased when the diet was optimized for maximum margin over feed cost. The

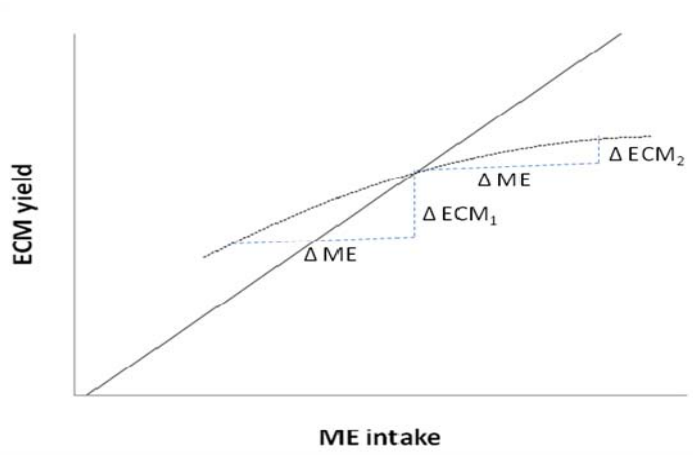

Figure 1 A schematic presentation of ECM responses within a herd to additional ME intake; with the same Δ ME the responses (ΔECM_1 and ΔECM_2) depend on the current feeding intensity. Solid line describes the response according to feeding standards (0.194 kg ECM/MJ ME).

changes depend on the current feeding intensity. Therefore it is important to use the standardized ECM yield in intake prediction model, since a greater production as a result improved diet does not increase the intake potential of the cow. It is also possible to optimize rations when the P (or N) surplus is constrained that allows to compare strategies to mitigate P (or N) emissions.

Conclusions

Developed ration formulation model Lypsikki is a flexible tool for the optimization of the economy of milk production under conditions with rapidly changing feed and milk prices. It also allows comparing strategies in mitigation of N and P surplus from dairy farms. There are several prerequisites of the model: (1) accurate and precise forage analysis, (2) feed evaluation systems that describe accurately and precisely relative productive values of feedstuffs, (3) an intake prediction model where animal and dietary factors are not confounded, (4) models that predict accurately production responses to the changes in nutrient supply and (5) accurate estimation of the current feed intake and production. As an example of (2), most feed protein evaluation systems predict a lower protein value (AAT/CP) for rapeseed meal than for soybean meal (relative value of rapeseed protein 0.80-0.90), but in a meta-analysis of data from milk production trials marginal milk protein yield response to increased CP intake was significantly greater (130 vs. 100 g/kg CP) for rapeseed meal (Huhtanen et al., 2011). With such failures in predicting the true productive values of feeds optimization according to margin over feed costs are not likely to improve traditional optimization for minimum diet costs.

Table 2 An example of ration formulation with Lypsikki in a herd with sECM 28.2 kg/d and LW 600 kg. Diets were optimized for minimum feed cost (R1) while meeting the requirements of current production or for maximal margin over feed cost without (R2) or with constraint for milk phosphorus surplus (R3, max 1.4 g P/kg).

Ration optimization	**Feed price cnt/kg DM**	**R1**	**R2**	**R3**
Feed intake, kg DM/d[a]				
Grass silage (D-value 690 g/kg DM)[b]	0	10.16	8.58	9.89
Whole crop silage (D-value 640 g/kg DM)[b]	0	4.27	3.94	
Barley	17	3.58	4.76	
Oats	17		1.65	8.56
Molassed sugar beet pulp[c]	22			2.00
Rapeseed meal	31	2.20		
Rapeseed expeller	31		2.79	
Soybean meal	45			0.92
NaCl	35	0.07	0.07	0.04
$CaCO_3$	35	0.11	0.11	0.11
Total DM intake		20.39	21.89	21.52
Predicted production				
Milk yield, kg/d		27.1	30.5	29.9
Protein concentration, g/kg		34.2	33.8	33.6
Fat concentration, g/kg		45.0	43.0	43.1
N surplus, g/kg		12.5	13.1	12.5
P surplus, g/kg		1.9	2.1	1.4
Feeding economy				
Milk income, €/d		10.75	11.88	11.64
Feed cost, €/d		2.98	3.64	4.01
Margin over feed cost, €/d		7.77	8.24	7.63

[a] Feed values from MTT (2010); [b] Variable cost 0 cnt/kg DM (feeds in storage with no alternative use); [c] Maximum amount allowed 2 kg DM/d.

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COMPARISON OF CARROTS AND RED PALM OIL AS SOURCE OF CAROTENOIDS IN FEED

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Introduction

Milk used for consumption or dairy processing should have a high antioxidative capacity to achieve high quality products. Feeding is one of the most significant factors influencing cow productivity, composition and quality of milk. It could be expected that milk from cows fed silage have higher concentration of β-carotene and α-tocopherol than milk from cows fed hay. Supplementing feed with carotenoids is a possibility to increase the content of antioxidants in milk, to increase milk fat quality and to prolong storage. Carotenoids are involved in the sensorial and nutritional values of dairy products. Content of α-tocopherol and β-carotene may be insufficient in the basal feeds at the end of indoor season. Presently, NRC (2001) recommends a β-carotene intake of 300 mg per cow per day, suggesting that the plasma β-carotene concentration in dairy cows should be >3mg L^{-1} to optimize udder health. Since animals cannot synthesize carotenoids and animal feed is generally poor in carotenoids, about 30-120 mgkg^{-1} of total carotenoids, are added to animal feed to improve animal health, enhance milk color and quality, and increase vitamin A levels in milk (Ananda and Vadlani, 2010). It has been demonstrated that carotenoids and retinol are able to reduce mastitis in dairy cows (Chew, 1995), although the effect of β-carotene was not systematic (Oldham et al., 1991). Amongst the richest sources of carotenoids are crude palm oil (0.05 to 0.2 %) (Stołyhvo, 2007) and carrots (0.006 to 0.055 %) (Kotecha et al., 1998) containing mainly α- and β-carotenes.

The aim of our investigation was to compare carrots and red palm oil as sources of carotenoids in cows feeding.

Materials and methods

Total carotenoids in feed samples of silage (39), haylage (13), hay (13), rapeseed cake (10) from 6 farms in Latvia from January till March were analysed. One of the farms where the content of total carotenoids in silage was insufficient (4.3 mg kg^{-1} DM) was selected for supplementations with feeds rich in carotenoids.

Milk samples were obtained from the selected dairy cow herd. Ten cows in 1st-3d month of lactation were selected and divided into two groups of five cows each. Cow breeds (Holstein, Latvian Brown and mixed) and lactation number (1-5) were similar in both groups. Milk samples were obtained from afternoon milking at 7, 24, 35 and 42 days after the beginning of feed supplementation and pooled per time and group.The basic feed was identical for both groups and consisted of silage ad libitum and rapseed meal - 2 kg per cow per day. In the carrot supplemented group (CG), additionally 7 kg carrots and rapeseed oil 100 g per cow per day was fed. In the palm oil supplemented group (POG), 100 g red palm oil "Carotino" was fed daily as a source of carotenoids. The composition of the basal feed and supplements is given in a Table 1.

Table 1. Diet ingredients and chemical composition

Item	Treatment	
	CG (carrots)	POG(red palm oil)
Diet ingredients (kg/d)		
Silage	ad lib. ~65-70kg	
Rapseed meal	2	2
Carrots	7	-
Rapseed oil	0.1	-
Red palm oil "Carotino"	-	0.1
Carotenoids: mg/day		
Total	1311	275
β-carotene	1090	252
α-carotene	221	22
lycopene	-	0.8
Total tocopherols	210	640

Milk samples were analyzed for fat, protein and lactose by automated infrared spectroscopy (method ISO 9622-1999) and somatic cell count (SCC) was determined by "Somacount 300" in the laboratory of Milk Quality Control of the Sigulda CMAS.

Milk (10 ml) was mixed with 10 ml of isopropanol and 5 ml of hexane: toluene (10: 8 vv^{-1}). The tubes were centrifuged at 4ºC and 2800 rpm for 5 min. The top layer was transferred to a clean container. The bottom layer was mixed with 3 ml of hexane: toluene mix, centrifuged and the extraction was repeated three times. Collected supernatants were evaporated under a gentle stream of N_2 on a warm plate at 40°C until dryness and dissolved in 5 ml of ethanolic butylhidroxitoluene (2 g l^{-1}) and 5 ml 2 *M* saturated potassium hydroxide solution. Samples were placed in a water bath at 40ºC for 30 min, than cooled in an ice water bath. The samples were added to 10 ml deionized water and centrifuged. The extraction was repeated 3 times, and supernatants were collected. The extracts were evaporated to dryness under N_2. The residues were dissolved in 2 ml of methanol, filtered, and 80 µl was injected on an HPLC (Waters Alliance 2695 HPLC equipped with 150x4.6mm, RP C18 column and diode array detector). Vitamin A was detected at 325 nm, tocopherols at 292 nm, and β-carotene at 475 nm.

Methods involving TBA as an analytical reagent are widely used to follow the progress of oxidation in dairy products. The method of King for the determination of 2-thiobarbituric acid reactive substances (TBARS) in milk (King, 1962) was used, and the result was expressed as the absorbance at 536 nm, The TBARS were measured at 1, 2, 3, 4 and 5 days of storage and with three replicates for each sample.

The microbiological examination of milk was performed at Day 1 and 5. Increase of the total plate count and psychrophilic bacteria of milk determined in accordance with "Standard Methods for the Examination of Dairy Products: 16th Edition", 1992.

Results and Discussion

Carotenoid content in cow feeds often is not sufficient, especially during the winter and spring months when they have become depleted. The content of total carotenoids in silage samples varied from 4.3 to 168.5 mg kg^{-1} DM with an average amount of 87.6±15.5 mg kg^{-1} DM. One third of the silage samples had total carotenoids contents lower than 50 mg

kg^{-1} DM at the end of indoor period and could therefore not provide cow sufficient levels of bioactive substance. The content of total carotenoids in haylage samples varied from 0.4 to 12.8 mg kg^{-1} DM. Results of hay samples showed total carotenoid below the detection level (<0.005 mg kg^{-1} DM) in 8 of 13 samples. Average content of carotenoids in feed samples of haylage, hay and rapeseed cake was 7.6±0.5; 1.8±0.5; 2.9±1.1 mg kg^{-1} DM respectively.

Comparison of average milk yield and composition in time of experiment are showed in Table 2.

Table 2 Comparison of milk yield and composition (CG=carrot group; POG=palm oil group).

Parameters	Treatments	
	CG	POG
Milk yield, kg day^{-1}	23.13±1.131	23.44±0.962
Fat content, g 100 g^{-1}	4.39±0.239	4.06±0.180
Protein content, g 100 g^{-1}	3.19±0.153	3.13±0.155
Somatic cell count, 10^3 ml^{-1}	171.20±12.300	109.00±22.365
β-carotene, μgL^{-1}	332±14	274±11
vitamin A, μgL^{-1}	488±12	377±12
vitamin E, μgL^{-1}	622±9	551±11

There were no differences ($p>0.05$) between groups in average milk yield, protein content and SCC. However, the fat content was higher ($p<0.05$) in milk samples where cows received carrots as supplement. Average content of β-carotene, vitamin A and vitamin E in milk was higher in carrot supplemented, than in red palm oil supplemented cows but the difference was not significant ($p>0.05$). Carotenoid availability for secretion in milk is governed by their transport into lymph and plasma, their metabolism within tissues (especially conversion into vitamin A and by utilization as pigments or antioxidants), as well as storage in adipose tissues or secretion into bile by the liver. Also, a specific effect of stage of lactation has not been clearly established, and this could be an important factor affecting concentrations of β-carotene, and other micronutrients, in milk (Noziere, et al., 2006).

The SCC in all milk samples did not exceed 400 000 ml^{-1}, and, during the experiment, SCC decreased in both groups (Figure 1). This tendency of SCC decrease is in accordance to previous findings (Chew, 1995), showing that carotenoids have positive role on cow health, especially mastitis prevention.

As seen from trend line slope coefficients (Figure 1), the tendency of milk SCC decrease was -51.46 in the CG and -38.91 in the POG group.

Storage time measurements of TBARs on milk sampled at 7, 24, 35 and 42 days after start of supplementation were similar for both groups. Results of TBARs measurements in milk samples collected at 24 and 35 days of experiment showed in Figure 2. As seen from Figure 2, the two groups did not differ. Amount of TBARs did also not seem affected by storage time.

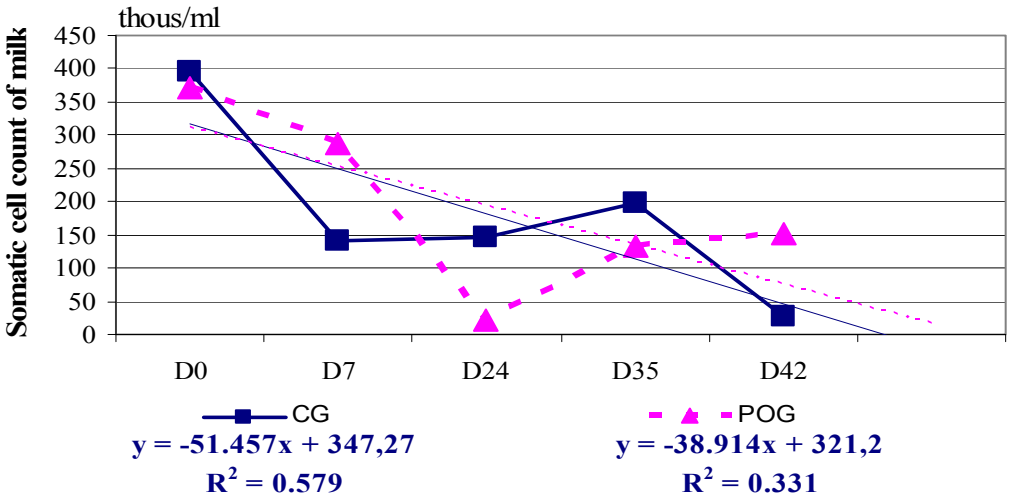

Figure 1. Somatic cell count in milk obtained from the two groups (CG=carrot supplemented group; POG=palm oil supplemented group).

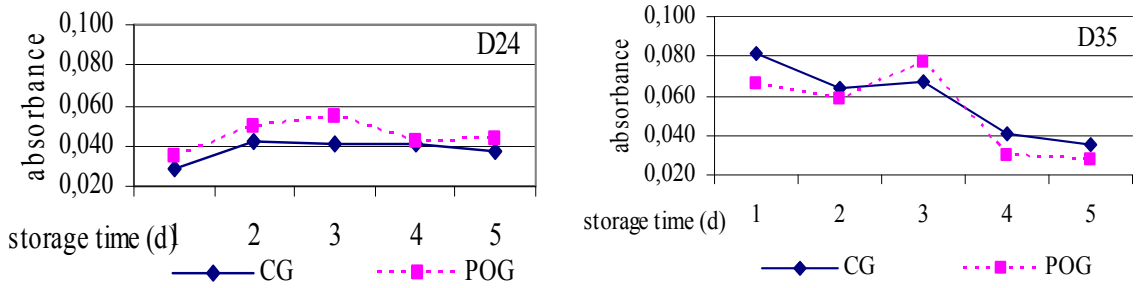

Figure 2. Results of TBARs measurements in milk samples collected at 24 (D24) and 35 (D35) days after start of feed supplementation as affected by storage time. (CG=carrot supplemented group; POG=palm oil supplemented group).

The total plate count after one and five days of storage in samples collected at 7, 24 and 42 days after start of supplementation are showed in Figure 3. After 5 days storage at the temperature 4-6^{0}C total bacterial count increased more slowly in the milk samples from cows received red palm oil. The growth of psychrophilic bacteria in milk sampled in D24 and D35 was less intensive in POG as in CG. This appears as a positive effect, because many

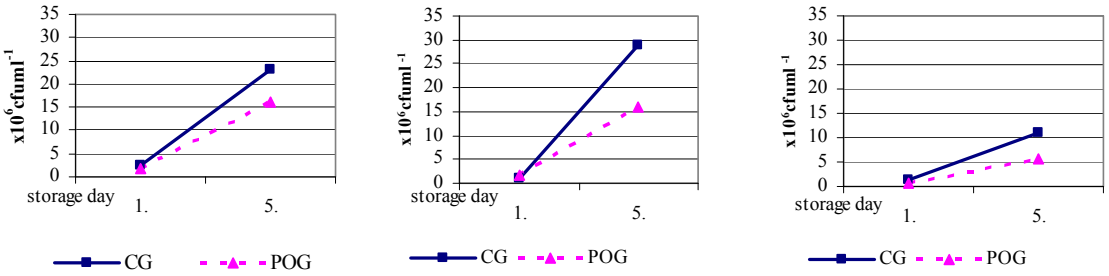

Figure 3. The total plate count difference at 1st and 5th day of storage in milk samples collected at 7 (left), 24 (middle) and 42 (right) days after starting feed supplementation. (CG=carrot supplemented group; POG=palm oil supplemented group)

microorganisms of this group are unfavourable for the dairy industry, leading to spoilage of milk products. Many of them are lypolytic, causing the development of rancid flavor. It could be explained with higher content of antioxidants α-tocopherol and lycopene in red palm oil in comparison with carrots.

From results of milk yield and composition we can see that carrots were more useful as a feed supplement at end of indoor season in comparison with red palm oil as a result of higher β-carotene content, same impact on milk quality and for being more readily available than palm oil.

Conclusion

Feeding carrots had similar effects on milk yield and composition as red palm oil despite having a higher content of β-carotene. Total plate counts and levels of psychrophilic bacteria in milk were lower in milk from cows receiving red palm oil as supplement.

Acknowledgements

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Buffering capacity and pH values of Swedish agricultural crops at harvest time

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Introduction

Chemical and microbiological composition of a crop determines its ensilability as a precondition for successful ensiling process. Buffering capacity (BC) is a commonly used parameter to predict an ensilability of crop. Weissbach et al. (1974) suggested BC, concentration of water soluble carbohydrates (WSC), and dry matter (DM) concentration of a forage crop to be markers to predict its ensilability. The influence of these parameters on forage conservation has been expressed in a fermentation coefficient (Weissbach et al., 1974). Besides these, there are other parameters, which were suggested as factors affecting ensilability of a crop, such as nitrate content of a crop or crop pH (Leibensperger & Pitt, 1987). The pH value of a fresh crop has not been extensively studied although it seems to be an important parameter when silage additives based on weak organic acids (sorbate and benzoate) are used (Internal report). The effectiveness of these silage additives is closely related to substrate pH. Since the use of silage additives means additional costs, precise estimation of an effective dosage has economical consequences for farmers as well as it may reduce possible environmental impact. The aim of the study was to survey pH of common fresh forages used for ensiling in Sweden.

Materials and Methods

A total of 33 samples were selected in a screening study of pH and BC in fresh legumes, grasses, and whole-crop cereals. Forages were harvested from May 24th to October 7th 2010, mostly nearby Uppsala. Different maturity stages within crop were obtained from the same plot, except for whole-crop oat which was harvested at milk stage. Forage samples were harvested manually using a pair of scissors and stored in plastic bags at 5°C, in case of immediate processing. Samples collected from long distance locations were transported frozen. Samples were then chopped in a stationary cutter to approximately 5 cm particle length. Samples were analysed for dry matter (DM), BC, ash, and pH. The concentration of DM was analyzed in 2 steps. First, fresh samples weighing approximately 150 g were dried for 18 h in a ventilated oven at 65°C and milled through a 1.0-mm sieve. Final DM concentration was achieved by drying at 103°C for 5 h. Ash was determined by combustion at 550˚C for 3 h in a muffle furnace. Forage pH was determined using a pH electrode (654 pH-meter Methrom AG, Herisau, Switzerland) in forage extract obtained by pressing. Dried and milled samples were used to determine buffering capacity of crops by titration with lactic acid according to (McDonald & Henderson, 1962)

Results and Discussion

Analyses of pH and BC in legumes (Table 1) confirmed that legumes have a high BC (McDonald et al. 1991), except for the red clover sample harvested at vegetative growing stage. Average pH value in legume crops was 5.7±0.2 without large variations, except for a lower pH in Alsike clover in comparison with other legumes. Grasses (Table 2) had slightly higher average pH value (5.9±0.1) than legumes. BC in grass crops was expectedly lower than in legumes (McDonald & Henderson, 1962), except for timothy harvested at early

heading stage. Unlike in legumes, pH and BC decreased with maturation in tall fescue and timothy. This BC decline corresponds to the results obtained by Muck et al. (1991).

Table 1 Buffering capacity (BC) and pH values in legume crops

Forage	pH	BC (g lactic acid/kg DM)	DM[a] (%)
Alsike clover, at flowering	5.4	67.3	17.2
Birdsfoot trefoil, at flowering	5.7	57.6	24.3
Peas, soft dough maturity	5.9	82.4	28.8
Red clover, before flowering	5.9	37.9	n.d.
Red clover, at flowering	5.8	53.5	24.8
White clover, at flowering	5.7	76.6	25.3
White clover, after flowering	5.8	80.0	n.d.
Mean	5.7	65.1	
Standard deviation	0.2	16.3	

[a]DM=dry matter; n.d.-not determined.

Table 2 Buffering capacity (BC) and pH values in grass crops

Forage	pH	BC (g lactic acid/kg DM)	DM[a] (%)
Cocksfoot, at flowering	6.0	36.4	48.7
Meadow fescue, after flowering	5.8	44.5	25.9
Tall fescue, at flowering	6.0	44.4	16.2
Tall fescue, after flowering	5.8	32.8	36.8
Timothy, at flowering	6.0	70.4	13.0
Timothy, after flowering	5.7	23.8	36.6
Mean	5.9	42.1	
Standard deviation	0.21	15.9	

[a]DM=dry matter.

Similar to grasses, BC of whole-crop cereals decreased as crop matured (Table 3). However, unlike to grasses, pH of the small grain whole-crop cereals increased with maturity. Fully ripened, this crop reached the highest pH values of all crops included in study. These values contrasted to those observed in whole-crop maize, where pH decreased as crop matured. All whole-crop cereals had a low BC, similar to McDonald et al. (1991).

Investigation of changes in pH and BC in clover-grass mixtures over the growing season is presented in Table 4. The most substantial change was observed in crop pH, where wilting of the

crop was reflected in a pH decrease. Similarly, McDonald & Henderson (1962) observed that wet crops had a higher pH than dry crops. However, the effect of wilting on pH reduction was not repeatedly observed in the 2nd cut. Wilting showed no clear effect on BC in clover-grass mixture.

Table 3 Buffering capacity (BC) and pH values in whole-crop cereal crops

Forage	pH	BC (g lactic acid/kg DM)	DM[a] (%)
Barley, soft dough maturity	6.2	38.9	38.3
Barley, at ripening	7.3	29.9	66.6
Oat, milk maturity	5.8	46.1	25.9
Oat, early milk maturity	5.7	43.9	34.4
Oat, dough maturity	6.0	31.9	44.2
Oat, at ripening	7.2	33.4	78.4
Wheat, milk maturity	6.1	30.2	38.7
Wheat, at ripening	7.2	22.0	73.9
Maize,whole-crop, milk maturity	5.8	21.4	22.4
Maize cobs, milk maturity	6.6	34.1	36.1
Maize stem + leafs, milk maturity	5.7	25.3	18.6
Maize,whole-crop , dough maturity	5.3	30.6	36.1
Mean	6.2	32.2	
Standard deviation	0.7	7.8	

[a]DM = dry matter.

Crop pH plotted against BC is shown in Figure 1. There was no significant correlation between crop pH and crop BC (P=0.2) across all samples. It could have been due to lack of samples for detecting such a correlation. On the other hand, there was a strong linear correlation ($P < 0.001$) between ash content and crop BC (Figure 2). Similarly, Behgar et al. (2009) observed a significant correlation between feed BC and ash content. It is suggested that the correlation between crop BC and ash is due to presence of minerals in crop ash which increase the BC.

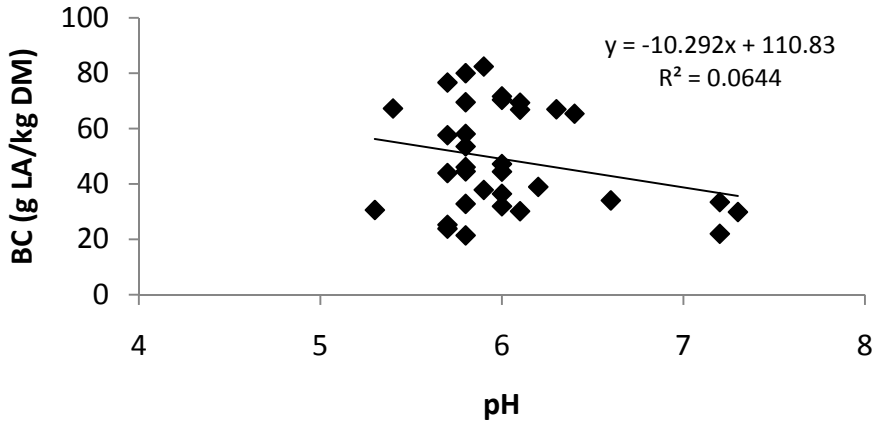

Figure 1 Relation between pH and buffering capacity (BC) in fresh forages.

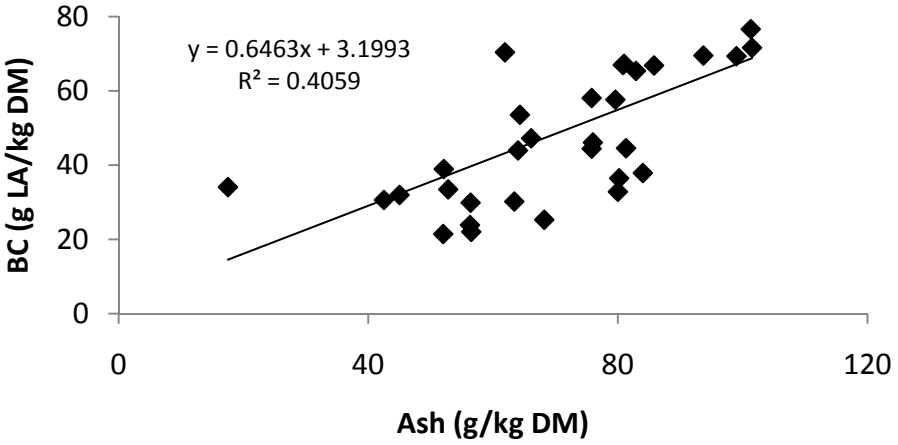

Figure 2 Relation between ash content and buffering capacity (BC) in fresh forages.

Table 4 Buffering capacity (BC) and pH values in a clover-grass ley over the growing season

Forage	Crop composition		pH	BC	DM[a]
	Clover proportion	Maturity		(g lactic acid/kg DM)	(%)
Early 1st harvest	24%	Vegetative	6.1	69.4	16
1st harvest	25%		6.4	65.4	19
1st harvest wilted	25%	Late vegetative	6.3	67.0	33
1st harvest wilted	25%		6.1	66.8	44
Late 1st harvest	35%	Bloom	5.8	58.1	23
Late 1st wilted	35%	Bloom	6.0	47.2	59
2nd harvest	54%	Late bloom	5.8	69.5	32
Mean			6.1	63.3	
Standard deviation			0.2	8.1	

[a]DM=dry matter.

Conclusions

Caution should be taken in drawing too far conclusions from the present screening study because of the limited number of observations from each sample type. Nevertheless, grasses and whole-crop cereals had expectedly a lower BC than legumes and legume containing crops. Maturity seems to influence both crop pH and BC but varying depending on forage type. The effect of wilting on reduction of crop pH was inconsistent. There was no correlation between pH and BC for the crop samples in this screening but there was a linear relationship between BC and ash content.

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Novel crop options for beef cow grazing – a review

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Introduction

Traditionally the length of grazing season in Finland is 3-4 months. Beef cow producers should aim for relatively long grazing period as in most cases grazed forage is cheaper than mechanically harvested feed. Our climatic conditions often impose restrictions for practical, long grazing season which is used in most large beef producing countries. Wet and muddy field conditions can pose a problem both for the beginning and for the extension of the grazing season. Tramping of soggy pasture should be avoided to maintain the growth aptitude of perennial pasture for next growing season.

The type of beef cattle and their production stage will determine their nutritional needs. Grazed forages must therefore produce enough dry matter per hectare and the yield should come at such a time as it can be used efficiently. The nutritional requirement of a spring calving beef cow is the highest during the lactation period. The peak of beef cow's lactation curve is reached approximately 9 weeks after calving (Jenkins & Ferrell, 1992). Beef cows that calve during 6-8 week period between March and April will use the rapid growth rate of pasture herbage most efficiently (Virkajärvi et al., 2003; Field, 2007).

Beef cow's dry matter intake increases as energy requirement increases due to milk production level (Figure 1). The growth rhythm of different forage crops varies. Beef cow operation could optimize grazing season extension by using several different forage crops (Figure 1).

In the beginning of the growing season the pasture herbage growth is relatively fast. The perennial herbage production declines in the point when calves dependency on herbage growth increases (Figure 1). Pasture quality should be maintained relatively high to meet the calves' growth potential. Sward height is considered as the main management criterion in with perennial grasses. Beef cows cannot gain body condition score efficiently and calves growth performance is depressed when the sward height is under 8 cm (Virkajärvi et al., 2006; Lively, 2007). There might be several crop options which could be used in to extend our beef cattle grazing season without compromising production goals.

The purpose of this work was to study the yield potential of a number of forage crops and find useful forage crop options for beef cow grazing systems, particularly for extension of the grazing season in autumn and early winter.

Annual ryegrasses

Ryegrasses are the most used perennial grasses in Europe. Ryegrasses are not very winter hardy in Finland therefore they are mainly used in annual seed mixtures (Peltonen et al., 2010). Annual ryegrasses could be used as summer annual from mid-summer to late fall to help extend the grazing season. Ryegrasses have rapid growth rate in spring and rotational grazing is most suited practice early in the grazing season (Phillips, 2010). Italian ryegrass grows more in mid-summer and into autumn. Italian ryegrass does not have stem forming in the first growing season so the nutritional value is remained even when grazed late in the autumn (Peltonen et al., 2010).

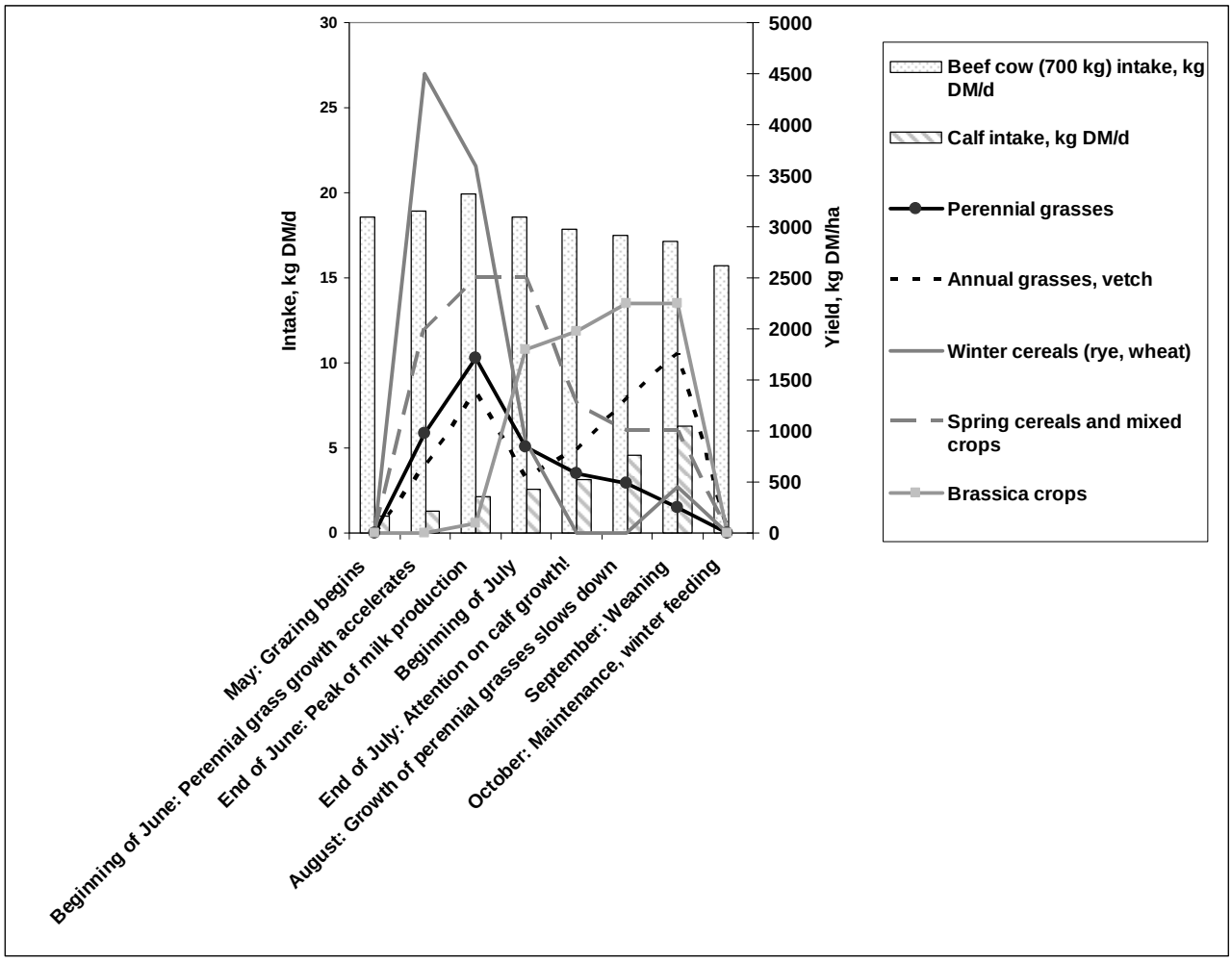

Figure 1 Daily DM intake, kg of a beef cow-calf pair in grazing season and yield potential of different forage crop options (Jarrige, 1989; Kangas & Harmoinen, 2010).

Brassica crops (fodder beet, fodder kale and fodder rape)

Brassica crops can have high yield potential per hectare and their growing period is longer than perennial crops. They are frost tolerant and can provide late-season grazing option for beef cow operation (Kunelius et al., 2003; McCartney et al., 2009). Grazing animals can consume both root and leaf portion of the plant. The tuber grows well above the ground surface in fodder beet varieties best suited for grazing purposes (Seed Force, 2010). The root or tuber is rich in energy and the leaf portion usually has more protein. The fibre content of these crops is too low to maintain normal ruminal function when fed as the sole forage for ruminants. The high moisture and low fibre content results in cattle becoming very dirty with loose manure. Feeding old hay or straw has been shown to increase the fibre intake and slow the rate of passage in the digestive tract (Andrews et al., 2004).

Brassica crops can cause animal health disorders if not grazed properly (Andrews et al., 2004). The best practice is to strip-graze *Brassica* crops behind an electric fence. Brassica pastures should be introduced for the animals slowly over 4-7 day period (Kunelius et al., 2003; McCartney et al., 2009). Health disorders can be avoided by complementing the ration

with hay, straw or provide ability for the animals to graze grass pasture at the same time (Kunelius et al., 2003).

Maize and millet

In Northern America there has been considerable interest in evaluating the grazing potential of warm season annual crops for their suitability in extending the grazing season because warm-season annuals tend to be seeded later and vary in days to maturity (May et al., 2007). This could be advantageous in grazing system for extending the grazing season. The interest in grazing standing maize is to avoid the costs of conventional harvesting and storage. In areas where maize is harvested as a grain crop, the stover or crop residue is used for late fall and winter grazing for weaned calves and dry pregnant beef cows. The quality of ration depends upon the amount of grain left in the field after harvesting (Field, 2007). Grazing standing maize is rarer. The lack of undergrowth and muddy field conditions has limited the adoption of standing maize grazing (McCartney et al., 2009).

Millets include numbers of cultivated semi-arid tropical annual grasses such as proso millet and foxtail millet. Proso millet is less leafy and has lower palatability for grazing than foxtail millets (McCartney et al., 2009). Proso millet is a short season crop with low water requirement and rapid maturity, it also grows further north than other millets (McCartney et al., 2009). Proso millet dry matter yield has been found to be similar to oat and barley (6 150 kg DM /ha) in Canada (May et al., 2007). Foxtail millet is generally taller, later maturing and better suited for forage production than proso millets. Foxtail millet is more palatable for grazing than proso millet, but cattle must need to adapt to it (McCartney et al. 2009). Foxtail millet maximizes yields as both growing degree days and rainfall increases (6 350 kg DM/ha) (May et al., 2007).

Millet and maize are sensitive to frost but swath grazing might be an option. Swath grazing of cereals is a low-cost system used for late fall and winter grazing for pregnant beef cows (McCartney et al., 2008). Cereals can be swathed at the soft dough stage; the swaths are left to the fields and later grazed limiting the access to the swaths by electric fencing (Agri-Facts, 2004). Swathing maize can be difficult due to the height and volume of the crop (McCartney et al., 2009). Low growth and late forage maize varieties could be more suited for swath grazing. Swathing near date of first frost could be best option for millet as millet seem to be even more sensitive to frost damage. This would reduce swath deterioration due to weather, cool weather also should maintain the nutritive value of forage for fall and winter grazing. Millet will resist some weathering in the swath due to a thick waxy coating over its leaves and stems (ARECA 2006).

Findings from Finland

Seven different forage crop options were planted on 20.5.2010 in three different experimental locations: Maaninka, Ruukki and Ylistaro. Seeding rate for faba bean-wheat mixture was either 49:195 seeds/m^2 (mixture 70:30) or 35:325 seeds/m^2 (mixture 50:50). Millet, kale and forage rape were seeded 5, 5-8 and 8-11 kg/ha, respectively. Fodder beet had 15 cm spacing between each crop. The crops were fertilized with N-P-K as follows (kg/ha): Fodder beet 140-43-220, forage rape 100-10-60, kale 100-70-170, faba bean-wheat mixture 50-15-30 and millet 100-15-70. Faba bean-wheat mixed crop and millet was harvested 6.8.2010 in Maaninka and 17.8.2010 in Ruukki. Millet was harvested after first frost because the frost damage for the crop was significant. Fodder beet, kale and forage rape was harvested 5.10.2010.

The dry matter (DM) yields of the harvested forage varied. Challenging growing conditions eg. very little rainfall in Maaninka area might have affected the results. Dry matter (DM) yields/ha (Figure 2) were highest for fodder beet (7 000-15 796 kg DM/ha). Kale and forage rape produced relatively high DM yields/ha (3 499-10 866 kg DM/ha). Faba bean-wheat DM yield/ha was average (6 760-7 952 kg DM/ha). Millet produced lower DM yield than was expected (2 194-4 745 kg DM/ha). Drought and early frost affected millet the most.

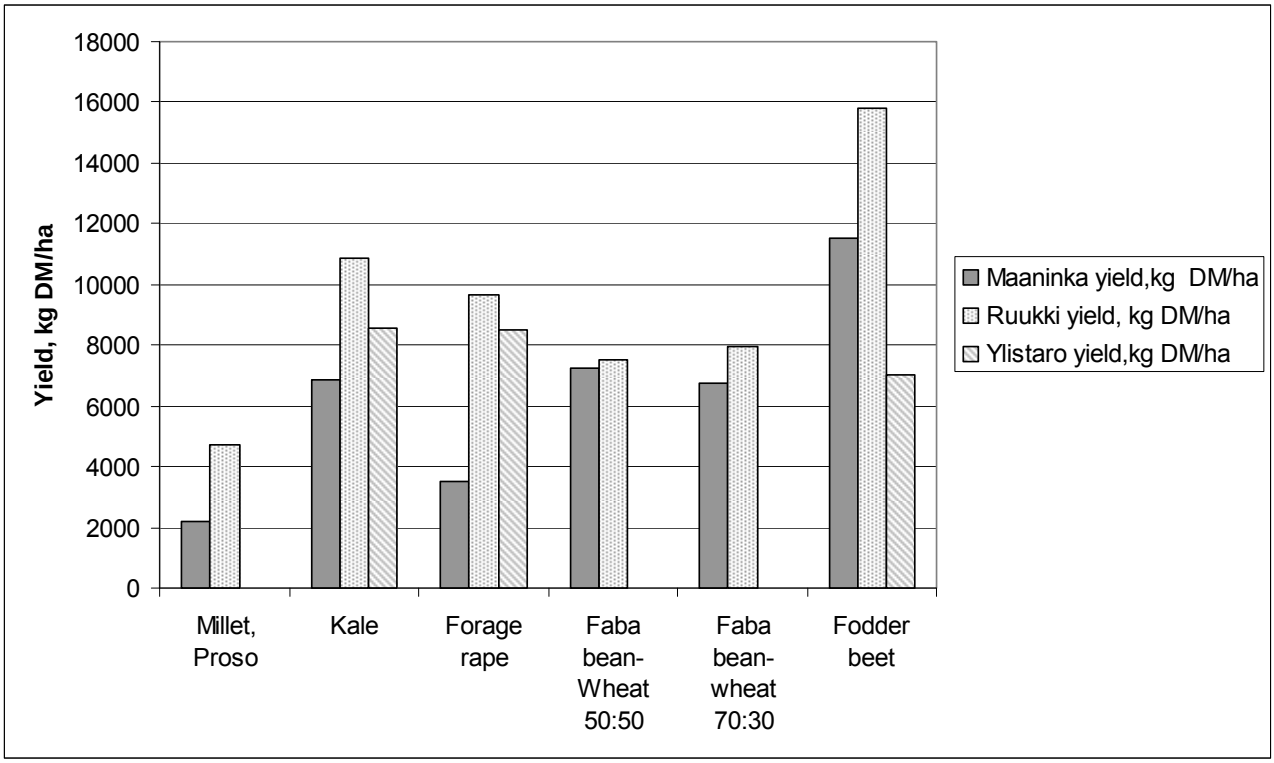

Figure 2 Dry matter (DM) yield/ha of six potential forage crops.

Conclusions

The growth potential and rhythm of different crop options could be advantageous for beef cattle grazing. Cool season *brassica* crops produce high DM yields/ha. They are frost tolerant and grazing could be an option late in autumn or early in winter. The problems can be muddy field conditions and animal health disorders especially if *brassica* crops are grazed as sole feed. Most easily grazing season can be extended with mixed cereal crops. The yield, protein content and harvesting date of cereal crops supplements perennial grazing practice which is mainly used in beef cow operations.

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Effects of increasing the proportions of high quality grass silage after peak lactation on milk yield and milk composition in Swedish Red Breed dairy cows

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Introduction

Swedish dairy cows are among the highest producing in Europe with an average milk yield of >9300 kg energy corrected milk (ECM)/year. During the last decades, the feeding has been intensified and more based on concentrate feeds. The use of forage has decreased and is at present 40-50% of dry matter (DM) of yearly consumed feed DM in conventional dairy production. Increased use of forage makes most dairy producers more independent of market fluctuations in concentrate prices. Therefore, diets with high proportions of forage are interesting to study. The objective of the present experiment was to evaluate milk production responses from dairy cows on high quality grass silage diets using gradually increasing forage proportions after peak lactation and until drying off.

Materials and Methods

Twenty-one primiparous and 33 multiparous Swedish Red Breed dairy cows were randomly assigned to three diets with different proportions of grass silage/concentrate on DM basis: low forage (L), medium forage (M) and high forage (H). The experiment ran through the whole lactation (305 days) starting in August 2007 and all cows calved between August and February. The cows were fed according to a predetermined scheme during the first month of lactation, with a gradual increase in concentrate intake up to a maximum of 14 kg, and grass/clover silage ad libitum. During the second and third lactation month, silage was fed ad libitum and the amount of concentrate was adjusted so that the forage proportion was 40% of DM in diet L and 50% of DM in diets M and H. From the fourth lactation month, feed intake was restricted when intake of the cow exceeded 110% of her energy requirement. The forage proportion was gradually increased during lactation month four to six from 50 to 60 and 50 to 70% of DM in diet M and H, respectively while it was left unchanged in diet L. From lactation month seven and until drying off was the proportion of forage increased from 40 to 50, 60 to 70 and 70 to 90% of DM in diet L, M and H, respectively.

The silage was made from an early first-cut ley of timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.) and red clover (*Trifolium pratense* L.) with 41% DM, 11.2 MJ metabolizable energy (ME), 14.6% crude protein (CP) and 44.3% neutral detergent fiber (aNDFom) /kg DM. The ingredients in the concentrate mixture were oats, barley, peas, rapeseed cake, beet fibre, wheat bran, whole rapeseed (crushed), minerals and vitamins (23.4, 23.2, 20.0, 12.5, 9.0, 7.0, 2.5 and 2.4% /kg feed, respectively) with 13.2 MJ ME and 17% CP /kg DM. The cows were housed indoors in an automatic milking system and feed intake was also registered automatically through electronic feeding gates and feeding throughs placed on balances. All groups had an average of 2.5 milkings/day. During the summer, the groups M and H were on pasture and fed concentrates indoors, while group L only had a paddock for exercise with all feeds offered indoors. The groups M and H received silage at pasture during periods of drought, when pasture allowance was less than 25 kg DM/day/cow. Milk yield was recorded automatically at each milking and milk samples for analyses of milk composition were taken once every two weeks. Data were analyzed using PROC GLM and means were separated using the LSMEANS/PDIFF option of SAS® (Version 9.1, SAS Institute Inc., Cary

NC, USA). Several independent variables and interactions were found non-significant and therefore the final model used was:
$Y_{ij} = \mu + D_i + L_j + e_{ij}$
where Y_{ij} = observed response, μ = overall mean, D_i = effect of diet (i = 1, 2, 3), L_j = effect of lactation number, primiparous or multiparous, (j = 1, 2) and e_{ij} = residual error.

Results and Discussion
The average proportions of forage over the entire lactation were 49, 62 and 71% of total DM, with an average daily DM intake of 19.9, 19.4 and 19.2 kg /cow in diet L, M and H, respectively. The nutritional quality of the silage was homogeneous during the winter period (11.2 MJ ME and 14.6% CP), while it was lower during the summer (10.3 MJ ME and 9.2% CP). Pasture grass quality was also lower (10.2 MJ ME and 14.1% CP) than the winter-fed silage. Daily milk yield in kg milk and kg ECM on diet H was lower compared with the other two groups, while milk yield on diets L and M did not differ (Table 1). The results of the present study are consistent with the study of Mäntysaari et al. (2003), who did not find any differences in average milk yield or milk composition of primiparous cows fed a constant proportion of grass silage (53% of DM) or a gradually increased proportion of grass silage (43 to 63% of DM) on 305 days of lactation. Other studies have shown lower milk yield of cows in mid lactation on diets with 80% forage compared with 60% forage of total DM (Dewhurst et al., 2003). In the present study, the primiparous cows had a lower daily milk and ECM yield compared with the multiparous cows: 26.7 *vs.* 31.3 kg (P=0.001) and 28.4 *vs.* 32.8 kg (P=0.001), respectively. Milk fat and milk protein did not differ among diets or between primiparous and muliparous cows. Milk lactose from diet L was higher compared with diet M and H.

Table 1 Milk yield and milk composition for the whole lactation (305 days) of Swedish Red Breed dairy cows fed gradually increased proportions of forage in the diet after peak lactation on dry matter basis. Low (40 to 50%), medium (50 to 70%) and high (50 to 90%) forage diet, least square means.

	Diet			SEM	*P*-value
	Low forage n=17	Medium forage n=16	High forage n=17		
Milk kg /day	30.6[a]	29.7[a]	26.7[b]	0.91	0.008
ECM kg /day	32.3[a]	31.2[a]	28.4[b]	0.77	0.002
Milk fat %	4.36	4.46	4.59	0.122	0.384
Milk protein %	3.48	3.50	3.43	0.050	0.612
Milk lactose %	4.85[a]	4.76[b]	4.74[b]	0.024	0.007
ECM kg/305 days	9846[a]	9528[a]	8667[b]	235.1	0.002

ECM, energy corrected milk (4%); SEM, standard error of means, highest value chosen
[a-b] within a row, different superscripts differ ($P \leq 0.05$)

The average ECM yield (kg/d) from weekly recordings are shown in Figure 1, and the lower lactation persistency on diet H compared with the other two groups is clearly visible through diverging curves and steeper slope on diet H from lactation week 15 and onwards. The divergence coincides in time with the increase in forage proportion from the fourth lactation month from 50 to 60% in diet M and 50 to 70% in diet H, and the increase from 40 to 50%, 60 to 70% and 70 to 90% in diet L, M and H from lactation month seven.

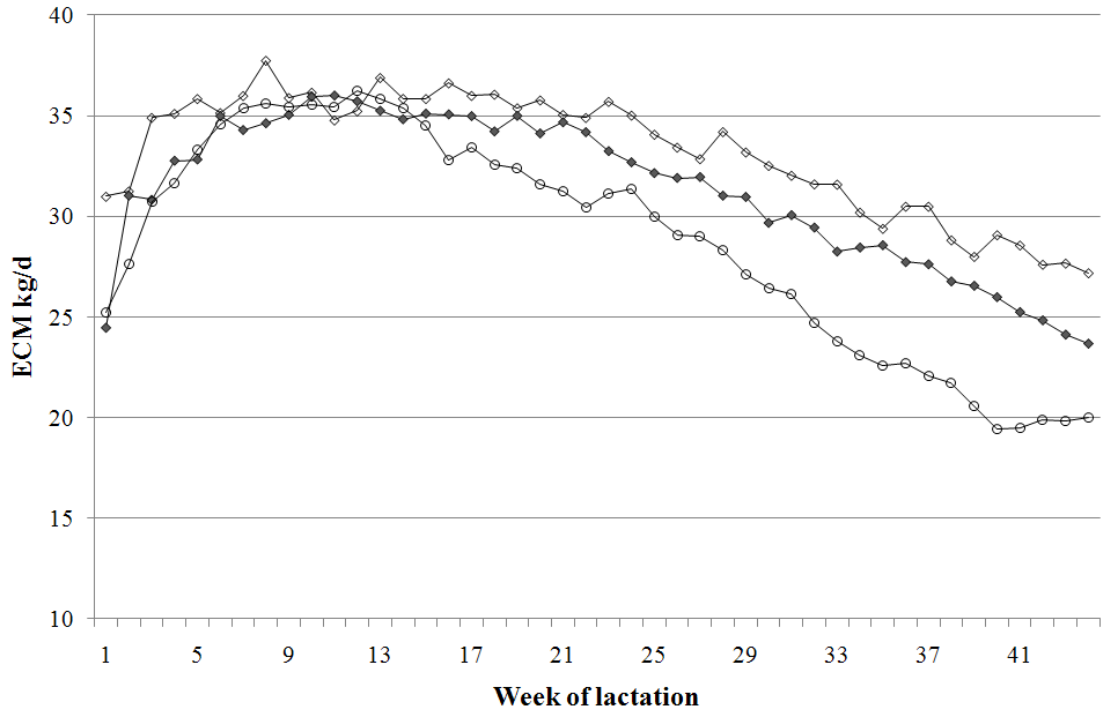

Figure 1 Energy corrected milk (ECM) yield (kg /d) of Swedish Red Breed dairy cows fed gradually increased proportions of forage in the diet after peak lactation until drying off (◊: low 40 to 50%; ♦: medium 50 to 70%; ○: high 50 to 90% forage).

Conclusions

The results suggest that when using high quality grass silage to dairy cows, the forage proportion can be gradually increased from 50% to 70% of DM in the period following peak lactation until drying off, without significantly reducing milk yield, milk fat or milk protein content. Economic evaluation of the results shows that this alternative is most attractive.

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Effects of RRR-α-tocopheryl acetate supplementation during the transition period on vitamin status in blood and milk of organic dairy cows during lactation

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Introduction

Organic dairy cow diets in Sweden normally include high amounts of grass-legume silage, which generally contains high levels of the antioxidants α-tocopherol and β-carotene (McDowell, 2000). Because of large variations in the vitamin content of feed rations due to forage species, harvest time and storage of the forage, supplementation with vitamins is recommended (NRC, 2001; Nadeau et al., 2004; Danielsson et al., 2008). The requirement is especially high during the transition period, 3 weeks before to 3 weeks after parturition (AP), due to secretion of vitamins into the udder, decreased dry-matter intake and physiological stress (Goff and Stabel, 1990; NRC, 2001).

Most vitamin supplements are provided in synthetic form, as natural vitamins are generally difficult and expensive to derive. The new EU regulations emphasize that synthetic vitamins should only be used in limited amounts when additional supplementation is necessary. However, several studies have shown that a high level of vitamin E supplementation (> 2000 IU/d) during the transition period is necessary to maintain α-tocopherol levels in blood plasma and milk (Weiss et al., 1997). Other studies show no or negative effects of high supplementation of vitamin E on health (Persson Waller et al., 2007; Bouwstra et al., 2010).

Natural vitamin E consists of a single stereoisomer, RRR-α-tocopherol, whereas synthetic α-tocopherol (all-rac-α-tocopherol) consists of a mixture of 8 stereoisomers of α-tocopherol; RRR-, RRS-, RSR-, RSS-, SRR-, SSR-, SRS- and SSS-α-tocopherol. RRR-α-tocopherol has the highest bio-availability and the 2S forms have very low or no bio-availability (Dersjant-Li and Peisker, 2010).

Our hypothesis was that organic dairy cows with a high inclusion of grass-legume silage in their diet fulfill their vitamin A and E requirements during their lactation period, without supplemental vitamins. However, during the transition period around calving, extra vitamin E may be required.

The aim of this study was to investigate the effect of a daily supplementation of 2400 IU RRR-α-tocopheryl acetate during the transition period around calving on concentrations of α-tocopherol in blood plasma and milk from prepartum to mid-late lactation in organic dairy cows fed a high dietary proportion of grass-legume silage. Additionally, effects of α-tocopherol and β-carotene contents in the feeds on vitamin A and E content in plasma and milk were evaluated.

Materials and methods

The study was performed at an organic farm in Western Sweden. The herd consisted of around 70 Swedish Holstein dairy cows with a rolling herd average of 10,452 kg milk/cow and year during the time of the experiment, with 3.8% fat and 3.2% protein in the milk. The cows were out on pasture from mid May to the end of October. The study was divided into

experiment (Exp.) 1, which included 44 cows, and Exp. 2, which included 32 cows. The experimental design was a randomized complete block, with 4 treatments in 11 (Exp. 1) and 8 (Exp. 2) blocks. Each block consisted of 4 cows with similar conditions. Within each block, the cows were randomly assigned to 1 of 4 dietary treatments in a 2 x 2 factorial arrangement of treatments with 2 levels of vitamin treatment and 2 levels of protein feed treatment. The 2 levels of vitamin treatment in Exp. 1 and 2 were natural vitamins from a 100% organic diet (C) and natural vitamins from a 100% organic diet supplemented with 2400 IU RRR-α-tocopheryl acetate (N-vet Inc., Uppsala) during the transition period (E). In Exp. 1, all cows consumed a diet without synthetic vitamins, but in Exp. 2 the synthetic vitamins A, D and E were supplemented to all cows throughout the lactation according to Swedish recommendations for lactating cows. The supplementation of synthetic vitamins in Exp. 2 was included due to ethical considerations, resulting from poor health status in the herd in Exp. 1, with some cows e.g. having low plasma levels of retinol and high levels of virus antibodies. The 2 levels of protein treatments were field beans and peas in both experiments. However, results from the protein feeds are reported elsewhere.

The vitamin concentrations of the feeds in Exp. 1 and 2 are presented in Table 1. During the first 3 months of lactation, the diet consisted of at least 50% silage and thereafter the diet consisted of at least 60% silage according to organic standards. Dry cows and heifers were offered a partly mixed ration with forage and barley from 3 weeks before expected parturition (BEP). Field beans, peas and cold-pressed rapeseed cake (CPRC), were included in the diet at parturition. The 2400 IU of RRR-α-tocopheryl acetate were offered from 3 weeks BEP to 3 weeks AP.

Diets were formulated to ensure similar composition between the treatments with 12.5 and 11.9 MJ/kg DM of metabolisable energy (ME), 162 and 167/kg DM of crude protein (CP), 363 and 372/kg DM of neutral detergent fibre (NDF), 181 and 138/kg DM of starch and 56.7 and 48.4/kg DM of crude fat in Exp. 1 and 2, respectively. The diets contained 11116 IU vitamin A and 61.5 IU vitamin E in Exp. 1. In Exp. 2, 5410 and 29.9 IU of the vitamin A and E, respectively in the diet came from the feed and 2977 and 22.3 IU of vitamin A and E, respectively were synthetic.

Feed samples were taken regularly for analysis of α-tocopherol and β-carotene as well as of *in vitro* OM digestibility, CP, NDF, starch, crude fat and fermentation characteristics (silage only) by conventional methods. Individual blood samples from the tail vein were collected at 3 weeks BEP, within 24 h AP, at 3 weeks AP and between 5-7 months AP. After centrifugation blood plasma was stored at -20ºC until vitamin analysis. Individual milk samples were taken within 24 h AP (colostrum), at 4 days AP, at 3 weeks AP and between 5-7 months AP and stored at -20ºC until analysis of vitamins.

Analyses of α-tocopherol and β-carotene were performed at Research Centre Foulum, Denmark. The α-tocopherol and β-carotene concentrations of feed were analyzed according to Jensen et al. (1998). Concentrations of retinol, β-carotene and α-tocopherol in plasma and milk were determined as previously described by Jensen (1994) and Jensen & Nielsen (1996).

The same statistical model, with cow treated as the experimental unit, was used for analysis of both experiments, but each experiment was analyzed separately. Vitamin concentrations in blood and milk were analyzed using proc mixed in SAS. In the statistical model, vitamin and protein treatment were treated as fixed effects and block as a random effect. Each sampling time was tested separately for the main effects of vitamin and protein and their interaction. Interactions between vitamin and protein treatments were excluded from both models as the overall *P*-value was > 0.10 for all variables.

Table 1 Average concentrations and standard deviations of vitamin content in feed used in Experiments (Exp.) 1 and 2 (n = number of samples).

Feed	n	α-tocopherol mg/kg DM	β-carotene mg/kg DM	Feed	n	α-tocopherol mg/kg DM	β-carotene mg/kg DM
Exp. 1				Exp. 2			
Silage	11	64.6 ± 18.9	57.7 ± 13.3	Silage	9	21.6 ± 4.0	20.7 ± 5.0
Pasture	4	29.8 ± 14.2	71.5 ± 22.3	Hay	1	9.0	3.1
Barley	1	29.0	0	Pasture	3	30.2 ± 3.6	71.6 ± 13.1
CPRC[a]	3	75.0 ± 13.6	0.7 ± 0.1	Barley	2	7.6 ± 2.1	0.05 ± 0.05
Field bean (43%)/barley (57%) pellets	2	12.2 ± 1.3	0.1 ± 0.1	Field bean (56%) / CPRC (44%) pellets	3	31.0 ± 10.0	1.5 ± 2.0
Pea (62%) /barley (38%) pellets	2	5.5 ± 3.4	0	Pea (64%) /CPRC (36%) pellets	3	19.7 ± 9.8	0.2 ± 0.1

[a] CPRC = cold-pressed rapeseed cake

Results and Discussion

The silage in Exp. 1 contained approximately 3 times as much α-tocopherol and β-carotene as the silage in Exp. 2 resulting in a higher content of natural vitamins from the feed in Exp. 1 than in Exp 2. The E cows in Exp. 2 had significantly higher concentrations of α-tocopherol in the colostrum, in the milk at 4 days AP and at 3 weeks AP than the C cows (Table 2). However, there were no significant differences in α-tocopherol, β-carotene or retinol contents in milk between treatments in Exp. 1. This is probably a result of the higher natural α-tocopherol concentration in the feed in Exp. 1. Cows can only secrete limited amounts of α-tocopherol into the milk (Jensen et al., 1999) and if there are high levels of natural α-tocopherol in the feed there may be lesser effect of the extra supplement. The *all-rac*-α-tocopherol that was supplemented in Exp. 2 did not have the same effect as the RRR-α-tocopherol from the feed in Exp 1 even though total content of IU of vitamin E in the diets were similar in both years. The higher concentrations of β-carotene in the colostrum and in the milk at 4 days AP and a tendency for higher β-carotene concentrations in the milk at 3 weeks AP from the E than from the C cows in Exp. 2 (Table 2), might be a result of lower oxidation in the milk from the E cows, as α-tocopherol acts as an antioxidant, protecting the β-carotene (McDowell, 2000). The E cows in Exp. 2 also had significantly higher concentrations of retinol in the milk 4 days AP than the C cows (Table 2).

Both treatment groups in both Exp. had low levels of α-tocopherol (< 3 mg/L) in the blood at parturition. The E cows tended, however, to have higher α-tocopherol concentrations than the C cows and they had more α-tocopherol in the blood at 3 weeks AP than the C cows (Table 3). This indicates that even if the increase was not as high as expected, there was an effect of supplementing the cows with extra α-tocopheryl acetate around parturition even though the diets contained high concentrations of natural α-tocopherol. The C cows in Exp. 1 had a higher concentration of β-carotene and there was a tendency for a higher retinol concentration in the blood at parturition than the E cows (Table 3). The retinol concentrations in plasma in Exp. 1 were quite low, as levels should be above 0.3 mg/L (Jensen 2011, personal communication) indicating that even if the diets contained high levels of β-carotene a supplement of synthetic vitamin A should be offered to the dairy cows. The C cows in Exp. 2 had higher plasma retinol concentration than the E cows at 3 weeks AP (Table 3).

Table 2 Mean levels of α-tocopherol, β-carotene and retinol in milk, mg/L, at 3 sampling occasions; colostrum, 4 days after parturition (AP) and 3 weeks AP, for Experiment 1, n[a] = 22 and Experiment 2, n = 16.

	Colostrum				4 days AP				3 weeks AP			
	E	C	SEM	*P*	E	C	SEM	*P*	E	C	SEM	*P*
Experiment 1												
α-tocopherol	9.20	7.58	1.05	NS	1.95	1.72	0.26	NS	1.22	0.97	0.17	NS
β-carotene	2.86	3.13	0.36	NS	0.51	0.52	0.10	NS	0.17	0.16	0.04	NS
Retinol	4.09	3.54	0.80	NS	0.63	0.70	0.09	NS	0.32	0.31	0.04	NS
Experiment 2												
α-tocopherol	11.5	7.16	1.58	0.041	2.24	0.73	0.29	0.001	0.90	0.32	0.13	0.001
β-carotene	2.86	1.92	0.28	0.018	0.40	0.18	0.08	0.023	0.14	0.10	0.02	0.082
Retinol	3.89	2.77	0.57	NS	0.79	0.50	0.08	0.024	0.36	0.35	0.05	NS

[a]n = number of cows. NS = not significant (P > 0.10).

Table 3 Mean levels of α-tocopherol, β-carotene and retinol in blood plasma, mg/L, at 3 sampling occasions; 3 weeks before expected parturition (BEP), at parturition and 3 weeks after parturition (AP), for Experiment 1, n[a] = 22 and Experiment 2, n = 16.

	3 weeks BEP				Parturition				3 weeks AP			
	E	C	SEM	*P*	E	C	SEM	*P*	E	C	SEM	*P*
Experiment 1												
α-tocopherol	2.59	2.55	0.28	NS	2.02	1.71	0.19	0.064	4.85	3.61	0.47	0.027
β-carotene	3.74	3.91	0.63	NS	2.34	3.46	0.37	0.013	3.06	2.83	0.41	NS
Retinol	0.22	0.21	0.02	NS	0.12	0.14	0.01	0.081	0.23	0.25	0.02	NS
Experiment 2												
α-tocopherol	3.25	2.82	0.16	0.066	2.76	2.06	0.27	0.080	5.43	4.45	0.34	0.047
β-carotene	5.46	4.84	0.36	NS	3.75	3.52	0.33	NS	4.56	4.18	0.28	NS
Retinol	0.29	0.30	0.02	NS	0.16	0.18	0.01	NS	0.28	0.33	0.02	0.062

[a]n = number of cows. NS = not significant (P > 0.10).

There were as expected no differences in vitamin concentrations in either plasma or milk between treatments in the sample taken between 5-7 months AP in Exp. 1 and 2. Levels of α-tocopherol, β-carotene and retinol were above 6, 9 and 0.31 mg/L, respectively.

Conclusions

Organic diets containing a high proportion of forage and oil-seed feeds, can potentially result in sufficient concentrations of α-tocopherol in blood and milk throughout the lactation. However, concentrations of α-tocopherol in forages vary and the diets are not reliable in providing dairy cows with sufficient concentrations of α-tocopherol around calving. Supplementation with a high dose of vitamin E, preferably as RRR-α-tocopheryl acetate, during a limited time around parturition, could secure the vitamin E supply for organic dairy cows in Sweden. According to this study, the diet of organic dairy cows also should be supplemented with vitamin A.

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Glycerol improves fluid balance in dehydrated calves

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Introduction

Glycerol can be derived from the production of biodiesel, and due to the growth of the biodiesel industry the availability of glycerol has increased as feed supplementation for cattle. It is mainly dairy cows in early lactation which have been supplemented with glycerol. Nevertheless, young calves may also benefit from addition of energy-rich compounds with glycogenic properties, especially when dehydrated and energy deprived due to diarrhea. Thus, glycerol may be useful in oral rehydration solutions (ORS) for calves.

Functional feed can be defined as a feed supplement which besides its nutritional value also contains components which can be beneficial for the health. *Lactobacillus reuteri* is a bacterium with probiotic properties which may produce a substance called reuterin in the presence of glycerol. Reuterin has a broad spectrum of antimicrobial qualities (Cleusix et al., 2008 and Spinler et al., 2008). *Lactobacillus reuteri* is naturally present in the gut of humans and animals and has the potential to inhibit the growth of pathogenic bacteria, e.g. *E. coli* and *Salmonella spp*.

Calves affected with diarrhea will rapidly become dehydrated and presumably often energy deficient. It is therefore of great importance that the calf acquires both milk and fluid replacer in order to maintain fluid and electrolyte balance as well as the energy balance (Phillips, 1985). Fluid replacements to calves contain mainly glucose and sodium chloride (Allen et al., 1999). We are not aware of any studies aiming at investigating effects of fluid replacer containing glycerol given to calves. However, studies performed with rats have shown that the water and sodium absorption was improved if two thirds of the glucose was replaced by glycerol (Allen et al., 1999). Furthermore, extra energy is provided if glucose is replaced by glycerol, since the latter has higher energy content (Liversey and Elia, 1988).

The aim of the present study was to investigate if glycerol has a positive effect on the intestinal flora in healthy young calves. The hypothesis is that glycerol stimulates the formation of reuterin and thus decreasing the amount of *E. coli* in calf faeces. Furthermore, the aim was to evaluate if glycerol can contribute to maintenance of the plasma volume and look into changes in plasma metabolites e. g. glucose and insulin, in young calves when deprived of feed and fluid for one day.

Material and Methods

The study was carried out at the Swedish University of Agricultural Sciences in Uppsala, Sweden and the experimental design and all handling of animals were approved by the Swedish Animal Ethics Committee.

The study was performed on 20 calves of the Swedish Red Breed, one to three weeks old, during a period of twelve days. The experimental period was divided into two parts, adaptation period (day 1-11) and deprivation period (day 11-12).

The calves were separated from their dams within 24 h after birth, and were kept into straw-bedded single pens (1.0×1.2 m^2) in a separate indoor calf barn during three days. All calves were fed colostrum, approximately 2.5 L from their dam, twice a day from a nipple bottle. They were subsequently moved to group pens with maximum 14 calves per pen (5.9×5.0 m^2) with concrete slatted floor and a straw-bedded area in the same calf barn. Milk replacer was provided by a transponder controlled automatic milk feeder (CF300A, DeLaval, Tumba, Sweden) with a rubber teat and available at all times. The ration of milk replacer was increased with 0.5 kg/d until the final ration of 10 L was reached, approximately at 15 days of age, starting with 5 L/d between 4-7 days of age. Concentrate ratio, supplied by a transponder controlled automatic feeder (CF300A, DeLaval, Tumba, Sweden), was 0.25 kg/d at start, and then gradually increased to maximum 1.5 kg/d. Forage was provided ad libitum and drinking water was available in automatic water bowls.

The calves were divided according to expected day of birth. Calves within the same block were kept in the same group and given the treatment simultaneously. Calves in each block were randomly assigned to three different oral supplementations: glycerol along with glucose (glycerol), glucose and water (control). During the adaptation period, day 1 to 11, the ORS (400 mL) was adjusted to body temperature and offered twice a day (08.00 and 16.00 h) from nipple bottles in excess of ordinary feeding ratio and water. The automatic milk feeder was blocked for two h before feeding to increase the calves' willingness to ingest the oral supplementation. Throughout, the deprivation period, day 11-12, the calves were offered body tempered ORS (800 mL) at 16.00 h day 11 and 08.00 h day 12 from nipple bottles without access to feed, milk and water.

Jugular blood 10 mL, was collected in heparinised tubes (Venoject, Terumo Europe N.V., Leuven, Belgium) day 0, 5, 11 and 12, respectively, between 08.30 and 09.30 h. The blood samples were analyzed for packed cell volume (PCV) and total plasma protein (TPP) within one hr after sampling. Further, the residual plasma samples were stored at -20°C until further analysis. Approximately 40 g of feces were obtained fresh from the calves upon rectal stimulation day 0, 5, 11 also between 08.30 and 09.30 h, and transferred into sterile 50 mL tubes. Approximately 1 g of feces from each sample were then transferred to sterile tubes and stored at -70°C until further analysis. Two markers were used to estimate potential intestinal reuterin production: 1.3 propanediol, a product of glycerol fermentation from reuterin reduction, using HPLC analysis, and concentration of *E. coli* and *Lactobacillus spp.* in pure cultures, determined by plate counts. The data were analyzed using the MIXED procedure of SAS (SAS 9.2, SAS Institute 2008) with unstructured covariance structure. The model accounted for effects of treatment, day, block and all 2-way interactions. The effects of treatment on plasma concentrations of glycerol, glucose, insulin and TPP, and concentration of PCV in blood, and amount of *E. coli* and *Lactobacillus spp.* were analyzed using repeated measures by day. None-significant interactions ($P>0.05$) were excluded from the model for each variable tested. The fixed effects were considered significant at $P<0.05$. Results from the statistical analyses are presented as least squares means and associated standard errors.

Results

Calves fed ORS with glycerol had a higher concentration of glycerol in plasma ($P<0.001$) day 11 and 12 compared to ORS with glucose or control (Table 1). The concentration of glucose in plasma differed among treatments day 12, with higher values for glycerol ($P=0.002$) and glucose ($P<0.001$), respectively, compared to the control. Further, calves provided ORS with glucose had higher concentration of insulin in plasma day 12 than glycerol ($P=0.04$) and control ($P=0.01$). Moreover, calves offered ORS with glycerol had

higher osmolality in plasma day 11compared to glucose (P=0.003) and control (P=0.004). The concentration of TPP and the amount of PCV did not differ between treatments, (P=0.23) and (P=0.40), respectively. Table 1 shows effect of treatment on blood and plasma metabolites at day 11 and 12.The precursor 1.3 propanediol was not found in feces. *E. coli* and *Lactobacillus* (log_{10} cfu/g) were not influenced by treatments.

Table 1 Effect of treatment on blood and plasma metabolites at day 11 and 12, least squares means ± standard error of the mean

Day	11			12		
Treatment[1]	Glycerol	Glucose	Control	Glycerol	Glucose	Control
Glycerol, m*M*/L	3.2^{a}±0.33	0.1^{b}±0.31	0.2^{b}±0.43	8.1^{a}±0.27	0.1^{b}±0.25	0.2^{b}±0.33
Glucose, m*M*/L	6.0^{b}±0.47	7.0^{a}±0.44	5.8^{b}±0.63	8.2^{b}±0.67	13.5^{a}±0.63	4.0^{c}±0.85
Insulin, µg/L	1.2±0.46	0.6±0.45	0.4±0.60	1.3^{b}±0.42	2.4^{a}±0.39	0.1^{b}±0.53
Osmolality, m*Osm*/kg	284.2^{a}±0.76	279.5^{b}±0.68	279.0^{b}±0.97	292.2^{a}±2.21	287.0^{a}±2.04	281.0^{b}±2.72
TPP[2], g/L	51.8±1.24	50.3±1.13	53.8±1.60	53.7±1.84	53.5±2.00	58.0±2.57
PCV[3], %	36.6±1.50	37.5±1.37	37.6±1.93	39.0±1.25	37.3±1.37	41.6±1.71

$^{a-c}$Means within a row with different superscripts differ (P<0.05) within day.
[1]Item: Glycerol=0.67 g glycerol/kg BW and 0.33 g glucose/kg BW; Glucose=1 g glucose/kg BW; control=water. [2]TPP=Total Plasma Proteins. [3]PCV=Packed Cell Volyme

Conclusions

These results suggest that the glycerol/glucose mixture was a better ORS than the pure glucose solution. Both solutions were able to prevent a drop in glucose after 24 h feed and fluid deprivation. However, calves receiving pure glucose ORS were clearly hyperglycemic and hyperinsulinemic, while calves receiving the glycerol containing ORS did not develop hyperglycemia and insulin fluctuated less. Changes in plasma volume may be determined by changes in plasma proteins. In the present study control calves numerically showed approximately 8% reduction of the plasma volume during the deprivation day. This drop appears to have been prevented by both ORS. Glycerol may be a good alternative to use in fluid replacers for calves in the future. More work is in progress to further establish the results of the present study. However, no positive effects of ORS containing glycerol were shown on the intestinal flora, since no evidence of increased reuterin production was observed and the *E. coli* numeral did not decrease in feces. Probably glycerol was efficiently absorbed in the small intestine since no glycerol was detected in the fecal samples while markedly elevated glycerol levels were detected in plasma.

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Feed and energy intake in standardbred yearlings in training on a forage only diet

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Introduction

Horses in training are generally fed high concentrate diets although such diets are associated with health problems (Tinker *et al*., 1997; MacLeay *et al*., 2000) and stereotypic behavior (Redbo *et al*., 1987). The reason might be that traditional forage produced for horses, tends to have an energy density too low to fulfill the energy requirements on a forage-only diet without challenging the feed intake capacity. According to studies by Edouard *et al.* (2008) and LaCasha *et al*. (1999) yearlings fed a grass hay (Costal Bermuda Grass/Matua Bromegrass) *ad libitum* may consume 1.7-2.8 % dry matter (DM) of body weight (BW). It has also been showed in a previous study by Forsmark (2006) that foals (average age 215 days) were able to consume 2.4 % DM of bodyweight and cover their energy requirements from a forage with 10.9 MJ metabolizable energy (ME) per kg DM. Adult horses in training fed a forage only diet in a study by Muhonen *et al*. (2009*)* consumed 1.8-2.0 % DM of BW of a grass forage with 11.6 MJ ME per kg DM. The aim of this study was to measure voluntary feed consumption in Standardbred yearlings in training fed a high-energy forage-only diet *ad libitum* and to compare energy intake with energy requirements suggested by NRC (2007).

Materials and Methods

Fifteen Standardbred stallions (age 15-19 months, 406-470 kg), adapted to a high energy grass haylage for two months, were used in a 7-week study. The study was conducted in the middle of Sweden (lat N 63°, long E 13°) from the middle of October till the end of November. Grass haylage (50 % DM, 11 MJ ME/kg DM and 150 g crude protein/kg DM) was offered *ad libitum*. The haylage was a first cut (8^{th} of June) fertilized with 126 kg N in spring and consisted mainly of meadow fescue and timothy. The diet was complemented with 1 kg of a pelleted Lucerne product (95 % Lucerne, 5 % molasses), 100 g molassed beet pulp and 150 g of a mineral feedstuff to ensure that mineral and vitamin requirements were met (NRC, 2007). The horses were kept individually in 9 m^2 boxes and spent approximately 8 hours per day outside in a paddock (with haylage and water available) except for the days when feed intake was measured when they were kept in their boxes. The training the first three weeks of the study consisted of trot (3 min/km) for 5 km three times a week on a flat racetrack. The fourth week horses were exercised twice for 5 km on the same racetrack and twice for 7.8 km on a slight hilly terrain track. In week 5-7 horses were trained once a week on the flat race track for 5 km and twice for 7.8 km in the terrain track. Daily feed intake was measured for three days the first and the last week of the study, and daily energy intake was calculated based on these values according to Lindgren (1979) and Jansson *et al.* (2004). Body weight was measured twice in the beginning and end of the first and last week respectively and an average of these measurements was used for the calculations. All data were subjected to analysis of variance (GLM procedure in the Statistical Analysis Systems package 9.2, SAS Inst., Cary, NC) using the following model; $Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ij}$ where Y_{ij} is the observation, μ the mean value, α_i the effect of animal, β_j the effect of period and e_{ijk} the residuals; $e_{ij} \sim IND(0, \delta^2)$. The *P* value for difference between treatments was < 0.05. Post-hoc analysis was made by a Tukey-test (difference at $P < 0.05$). Values are presented as least square means ± standard error of the mean.

Results and Discussion

As far as we know this is the first study measuring the voluntary haylage intake in young horses in training fed a forage-dominated diet. During the experimental period BW increased from 436 ± 1 kg to 451 ± 1 kg and the daily dry matter and energy intake increased from 10.6 ± 0.1 kg DM/horse and 120 ± 1 MJ ME to 11.0 ± 0.1 kg DM/horse and 126 ± 1 MJ ME ($P<0.05$) by the end of the study. DM intakes corresponded to 2.5-2.6 % of the body weight and were in accordance with earlier observations on young horses at rest but higher than previous reported in adult horses in training fed forage with a higher energy content (Muhonen *et al*., 2009). An increase in the energy requirement during the experiment could be due to both an increased body weight and exercise load. The energy intake corresponded to 130-140 % of the requirements suggested by NRC (2007) for young horses (18 months) in moderate exercise and was even slightly higher than the requirements suggested for 24 months old horses in very heavy exercise.

Conclusions

This study shows that voluntary feed intake of high energy forage in Standardbred yearlings may correspond to > 2.5 % of BW and meet the requirements of very heavy exercising growing horses according to NRC (2007).

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Urinary phosphorus excretion in growing horses in training fed forage *ad libitum* and two levels of phosphorus

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Introduction

Phosphorus among other macro minerals is important for the development of skeleton in growing horses, and dietary mismanagement may be one of the factors predisposing young horses for developmental orthopedic disease (Pagan, 2011). In horses at pasture or fed forage *ad libitum* the intake of nutrients, including minerals, can only be estimated (Pagan, 2011). It would therefore be valuable to find simple methods for detecting the intake of minerals in horses. Schryver (1971) and Meyer (1990) reported a relationship between P intake and urinary P excretion, while van Doorn (2004) and Stephens (2004) found large variation in P excretion in urine among individuals. Cymbaluk (1989) compared two low P diets (one forage diet and one concentrate diet) and concluded that horses fed forage diet low in P might have absorbed insufficient amounts of P. Therefore P supplementation might be needed on high-forage diets but not on concentrate diets. The aim of this study was to investigate if P in a spot sample of urine reflects P intake in growing horses in training fed forage-only diets.

Materials and methods

Fourteen healthy Standard-bred trotters (thirteen geldings and one stallion, bodyweight 450 ± 54 kg, age 20 ± 0.3 month) were randomly blocked into two dietary treatments: with (C) and without (T) P supplements for 6 days in a cross-over design. Horses were fed forage *ad libitum* (minimum 10 % left overs) and 250 g pelleted lucerne (Krafft AB, Sweden) once per day. In treatment C 150 g of mineral supplements (Krafft Miner Vit) was offered daily. Horses were fed individually in the stable with free access to water from buckets. Crude protein, metabolizable energy, Ca and P content of the feeds are found in Table 1. All horses were exercised (trot at 2-3 min/km for 4-7 km on a race track) four days per week. On days off training all horses were exercised in a walker for 30-60 min. once or twice per day. One spot sample of urine per horse was collected once during the last three days in each treatment period. All feed and left-overs were weighed daily, and samples of feed and refusals were collected daily from each horse. Samples were frozen at -20°C for further analysis. Feed samples were mixed and dried for 16 h in 65°C and ground in a hammer mill using a 1-mm screen (KAMAS, Slagy 200 B, Malmö, Sweden). Dry matter content was determined by overnight drying at 105°C. P content were analyzed using a spectrophotometer (ICP-AES, ICP Spectro Flame, Spectro Analytical Instruments) after extraction in HCl (7N) and dilution. P analyses were made by AgriLab, Uppsala, Sweden. Urine was analyzed for creatinine by Segmented Flow Analysis with an Autoanalyser III system (Seal, USA), using the Jaffee reaction, a colorimetric method with picric acid and wavelength 505 nm (Technicon method No. SE4-0011FH4, Technicon, Dublin, Ireland). Creatinine was used for calculating the amount of urine excreted each day, assuming a creatinine excretion in urine of 1.15 mg/ kg BW/h (Meyer, 1990). Inorganic P in urine was analyzed by UV method (PH 1016, Randox, Antrim, UK) with molybdate as reagent and measured by a spectrophotometer (340 nm) (Kinetics Spectrophotometer, LKB Biochron, Ultrospec K 4053, Cambridge, England).

All data were subjected to analysis of variance (GLM procedure in the Statistical Analysis Systems package 9.2, SAS Inst., Cary, NC) using the following model; $Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk}$ where Y_{ijk} is the observation, μ the mean value, α_i the effect of animal, β_j the effect of treatment, γ_k the effect of period and e_{ijk} the residuals; $e_{ijk} \sim$ IND $(0, \delta^2)$. The *P* value for significance between treatments was < .05. Post-hoc analysis was made by a Tukey-test (significance P < .05). Values are presented as least square means ± standard error of the mean.

Table 1. Nutrient composition of feed used (of dry matter)

Item	Forage	Lucerne	Supplement (Krafft Miner Vit)
CP %	14.3[a]	15.0[b]	0
Ca %	.56	1.76	6.76
P %	.26	.25	6.44
Metabolizable energy, MJ	10.6[a]	8.5[b]	0

[a] nutrient analyzed by AgriLab before the trials [b] values from the manufacturer

Results and Discussion

Intake

As expected, mean P intake was lower for horses on diet T (P<.0001) compared to diet C (Table 2). Based on the daily energy intake it was estimated that daily P intake corresponded to 130 % and 70 % of the NRC requirement on diet C and T, respectively. There was a lower (P<.0001) DM intake for the T-diet compared to the C-diet.

Excretion in urine

There was no correlation between P intake and urinary P excretion in individual spot samples (Fig.1), and there was no difference between diets in daily urinary P excretion. These data are in agreement with Nielsen (1998) and Stephens (2004) using growing horses, and Buchholz-Bryant (2001) using young, mature and aged horses. In contrast, both Schryver (1971) and Meyer (1990) found that renal P excretion was positively related to P intake. However, at low levels of P intake this relationship may not be an adequate description (Schryver, 1971). In our study, estimated loss of P via urine was less than 1.5 % of intake on both diets. Nevertheless, urine P % of P intake was higher (p<.0001) in non-supplemented horses.

Table 2. Mean values ± stddev of daily feed and P intake, and P excreted in urine

Item, unit	Control (supplemented)	Treatment (non-supplemented)
DM intake, kg	9.00 ± 0.17	6.60 ± 0.17*
P intake, g/d	32 ± 1	17 ± 1*
P urine, mg/d	170 ± 9	190 ± 9
P urine % of P intake	0.5 ± 0.05	1.1 ± 0.05*

* difference between treatments ($p < .05$)

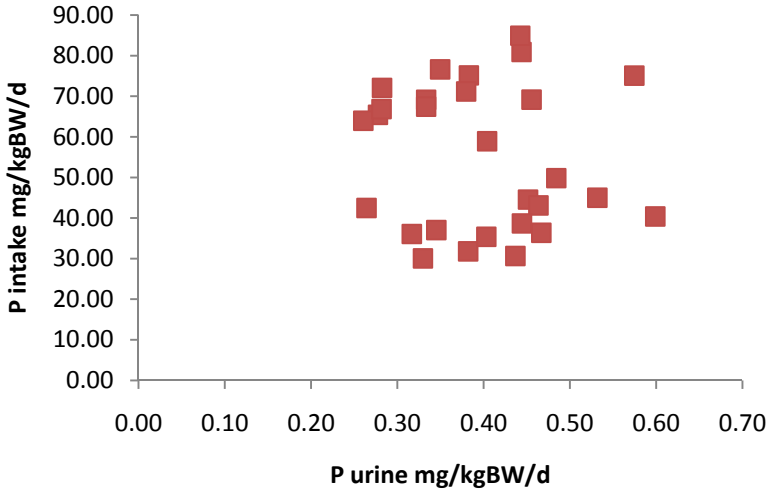

Figure 1. P excreted in urine versus P intake in 14 young Standardbred horses fed a forage diet supplemented or not supplemented with P.

Conclusion

A single spot sample of urine does not reflect P intake in young horses fed forage-only diets and P levels ± 30% of the NRC recommendations. Estimated loss of urinary P excretion was low in this trial, about 1%. DM intake was 34 % higher in supplemented horses compared to non-supplemented. This result was surprising and further studies are needed to clarify if there is a relationship between DM intake and mineral supplement intake.

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