The effects of high-sugar ryegrass/red clover silage diets on intake, production, digestibility, and N utilization in dairy cows, as measured in vivo and predicted by the NorFor model

J. Bertilsson,^{*1} M. Åkerlind,[†] and T. Eriksson^{*}

^{*}Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, PO Box 7024, SE-75007 Uppsala, Sweden

[†]Växa Sweden, Box 288, 75105 Uppsala, Sweden

ABSTRACT

Grass silage-based diets often result in poor nitrogen utilization when fed to dairy cows. Perennial ryegrass cultivars with high concentrations of water-soluble carbohydrates (WSC) have proven potential for correcting this imbalance when fed fresh, and have also been shown to increase feed intake, milk production, and N utilization. The possibility of achieving corresponding effects with silage-based diets was investigated in change-over experiments in an incomplete block design with 16 (yr 1) or 12 (yr 2) Swedish Red dairy cows in mid lactation. Measurements on N excretion and rumen parameters were performed on subgroups of 8 and 4 cows, respectively. In yr 1, 2 ryegrass cultivars (standard = Fennema; high-WSC = Aberdart) and 2 cuts (first and second) were compared. In all treatments, ryegrass silage was mixed 75/25 on a dry matter (DM) basis, with red clover silage before feeding out. In yr 2, 1 basic mixture from the different cuts of these 2 cultivars was used and experimental factors were red clover silage inclusion (25 or 50%) and sucrose addition (0 or 10%) on a silage DM basis. Differences in WSC concentration in the silage mixtures in yr 1 were minor, whereas the differences between cuts were more substantial: 100 compared with 111 g/kg of DM for first-cut silage and 39 compared with 47 g/kg of DM for second-cut silage. The silages fed in yr 2 had a WSC concentration of 115 or 102 g/kg of DM (25 or 50% red clover, respectively), but when sucrose was added WSC concentration reached 198 and 189 g/kg of DM, respectively. Milk production (kg/d) did not differ between treatments in either year. Red clover inclusion to 50% of silage DM increased milk protein. Nitrogen efficiency (milk N/feed N) increased from 0.231 to 0.254 with sucrose inclusion in yr 2 (average for the 2 red clover levels). Overall rumen pH was 5.99 and increased sucrose level did not affect pH level or daily

pH pattern. Sucrose addition reduced neutral detergent fiber digestibility, particularly at higher inclusion rates of clover. Rumen pool of total purines did not differ between treatments, nor did protein production assessed from urinary allantoin. The NorFor feed evaluation model overestimated digestibility of neutral detergent fiber and N, but underestimated N excretion in feces. We concluded that addition of WSC to dairy cow diets at levels up to 3 kg of WSC per day (>14% of DM) does not dramatically affect cow performance.

Key words: dairy cow, sugar, nitrogen efficiency, microbial protein

INTRODUCTION

Water-soluble carbohydrates (WSC), commonly called sugars, constitute, on average, 10% (range 0–25%) of DM in grasses harvested for silage in Sweden (Åkerlind et al., 2015). In a typical dairy cow diet based on grass silage, an imbalance may exist between N and energy, leading to low N efficiency. When we planned this study a decade ago, we hypothesized that an increase in WSC content in grasses would be beneficial for N efficiency through increased microbial activity in the rumen, and could possibly lead to higher milk production. Miller et al. (2001) showed positive effects on milk production and apparently less N to urine and more to milk when zero-grazed, high-WSC grass was fed to dairy cows in late lactation. When similar diets were fed in early lactation, feed intake increased but milk yield was not affected, although less N was partitioned to urine (Moorby et al., 2006). Merry et al. (2006) fed grass-clover diets to steers and found that N flows to the duodenum were greater for steers fed high-WSC silage and increased further for steers fed mixes of red clover and grass; their data suggested that grasses with a high WSC content enable a forage-based strategy for balancing energy and protein. Later experiments have shown more varying results for high-sugar grasses. Ellis et al. (2011) evaluated literature data with a dynamic model and showed that the greatest benefit in terms of N utilization ratio and urine

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¹Corresponding author: Jan.Bertilsson@slu.se

N levels was obtained when the WSC content of grass increased at the expense of CP. When sugar replaces starch in the diet, it has been found that this increases DMI, butyrate concentration in the rumen, and milk fat yield, but does not improve N utilization and milk protein yield in dairy cows (Oba, 2011). High dietary concentrations of readily available carbohydrates may bring some nutritional drawbacks, with low rumen pH and impaired fiber digestibility (Hoover, 1986). The main objective of our study was to investigate whether increased WSC content in silage improved dairy cow performance in terms of milk production and N utilization of typical northern European diets characterized by highly digestible grass-clover silage with grain-based concentrate supplementation. Our project was a part of the European Union-funded project Sweetgrass, where perennial ryegrass with enhanced WSC content was cultivated, conserved, and fed to animals in 5 countries in north and western Europe. Postexperimental simulations with the NorFor model (Volden, 2011) were also performed to evaluate how well the dietary effects were predicted by the model.

MATERIALS AND METHODS

Animals, Feeding, and Housing

The experimental design and all handling of the animals, including surgical procedures, were approved by the Uppsala Local Ethics Committee. Two incomplete change-over experiments with 4 treatments and 3 periods were performed during the period January to April 2003 (yr 1) and 2004 (yr 2). Both experiments used cows of the Swedish Red breed (mean $\pm$ SD at experimental onset); yr 1 included 16 cows (parity = 2.0 ± 0.6 ; BW = 650 ± 49.4 kg; DIM = 145 ± 40.0), whereas yr 2 included 12 cows (parity = 2.7 ± 0.7 ; BW = 676 ± 72.1 kg; DIM = 203 ± 34.5). Period length was 4 wk in yr 1 and 3 wk in yr 2, with measurements and sampling carried out in the last week of each period. Measurements of N excretion and rumen parameters were performed on subgroups of 8 and 4 cows in yr 1 and 2 respectively, where cows for rumen sampling were fitted with cannulas with 100 mm i.d. (Bar Diamond Inc., Parma, ID). The cows were arranged in 4 blocks in yr 1 and 3 blocks in yr 2, based upon parity and DIM. The cows were kept in individual tiestalls with sawdust bedding throughout the experiment and were milked in their stalls at 0600 and 1530 h.

Experimental Treatments and Feeds

The 4 treatments in each year were applied in a 2×2 factorial design, with the experimental factors in yr 1

being ryegrass cultivar Fennema (standard cultivar) or Aberdart (high-WSC cultivar) and cut (first or second). Before feeding out, ryegrass silage for all treatments in yr 1 was mixed with first-cut red clover silage (75:25, DM basis). In yr 2, the experimental factor was inclusion level of second-cut red clover (25 or 50%, DM basis) in the grass silage before feeding. In addition, food-grade sucrose (Danisco Sugar AB, Malmö, Sweden) was mixed into the grass-clover forage in yr 2 (0 or 10%, DM basis). Pure swards of the 2 ryegrass cultivars and of red clover (cultivar Vivi) were established in spring 2001 near Uppsala, Sweden (18°E , 60°N), and harvested 2 or 3 times per year in 2002 and 2003 for the feeding experiments in yr 1 and 2, respectively. The ryegrass swards were fertilized with a total of 200 kg of N/ha, with 90 kg in spring, 70 kg after the first cut, and 40 kg after the second cut. Cutting dates in yr 1 were June 4 and July 15 for first- and second-cut ryegrass, respectively, and June 18 for the red clover. The harsh winter before experimental yr 2 severely harmed the grasses. The first cut yielded very little, but the second and third cuts also gave low yields. Cutting dates in yr 2 for ryegrass were June 16 (not used in feeding experiment), July 29, and October 7, with equal proportions of the latter 2 cuts from both cultivars used in the trial. The first-cut red clover sward used in the mixes was cut on June 29. All swards were cut with a mower-conditioner, wilted for 24 h, baled (round bales yr 1, square bales yr 2, approximate bale fresh weight 500 kg), and wrapped in 8 layers of plastic. An alkaline additive containing sodium nitrite, hexamethylene, tetraamine sodium benzoate, and sodium propionate (Kofasil Ultra, AD-DCON, Bonn, Germany) was applied at a rate of 4 L/t of fresh weight. The silage was fed ad libitum with 2 meals and allowance was continuously adjusted to result in 10% orts. First feeding (40% of allowance) was at 0600 h in both years and second feeding (60% of allowance) was at 1730 h in yr 1 but at 1430 h in yr 2. A grain-based, custom-manufactured pelleted concentrate feed was fed at a flat rate of 8 kg (as fed) per day, in 4 equal meals at 0600, 0900, 1300, and 1700 h. A mineral-vitamin supplement (Kvarnby VM 0.9, Kvarnbyfoder, Staffanstorps, Sweden) was fed separately at 0.15 kg/d. Orts were weighed once daily. Every 2 cows shared a water bowl, with free access to water.

Milk Sampling and Analysis

Milk yield was recorded with the Tru-Test technique (Tru-Test Ltd., Auckland, New Zealand), and milk was sampled on 3 d (6 consecutive milkings) in the sampling week. Each sample was analyzed for fat, protein, and lactose monohydrate by infrared spectroscopy (DairyLab2 A7S Foss Electric, Hillerød, Denmark).

The milk analyses were weighted to a daily mean using the separate analyses and corresponding milk amounts. Cows were weighed on 2 consecutive days immediately before the intensive rumen sampling started in the last week of each period.

Feeds

Three samples from each crop were taken from the swath immediately before baling. Each sample was a composite of grass picked at 10 different places adding to a total of 2 kg. Before feeding out, core samples were taken from the bales at 8 different places, drilling to the center of the bale and giving a sample of 0.5 kg. All samples were frozen and stored at -20°C before analysis. The silages and concentrates fed were sampled daily on weekdays and the samples were stored at -20°C until analysis. Silage samples from each measurement week were then pooled within feed and period, milled frozen in a meat mincer through a 10-mm screen, mixed, and sampled. Kjeldahl N was analyzed directly in the fresh silage by a fully automated procedure (Kjeltec 1030, Tecator, Höganäs, Sweden). Silage for other analyses and pooled concentrate samples from each measurement week were dried at 60°C and milled in a hammer mill (Slagy 200 B; Kamas, Malmö, Sweden) through a 1-mm screen. Dry matter was determined at 105°C and volatilization losses in silage samples were compensated for by addition of 14 g/kg of uncorrected DM (Rammer, 1996). Ash content was determined by combustion at 550°C for 3 h (EC No. 152/2009). Ash-free NDF was analyzed according to Chai and Uden (1998), and ADF and permanganate lignin according to Van Soest et al. (1991); WSC (sum of glucose, fructose, sucrose, and fructans reported as hexoses, equivalent to WSC determination) and starch (concentrates only) were determined enzymatically after extraction with acetate buffer (Larsson and Bengtsson, 1983). Ether extract in concentrates was analyzed according to European Communities (2009), using a 1047 hydrolyzing unit and a Soxtec system HT 1043 extraction unit (Foss Analytical A/S, Hillerød, Denmark). Feed samples were also analyzed for acid-insoluble ash (**AIA**) for digestibility calculations (Van Keulen and Young, 1977). Silage juice was obtained by applying hydraulic pressure to resealable bags containing minced silage that had been soaked with an equal weight of deionized water and refrigerated overnight. The juice was analyzed for pH, fermentation acids with HPLC (Ericson and André, 2010), and ammonia-N ($\text{NH}_3\text{-N}$) by the phenol-hypochlorite method (Broderick and Kang, 1980). Silage in vitro digestible OM was determined by the Swedish standard method, with 96 h of incubation with 2%

rumen liquid (Lindgren, 1979). In sacco degradation parameters of CP and NDF were determined by time-series incubations for 0, 2, 4, 8, 16, 24, 48, and 96 h (NDF only) and followed the protocol later adapted as standard for the NorFor model (Åkerlind et al., 2011), except that bag pore size was 28 rather than $37\text{ }\mu\text{m}$ and that no 288-h incubation for determination of indigestible NDF (**iNDF**) was performed.

Feces Sampling

Spot samples of feces were taken twice daily for 5 consecutive days during each sampling week. The samples were frozen and stored at -20°C . After thawing, the samples were pooled within cow and period, homogenized, freeze-dried, milled, and analyzed for DM, ash, AIA, NDF, and Kjeldahl N as described above for feeds. The reason for freeze-drying these samples was to facilitate grinding.

Urine Sampling

Quantitative urine collection was carried out for 72 h as described by Eriksson et al. (2004), with a natural rubber/latex mold tightly secured over the vulva by attachment to a harness. The samples were collected in 1.8 M H_2SO_4 (2–3 L/d, individually adapted after initial pH measurements) to keep pH < 3. Total urine was weighed daily, and subsamples representing 10% of daily weight were frozen and stored at -20°C , then thawed, pooled, and analyzed. The urine samples were analyzed for Kjeldahl N as described for feeds and for allantoin (Lindberg and Jansson, 1989) and creatinine (Technicon, 1974) in a Technicon AutoAnalyzer.

Rumen Sampling

Rumen liquid samples were obtained at 23 different hours spread equally over the day. Sampling was performed through the open rumen cannula, by manually pressing a 50-mL test-tube down approximately 20 cm into the rumen digesta. The pH was measured immediately (Mettler Toledo MP 125, Mettler-Toledo AG, Schwerzenbach, Switzerland) and the test-tube was then immediately frozen and stored at -20°C until analysis of VFA and ammonia N, as described for silage. Total rumen evacuations were performed at 1300 h on d 3 and 5 in the sampling week. Rumen contents were weighed and sampled as described by Eriksson et al. (2004) and immediately returned to the rumen. Samples were dried at 60°C , milled, and analyzed for DM, ash, Kjeldahl N, and NDF as described for feeds. In addition, rumen evacuation samples were analyzed

for total purines with yeast RNA as the standard (Zinn and Owens, 1986; Aharoni and Tagari, 1991).

NorFor Model Simulations

Feed and animal characteristics of the cows with urine collection data were entered into the NorFor software version 1.22.0.427 and FRC version 1.81 (Volden, 2011) on an individual basis to predict animal performance and underlying processes, such as digestibility and microbial protein production. Because all animal experiments and chemical analyses were performed before standardization of analytical protocols for the NorFor model (Åkerlind et al., 2011), a few input parameters had to be substituted. For example, the a value from in sacco determinations was used as soluble CP rather than buffer-soluble CP, and the iNDF value for silages was calculated by regression as

$$\text{iNDF (g/kg of OM)} = \\ (96 \text{ h in vitro digestible OM value}) \times 0.61 - 7.4,$$

the equation being based upon 476 samples with a coefficient of determination = 0.87 and root mean squared error = 17.6 g/kg of OM (Eriksson, 2010). For the concentrates, the iNDF value was calculated from ingredient proportions and 288-h in sacco incubations in our laboratory of the same ingredients (although not the actual samples).

Calculations and Statistical Analysis

Energy-corrected milk was calculated according to Sjaunja et al. (1991). Apparent digestibility was calculated from AIA concentration in feces, assuming that all AIA in feeds was recovered. One cow during 1 period in yr 1 was regarded as an outlier (>2 SD) and treated as a missing value in the calculations. For comparison with NorFor predictions of microbial protein production, allantoin measurements were calculated further to microbial protein production using literature data. It was assumed that allantoin excretion (mmol/d) was equal to $93.5 + 0.772 \times \text{intestinal purine flow}$ (Vagnoni et al., 1997). A microbial CP content of 6.125 g/mmol of purines was assumed, arrived at by pooling analyses from Scandinavian dairy cows (Volden et al., 1999). For variables with multiple observations during the measurement week, an arithmetic mean was calculated and used in statistical analysis. Animal performance data were analyzed separately for the 2 experimental years. Statistical analyses were performed using the MIXED procedure in SAS (Littell et al., 1996). In both

years, cow was regarded as a random factor. Remaining effects from the previous period were tested in all cases, but they were found to be nonsignificant ($P > 0.10$) and omitted from the final model. When rumen parameters were tested block was not included in the model, as all rumen-fistulated cows were in one block. The general SAS model was

- Year 1: $Y = \text{period, block, cultivar, cut, cultivar} \times \text{cut}; \text{ and}$
- Year 2: $Y = \text{period, block, sucrose (added or not), clover (25 or 50\% inclusion), sucrose} \times \text{clover}.$

Rumen parameters and how they are calculated are explained by Volden (2011). The NorFor model for diet formulation (Volden, 2011) includes a rumen load index (**RLI**) that predicts the acid load of the diet as well as the digestibility of chemical constituents in the diet. It is calculated as the ratio between the sum of rumen-degraded starch and WSC intake and sum of intake of NDF and soluble fiber, where rumen-degraded starch is estimated by the NorFor model and soluble fiber is calculated as the WSC-free residual fraction. Predictions from the NorFor model were compared by regression of observed values on predicted values. Comparisons were made as simple regressions.

RESULTS

Composition of Crops at Harvest and in Silage

Differences in WSC content between the herbage of the 2 contrasting grass cultivars were minor in yr 1, with 12 g of WSC/kg of DM higher in the first cut and 32 g higher in the second cut (Table 1) for the high-WSC cultivar compared with the standard cultivar. Ensiling decreased WSC further and, after mixing the grasses with clover, the WSC content of the contrasting silages as fed differed by only 11 g for the first-cut silage and 8 g for the second-cut silage (Table 2). The CP content in all silage mixes was within the range of 160 to 170 g/kg of DM silage. Due to the low survival of crops in the winter preceding yr 2 and to the minor differences in WSC content found between the grasses, it was decided to mix all grass silages to a basic mix and add sucrose to ensure significant differences in WSC content between treatments (Table 2). This would not entirely mimic the natural abundance of fructans in high-sugar ryegrass (Harrison, 2012), but create a substitute treatment of WSC with a fermentation rate intermediate between inulin and glucose (Hall and Weimer, 2016). Content of ammonia-N and pH in silage as fed indicated good-quality silage in both years

(Table 2), as did the concentrations in silage DM of lactic (1–3%), acetic (0.2–0.6%), and butyric (<0.06%) acid.

Intake and Production

We found only minor differences in feed intake (kg of DM) when comparing different treatments. In yr 1, however, feeding second-cut silage resulted in lower ($P < 0.03$) silage DMI, mainly due to low intake of the high-WSC ryegrass (Table 3). However, intake of WSC more than doubled when the first-cut silage was fed in yr 1, independent of cultivar. In yr 2, silage intake was similar in terms of DMI when comparing different sucrose levels, but WSC intake increased by more than 50% when sucrose was added to the silage. The cows consumed all concentrates fed in yr 1, but in yr 2 the addition of sucrose to the silage resulted in a numerically small, but significant ($P < 0.02$), decrease in concentrate DMI. The CP content in all diets was, on average, around 17% of DM. Feeding differing WSC levels resulted in no differences in milk yield or milk composition in either year. Increasing clover proportion in yr 2 tended to increase milk yield (as kg/d; $P = 0.08$) and increased protein yield (as kg/d; $P = 0.04$), whereas fat content in milk decreased ($P < 0.05$).

Nitrogen Utilization

Silage with a higher WSC content (i.e., first-cut silage in yr 1 and sucrose-fortified silage in yr 2) resulted in reduced N intake (Table 4). This resulted in larger proportions of feed N being recovered in milk and in feces with the high-WSC silages. We also noted a cultivar $\times$ cut interaction for N intake with second-cut forage in yr 1, where the standard cultivar resulted in higher N intake and the high-WSC variety in lower N intake. The yr 1 effects of cut and the cultivar $\times$ cut interaction were also evident in NorFor estimates of rumen-degraded and undegraded protein intake, but the effect of lowered N intake with sucrose addition observed in yr 2 was no longer significant when split into degraded

and undegraded protein by NorFor (Figure 1). Creatinine excretion was slightly higher with the second-cut silage in yr 1 (Table 4), whereas no treatment effect was observed in yr 2. Creatinine levels were numerically different (although not statistically tested) between years (25.9 mg/kg of BW in yr 1 and 21.0 mg/kg of BW in yr 2). Allantoin excretion in yr 1 was slightly higher with the second-cut silage, but we found no differences in yr 2 (Table 4). Microbial protein estimates made with the NorFor model ranked the diets differently from the allantoin measurements, with a positive correlation ($r = 0.55$) to N intake but a negative correlation to allantoin excretion ($r = -0.31$). The arithmetic mean of observations ($n = 6$ per treatment) and NorFor prediction of N excreted in urine had a mean prediction error (MPE) of 26% (Figure 1d). When the calculated N balance was added to the observed amount of N recovered in urine and compared with the NorFor prediction of N excreted in urine, the MPE decreased to 13% and the root mean square error of prediction was 28 g/d.

Apparent Digestibility

We noted only minor differences in digestibility between the different grass cultivars and cuts in yr 1, with the exceptions that the second-cut silage showed higher NDF digestibility. In yr 2, more sucrose gave lower digestibility of NDF ($P < 0.006$) and CP ($P = 0.07$). Increased clover content decreased DM digestibility ($P = 0.03$) and tended to decrease OM digestibility ($P = 0.06$).

Arithmetic means of each treatment for observed values and predictions of NorFor regarding total apparent digestible NDF (tdNDF) and N (tdN) and N excretion in feces are shown in Figure 1a to c. There were 6 observations per treatment, except for the high-WSC, cultivar, second-cut treatment, where 1 observation in period 3 was deleted due to unreasonable results of AIA analyses on feces. NorFor overestimated tdNDF and tdN and underestimated N excretion in the feces (Figure 1). The MPE calculated on the arithmetic means for tdNDF, tdN, and N excretion in feces was 17, 5, and

Table 1. Water-soluble carbohydrate (WSC) content [g/kg of DM; means (SD)] in herbage and basic silages (n = 3)

WSC content	Yr 1					Yr 2	
	Standard ryegrass		High-sugar ryegrass		Red clover	Ryegrass ¹	Red clover
	1st cut	2nd cut	1st cut	2nd cut	2nd cut	2nd and 3rd cut	2nd cut
Herbage	182 (12)	100 (9)	194 (11)	132 (10)	84 (3)	161 (42)	49 (0.2)
Silage	155 (12)	69 (13)	167 (21)	66 (14)	69 (11)	151 (45)	60 (1.0)

¹Ryegrass from 2 cultivars and 2 cuts were mixed to a basic grass silage for each treatment and period (n = 12).

Table 2. Chemical composition and calculated nutrient content in feeds [g/kg of DM, unless otherwise stated; means (SD), n = 3]

Item	Yr 1					Yr 2				
	Standard ryegrass ¹		High-sugar ryegrass ¹			No sucrose added		Sucrose added		
	1st cut	2nd cut	1st cut	2nd cut	Concentrate	RC 25 ²	RC 50	RC 25	RC 50	Concentrate
DM, g/kg of feed	468 (3)	370 (1)	440 (81)	406 (2)	872 (10)	320 (8)	325 (15)	347 (2)	334 (5)	874 (9)
Ash	86 (3)	103 (1)	90 (1)	104 (2)	36 (2)	89 (1)	87 (4)	82 (1)	84 (1)	33 (1)
Water-soluble carbohydrates	100 (3)	39 (12)	111 (14)	47 (4)	40 (2)	115 (4)	102 (6)	198 (16)	189 (6)	49 (4)
Starch	—	—	—	—	467 (13)	—	—	—	—	490 (2)
ME, MJ/kg of DM	10.9 (0.1)	11.0 (0.1)	11.0 (0.1)	10.8 (0.1)	13.9 ³	10.7 (0.1)	10.6 (0.1)	11.1 (0.0)	10.8 (0.1)	13.9 ³
CP	161 (0.3)	171 (1)	159 (1)	161 (0.3)	173 (3)	175 (3)	169 (4)	162 (2)	158 (4)	177 (0.2)
NH ₃ -N, % of total N	7.2 (0.11)	7.5 (0.49)	6.2 (0.37)	8.1 (0.48)	—	10.1 (0.65)	10.9 (1.39)	9.3 (0.31)	9.6 (1.00)	—
pH	5.6 (0.04)	5.4 (0.11)	5.6 (0.07)	5.3 (0.04)	—	5.0 (0.07)	5.0 (0.04)	5.3 (0.07)	5.2 (0.03)	—
NDF	404 (3)	434 (2)	413 (11)	422 (3)	202 (7)	388 (8)	386 (9)	360 (9)	348 (8)	179 (5)
ADF	253 (1)	281 (1)	263 (1)	281 (4)	113 (5)	256 (8)	274 (5)	233 (6)	246 (6)	102 (5)
Lignin	42 (2)	41 (1)	40 (2)	37 (1)	24 (2)	56 (5)	64 (1)	49 (1)	57 (1)	26 (3)
Indigestible NDF, g/kg of NDF	154 (5)	138 (4)	160 (6)	132 (3)	265 ³	192 (11)	211 (7)	176 (7)	207 (12)	265 ³
Degradation coefficient of NDF, %/h	4.4 (0.3)	4.8 (0.1)	4.3 (0.2)	4.9 (0.3)	5.1 ³	5.3 (0.6)	5.6 (0.2)	5.6 (0.5)	5.1 (0.4)	5.3 ³

¹Ryegrass mixed with 25% red clover (DM basis) in yr 1.²Red clover (RC) percentage (25 or 50%) on a DM basis in yr 2.³Declared by the manufacturer.

10%, respectively, and coefficient of determination was 0.55, 0.55, and 0.08, respectively.

Rumen Fermentation

The overall mean rumen pH was 5.99, and increased WSC level had no effect on pH expressed in different ways (Table 5). In yr 1, RLI calculated by the NorFor model was higher with first-cut than second-cut silage. In yr 2, RLI increased with sucrose addition and also with increasing clover proportion. In yr 2, the ammonia level in rumen liquid was significantly lower when more sucrose was fed ($P < 0.01$), and in yr 1 there was a similar tendency ($P < 0.08$). No significant differences in total ruminal VFA concentration were seen in either year. The only effect on proportions of the 3 principal VFA in yr 1 was a tendency for higher butyrate proportion with first-cut silage. In yr 2, sucrose addition decreased acetate proportion but increased the proportions of both propionate and butyrate. In yr 1, isoacid (isobutyrate and isovalerate) proportions were larger with second-cut silage than with first-cut silage; in yr 2, the isoacid proportions decreased with sucrose addition.

Rumen Pools

There were no treatment effects on ruminal pools of DM, OM, CP, NDF, or total purines in either year (Table 6). Pool size was similar between years except for the CP pool, which was numerically larger in yr 2 (by 500 g, corresponding to 27%).

DISCUSSION

The general conclusion from the agronomy part of the Sweetgrass project was that, when averaged across all cuts and years, there was no significant difference in WSC content between ryegrass bred for high WSC content and a control cultivar (O'Kiely et al., 2005). Taking into account the lower DM yield of the high-WSC cultivars, total yield of WSC (kg/ha) was similar for the standard and high-WSC cultivars (Halling et al., 2004). These findings explain the small differences in WSC content found between cultivars on harvesting grasses for the present feeding study. Although general interest exists in the effects of sugar in the diet of dairy cows, comparing diets with only minor differences in WSC content did not seem to be meaningful. It was necessary to add sucrose to the silages in yr 2 to achieve the desired variation in WSC content between treatments. Sucrose addition would give a somewhat different WSC composition compared with a natural abundance of WSC of the same magnitude, because the

Table 3. Feed intake, BW, and milk production for cows fed different silage mixtures [LSM and SEM; n = 16 in yr 1, n = 12 in yr 2]

	Yr 1						Yr 2						Effects, yr 1				Effects, yr 2			
	Standard ryegrass			High-sugar ryegrass ¹			No sucrose			Sucrose added			P-value				P-value			
	1st cut	2nd cut		1st cut	2nd cut		RC 25 ²	RC 50		RC 25	RC 50		SEM	Cultivar	Cut	Cultivar × Cut	SEM	Sucrose	Clover	Sucrose × Clover
Item																				
Feed intake																				
Silage, kg of DM/d	14.8	15.1		15.5	13.9		13.9	14.4		14.5	14.3		0.5	0.40	0.03	0.002	0.7	0.45	0.62	0.27
Concentrate, kg of DM/d	7.0	7.0		7.0	7.0		6.7	6.7		6.4	6.7		0.05	—	—	—	0.1	0.02	0.34	0.49
Total DM, kg/d	21.8	22.1		22.5	20.9		20.6	21.1		20.9	20.9		0.5	0.40	0.03	0.002	0.8	0.90	0.47	0.37
Water-soluble carbohydrates, kg/d	1.75	0.85		2.01	0.94		1.92	1.78		3.15	3.03		0.06	<0.001	<0.001	0.003	0.12	<0.001	0.08	0.96
CP, kg/d	3.58	3.78		3.54	3.44		3.50	3.56		3.37	2.47		0.5	<0.001	0.26	0.007	0.14	0.20	0.39	0.65
BW, kg	650	646		652	671		671	679		680	683		10.7	0.27	0.10	0.95	22	0.01	0.02	0.27
Milk production																				
Milk, kg/d	28.4	28.1		28.3	27.8		24.1	25.4		24.7	25.9		0.9	0.73	0.44	0.89	1.1	0.39	0.08	0.17
ECM, kg/d	30.2	29.9		30.0	29.4		27.1	26.8		26.6	28.1		1.0	0.60	0.50	0.75	1.0	0.63	0.47	0.82
Protein, kg/d	0.95	0.93		0.94	0.93		0.81	0.85		0.83	0.90		0.03	0.71	0.32	0.96	0.03	0.16	0.04	0.22
Fat, kg/d	1.25	1.25		1.24	1.21		1.17	1.10		1.10	1.18		0.05	0.61	0.66	0.65	0.04	0.97	0.96	0.24
Lactose, kg/d	1.38	1.36		1.37	1.35		1.17	1.24		1.21	1.26		0.05	0.54	0.29	0.99	0.06	0.40	0.12	0.20
Protein, g/kg	33.7	33.5		33.7	33.6		33.8	33.6		34.0	34.7		0.7	0.75	0.46	0.64	0.6	0.16	0.61	0.70
Fat, g/kg	44.2	44.8		44.4	43.9		48.0	43.6		46.7	450.0		1.5	0.69	0.97	0.46	1.4	0.99	0.005	0.004
Lactose, g/kg	48.9	48.5		48.5	48.5		48.6	48.8		49.1	48.8		0.4	0.17	0.29	0.22	0.6	0.53	0.94	0.64

¹Ryegrass mixed with 25% red clover (DM basis) in yr 1.²Red clover (RC) percentage on a DM basis (25 or 50%) in yr 2.

Table 4. Nitrogen intake, N partitioning, and N digestibility in cows fed different silage mixtures [LSM and SEM; n = 8]

Item	Year 1				Year 2				Effects, yr 1			Effects, yr 2		
	Standard ryegrass		High-WSC ¹ ryegrass		No sucrose		Sucrose added		P-value			P-value		
	1st cut	2nd cut	1st cut	2nd cut	RC 25 ²	RC 50	RC 25	RC 50	SEM	Cultivar	Cut × Cut	SEM	Sucrose Clover × Clover	Sucrose Clover × Clover
N intake, g/d	561	595	558	542	577	568	540	546	14.3	0.01	0.34	24	0.02	0.91
Fraction of N intake														
Milk N	0.258	0.242	0.260	0.251	0.232	0.230	0.247	0.260	0.007	0.33	0.05	0.01	0.02	0.52
Fecal N	0.327	0.314	0.356	0.308	0.303	0.323	0.349	0.410	0.011	0.31	0.02	0.02	<0.001	0.01
Urinary N	0.314	0.336	0.272	0.370	0.320	0.331	0.315	0.293	0.016	0.81	<0.001	0.02	0.26	0.76
N balance	0.100	0.110	0.111	0.070	0.137	0.121	0.093	0.042	0.022	0.48	0.45	0.03	0.01	0.14
Urinary marker excretion and protein supply estimates														
Creatinine, g/d	15.7	16.3	15.6	17.0	14.3	14.0	13.5	13.6	0.5	0.21	0.009	1.1	0.52	0.93
Creatinine, mg/kg of BW	24.8	26.2	25.0	27.7	21.8	21.1	20.4	20.6	0.6	0.21	0.009	1.4	0.46	0.84
Allantoin, g/d	44.1	53.8	45.7	50.6	50.9	53.6	48.0	55.9	2.33	0.45	0.02	4.4	0.84	0.16
MCP synthesis, ³ g/d	1,468	1,960	1,554	1,796	1,813	1,948	1,669	2,064	143	0.77	0.02	246	0.95	0.21
MCP synthesis, ⁴ g/d	2,163	2,223	2,295	2,086	2,122	2,135	2,077	2,101	72.7	0.96	0.14	17.9	0.70	0.96
MCP absorption, ⁴ g/d	1,838	1,889	1,950	1,772	1,799	1,802	1,778	1,781	61.8	0.96	0.14	95.0	0.48	0.74
RDP, ⁴ g of CP	2,692	2,790	2,608	2,642	2,866	2,922	2,756	2,789	52.6	0.002	0.30	114.2	0.05	0.83
RUP, ⁴ g of CP	734	805	774	695	680	669	726	700	27.0	0.04	0.82	41	0.11	0.43
Digestibility														
DM	0.682	0.688	0.674	0.716	0.708	0.701	0.710	0.670	0.02	0.57	0.18	0.01	0.13	0.03
OM	0.703	0.705	0.696	0.732	0.721	0.719	0.722	0.687	0.01	0.57	0.26	0.01	0.11	0.06
NDF	0.529	0.594	0.534	0.612	0.613	0.614	0.588	0.536	0.02	0.65	0.01	0.02	0.006	0.13
CP	0.677	0.621	0.621	0.689	0.681	0.674	0.656	0.593	0.02	0.81	0.02	0.03	0.07	0.22

¹WSC = water-soluble carbohydrates; ryegrass mixed with 25% red clover (DM basis) in yr 1.²Red clover (RC) percentage on a DM basis (25 or 50%) in yr 2.³MCP = microbial CP, calculated from allantoin excretion.⁴Rumen-degradable and rumen-undegradable protein estimated by NorFor model (Volden and Larsen, 2011).

Figure 1. Observed data and NorFor predictions (Volden and Larsen, 2011) in cows fed ryegrass silage with different sugar concentrations and red clover inclusion. Markers indicate means from (n = 6) observations with the respective root mean squared error of prediction (RMSPE) from all observations of total apparently digested NDF (tdNDF, 0.77 kg/d), total apparently digested nitrogen (tdN, 19 g/d), fecal N (19 g/d), and urinary N (62 g/d). Year 1 treatments all included 25% red clover: standard cultivar 1st cut (□), standard cultivar 2nd cut (○), high-sugar cultivar 1st cut (■), and high-sugar cultivar 2nd cut (●). Year 2 treatments were 25% red clover no sugar (△), 50% red clover no sugar (◇), 25% red clover sugar added (▲), and 50% red clover sugar added (◆). The dashed line is X = Y.

breeding of high-sugar ryegrass varieties has mainly led to fructan accumulation in the leaves (Harrison, 2012). Hall and Weimer (2016) reported that grass fructans had a disappearance rate by mixed rumen bacteria

slightly lower than for glucose but twice that of inulin, whereas there was a lag phase for both grass fructan and inulin but not for glucose. The sucrose added in our experiment would probably have a somewhat more

Table 5. Rumen fluid characteristics for cows fed different silage mixtures (LSM; n = 4)

Item	Yr 1				Yr 2				Effects, yr 1				Effects, yr 2			
	Standard ryegrass		High WSC ryegrass ¹		No sucrose		Sucrose addition		SEM		P-value		SEM		P-value	
	1st cut	2nd cut	1st cut	2nd cut	RC 25 ²	RC 50	RC 25	RC 50	SEM	Cultivar	Cut	Cultivar × Cut	SEM	Sucrose	Clover	Sucrose × Clover
pH, mean	5.98	6.03	5.98	6.05	6.04	5.97	5.90	5.94	0.04	0.66	0.11	0.77	0.09	0.25	0.82	0.40
pH, minimum	5.55	5.44	5.62	5.70	5.63	5.66	5.30	5.49	0.14	0.30	0.90	0.54	0.12	0.21	0.42	0.52
pH, maximum	6.69	6.53	6.52	6.68	6.73	6.57	6.56	6.73	0.06	0.79	0.98	0.02	0.07	0.96	0.99	0.17
Area pH <5.8, min × pH/d	34.5	21.2	26.1	19.2	22.6	29.8	30.0	40.1	15.5	0.61	0.35	0.75	27.2	0.76	0.76	0.96
pH <5.8, min/d	399	309	297	245	264	296	432	470	123	0.38	0.45	0.83	199	0.50	0.87	0.99
pH <5.8, episodes	2.44	1.76	2.28	1.52	2.1	2.0	2.5	2.9	0.62	0.73	0.26	0.94	0.9	0.59	0.89	0.82
Rumen load index ³	0.34	0.23	0.34	0.27	0.45	0.41	0.64	0.61	0.02	0.02	<0.001	0.04	0.02	<0.001	0.03	0.77
NH ₃ -N, mg/L	159.7	168.0	130.1	151.4	178.1	163.7	149.9	136.6	17.2	0.08	0.18	0.50	12.8	0.01	0.07	0.91
Total VFA, mM	154.9	150.2	150.0	148.7	147.0	152.2	153.7	154.6	3.97	0.11	0.12	0.32	5.1	0.41	0.58	0.70
Lactate, mM	<0.6	<0.6	<0.6	<0.6	0.53	1.16	0.81	1.00	— ⁴	— ⁴	— ⁴	— ⁴	0.17	0.57	0.02	0.09
Fraction of VFA, mol/100 mol																
Acetate	64.3	64.4	64.3	64.9	63.8	63.9	62.0	61.6	0.6	0.67	0.57	0.66	0.5	0.002	0.657	0.27
Propionate	18.5	19.4	18.8	18.6	18.3	18.0	19.0	19.7	0.5	0.67	0.53	0.33	1	0.05	0.60	0.70
Butyrate	13.4	12.3	13.0	12.8	14.1	14.0	15.3	15.0	1	0.90	0.10	0.22	0.7	0.02	0.52	0.17
Isobutyrate	0.8	1.0	0.8	1.0	0.9	0.8	0.7	0.6	0.03	0.43	0.004	0.77	0.04	0.02	0.07	0.44
Valerate	1.9	1.7	1.9	1.8	1.5	1.4	1.4	1.5	0.02	0.94	0.01	0.10	0.04	0.006	0.02	0.01
Isovalerate	1.0	1.2	1.0	1.2	1.1	1.0	0.9	0.9	0.09	0.07	0.005	0.24	0.07	0.001	0.09	0.15

¹Ryegrass mixed with 25% red clover (DM basis) in yr 1.²Red clover (RC) percentage on a DM basis (25 or 50%) in yr 2.³Estimated by NorFor model (Volden and Larsen, 2011).⁴Below detection level. No statistics possible.

Table 6. Rumen contents of cows fed different silage mixtures (LSM; n = 4)

Item	Yr 1				Yr 2				Effects, yr 1		Effects, yr 2	
	Standard ryegrass		High WSC ryegrass ¹		No sucrose		Sucrose addition		P-values		P-values	
	1st cut	2nd cut	1st cut	2nd cut	RC ² 25 ²	RC 50	RC 25	RC 50				
	SEM	Cultivar	SEM	Cultivar	SEM	Cultivar	SEM	Cultivar	Cut	Cultivar	SEM	Sucrose × Clover
Fresh weight, kg	85.3	91.6	84.8	79.9	86.4	91.0	84.4	90.3	5.57	0.11	4.7	0.46
DM, kg	13.50	13.24	13.08	12.85	13.1	13.9	13.7	13.3	0.41	0.33	0.7	0.94
OM, kg	11.44	12.01	11.07	10.11	11.9	12.6	12.5	12.1	0.98	0.14	0.6	0.92
CP, kg	2.04	2.14	1.91	1.79	2.4	2.5	2.6	2.5	0.16	0.12	0.1	0.27
NDF, kg	6.11	6.26	6.07	5.37	6.5	7.2	6.8	6.6	0.60	0.22	0.4	0.66
RNA, g	200	213	191	181	210	199	217	225	16	0.16	12	0.13
									0.93	0.37		0.30
												0.73
												0.23
												0.20
												0.50
												0.20
												0.82

¹Ryegrass mixed with 25% red clover (DM basis) in yr 1.
²Red clover (RC) percentage on a DM basis (25 or 50%) in yr 2.

pronounced effect on fermentation intensity than the natural abundance of WSC in high-sugar ryegrass, as in sucrose-rich feed such as molasses. Addition of inulin, which would have been a way to supply fructan, would probably give less intensive fermentation than naturally abundant grass fructan (Hall and Weimer, 2016).

Production Response to Elevated WSC Content

According to a review by Oba (2011), inclusion of WSC in the diet of cows often increases DMI. It has also been speculated that cows generally prefer sweetness over other tastes, and this has occasionally been demonstrated experimentally (Murphy et al., 1997). In the present study, WSC consumption varied from <1 to 3.15 kg/cow per day, representing a range from 4 to 15% of DM, which is higher than in most experiments presented in the literature. In our study, silage DMI and total DMI did not differ even between the extremes of WSC in the diet. This is in contrast to the increased intake observed when replacing starch with sucrose or lactose (Gao and Oba, 2016). An interesting observation was that when sucrose was added to the silage (yr 2), more residuals of concentrates were seen. This could be attributed to a higher metabolic load when more WSC is fed and might be worth considering when feeding high-producing dairy cows.

The lack of milk production response to WSC in our study was in line with findings by Moorby et al. (2006), who found no difference in milk production when high-WSC grass was fed to cows in early lactation. It is also in line with findings by Broderick and Radloff (2004) and reviews by Oba (2011) and Penner (2015), all of whom concluded that increased WSC in the diet does not increase milk production in general, with the exception of a moderate increase in milk fat yield. In fact, Broderick and Radloff (2004) found that feeding more than 6% WSC actually depressed milk production. It should be noted that, in the studies cited, sugar was increased at the expense of starch, whereas in the present study sugar was increased at the expense of fiber and protein.

The nutritional status of the cows is, of course, also of interest. The calculated energy intake for all treatments indicated that the cows had a positive energy balance in the range 105 to 110% for net energy and even higher for ME and that cows deposited body reserves during the experimental period (data not shown).

Nitrogen Efficiency

Contrary to experiments exchanging a carbohydrate source, typically starch, for WSC (Broderick and Radloff, 2004), replacing a standard ryegrass variety with

a high-WSC variety and addition of sucrose before feeding give a dilution effect on other constituents of the feed, including CP. This would improve N utilization even without any specific sugar effect. Although numerically small, there were indications that a higher percentage of intake N was directed to milk when sugar content increased in the diet. We also noted advantages in excretion of the nonmilk N portion when WSC intake increased (i.e., more N was directed to feces and less to urine), providing the conditions for lower N losses to the environment (Hristov et al., 2011). The average daily creatinine excretion of 23.4 mg/kg of BW in urine was similar to the 24.1 mg/kg of BW found by Chizzotti et al. (2008), and is in agreement with the bulk of previous results from our laboratory. However, we have no obvious explanation for the different levels in the 2 study years, but treatment or period effects of larger magnitude are known from other studies (Gonda et al., 1996; Moorby et al., 2016).

Microbial Production

Ruminal formation of microbial protein requires N and energy in the form of readily available carbohydrates. Sucrose would fulfill the energy demand, being entirely available for rumen fermentation and with a high fermentation rate (Weisbjerg et al., 1998). A faster fermentation rate is assumed to promote microbial efficiency through reduced microbial maintenance requirement, as long as this does not result in a detrimentally low pH (Russell et al., 1992). Thus, sucrose supplementation should increase microbial protein production, which should be detectable by increased duodenal purine flow and increased urinary allantoin excretion. Duodenal flow of purines was not measured in our study, but rumen pools were only numerically larger ($P = 0.13$) with sucrose addition, whereas the only allantoin effect was higher excretion with the second-cut silage in yr 1; this is contrary to expectations, because this silage provided the least WSC of all experimental diets. The NorFor model also did not suggest improvements in microbial protein production with sucrose additions. The factors most influential on NorFor predictions of microbial protein production were DMI and the amount of ruminally degraded carbohydrates. The lower concentration of branched-chain VFA in the rumen with increased dietary WSC concentration suggests less ruminal fermentation of AA (Van Soest, 1994) and, thereby, a potential improvement in the proportion of feed protein contributing to protein supply on an individual-animal level. This change could be expected when readily available carbohydrates in the form of WSC increase at the expense of CP in the ration.

Effect on Rumen Environment

Calculations using NorFor gave an RLI value that was at most around 0.6, which is the maximum value tolerated for optimizations in NorFor. However, the pH in the rumen, calculated in different ways (mean, minimum, maximum time below 5.8, number of episodes below 5.8), did not show any significant effect of WSC content in the diet. Total VFA concentration was around 150 mM, indicating a well-functioning rumen. An increased butyrate level was the most noticeable effect of increased dietary WSC on VFA in the rumen. Although the levels were numerically low, we observed a decrease in isobutyrate and isovalerate when more sugar was consumed. This is an indication of better supply of energy and more favorable conditions for AA supply to the cow, through increased rumen escape of protein supply (Van Soest, 1994); it also indicates faster fermentation rate. However, there may be a drawback, in that rumen fiber digestion could be impaired.

Apparent Digestibility

The clearest effect of adding sugar on apparent digestibility was that NDF digestibility decreased, with a tendency for the largest decrease at the higher clover inclusion rate (50%). Decreased fiber digestibility with sugar inclusion is in line with previous findings (Khalili and Huhtanen, 1991; Piwonka and Firkins, 1993); it has been attributed to reduced activity of fibrinolytic enzymes, either pH-dependent (Huhtanen and Khalili, 1992) or nondependent on pH, and to proteinaceous end-products from glucose fermentation (Piwonka and Firkins, 1996). Khalili and Huhtanen (1991) speculated that reduced ruminal digestibility of cell walls might be partially compensated for by increased microbial fermentation in the hindgut when sucrose was added. The increased fecal N output with sugar WSC in Year 2 supports increased hindgut fermentation, with subsequent excretion of the microbial N produced.

The prediction of digested N by the NorFor model corresponded well to the observed N digestions determined with AIA as a marker in the present experiment. Compared with the evaluation of the model by Volden et al., (2011), where the root mean square error of prediction of digested CP was 167 g, corresponding to 26.7 g of N. But the prediction error of digested NDF by the NorFor model deviated with 0.77 kg from the observed data, and was larger than the prediction error presented by Volden et al. (2011), which was 0.26 kg. The deviation might depend on measure uncertainties of determination of OMD, NDF, degradation coefficient of NDF, or estimation of iNDF and passage coefficient of NDF. The backward calculation of the

degradation coefficient of NDF of roughages (Volden, 2011) is slightly lower than the degradation coefficient of NDF that was determined with nylon bags of 27 μm , but does not explain the whole deviation. Simulations were done regarding measure uncertainties, such as decreased OM digestibility and increased NDF of the forages. Input values were changed 2 standard deviations of the internal standard sample used by the laboratory, 1.1 percentage units of in vitro OM digestibility and 5% of NDF, to decrease the rumen degradation rate and increase ruminal passage rate of forage NDF in the NorFor model. The MPE decreased below 8% and the general bias dropped in favor of the random bias. The high digestibility of the forage might fit better to a 1-compartment pool passage rather than the 2-compartment pool passage as is in the NorFor model (Volden, 2011). Simulations of a 1-pool scenario increased the value of passage coefficient of NDF and also decrease the MPE; the model must be evaluated by several experiments before equations will be changed.

CONCLUSIONS

Elevated WSC levels in forage were achieved at the expense of CP and fiber, but only minor effects on feed intake and milk production were found in this experiment. Positive effects observed included a more favorable distribution of N (i.e., more N to milk and feces and less to urine). In summary, a high-WSC perennial ryegrass cultivar gave only slightly higher sugar content than a standard cultivar and, even when extra WSC (as sucrose) was added, the effects on dairy cow performance were minor.

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REFERENCES

- Aharoni, Y., and H. Tagari. 1991. Use of nitrogen-15 determinations of purine nitrogen fraction of digesta to define nitrogen metabolism traits in the rumen. *J. Dairy Sci.* 74:2540–2547.
- Åkerlind, M., J. Bertilsson, and T. Eriksson. 2015. Har norra Sverige mer socker i vallen? [Are there more sugar in ley from Northern Sweden?] Svenska Vallbrev Nr. 5. Svenska Vallforeningen, Uppsala, Sweden (In Swedish).
- Åkerlind, M., M. Weisbjerg, T. Eriksson, R. Tøgersen, P. Udén, B. L. Ólafsson, O. M. Harstad, and H. Volden. 2011. Feed analyses and digestion methods. Pages 41–54 in *NorFor—The Nordic Feed Evaluation System*. H. Volden, ed. EAAP publication No. 130. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63:64–75.
- Broderick, G. A., and W. J. Radloff. 2004. Effect of molasses supplementation on the production of lactating dairy cows fed diets based on alfalfa and corn. *J. Dairy Sci.* 87:2997–3009.
- Chai, W., and P. Uden. 1998. An alternative oven method combined with different detergent strengths in the analysis of neutral detergent fibre. *Anim. Feed Sci. Technol.* 74:281–288.
- Chizzotti, M. L., S. C. Valadares Filho, R. F. D. Valadares, F. H. M. Chizzotti, and L. O. Tedeschi. 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. *Livest. Sci.* 113:218–225.
- Ellis, J. L., J. Dijkstra, A. Bannink, S. Parsons, G. R. Edwards, E. Kebreab, and J. France. 2011. The effect of high-sugar grass on predicted nitrogen excretion and milk yield simulated using a dynamic model. *J. Dairy Sci.* 94:3105–3118.
- Ericson, B., and J. André. 2010. HPLC—Applications for agricultural and animal science. Pages 23–26 in *Proc. 1st Nordic Feed Sci. Conf.*, 22–23 of June 2010. Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Eriksson, T. 2010. In vitro methods for indigestible Neutral Detergent Fiber (iNDF). Pages 41–44 in Report no. 274, Proceedings of the 1st Nordic Feed Science Conference. Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Eriksson, T., M. Murphy, P. Cizuk, and E. Burstedt. 2004. Nitrogen balance, microbial protein production, and milk production in dairy cows fed fodder beets and potatoes, or barley. *J. Dairy Sci.* 87:1057–1070.
- European Community. 2009. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. *Official Journal of the European Communities*, EC No. 152/2009. L54:1–130. <http://eur-lex.europa.eu/LexUriServ.do?uri=OJ:L:2009:054:0001:0130:EN:PDF>.
- Gao, X., and M. Oba. 2016. Effect of increasing dietary nonfiber carbohydrate with starch, sucrose, or lactose on rumen fermentation and productivity of lactating dairy cows. *J. Dairy Sci.* 99:291–300.
- Gonda, H. L., M. Emanuelson, and M. Murphy. 1996. The effect of roughage to concentrate ratio in the diet on nitrogen and purine metabolism in dairy cows. *Anim. Feed Sci. Technol.* 64:27–42.
- Hall, M. B., and P. J. Weimer. 2016. Divergent utilization patterns of grass fructan, inulin, and other nonfiber carbohydrates by ruminal microbes. *J. Dairy Sci.* 99:245–257.
- Halling, M. A., A. C. Longland, S. Martens, L. Nesheim, and P. O'Kiely. 2004. Accumulation of water-soluble carbohydrates in two perennial ryegrass cultivars at nine European sites. Pages 954–956 in *Proc. 20th General Meeting of the European Grassland Federation*, Luzern, Switzerland, 21–24 June 2004.
- Harrison, S. 2012. Developing metabolomics-type techniques for the high-throughput analysis of carbohydrate polymers to improve our understanding of fructan biosynthesis in grasses. PhD thesis. University of Auckland, New Zealand.
- Hoover, W. H. 1986. Chemical factors involved in ruminal fiber digestion. *J. Dairy Sci.* 69:2755–2766.
- Hristov, A. N., M. Hanigan, A. Cole, R. Todd, T. A. McAllister, P. M. Ndegwa, and A. Rotz. 2011. Review: Ammonia emissions from dairy farms and beef feedlots. *Can. J. Anim. Sci.* 91:1–35.
- Huhtanen, P., and H. Khalili. 1992. The effect of sucrose supplements on particle-associated carboxymethylcellulase (EC 3.2.1.4) and xylanase (EC 3.2.1.8) activities in cattle given grass-silage-based diet. *Br. J. Nutr.* 67:245–255.
- Khalili, H., and P. Huhtanen. 1991. Sucrose supplements in cattle given grass silage-based diet. 2. Digestion of cell wall carbohydrates. *Anim. Feed Sci. Technol.* 33:263–273.
- Larsson, K., and S. Bengtsson. 1983. Bestämning av lätt tillgängliga kolhydrater i vaxtmaterial (Determination of nonstructural carbohydrates in plant material). Method description no. 22. National Laboratory for Agricultural Chemistry, Uppsala, Sweden.

- Lindberg, J. E., and C. Jansson. 1989. A rapid automated analysis of allantoin in ruminant urine. *Swed. J. Agric. Res.* 19:163–167.
- Lindgren, E. 1979. Vallfodrets näringsvärde bestämt in vivo och med olika laboriemetoder. (Feed value of forage determined in vivo and by different laboratory methods). Rapport 45. Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden (in Swedish with English summary).
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Institute Inc., Cary, NC.
- Merry, R. J., M. R. F. Lee, R. J. Davies, R. J. Dewhurst, J. M. Moorby, N. D. Scollan, and M. K. Theodorou. 2006. Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. 1. In vitro and in vivo studies of nitrogen utilization. *J. Anim. Sci.* 84:3049–3060.
- Miller, L. A., J. M. Moorby, D. R. Davies, M. O. Humphreys, N. D. Scollan, J. C. MacRae, and M. K. Theodorou. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): Milk production from late-lactation cows. *Grass Forage Sci.* 56:383–394.
- Moorby, J. M., N. M. Ellis, and D. R. Davies. 2016. Assessment of dietary ratios of red clover and corn silages on milk production and milk quality in dairy cows. *J. Dairy Sci.* 99:7982–7992.
- Moorby, J. M., R. T. Evans, N. D. Scollan, J. C. MacRae, and M. K. Theodorou. 2006. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in early lactation. *Grass Forage Sci.* 61:52–59.
- Murphy, M. R., A. W. P. Geijssels, E. C. Hall, and R. D. Shanks. 1997. Dietary variety via sweetening and voluntary feed intake of lactating dairy cows. *J. Dairy Sci.* 80:894–897.
- Oba, M. 2011. Review: Effects of feeding sugars on productivity of lactating dairy cows. *Can. J. Anim. Sci.* 91:37–46.
- O'Kiely, P., P. Conaghan, H. Howard, A. Moloney, and A. Black. 2005. Grazing and Ensiling of Energy-Rich Grasses with Elevated Sugar Contents for the Sustainable Production of Ruminant Livestock. Beef Production Series No. 80, Teagasc, Carlow, Ireland.
- Penner, G. B. 2015. Optimal use of sugar in diets for dairy cattle. 77th Annual Cornell Nutrition Conference for Feed Manufacturers. East Syracuse, NY. Accessed Sep. 6, 2016. <https://ecommons.cornell.edu/handle/1813/41222>.
- Piwonka, E. J., and J. L. Firkins. 1993. Effects of glucose on fiber digestion and particle-associated carbomethylcellulase activity in vitro. *J. Dairy Sci.* 76:129–139.
- Piwonka, E. J., and J. L. Firkins. 1996. Effect of glucose fermentation on fiber digestion by ruminal microorganisms in vitro. *J. Dairy Sci.* 79:2196–2206.
- Rammer, C. 1996. Quality of grass silage infected with spores of *Clostridium tyrobutyricum*. *Grass Forage Sci.* 51:88–95.
- Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.* 70:3551–3561.
- Sjaunja, L. O., L. Baevre, L. Junkkarinen, J. Pedersen, and J. Setälä. 1991. A Nordic proposal for an energy corrected milk (ECM) formula. Pages 156–157 in Performance Recording of Animals: State of the Art, 1990: Proceedings of the 27th Biennial Session of the International Committee for Animal Recording. P. Gaillon and Y. Chabert, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Technicon. 1974. Technicon method No. SE4-0011FH4. Technicon Instruments Corp., Tarrytown, NY.
- Vagnoni, D. B., G. A. Broderick, M. K. Clayton, and R. D. Hatfield. 1997. Excretion of purine derivatives by Holstein cows abomasally infused with incremental amounts of purines. *J. Dairy Sci.* 80:1695–1702.
- Van Keulen, J., and B. A. Young. 1977. Evaluation of acid insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* 41:282–287.
- Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Comstock Publishing Associates, Ithaca, NY.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Volden, H., ed. 2011. NorFor—The Nordic Feed Evaluation System. EAAP publication No. 130. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Volden, H., and M. Larsen. 2011. Digestion and metabolism in the gastrointestinal tract. Pages 59–80 in NorFor—The Nordic Feed Evaluation System. EAAP publication No. 130. H. Volden ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Volden, H., L. T. Mydland, and O. M. Harstad. 1999. Chemical composition of protozoal and bacterial fractions isolated from ruminal contents of dairy cows fed diets differing in nitrogen supplementation. *Acta Agric. Scand. Anim. Sci.* 49:235–244.
- Volden, H., N. I. Nielsen, M. Åkerlind, and A. J. Rygh. 2011. System Evaluation. Pages 141–165 in NorFor—The Nordic Feed Evaluation System. EAAP publication No. 130. H. Volden ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Weisbjerg, M. R., T. Hvelplund, and B. M. Bibby. 1998. Hydrolysis and fermentation rate of glucose, sucrose and lactose in the rumen. *Acta Agric. Scand. Anim. Sci.* 48:12–18.
- Zinn, R. A., and F. N. Owens. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *Can. J. Anim. Sci.* 66:157–166.