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Milk production of Norwegian Red dairy cows on silages presumed either low or optimal in dietary crude protein content

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ABSTRACT

Dairy cow diets often exceed protein requirements for milk production. At the same time, dietary protein use efficiency for milk production is low leading to excretion of large amounts of nitrogen to the environment. We assessed the effects of feeding two grass/clover silages mainly differing in crude protein (CP) content on milk and component yields, nitrogen and gross feed utilization efficiency with Norwegian Red dairy (NRF) cows. Forty-eight early- to mid-lactation NRF dairy cows were randomly allocated to two dietary treatments (n = 24) after blocking by initial milk yield, stage of lactation, body weight and parity. Cows were fed silages either low in CP (late cut silage, LCPS; 112 g CP per kg dry matter (DM)) or optimal (mixture of 4 different silages, OCPS; 142 g per kg DM) *ad libitum* for a period of 54 days. These basal diets were augmented with a fixed level of concentrate feed (160 g CP per kg DM). This was estimated using the Nordic Feed Evaluation System (TINE Optfor KU optimization program) assuming OCPS as an available silage. We hypothesized that OCPS would support higher milk yield than LCPS, and the LCPS cows would consume more feed if they were to achieve similar level of milk yield as their OCPS counterparts. Contrary to our hypothesis, milk (23.7 vs. 24.5 kg per day), energy corrected milk (25.2 vs. 25.6 kg per day) yields and milk components were not significantly different between the groups (LCPS vs. OCPS). Furthermore, LCPS cows sufficiently matched DMI from the silage part of the diet to that of the OCPS cows (12.7 vs. 12.4 kg per day). However, OCPS decreased ($P < 0.01$) nitrogen use efficiency compared to the LCPS (30 vs. 33%). Our results confirm that reduction in dietary CP levels (ca 130 g per kg DM) can be achieved without loss of production, with reduced N excretion to the environment and reduced cost of milk production in moderately yielding cows. This would have a positive contribution to the current efforts being made to base our dairy production on locally available resources with minimal recourse to imported inputs.

1. Introduction

Ruminant production largely depends on the use of grazed and conserved forages. The digestibility and nutritive value of these forages vary considerably depending on stage of maturity of the crop (Rinne et al., 1999; Schroeder, 2012). Therefore, pasture management, feed conservation and feeding/grazing strategy have large effects on dry matter intake, animal performance (e.g. weight gain or milk yield) (Abrahamse et al., 2008; Kidane et al., 2014; Rinne et al., 1999) and nutrient use efficiency (e.g. nitrogen) (Kebreab et al., 2000).

In Norway, current efforts to improve milk production from dairy cows have largely relied on increased use of imported protein (more than 90%) ingredients (Landbruksdirektoratet, 2016). There is large variation between production systems in protein use efficiency (Whelan et al., 2013), and the extensive use and associated excretion of nitrogen (N) to the environment is challenged. Thus, reducing dietary N content in the feed is one of the potent strategies to improve efficiency, mitigate N emission (Dijkstra et al., 2013; Godden et al., 2001; Hristov and Huhtanen, 2008) and reduce feed costs (Godden et al., 2001) in ruminant production systems.

However, this should not be at the expense of production, as the global food demand is increasing mainly due to the projected human population growth in the years to come. In Norway, the human population is predicted to grow by 20% by the year 2030, and a similar increase in Norwegian agricultural production has been called for. At the same time, a greenhouse gas (GHG) emission reduction of 30% (equivalent to the 1990 level) by 2020 is targeted (Government.no, 2017). Furthermore, Norway aims to be carbon neutral by 2050. Such commitments require increased degree of self-sufficiency based on domestic feed resources (Aaby et al., 2014), through improved feed use efficiency and reduced recourse to imported feed resources.

Efficiency of dietary protein utilization by dairy cows varies considerably ranging from 10–40% with an average of about 25%

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(Calsamiglia et al., 2010; Hristov and Huhtanen, 2008). Therefore, there is a potential for substantial improvements to be made on many commercial dairy farms (Powell et al., 2010). The objective of this experiment was to assess protein and gross feed utilization efficiency and performance of Norwegian Red dairy (NRF) cows fed grass/clover silages considered to be either low in CP (LCPS) or optimal in CP (OCPS). The OCPS was a standard silage used at the experimental farm (Animal Production Experimental Centre, Norwegian University of Life Sciences, Norway) for lactating dairy cows at the time. The LCPS was selected for the purpose of this experiment and was of sub-optimal quality relative to OCPS for lactating cows. We hypothesized that OCPS would support higher milk yield than LCPS, and if the LCPS cows were to achieve similar level of milk yield as their OCPS counterparts, they would need to consume more of the silage part of their diet, on top of the fixed level of concentrate. The outcomes were expected to benefit the strategic allocation of available local feed resources to dairy cows.

2. Materials and methods

2.1. Animal grouping, housing, and feeding treatments

The experiment was carried out at the Animal Production Experimental Centre at the Norwegian University of Life Sciences following the laws and regulations controlling experiments on live animals in Norway under the surveillance of the Norwegian Animal Research Authority.

Forty-eight early- to mid-lactation NRF dairy cows were used in the experiment that lasted for 54 days. At start of the experiment, the cows had mean days into milk (DIM ± SD) of 126 ± 60, body weight (BW ± SD) of 566 ± 46.7 kg, and milk yield (mean ± SD) of 27.8 ± 5.4 kg/day. The herd was composed of cows from 1st to 4th lactation in the order of 21%, 46%, 21% and 13%, respectively. The cows were housed in a free-stall accommodation with concrete slatted floors and rubber mat beds with regularly applied sawdust in the resting areas. All cows had free access to drinking water and to mineral blocks.

The cows were blocked by parity, pre-experimental milk yield and composition, and BW at the start of the experiment. Thereafter, cows within a block were assigned to one of the two groups (later termed as LCPS or OCPS) balancing for the above parameters. The LCPS was a grass/clover mixture silage with a CP content of 112 g/kg DM whereas, the OCPS was a silage blend from different grass/clover swards used on the farm (Animal Production Experimental Centre, Norwegian University of Life Sciences) with a CP content of 142 g/kg DM. Chemical composition of the silages and concentrate feed is reported in Table 1 in detail.

The cows in each group received *ad libitum* access to their respective grass/clover silages prepared from timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) dominated swards. The silages were fed from individual automatic feeders (BioControl AS, Rakkestad, Norway) equipped with vertically moving gates where electronic cow identification ensured each cow's access to the correct silage source. The 24 feed troughs for each silage type were placed adjacent to each other so that cows in each treatment could get easy access to the correct silage type. The available feed troughs were matched to the number of cows. This ensured cow access to the feed at any given time. This was also meant to minimize cow displacement during feeding (Huzzey et al., 2006) and to avoid the ambiguity of using individual cow per feed trough as an experimental unit.

For daily dry matter intake (DMI), the automatic feed troughs registered summed daily intakes from multiple feeding episodes during a day. Feed troughs were filled with fresh silages twice a day (during morning and afternoon milking) after moving the cows to a resting and milking area. A manually controlled gate separated the milking and silage feeding zones. Feed refusal was cleared every Monday and Friday each week. Furthermore, cows were given a concentrate feed (FORMEL Favør 90, Felleskjøpet Agri, Gardermoen, Norway) (Table 1) that was estimated to meet the requirements for the expected milk yield based on 305 days lactation curve, and nutrient balance according to the Nordic Feed Evaluation System (NorFor) feeding standards (NorFor, 2011). For individual cow, the estimation took into account-along with the OCPS quality- expected milk yield at mid-point of the experiment, rumen fill, energy balance, amino acid supply to the small intestine (AAT), and rumen protein balance (PBV). For the 24 LCPS cows, the concentrate proportion of the diet was estimated by the NorFor system assuming the OCPS was the only available silage. This estimated level of concentrate feed was fixed for the whole experimental period and was fed from two central feeders on split basis (maximum 2.0 kg per cow per visit) throughout the day.

Table 1
Chemical composition and feed values (g/kg DM, unless otherwise mentioned) of the two silages and concentrate feed used for the experiment.

	Diet components and their chemical composition		
Characteristics	LCPS	OCPS	(FF90)[a]
Dry matter, g/kg	457	371	874
Crude protein	112	142	160
Neutral detergent fiber	550	457	198
Acid detergent fiber	349	300	82
iNDF[c]	183	195	385
pdNDF[c]	817	805	615
Crude fat	39	36	34
Starch	–	–	394
Residual CHO[b]	206	211	171
Ash	57	83	70
sCP (g/kg CP)	554	583	144
NEL at 20 kg DMI, MJ/kg[d]	6.09	6.49	6.82
AAT at 20 kg DMI[d]	85.8	82.3	117.0
PBV at 20 kg DMI[d]	−16	20	−4
CAD, meq/kg DM[e]	452	375	–
OM digestibility, %[d]	73.8	76.9	–
Additional silage parameters			
Ammonia-N, g/kg N	49.0	67.0	–
Lactic acid	19.6	48.0	–
Acetic acid	3.5	8.9	–
Butyric acid	1.0	<0.0	–
Formic acid	5.0	6.8	–
Propionic acid	2.0	1.0	–
Ethanol	5.1	6.3	–
pH	4.9	4.5	–

sCP = buffer soluble CP.
[a] Commercial concentrate feed – FORMEL Favør 90 (Felleskjøpet Agri, Gardermoen, Norway).
[b] Residual CHO = 1000 – [Crude fat + Crude protein + Neutral detergent fiber + Ash + Fermentation products from feeds] (NorFor method, (NorFor, 2011)).
[c] iNDF (g/kg NDF) is total indigestible NDF as determined using 288 h in situ incubation according to NorFor (2011) standards and pdNDF is potentially degradable NDF (g/kg NDF) calculated as (1000-iNDF).
[d] Estimations made by Eurofins on net energy for lactation (NE_L), metabolizable protein (AAT), protein balance in the rumen (PBV), and organic matter (OM) digestibility.
[e] CAD is dietary cation anion difference calculated according to NorFor (2011).

2.2. Body weight and body condition score

The cows were weighed and body condition was scored by a trained assessor following morning milking every week. Body condition score (BCS) was taken on a scale from 1.0 to 5.0 (to the nearest 0.25, with 1.0 = emaciated and 5.0 = obese), using a scoring scheme prepared by GENO (GENO Global LTD.) for NRF cows.

2.3. Feed intake, chemical composition, and digestibility

Daily feed intakes of concentrate and silage were retrieved from the automated feeding system each morning starting from the second day of the experiment. Mean daily feed intake was reported as the sum of DMI from both concentrate and silages.

Feed samples were taken for chemical analysis once a week during the first two weeks and twice a week for the last six weeks of the experiment. Silages were sampled by taking a handful of grab sample from each feed trough immediately following the morning filling. Silage refusal and concentrate feed samples were taken twice a week (on Mondays and Fridays). The samples were thoroughly mixed by type, and split into two portions. One portion of the sample was stored in an air-tight plastic bag and preserved at or below −20 °C whereas a second sample was dried overnight at 103 °C to estimate DMI. At completion of the experiment, the frozen feed samples were thawed overnight, mixed thoroughly and divided in two portions (for concentrate feed just one subsample was taken from a bulk sample). One silage subsample was used for analysis of volatile components and mineral composition. Silage and refusal samples meant for chemical analysis were dried at 59 °C for 48 h.

Concentrate samples for starch analysis, and concentrate and silage samples for indigestible neutral detergent fiber (iNDF) were milled through 0.5 and 1.5 mm sieve size, respectively, using Retsch SM 200 cutting mill (Retsch GmbH, Haan, Germany). Samples for other analysis, except for silage volatile components, were milled using 1.0 mm sieve size.

Feed samples were analysed for ash using ISO 5984 method (550 °C for a minimum of 4 h) and Kjeldahl N using Method 2001.11 (AOAC, 2002) according to Thiex et al. (2002) with Kjeltec 2400/2460 Auto Sampler System (Foss Analytical, Hellroed, Denmark). The CP was estimated as N*6.25. Total starch content of the concentrate feed was analysed using AACCI Method 76–13.01 (Megazyme amyloglucosidase/α-amylase method). The NDF was determined with an ANKOM[220] fiber analyser (ANKOM Technology, Fairport, NY, USA) according to Mertens (2002) using sodium sulphite and alpha amylase. Similarly, the acid detergent fiber (ADF) was determined according to Method 973.18 (AOAC, 2000) with the modification that the samples were not washed with acetone and were corrected for ash (expressed as ADFom). Buffer soluble CP was determined according to Licitra et al. (1996) with the modification that incubation with borate–phosphate buffer was done at 39 °C for 1 h and that soluble N was analysed in the supernatant after centrifuging. Silage fermentation products and ammonia nitrogen were analysed on fresh samples (by Eurofins Food & Feed Testing Norway AS, Moss, Norway) as described in Dønnem et al. (2011). The refusal samples were analysed for NDF and CP content as described for the silage samples.

Whole tract apparent DM digestibility was estimated using iNDF as an internal marker in the feeds. For this purpose, fresh faecal grab samples of about 800 g per cow were taken on aluminium trays on three consecutive days in week 4 and week 8 of the experiment. On each sampling date, faeces was collected after morning milking (between 10:00 to 11:30 h local time). These samples were kept frozen at −20 °C until later dried at 60 °C for 48 h. For iNDF, the three samples per cow per week were combined on equal proportions on DM basis.

The iNDF content in feeds and faeces samples was determined as NDF after 288 h *in sacco* rumen incubation according to NorFor standards (NorFor, 2011) using bags made from Sefar Nitex cloth (Sefar Ag, Heiden, Switzerland), with 15 µm pores and about 200 cm^2 surface area.

2.4. Milk yield, composition, and nitrogen use efficiency

Cows were milked twice a day (between 06:15 to 08:15 h = a.m. and 15:00 to 17:00 h = p.m.) using milking machines. During each milking, individual milk yield was registered for a.m. and p.m. milking. Milk samples were taken regularly for standard chemical analysis. In brief, samples were taken during both a.m. and p.m. milking every Monday on week 1 and 2, and every Monday and Thursday on weeks 3 to 8 of the experiment. These sampling points matched to days 1, 8, 15, 18, 22, 25, 29, 32, 36, 39, 43, 46, 50 and 53 of the experiment. Samples were taken using sampling bottles containing Bronopol tablets (2-Bromo-2-nitropane-1,3 diol, Broad Spectrum Microtabs® II) and stored in a cold room (4 °C) until analysis. Milk protein, fat, lactose and urea contents were analysed using infrared milk analyser (MilkoScan 6000; Foss Analytical, Hilleroed, Denmark).

2.5. Calculations and statistical analysis

Some response variables and parameters not directly measured were calculated or estimated as indicated hereunder. Apparent total tract DM digestibility was estimated using iNDF in faeces and feeds according to Mehtio et al. (2016):

$$DMD_{iNDF}=\left(\frac{i\text{NDF in faeces} - i\text{NDF in feeds}}{i\text{NDF in faeces}}\right)*100$$

where, DMD_{iNDF} is dry matter digestibility based on iNDF as a marker and iNDF is the indigestible NDF content of feeds and faeces.

Residual feed intake (RFI) was calculated as the difference between actual and expected DMI for the achieved level of production. Gross feed use efficiency (FUE) was calculated as the ratio of energy corrected milk yield to total achieved DMI. Similarly, gross nitrogen use efficiency (NUE) was calculated as milk N (milk protein/6.38) divided by total N (dietary CP/6.25) intake.

Energy-corrected milk (ECM; kg) yield was calculated for individual cow based on mean milk chemical composition (g/kg) and fresh milk yield (MY; kg) according to Sjaunja et al. (1991) as $MY \times [(38.3 \times fat + 24.2 \times protein + 16.54 \times lactose + 20.7)/3140]$.

Data collected over the experimental days on each cow was assumed not independent (Littell et al., 1998). Therefore, DMI, BW, BCS, milk yield (fresh and ECM), milk component yields (fat, protein and lactose), MUN, and other parameters were analysed as repeated measurements by using Mixed Models ANOVA in SAS (SAS 9.4 for Windows, SAS Institute Inc. 2002–2012; Cary, NC, USA) with first order autoregressive AR(1) covariance structure. The full model used, for example, for milk yield was:

$$Y_{ijklm} = \mu + T_i + D_j + P_k + (TD)_{ij} + DIM_l + PreMY_m + e_{ijklm}$$

where: Y_{ijklm} = the response variable; μ = overall mean; T_i = the effect of treatment; D_j = the effect of day of measurement; P_k = the effect of parity; $(TD)_{ij}$ = the interaction effect between treatment and day of measurement; DIM_l = the effect of stage of lactation expressed as days into milk at start of the trial; $PreMY_m$ = the random effect of pre-experimental week milk yield used as a covariate and e_{ijklm} = the random error term.

Total tract DM digestibility (DMD_{iNDF}), mean daily body weight gain (ADG), mean DMI per kg BW, DMI per kg BW$^{0.75}$, concentrate DMI per day, and DMI as a multiple of maintenance were analysed using one way ANOVA. Statistical significance was declared at $P < 0.05$. Shorthand notations were used in tables with only tendencies ($0.05 \le P \le 0.1$) expressed in full for the judgement by the reader.

3. Results

3.1. Body weight and body condition score change

Mean BW development and BCS data are presented in Fig. 1 and Table 2, respectively. Mean BW and BCS adjusted for the starting BW and BCS as covariates did not differ between the treatments ($P > 0.05$). Cows in both groups gained BW with advancing days in the experiment (effect of date, $P < 0.05$). As a result, linear estimates of average daily BW gain and changes in BCS were positive, but were not significantly

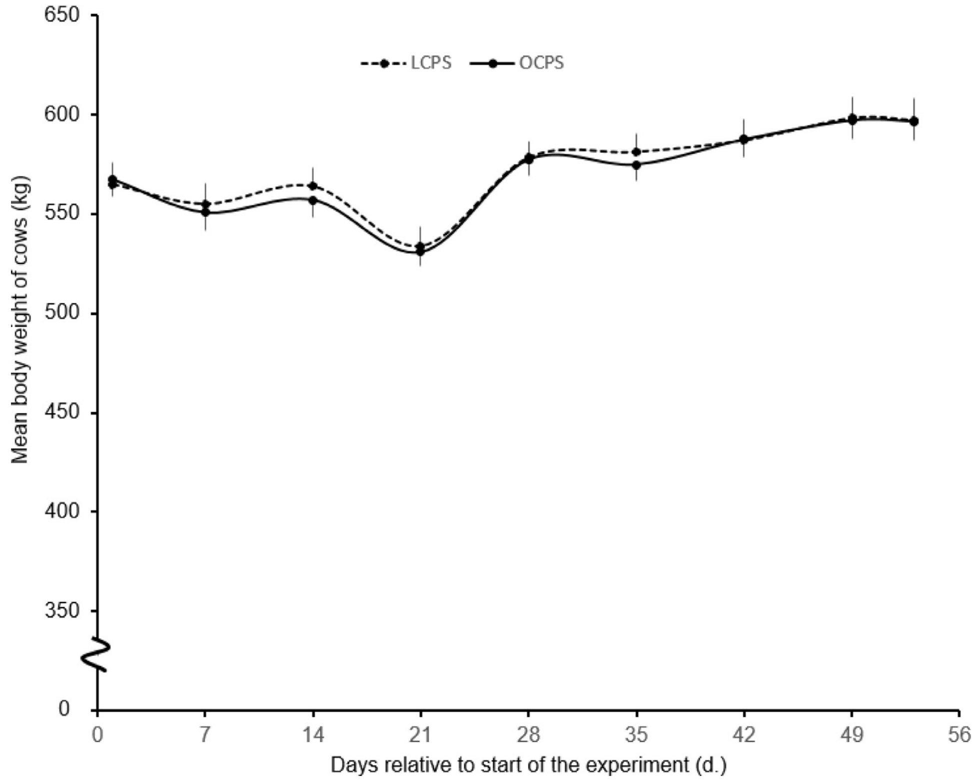

Fig. 1. Body weight change of two groups of cows fed grass/clover silages either low in CP (LCPS) or optimal in CP (OCPS) and supplemented with a fixed level of commercial concentrate (FF90).

different between the treatments.

3.2. Dry matter and nutrient intake

Data on DM and nutrient intake are presented in Table 2. Total achieved DMI, intakes partitioned into silage and the concentrate feed, mean DMI per kg live-weight, DMI per kg metabolic body weight ($BW^{0.75}$) and estimated NE_L intake as a multiple of maintenance were not significantly different ($P > 0.05$) between the two treatments. However, daily CP intake was significantly higher in the OCPS than the LCPS cows as planned. On the other hand, the LCPS cows achieved significantly higher NDF intake per kg live-weight compared to the OCPS cows.

Estimated intake of metabolizable protein calculated as a function of silage and concentrate feed DMI and their respective AAT (at 20 kg DMI) values was not different between the silage groups. However, the estimated total PBV was significantly higher for the OCPS than LCPS cows.

Intakes of silage DM and total DMI showed a slight fluctuation between days over the experimental period as manifested with a significant day of measurement by treatment interaction effects ($P < 0.001$). Furthermore, the estimated DMD_{iNDF} of the LCPS diet was significantly ($P < 0.01$) higher than that of the OCPS diet.

Silage refusal samples were analysed for NDF and CP contents as an index for feed sorting activity by the cows. This showed that, on average, NDF was 6.7% higher (6.4% for OCPS vs. 6.9% for LCPS) in the refusal DM relative to the offered feed. The CP contents decreased by 0.14% and 0.85% for LCPS and OCPS refusals, respectively.

3.3. Milk yield and composition

Mean milk yield, milk composition and component yields are presented in Table 3. Furthermore, observed individual cow daily milk yield and its distribution around mean predicted 305 days lactation curve, and silage group mean milk yields adjusted for stage of lactation are presented in Fig. 2.

Fresh milk and its chemical composition (lactose, protein and fat) were not affected by the silage qualities used for the dairy cows. As a result, milk components and ECM yields were not different between the two groups of cows. However, MUN was higher for cows fed the OCPS (9.76 vs. 8.19 mg/dl; S.E. = 0.304) with some fluctuations among the tested days as indicated by the interaction effect of day of measurement by treatment.

3.4. Feed and dietary nitrogen use efficiency

Gross FUE was not affected by the two silage qualities. However, cows in the OPCS exhibited significantly lower NUE compared to the LCPS cows. Similarly, calculated RFI values (Table 2) showed large variations among cows but were not different between the two silage groups compared.

Regardless of the dietary treatments, the daily DMI among individual cows showed large variation between measurement days. As a result, DMI fluctuated over the days for individual cows resulting in differences in daily quantitative CP intake (effect of experimental day; $P < 0.01$). The NUE of individual cows plotted against the quantitative daily CP intake over the experimental days showed a declining trend with increasing dietary nitrogen intake (Fig. 3). The lower NUE of OCPS cows was accompanied by a consistent and higher MUN (Fig. 4) over the milk sampling days of the experiment.

4. Discussion

The effects of feeding two types of glass/clover silages which differed mainly in CP on milk and component yields, NUE, FUE, and BW changes of NRF cows in the early- to mid-lactation stage were assessed. Contrary to our hypothesis, we observed only minor differences in milk yield and quality between cows fed LCPS and OCPS. However, reducing

Table 2
Mean body weight (kg), body condition score (x/5.0), linear estimate of average daily BW gain (AGD, kg/day), total DM and its component intake with other calculated values for cows fed two types of grass/clover silages either low in CP (LCPS) or optimal in CP (OCPS) and supplemented with a fixed level of commercial concentrate (FF90).

Parameters	Treatments		SEM	Statistical significance		
BW and BCS	LCPS	OCPS		Treatment	Date	Treatment* Date
BW	575	571	2.5	ns	***	ns
BCS	3.27	3.24	0.023	ns	**	ns
ADG	0.877	0.898	0.046	ns	–	–
Dietary characteristics, g/kg DM consumed unless otherwise mentioned						
Ration DM content, %	59.6	54.5	4.58	**	–	–
Silage DMI, % total DMI	66.6	65.5	10.09	ns	–	–
FF90 DMI, % total DMI	33.4	34.5	10.09	ns	–	–
CP	128.2	148.2	3.91	**	–	–
NDF	432.3	367.6	32.1	**	–	–
Starch + residual carbohydrate	320.0	325.3	31.6	ns	–	–
Crude fat	37.3	35.3	0.41	**	–	–
Ash	61.3	78.5	1.31	***	–	–
Achieved DM and its component intake, kg/day						
Total DMI	19.1	18.8	0.79	ns	***	***
Silage DMI	12.7	12.4	0.56	ns	***	***
Concentrate DMI	6.4	6.4	2.31	ns	–	–
CP intake	2.48	2.91	0.12	**	***	***
Other mean calculated intake values						
AAT[a]	1835	1770	42.0	ns	–	–
PBV[a],	−228	221	33.7	***	–	–
DMI, g/kg $BW^{0.75}$	162.5	164.0	6.77	ns	–	–
DMI, g/kg BW	33.2	33.0	1.42	ns	–	–
NDF, g/kg BW	13.8	11.6	0.35	**	–	–
Est. NEL intake X maint.[b]	3.49	3.64	0.16	ns	–	–
DMD_{iNDF}, %[c]	64.9	61.7	0.55	**	–	–
RFI[d]	*0.30*	*−0.78*	*1.212*	*ns*	–	–

FF90 is commercial concentrate feed used to augment the basal diets.
[a] Mean AAT and PBV (g/day) calculated based on diet composition and its estimated AAT or PBV at 20 kg DMI values made by Eurofins (Eurofins Food & Feed Testing Norway AS, Moss, Norway).
[b] Total estimated net energy lactation intake achieved as a multiple of maintenance.
[c] Total tract DM digestibility based on indigestible NDF as an internal marker.
[d] Residual feed intake – calculated as the difference between mean achieved intake and expected intake based on achieved level of production. ns = $P > 0.1$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$; – not tested.

the CP intake by feeding the LCPS silage improved NUE compared to feeding the OCPS silage. These results are discussed hereunder in relation to current efforts being made to reduce dietary CP level, improve NUE, and reduce N excretion to the environment without adverse effect on milk yield and quality.

4.1. Feed intake and milk yield

Dry matter intake between the two silage groups was similar. If CP intake were limiting in the LCPS group, this would have restricted rumen microbial activity with reduced DM digestibility and reduced DMI. The latter was not observed. Assuming that dietary CP was not limiting DMI, we expected the LCPS group to consume more silage if they were to achieve similar level of milk yield as the OCPS group. This was not observed either. The LCPS group matched the DMI to their OCPS counterparts. The higher estimated OM digestibility of the LCPS relative to the OCPS suggests that the achieved level of dietary CP intake was not limiting DMI. The NDF constituted close to half of the DM content of the silages. As such, differences between the silages in digestibility of the NDF component would influence total DMI and digestibility. The NDF in the LCPS had 12 g more pdNDF per kg NDF than the OCPS silage. This would further support higher passage rate and improved DMI (Oba and Allen, 1999) from LCPS relative to OCPS group. In our follow-up digestibility experiment with sheep fed at maintenance, OM digestibility in OCPS and LCPS was determined to be 73.6% and 72.1%, respectively, in contrast to the DM digestibility based on iNDF as an internal marker. The concentrate feed and silages were fed in equal proportions in the sheep digestibility experiment. Therefore, the estimated higher DM digestibility of the LCPS (Table 2) could be attributed to the higher pdNDF of the LCPS silage along with indications that cows from this group might have sorted the silage. Previous reports indicate that cows could easily sort against diet components when given a feed free of choice (Leonardi and Armentano, 2003; DeVries et al., 2007). The silage part of the ration was fed *ad libitum*, which would allow sorting. If selective feeding had occurred with LCPS, the quality of consumed DM would be better than what was offered. To this end, analysis on NDF and CP contents of the refusal samples support sorting for dietary components low in NDF in both groups with feed refusals consisting more NDF per kg DM compared to the silages offered. This increment in NDF of the refusal was accompanied by lower CP content (close to one percentage unit) for the OCPS silage. However, the CP content of the LCPS silage offered and refused were similar (a difference of 0.14% units), which was not easy to explain.

Under such high forage and high fiber diets, optimal DMI is

Table 3
Milk yield (kg/day), its chemical composition (g/kg) and other calculated and estimated parameters of cows fed two types of grass/clover silages either low in CP (LCPS) or optimal in CP (OCPS) and supplemented with a fixed level of commercial concentrate (FF90).

Milk yield and composition	Treatments			Statistical significance		
	LCPS	OCPS	SEM	Treat.	Date	Treat.* Date
Milk yield	23.7	24.5	1.43	ns	***	0.072
ECM[a]	25.2	25.6	1.26	ns	***	**
Fat	45.7	44.6	1.12	ns	***	***
Protein	35.3	35.4	0.69	ns	***	ns
Lactose	47.1	46.6	0.39	ns	***	***
Milk component yields, g/day						
Fat	1052	1058	49.3	ns	***	***
Protein	825.8	852.2	39.2	ns	***	ns
Lactose	1095.1	1106.7	63.1	ns	***	ns
Gross feed use efficiency						
FUE[b]	1.32	1.39	0.044	ns	***	***
NUE[c]	0.33	0.30	0.010	**	***	***

Statistical significance: ns = $P > 0.1$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
[a] Energy corrected milk yield calculated as ECM (kg) = Milk yield (kg) × (383 × fat-% + 242 × protein-% + 165.4 × lactose-% + 20.7)/3140].
[b] FUE is gross feed use efficiency calculated as kg ECM divided by kg DMI.
[c] NUE is gross nitrogen use efficiency calculated as g milk N divided g feed N.

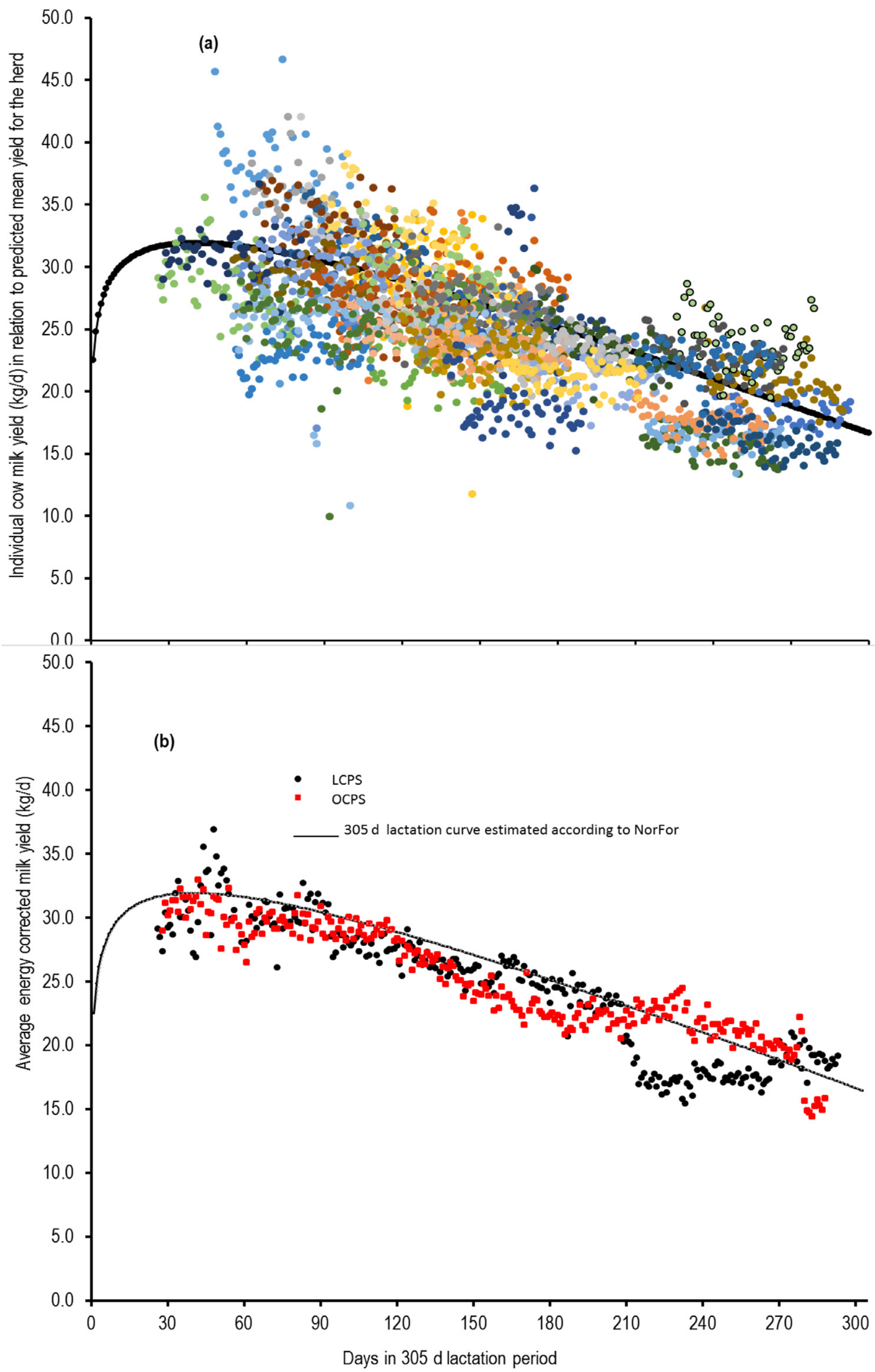

Fig. 2. Achieved milk yield in relation to 305 days lactation curve: (a) Individual cow energy corrected milk yield (kg/day) around 305 days lactation curve after adjusting for stage of lactation (DIM) and, (b) Mean energy corrected milk yield (kg/day) of two groups of cows fed grass/clover silages either low in CP (LCPS) or optimal in CP (OCPS) and supplemented with a fixed level of commercial concentrate (FF90).

supposed to be restricted by the bulkiness and slow disappearance of insoluble fiber from the gastrointestinal tract (Mertens, 2003). As such, optimal NDF intake for high yielding dairy cows is suggested to be about 1.25% of BW at mid-lactation (Mertens, 1997, 2003) with variations due to parity and stage of lactation (e.g. range for 2nd lactation high yielding cows; 0.87 to 1.30) (Mertens, 2009). Here, we observed mean values (± SD) of 11.6 ± 1.72 g/kg BW for OCPS and 13.8 ± 1.72 g/kg BW for LCPS. The values suggest that the latter group has maximized the potential DMI under restrictive rumen fill conditions, whereas for the OCPS group, rumen fill probably did not restrict DMI. As discussed above, the higher pdNDF of the LCPS could have contributed to this higher intake of NDF in the group.

Under the conditions of the above DM and nutrient intake, milk yield and composition did not differ between the two silage groups. Comparable results were shown by Hristov et al. (2015) where feeding high-producing dairy cows with diets low versus high in CP (15.4 vs.

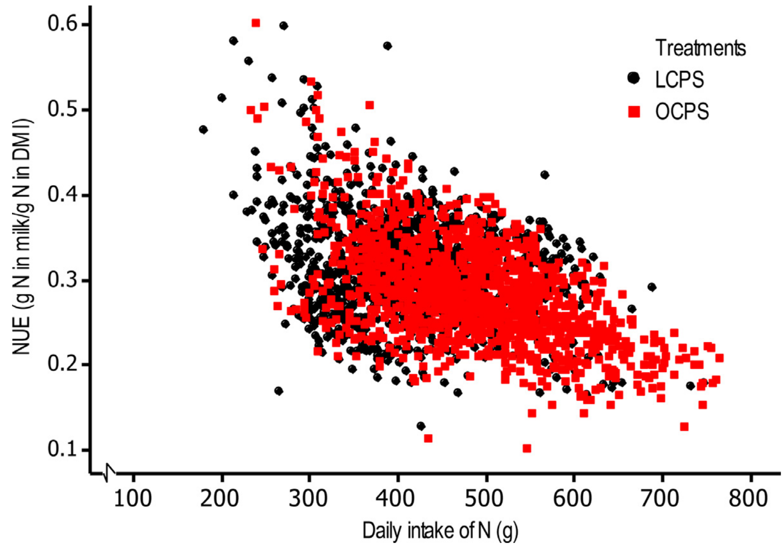

Fig. 3. The relationship between daily quantitative dietary nitrogen intake and apparent nitrogen use efficiency (g milk N per g dietary N intake) of two groups of cows fed grass/clover silages either low in CP (LCPS) or optimal in CP (OCPS) and supplemented with a fixed level of commercial concentrate (FF90).

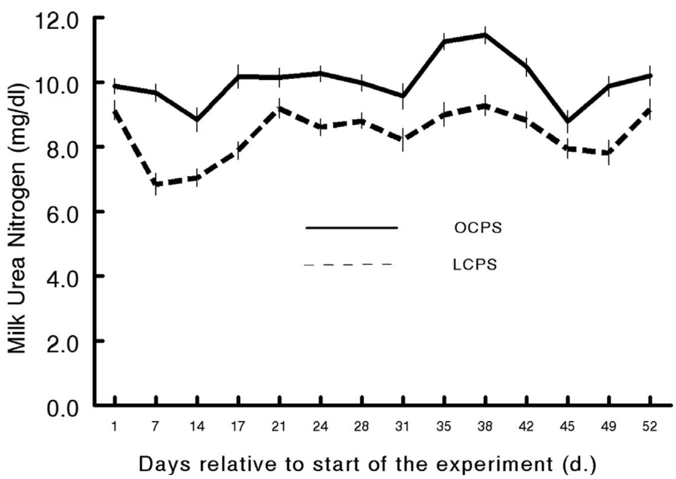

Fig. 4. Milk urea nitrogen over the milk sampling days of the experiment with two groups of cows fed grass/clover silages either low in CP (LCPS) or optimal in CP (OCPS) and supplemented with a fixed level of commercial concentrate (FF90). MUN was calculated as milk urea multiplied by 2.8011.

16.5%) did not affect milk yield and composition. Furthermore, Monteils et al. (2002) reported similar level of milk yields among three groups of cows fed with diets differing in CP (130, 145 and 160 g/kg DM). These findings support the often variable and weak marginal milk yield responses to increasing dietary CP (Bach, 2013; Broderick, 2003) at a higher CP level. In our experiment, the levels of achieved dietary CP were low and the margins between these levels were very narrow. At such levels, unless the LCPS silage limited feed intake due to reduced fiber digestibility and slow rate of passage, expected differences in milk yield would also be limited. Indeed, the level and balance of intestinally absorbable amino acids (AAT_N) is crucial in sustaining milk yield and composition with minimal dietary protein (Sinclair et al., 2014), in addition to energy-yielding metabolites. The estimate of this AAT_N (based on standard feed value for AAT at 20 kg DMI; (NorFor, 2011)) for the LCPS was equivalent to, if not better than, the OCPS. However, the estimated AAT_N assumes optimal protein balance in the rumen for microbial growth. Due to the low PBV values of the silage and concentrate feed used, AAT_N for the LCPS group could very well be an overestimate. The LCPS silage would have required a concentrate feed higher in PBV (and NE_L) for augmentation than the one used here if we had not wanted to challenge the group to meet their requirements from the *ad libitum* supply of the basal diet. Nonetheless, the matched DMI and estimated higher DM and NDF digestibility from the LCPS would mean that the actual differences in energy supply may have been less than what we expected. Therefore, despite the differences in dietary CP and NDF, the differences in the achieved levels of AAT_N and NE_L between the silage groups probably have not been strong enough to affect milk yield and composition.

4.2. Nitrogen use efficiency

Milk urea is a product of N metabolism and is influenced by diet composition such as dietary protein level (Broderick, 2003; Jonker et al., 1998), adequacy of energy (Broderick and Clayton 1997) and, as such, protein to energy ratio (Oltner, 1985). It is often used as an indicator of the level of diet adequacy (e.g., dietary N) (Broderick and Clayton, 1997) and as a non-invasive measurement to monitor N excretion and environmental impacts from dairy farms (Jonker et al., 1999; Jonker and Kohn, 2001). In the current experiment, in both silage groups, some calculated apparent NUE values at lower levels of estimated daily CP intake appeared to be unrealistic (e.g., ≥ 50% dietary N recovered in milk). Nevertheless, the LCPS cows had lower MUN and higher NUE compared to their OCPS counterparts. This fits the notion that reducing dietary CP level for dairy cows can improve NUE, and agrees with other reports (Agle et al., 2010; Broderick, 2003; Kalscheur et al., 2006; Monteils et al., 2002). The observed level of MUN in both silage groups is in the lower range of target MUN, at least as reported for the Holstein-Friesian cows (12–16 mg/dl by Jonker et al., 1999, and 8.5–11.5 mg/dl by Kohn et al., 2002). However, the declining NUE with increasing daily quantitative dietary CP intake (Fig. 2) by individual cows over different days clearly indicates its sensitivity to changing patterns of daily CP intake. Unfortunately, urinary urea excretion was not measured in this trial, but it has been reported that it is directly related to the MUN concentrations (Jonker et al., 1998, 1999; Kauffman and St-Pierre, 2001; Kohn et al., 2002; Roseler et al., 1993). Hristov et al. (2015) showed that decreasing crude protein concentration of the high yielding cow rations from 16.5% to 15.4% reduced laboratory level ammonia emission of reconstituted manure on average by 23%. Lee et al. (2012) reported similar findings of reduced N excretions when dairy cows diets were decreased in CP concentrations. This would suggest that the LCPS cows have excreted less urinary N based on our MUN values. Thus, the outcomes observed here on NUE and MUN with LCPS imply the possibility to reduce N excretion to the environment through reduced dietary CP in basal diets without adverse effect on milk yield, or its composition in mid-lactation dairy cows.

4.3. Changes in BW and BCS

The observed similar BW change and BCS over the experimental period with milk yield that approached to what is predicted suggested that both groups of cows achieved similar level of dietary nutrients. Even though silage was fed *ad libitum* from day one of the experiment, the offered fixed level of concentrate diet was estimated assuming the mid-point of the experiment as a starting point. This could have influenced DMI and, therefore, milk yield in two ways. First, this might have negative effects at the early part of the experiment when the cows were expected to have relatively heightened nutrient demands compared to the later part of the experiment. Second, this should mean that cows probably had more than their requirements towards the 2nd half of the experiment with declining nutrient demands due to advancing lactation. The latter effect could also be seen from the observed similar pattern in BW gain from mid-point of the experiment with both groups of cows.

5. Summary

The observed large variation among cows in daily milk yield relative to NorFor predictions provides opportunities for selective feeding and management of cows for improved feed efficiency. Dietary CP levels can be reduced in dairy cows with moderate yields to increase our reliance on local feed resources. Furthermore, this will reduce the environmental impacts of producing and transporting protein supplements, wastage of excess N to the environment [especially urinary N, which is the most labile component of excreted N], and more importantly reduce the cost of milk production. Balancing rations to achieve greater NUE, lower milk urea concentrations and lower feed costs whilst achieving high milk production could be achieved by fine-tuning feeding at individual cow level. Feeding a fixed level dietary CP at a herd level, for instance, as total mixed ration to reduce feed sorting and synchronize nutrient supply in the rumen, might not optimize the overall NUE as could be seen from the wide variation in NUE by individual cows. Our results suggest that dietary CP levels of 130 g/kg DM can be used for moderately yielding early- to mid-lactation NRF dairy cows, as long as the basal diet is provided *ad libitum*, with gains in NUE and without adverse effects on milk yield and composition.

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Conflict of interest

The authors do not have any conflict of interest and this submission has been done upon agreement of all the co-authors.

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