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Feed intake, milk yield and metabolic status of early-lactation Swedish Holstein and Swedish Red dairy cows of different parities fed grass silage and two levels of byproduct-based concentrate

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ABSTRACT

Dairy cows can produce highly nutritive food products (milk, meat etc.) from fibrous feed *e.g.* grass and different byproducts from the food and fuel industry, that cannot be consumed directly by humans. However, as there are limited amounts of byproducts available, decreasing the amount of byproduct-based concentrate in the diet could be a strategy for improving sustainability within dairy production if high milk production should be maintained. In this study, 26 multiparous (n = 14) and primiparous (n = 12) dairy cows of the breeds Swedish Red (n = 14) and Swedish Holstein (n = 12) were followed between lactation weeks 1 and 6. They were fed either a low-concentrate (n = 13; LC) or high-concentrate (n = 13; HC) byproduct-based (sugar beet pulp, rapeseed meal, distiller's grain, wheat bran) ration, in combination with highly digestible grass-clover silage *ad libitum*. To achieve similar concentrate intake per kg body weight in primiparous and multiparous cows, multiparous cows were offered 5 kg concentrate on the LC diet and 15 kg concentrate on the HC diet, while primiparous cows were offered 4 kg concentrate on the LC diet and 14 kg concentrate on the HC diet as target concentrate rations. We found no overall differences in dry matter intake, energy-corrected milk yield, energy balance, blood plasma metabolites, blood hormones or milk fatty acids between cows on the LC and HC diets. However, HC cows had a higher yield of ECM in lactation week 6 and gained body weight compared with LC cows. As expected, multiparous cows had higher dry matter intake and energy-corrected milk yield, but we found no difference in energy balance between parities. However, multiparous cows lost more body condition and had higher blood plasma concentrations of non-esterified fatty acids, indicating that they used more body tissue to support milk production. In conclusion, both multiparous and primiparous Swedish Red and Swedish Holstein dairy cows seem able to adapt to low-concentrate diets in early lactation when the diets are based on byproducts and grass-clover silage, providing the potential to increase sustainability in dairy production.

1. Introduction

Human population growth, in combination with greater per-capita income, are driving global increases in demand for food, especially of livestock origin. For several reasons, including climate change and ecosystem services, food security cannot be met by increasing the area of arable land. A more sustainable way of increasing food security could be by changing the diet of humans (IPCC, 2019) and farm animals (Van Zanten et al., 2019) in high-income countries. High-yielding dairy cows are often fed high proportions of cereal grain and pulses (FAO, 2014), which could be consumed directly by humans. Replacing cereal grain and pulses in the diet of dairy cows with byproducts and forage not suitable for human consumption would increase net food production (Ertl et al., 2015, 2016; Karlsson et al., 2018). Replacing human-edible products in dairy cow diets with byproducts such as sugar beet pulp, wheat bran, distiller's grain and rapeseed meal has been shown to have no negative effects on milk production in mid-lactation (Ertl et al., 2016; Karlsson et al., 2018; Pang et al., 2018). However, the strategy of replacing cereal grain and pulses with byproducts in high concentrate to forage ratios might not be possible if the supply of byproducts are insufficient on local or global scale. High-quality forage cannot be consumed by humans, but has the potential to maintain high milk production in dairy cows (Randby et al., 2012). Some forage is produced on land that could be used for cultivating crops directly consumed by humans. However, grass and legume leys for forage production are important in crop rotations and improve soil

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quality, increase soil carbon sequestration (Jarvis et al., 2017) and improve the ability of soil to supply other ecosystem services (Albizua et al., 2015).

Studies have found that dairy cows can maintain high milk production on byproduct-based diets (Ertl et al., 2016; Karlsson et al., 2018; Pang et al., 2018) and high-forage diets (Aguerre et al., 2011; Randby et al., 2012), but a combination of the two has not been well studied. The first weeks of lactation are demanding due to the lagging energy intake compared to the demand for milk production (Bauman and Currie, 1980; Ingvartsen, 2006). The consequences of high-forage diets in combination with byproduct-based concentrate on metabolic status and performance of early lactating cows have apparently not been investigated. Primiparous cows differ in many ways from multiparous cows. Primiparous cows have lower body weight (BW) and allocate some of the nutrients ingested to body growth, which influences metabolism, milk production and fertility differently in primiparous cows than in multiparous cows (Wathes et al., 2007). Apart from diet and parity, breed differences can also have a significant impact on metabolism and milk production (Ntallaris et al., 2017; Andrée O'Hara et al., 2018; 2019). Swedish Holstein (SH) cows might be more negatively affected regarding metabolic status and energy balance (EB) from high forage diets than Swedish Red (SR) due to the higher genetic potential for milk production of SH than SR cows (Interbull, 2020).

In the present study, the aim was to compare the response of primiparous and multiparous cows of the SH and SR breeds within the first six weeks of lactation in terms of feed intake, milk production and metabolic status when fed diets high or low in byproduct-based concentrate in combination with grass-clover silage *ad libitum*.

2. Material and methods

2.1. Animals, experimental design and housing

The study was conducted at the Swedish Livestock Research Centre, Uppsala, Sweden, from February to April 2016. It was performed in accordance with Swedish regulations on experimental use of dairy cows, was approved by the local Ethics Committee on Animal Research, Uppsala, Sweden (diary number C98/15) and complied with EU Directive 2010/63/EU on animal experiments.

A total of 26 dairy cows were used, where half of the cows were allocated to a low-concentrate (LC) ration and half to a high-concentrate (HC) ration (control). The cows were of SH and SR breed and both multiparous and primiparous. Every other cow that calved per parity class (primiparous *vs* multiparous) was allocated to the LC diet (6 primiparous and 7 multiparous cows, 6 SR and 7 SH cows) and the remaining cows were allocated to the HC diet (6 primiparous and 7 multiparous cows, 8 SR and 5 SH cows). The cows entered the study (mean ± SD) at 4.2 ± 2.4 days in milk (DIM) after calving and stayed in the study until 40–42 DIM.

All cows were housed in an insulated loose-house with rubber mats and sawdust-bedded cubicles. The cows were milked morning and evening in an automatic milking rotary system (AMR, DeLaval International AB, Tumba, Sweden). The experimental cows were housed together with other cows in a group of up to 64 animals that shared 22 forage troughs, four feed dispensers, 64 cubicles and eight water cups.

2.2. Diets and feeding

The concentrate was pelleted and based on byproducts of low nutritional value for humans (Table 1), while the forage was a grass-clover silage fed *ad libitum*. Chemical composition of silage and concentrates is shown in Table 2. All silage was first-cut silage harvested in early June 2015 from perennial swards sown with mainly timothy (*Phleum pratense* L.), with inclusion of perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* Schreb.), and red clover (*Trifolium pratense* L.). The herbage was precision-chopped, wilted, preserved using an acid-based additive (Promyr NT 570, Perstorp, Sweden) and stored in bunker silos. A stationary feed mixer, conveyor belt and automatic feed waggon (DeLaval International AB, Tumba Sweden) were used to distribute the forage to the cows up to seven times per day to ensure *ad libitum* access. Salt was mixed with the silage at 3.75 g per kg DM before distribution into the forage troughs. The cows had free access to water in water cups.

All cows in the study were fed the experimental concentrate from two weeks before expected calving, starting with 0.5 kg/d and increasing by 0.5 kg/d until 3 kg/d was reached. When the cows entered the group of milking cows after calving, the concentrate ration was increased by 0.5 kg/d for both treatments, to 4.0 (primiparous) and 5.0 (multiparous) kg/d for LC cows and 14.0 (primiparous) and 15.0 (multiparous) kg/d for HC cows. The higher concentrate ration fed to multiparous cows compared with primiparous cows was designed to

Table 1
Formulation of the byproduct-based concentrate (g/kg DM unless otherwise stated).

Ingredient	Concentrate
Sugar beet pulp[1]	500.7
Rapeseed meal[2]	167.5
Distiller's grain[3]	150.0
Wheat bran	80.0
Palm kernel expeller	40.0
Feed fat[4]	39.8
Molasses	20.0
Premix[5]	2.00

[1] Dried with no inclusion of molasses (Nordic Sugar AB, Eslöv, Sweden).
[2] Solvent-extracted and heat-moisture treated and with low levels of glucosinolates and erucic acid (ExPro®, AAK Sweden AB, Karlshamn, Sweden).
[3] Fibre and yeast cells from ethanol manufacturing (Agrow Drank 90, Lantmännen Agroentanol, Norrköping, Sweden).
[4] Fatty acids (99% fat; 45% C16:0, 37% C18:1) (AkoFeed® Cattle, AAK Sweden AB, Karlshamn, Sweden).
[5] Containing minerals (g/kg) Ca 61.9, P 0.4, Mg 408.9, K 1.0, Na 0.2, S 3.2, vitamin A 2,000,030 IE/kg, vitamin D3 1,000,090 IE/kg and vitamin E 20,011 mg/kg, and trace elements (mg/kg) Cu 5, Mn 10, Zn 25, I 0.35, Se 0.2 and Co 0.09.

Table 2
Chemical composition (mean ± SD) of experimental feeds (g/kg DM unless otherwise stated). Where standard deviation is reported, the number of samples used for analyses of chemical composition was $n = 6$ for silage and $n = 4$ for concentrates.

Item	Silage[1]	Concentrate
DM, g/kg	371 ± 28	868 ± 3
Ash	83.1 ± 1.9	56.0 ± 3.2
Crude protein	136 ± 7	184 ± 3
Crude fat	–[2]	58.7
NDF	450 ± 28	340 ± 7
Starch	–[2]	69.5
NE, MJ/kg DM[3]	6.56 ± 0.16	6.95 ± 0.04
ME, MJ/kg DM	11.7 ± 0.2	13.2[4]

[1] Grass-clover silage had pH 4.28 ± 0.10, NH_3–N concentration 15 ± 3 g/kg N and estimated *in vivo* digestibility of organic matter 79.0 ± 1.8% of OM.
[2] Not analysed.
[3] Calculated in NorFor (Åkerlind and Volden, 2011) based on chemical composition, and tabulated values and estimates where analytical data were lacking.
[4] Calculated based on concentrate formulation and tabulated values according to the Swedish Board of Agriculture (SJVFS, 2011).

approximately reflect a similar ratio of kg concentrate/kg BW, as multiparous cows weighed more.

2.3. Measurements and sample collection

Individual forage intake was recorded automatically from forage troughs (CRFI, BioControl, Ås, Norway), while concentrate dispensers (FSC400, DeLaval International. Tumba, Sweden) automatically recorded daily concentrate intake for each cow. The equipment used for forage intake recording was calibrated weekly, while that used for concentrate was calibrated monthly. The individual daily forage intake raw data showed improbably high feed intake for some cows and days, mainly because some cows threw silage out of the forage troughs. Therefore the intake value for feeding occasions with fresh matter intake rate >30 g/s (95% confidence level of all eating occasions for all cows included in the study) was replaced with individual intake estimates derived from daily average intake rate <30 g/s. Data on duration and weight of consumed feed was extracted from log-files from the CRFI system to calculate fresh matter intake rate from each feeding occasion. Cows that had been visually observed throwing forage matched those cows that had many feeding occasions with very high intake rates. Forage dry matter intake (DMI) and total DMI were also treated as missing values for those days when total DMI was above 34 kg dry matter (DM) (95% confidence level).

Silage was sampled five times a week, stored frozen and pooled to three-week periods before analysis. Concentrate was sampled once weekly, stored dry at room temperature and pooled to three-week periods before analysis of chemical composition. On two different occasions, three weeks apart, spot samples of faeces were collected from each cow once daily for three consecutive days (Mehtiö et al., 2016). The faeces samples were stored frozen before being pooled for each cow and occasion.

Milk was sampled for composition analysis at afternoon milking and the following morning in lactation week 2, 4 and 6 for each cow. The equipment used in milk sampling (MM6, DeLaval International AB, Tumba, Sweden) and measurement of milk yield has been certified by the International Committee for Animal Recording (Rome, Italy). Milk samples were preserved with bronopol, stored at 8 °C and analysed within three days.

The cows were weighed automatically when passing through a sort gate after milking, and mean daily body weight was recorded (originally from DeLaval International AB, Tumba, Sweden, but rebuilt by BioControl, Ås, Norway). The weight scale was calibrated monthly. Body condition scoring (BCS; scale 1–5) was recorded automatically with a 3-D camera (DeLaval International AB, Tumba, Sweden; Hallén Sandgren and Emanuelson, 2016) when cows passed through the sort gate after milking. Weekly mean BW and BCS were calculated from daily mean BW and BCS, respectively. The system used for identifying individual cows at milking, feeding, weighing and BCS has been certified by the International Organization for Standardization (Geneva, Switzerland).

In lactation week 2, 4, and 6, blood was collected from the coccygeal artery or vein into 10-mL vacuum tubes with lithium heparin as anticoagulant (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ). The blood samples were centrifuged at +4 °C for 10 min at 4000 × *g* within one hour after sampling. After separation, plasma samples were frozen and stored at −20 °C.

2.4. Chemical analysis and calculations

All analyses were performed by the laboratory at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, unless otherwise stated. Silage DM content was determined by a two-step procedure, first drying at 60 °C overnight and milling, then drying at 60 °C overnight, according to Åkerlind et al. (2011). The DM content of the concentrate mixture was determined by drying at 103 °C overnight (EC, 2009). Ash content for all feeds was determined by ignition at 550 °C for 3 h (EC, 2009). Acid-insoluble ash (AIA) content of all feeds was analysed according to Van Keulen and Young (1977). Feeds were analysed for crude protein (CP) in an automated Kjeldahl procedure (Foss, Hillerød, Denmark) and for crude fat according to European Commission Regulations (EC, 2009). The concentrate was analysed enzymatically for starch (including maltodextrin) according to Larsson and Bengtsson (1983). All feeds were analysed for neutral detergent fibre (NDF) according to Chai and Udén (1998). The silage samples were pressed and the silage juice was analysed for pH and ammonia-nitrogen (Broderick and Kang, 1980). The estimated *in vivo* digestible organic matter of the silage was analysed by the VOS (rumen fluid digestible organic matter) method according to Lindgren (1979, 1983) as:

$$OMD_{in\ vivo} = 0.90 \times VOS - 2.$$

Metabolisable energy (ME) content in the silage was estimated according to Lindgren (1983) as:

$$ME[MJ/kg\ OM] = 0.160 \times VOS[\%] - 1.91.$$

Metabolisable energy was then converted to MJ/kg DM.

Metabolisable energy content in the concentrate was calculated using tabulated values of the ingredients according to the Swedish Board of Agriculture (SJVFS, 2011). Net energy (NE) content in the feed and energy intake were estimated according to the NorFor system (Volden and Nielsen, 2011). Energy balance and residual feed intake (RFI) were calculated as difference between net energy intake and net energy requirements:

$$EB = NE_{intake} - (NE_{maintenance} + NE_{milk})$$

$$RFI = (NE_{intake}) - (NE_{maintenance} + NE_{milk} - NE_{mobilisation} + NE_{deposition})$$

with NE_{intake}, $NE_{maintenance}$, NE_{milk}, $NE_{mobilisation}$ and $NE_{deposition}$ calculated according to the NorFor system (Volden and Nielsen, 2011).

The faeces were freeze-dried, milled and determined for DM, ash and AIA. Total amount of faeces was estimated from total intake of AIA and the content of AIA in the faeces, assuming that all AIA in feed intake ends up in faeces (Van Keulen and Young, 1977). Total tract apparent dry matter digestibility (DMD) and organic matter digestibility (OMD) was calculated from estimated intake and excretion of DM and organic matter (OM) from feed intake and faeces, as (DM_{intake} - DM_{faeces})/DM_{feed} and (OM_{intake} - OM_{faeces})/OM_{feed}. The calculation was based on faeces samples taken once daily for three consecutive days and on feed intake data from these three days of faeces sampling and the previous day.

Milk samples were individually analysed for fat, fatty acids (FA), protein and lactose, using infrared Fourier transform spectroscopy (CombiScope FTIR 300 HP, Delta Instruments B.V., Drachten, the Netherlands). The FA analysed by spectroscopy were the four most abundant in milk (C14:0, C16:0, C18:0, C18:1 cis-9). Lactose was corrected for lactase monohydrate by division by 1.053. Energy corrected milk was calculated based on fat, protein and lactose content, according to Sjaunja et al. (1990). Values from the two milkings from each sampling occasion regarding both composition and yield were used for yield of milk, ECM, milk fat, milk protein and lactose in statistical analyses.

Glucose in blood plasma was analysed enzymatically (D-Glucose UV-method, R-biopharm AG, Darmstadt, Germany). Insulin concentration was analysed using an enzyme immunoassay method adapted for bovines (Mercodia Bovine Insulin ELISA, Mercodia AB, Uppsala, Sweden) and the concentration of non-esterified fatty acids (NEFA) using an enzymatic colorimetric method (NEFA-HR, FujifilmWako Diagnostics U.S.A. Corporation, CA). Concentration of beta-hydroxybutyrate (BHB) in plasma was analysed with a colorimetric test (MAK041, Sigma-Aldrich, St. Louis, MO), while insulin-like growth factor 1 (IGF-1) concentration was analysed with an enzyme

immunoassay (Mediagnost E20, Mediagnost, Reutlingen, Germany).

2.5. Statistical analyses

All statistical analyses were performed in SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). The treatment effects of feed and nutrient intake, milk yield and composition, BCS and BW variables, and blood plasma parameters were analysed using PROC MIXED, with lactation week as autoregressively repeated:

$$Y_{ijklm}=\mu+C_i + P_j + B_k + L_l + W_m+BW_{km}+LW_{lm} + \varepsilon_{ijklm}$$

where Y_{ijklm} is the dependant variable, μ is the overall mean, C_i is the random effect of cow i, P_j is the effect of parity j, B_k is the effect of breed k, L_l is the effect of concentrate level l, W_m is the effect of lactation week m, BW_{km} is the breed × lactation week interaction effect of breed k and lactation week m, LW_{lm} is the concentrate level × lactation week interaction effect of concentrate level l and lactation week m, and ε_{ijklm} is the random error. Primiparous cows formed one parity class and all multiparous cows (parity 2 or older) formed another. For treatment effects of digestibility (with only two measures per cow), the model was modified in that cow was not treated as a random effect and lactation week was set as repeated unstructured.

Treatment effects of weekly change in BCS and BW were analysed by PROC GLM with the model:

$$Y_{ijklm}=\mu+C_i + P_j + B_k + L_l + W_m + \varepsilon_{ijklm}$$

where Y_{ijklm} is the dependant variable, μ is the overall mean, C_i is the random effect of cow i, P_j is the effect of parity j, B_k is the effect of breed k, L_l is the effect of concentrate level l, W_m is the effect of lactation week m, and ε_{ijklm} is the random error.

Several models were tested to combine and account for interactions between variables and those with the lowest Akaike information criterion (AIC) were used. We did not find any interaction effect of parity × concentrate level, breed × concentrate level or breed × parity on any of the parameters analysed.

All residuals were tested for normality and those that did not follow a normal distribution were log-transformed (milk SCC and blood plasma insulin, NEFA, BHB and IGF-1). Values presented in text and tables are least square means, calculated using the LSMEANS/PDIFF option. Statistical differences were determined following Tukey's adjustment declared at $P \leq 0.05$.

3. Results

3.1. Concentrate level

We found no difference in total DMI between cows offered LC or HC diets during the first six weeks of lactation, where LC cows had lower concentrate intake but compensated with higher forage intake (Table 3; Fig. 1). There was no interaction of concentrate level × lactation week for total DMI, but the cows on the HC diet had a higher concentrate intake than the LC cows ($P<0.001$) during lactation week 2, 4 and 6 (as planned). For forage intake, the LC cows had a higher intake in lactation week 4 and 6 compared with HC cows ($P<0.001$). The cows on LC diet reached maximum concentrate ration at (mean ± SD) 8 ± 2 DIM, while the HC cows reached it at 27 ± 3 DIM. Cows fed the LC diet had lower DMI/BW and DMI/$BW^{0.75}$ than HC cows. There was no overall difference in total tract OMD or DMD between concentrate levels. Neither were there any differences between LC and HC cows regarding yield of milk and ECM, along with yield and concentration of milk fat, protein and lactose (Table 4). However, there was an interaction of concentrate level × lactation week for ECM ($P = 0.01$), with no difference between HC and LC in lactation week 2 and 4 but a difference in lactation week 6 (38.5 vs 34.5 kg ECM/d for HC and LC, respectively).

We found no differences regarding the most abundant FA in milk, or in total milk FA, between LC and HC cows. There was no effect of concentrate level in this first period of early lactation on blood plasma concentrations of glucose, insulin, BHB, NEFA and IGF-1 (Table 5). The two different dietary regimes did not affect EB or change in BCS. However, there was a difference as regards change in BW, as HC cows appeared to have gained weight while LC cows appeared to have lost weight (Table 6).

We found no differences between the LC and HC diets as regards the efficiency measures RFI and milk production in relation to energy intake (ECM/NE) (Table 6). Cows fed the LC diet had higher N-efficiency than HC cows. The LC diet contained 147 g CP/kg DM, while the HC diet contained 158 g CP/kg DM.

3.2. Parity

Multiparous cows had higher total DMI than primiparous cows. In addition, our multiparous cows also had higher intake per kg BW and per kg metabolic BW ($BW^{0.75}$) than primiparous cows. The higher DMI of multiparous cows was accompanied by a lower total tract apparent OMD of multiparous cows compared with primiparous cows. There were differences related to parity during the first six weeks of lactation for most milk parameters examined in this study. Multiparous cows yielded more ECM and milk, and had higher yield of fat, protein and lactose than primiparous cows. There were no differences in the concentrations of fat and protein in milk between parities.

Primiparous cows had higher levels of glucose, insulin and IGF-1, and lower levels of NEFA in blood plasma, compared with multiparous cows. Multiparous cows lost more body condition (Table 4). However, we found no differences in EB or BW change between parities. No differences between parities were found for the efficiency measures RFI and ECM/NE in the present study.

3.3. Breed

Swedish Holstein cows had higher total DMI than SR cows, and there were no difference in concentrate intake between the breeds (Table 3). Swedish Holstein cows had a higher milk, milk fat and lactose yield than SR cows in the present study. In addition, SH cows had a lower milk protein content compared with SR cows.

Swedish Holstein cows had a lower concentration of IGF-1 in blood plasma than SR cows. We found no breed differences for any of the other blood parameters analysed. There were no differences between SH and SR cows in EB, BCS change or BW change (Table 6). Thus, SR cows had higher BCS than SH cows. However, that was not reflected in BW as we saw no difference in BW between breeds. We found no breed differences for the efficiency measures RFI and ECM/NE.

4. Discussion

We investigated the effects of feeding byproduct-based concentrate at a high or low level in combination with *ad libitum* access to grass-clover silage of high digestibility on feed intake, milk production and metabolic status in primiparous and multiparous SH and SR dairy cows during the first six weeks of lactation. This was a rather short experimental period. However, this period is critical for metabolic status and EB of the cow (Ingvartsen, 2006). Concentrate level only affected some few of the studied aspects, while the impact of parity was significant for feed intake, milk yield and metabolic indicators in blood plasma. Breed had an impact on both feed intake and milk yield parameters, but not on most indicators of metabolic status or efficiency. The lack of interaction effect of parity × concentrate level and breed × concentrate level for any of the parameters analysed indicates that primiparous cows managed to perform just as well as multiparous cows on the LC diet, and with SH cows being as well adapted to LC diets as SR cows. However, all the results in the present study should be interpreted with care as it was a relatively low number of animals included which affected the statistical power of the study.

Table 3
Intake of feed, nutrients and energy, intake per kg body weight (BW) and metabolic BW ($BW^{0.75}$) in lactation week 2, 4 and 6, along with total tract apparent organic matter digestibility (OMD) and dry matter digestibility (DMD) sampled twice between lactation week 2 and 6, presented as least square mean with standard error of the mean (SEM) and P-value, of primiparous and multiparous Swedish Red (SR) and Swedish Holstein (SH) cows fed a low (LC) or high (HC) ration of byproduct-based concentrate in combination with grass-clover silage *ad libitum*.

		Diet			Parity			Breed			P-value		
Item	No. of obs.	LC	HC	SEM	Primiparous	Multiparous	SEM[1]	SR	SH	SEM[1]	Diet	Parity	Breed
Number of cows		13	13	–	12	14	–	14	12	–	–	–	–
Intake (kg DM/d)													
Total dry matter	76	20.4	21.5	0.63	18.5	23.4	0.63	19.9	22.0	0.64	0.26	<0.001	0.04
Forage intake	77	16.6	12.0	0.59	12.4	16.2	0.59	13.3	15.3	0.60	<0.001	<0.001	0.03
Concentrate intake	77	3.81	9.46	0.18	6.05	7.21	0.18	6.59	6.67	0.18	<0.001	<0.001	0.76
NE (MJ/d)	77	133	141	4.0	122	153	4.0	131	144	4.0	0.19	<0.001	0.04
DMI/BW (kg/kg)	76	0.031	0.035	0.0009	0.031	0.035	0.0009	0.032	0.034	0.0009	0.01	0.002	0.10
$DMI/BW^{0.75}$(kg/kg)	76	0.16	0.17	0.004	0.15	0.18	0.004	0.16	0.17	0.004	0.01	<0.001	0.05
OMD (%)	43	69.5	70.1	0.38	70.5	69.1	0.38	70.2	69.3	0.38	0.13	0.03	0.24
DMD (%)	43	67.6	68.3	0.35	68.5	67.3	0.35	68.4	67.4	0.35	0.09	0.04	0.19

[1] SEM values are weighted averages to adjust for the imbalance of observations between parities and breeds.

4.1. Concentrate level

Total dry matter intake between LC and HC diets was similar. Due to the linear increase of 0.5 kg concentrate per day from 3 kg to maximum concentrate rations of 5 kg (LC diet) or 15 kg (HC diet), it took much longer for the cows on the HC diet to reach maximum concentrate ration. Due to this, the difference in concentrate intake was greater between HC and LC diets the further into lactation the cows were. This is probably the reason why differences in ECM between diets only appeared in lactation week 6. The lower DMI/BW and $DMI/BW^{0.75}$ of LC cows than HC cows was probably mainly an effect of unintended numerical differences in BW between LC and HC cows since there were no differences in DMI. A more energy-dense diet with more concentrate usually leads to higher total diet digestibility (Tyrrell and Moe, 1975). In the present study, the byproduct-based concentrate was relatively low in net energy for lactation, partly due to high NDF content, while the silage had a high net energy content (Table 2). Thus, the higher concentrate proportion in the HC diet did not improve DMD and OMD.

The four most abundant milk FA were analysed as it has been suggested that they can be used to indicate the energy balance of dairy cows (Gross et al., 2011). The lack of differences in these FA in milk, or in total milk FA, between LC and HC cows, indicates that there were no differences in use of adipose tissue to support milk production between diets (Table 4). Blood plasma concentrations of glucose, insulin, BHB, NEFA and IGF-1 reflect metabolic status in dairy cows (Adewuyi et al., 2005). We found no effect of concentrate level during the first six weeks of lactation on these compounds, indicating that metabolic status was not compromised in cows fed the LC diet during this period. Previous findings on metabolic responses to low-concentrate diets in early lactation are inconsistent, *e.g.* Andersen et al. (2004) observed lower glucose and insulin levels and higher BHB, while Lawrence et al. (2015) observed lower BHB and higher NEFA. However, it is difficult to compare results from different studies, since dietary regimes can vary substantially. The metabolic plasma profile and milk FA profile confirmed the lack of treatment differences in EB and BCS change. It is possible that the changes in BW partly reflect weight of digesta. Taken together, the results indicate that there was no effect of dietary treatment on cow metabolism.

The higher N-efficiency of LC cows than HC cows can be explained as dietary CP is the most important factor influencing N-efficiency (Huhtanen and Hristov, 2009) and as the LC cows consumed less CP while there were no difference in milk protein yield.

4.2. Parity

The higher total DMI of multiparous cows was expected and can be partly explained by the 1 kg/d higher concentrate ration offered compared with primiparous cows and partly by higher BW, related to larger digestive volume (Allen, 1996). That multiparous cows yielded more ECM and milk than primiparous cows have also been found by others (Ray et al., 1992; Patel et al., 2016) and is most probably related to the higher DMI. However, the higher DMI of multiparous cows might also be related to differences in metabolism or digestibility as multiparous cows had a higher intake per kg BW and per kg metabolic BW than primiparous cows in the present study. Higher DMI increases the passage rate, in turn lowering digestibility (Tyrrell and Moe, 1975), which could explain the lower OMD in multiparous cows than primiparous cows. Similarly, Neave et al. (2017) found that DMI was lower in primiparous cows after controlling for BW and milk production during the first weeks of lactation, and suggested that the difference was due to

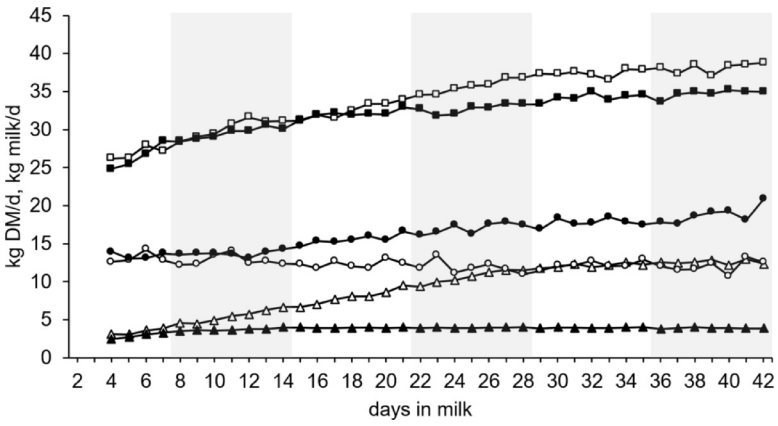

Fig. 1. Milk yield (kg/d; squares), forage DMI (kg/d; circles), and concentrate DMI (kg/d; triangles), as means, per day for primiparous and multiparous cows of Swedish Red and Swedish Holstein fed a daily ration of up to 5 kg of concentrate (LC; filled; $n = 13$) or up to 15 kg of concentrate (HC; open; $n = 13$). Lactation weeks 2, 4 and 6 are marked as grey areas. There were no diet × DIM interaction for milk yield, but for forage and concentrate DMI. *Ad libitum* forage DMI differed between diets at DIM 5–6, 19, 24, 26–31, 33–34, 36–40 ($p < 0.05$), and concentrate intake differed from 10 to 42 DIM ($p < 0.05$) The concentrate was byproduct based and fed together with high-quality forage.

Table 4
Milk performance in lactation week 2, 4 and 6, presented as least square mean with standard error of the mean (SEM) and P-value, of primiparous and multiparous Swedish Red (SR) and Swedish Holstein (SH) cows fed a low (LC) or high (HC) ration of byproduct-based concentrate in combination with grass-clover silage *ad libitum*.

		Diet			Parity			Breed			P-value		
Item	No. obs.	LC	HC	SEM	Primiparous	Multiparous	SEM[1]	SR	SH	SEM[1]	Diet	Parity	Breed
Number of cows		13	13	–	12	14	–	14	12	–	–	–	–
Yield (kg/d)													
Milk	78	32.3	34.0	1.13	28.8	37.5	1.14	31.2	35.1	1.15	0.30	<0.001	0.03
ECM	78	33.8	34.9	0.94	29.4	39.3	0.95	32.5	36.1	0.95	0.42	<0.001	0.02
Fat	78	1.44	1.47	0.046	1.22	1.69	0.046	1.38	1.53	0.046	0.65	<0.001	0.03
Protein	78	1.08	1.12	0.036	0.94	1.26	0.036	1.06	1.13	0.036	0.37	<0.001	0.21
Lactose	78	1.47	1.53	0.048	1.33	1.67	0.048	1.41	1.58	0.048	0.35	<0.001	0.02
Concentration (%)													
Fat	78	4.46	4.40	0.139	4.32	4.54	0.141	4.45	4.41	0.141	0.74	0.28	0.83
Protein	78	3.36	3.33	0.040	3.32	3.38	0.040	3.43	3.27	0.040	0.62	0.26	0.01
Lactose	78	4.55	4.51	0.031	4.61	4.44	0.031	4.54	4.51	0.031	0.42	0.001	0.49
Milk FA (g/100 g milk FA)													
C14:0	76	10.7	11.0	0.26	10.4	11.3	0.26	11.3	10.4	0.26	0.41	0.03	0.03
C16:0	76	26.7	27.5	0.59	26.6	27.5	0.59	27.4	26.8	0.60	0.38	0.31	0.51
C18:0	76	9.6	9.6	0.36	10.2	9.0	0.36	9.4	9.9	0.37	0.95	0.03	0.32
C18:1 cis-9	76	24.0	23.5	0.60	24.4	23.1	0.60	23.2	24.3	0.60	0.51	0.14	0.23
SCC (log10)	78	1.67	1.57	0.14	1.56	1.68	0.15	1.50	1.74	0.15	0.65	0.58	0.28
SCC antilog, 10^3 cells/mL	78	47	37	–	37	48	–	32	55	–	–	–	–

[1] SEM values are weighted averages to adjust for the imbalance of observations between parities and breeds.

Table 5
Blood plasma concentrations of glucose, insulin, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB) and insulin-like growth factor 1 (IGF-1) in lactation week 2, 4 and 6, presented as least square mean with standard error of the mean (SEM) and P-value, of primiparous and multiparous Swedish Red (SR) and Swedish Holstein (SH) cows fed a low (LC) or high (HC) ration of byproduct-based concentrate in combination with grass-clover silage *ad libitum*.

		Diet			Parity			Breed			P-value		
Item	No. obs.	LC	HC	SEM	Primiparous	Multiparous	SEM[1]	SR	SH	SEM[1]	Diet	Parity	Breed
Number of cows	–	13	13	–	12	14	–	14	12	–	–	–	–
Glucose, mmol/L	71	3.51	3.47	0.084	3.73	3.25	0.084	3.47	3.51	0.085	0.73	<0.001	0.75
Insulin (log10)	70	−0.89	−0.69	0.092	−0.55	−1.03	0.092	−0.80	−0.79	0.093	0.14	0.001	0.91
Insulin antilog, μg/L	–	0.13	0.20	–	0.28	0.09	–	0.16	0.16	–	–	–	–
NEFA (log10)	71	−0.56	−0.59	0.047	−0.65	−0.50	0.047	−0.57	−0.58	0.048	0.72	0.05	0.85
NEFA antilog, mmol/L	–	0.27	0.26	–	0.23	0.31	–	0.27	0.26	–	–	–	–
BHB (log10)	71	−0.40	−0.43	0.045	−0.48	−0.35	0.045	−0.40	−0.42	0.046	0.59	0.06	0.76
BHB antilog, mmol/L	–	0.40	0.37	–	0.33	0.45	–	0.39	0.38	–	–	–	–
IGF-1 (log10)	71	1.90	1.91	0.034	2.02	1.79	0.034	1.96	1.85	0.035	0.93	<0.001	0.03
IGF-1 antilog, ng/ml	–	80.2	80.9	–	105	61.6	–	92.1	70.4	–	–	–	–

[1] SEM values are weighted averages to adjust for the imbalance of observations between parities and breeds.

Table 6
Energy balance (EB), residual feed intake (RFI), feed conversion, N-efficiency, body condition score (BCS), and body weight (BW) in lactation week 2, 4 and 6, and weekly change in BCS and BW between lactation week 6 and 2, presented as least square mean with standard error of the mean (SEM) and P-value, of primiparous and multiparous Swedish Red (SR) and Swedish Holstein (SH) cows fed a low (LC) or high (HC) ration of byproduct-based concentrate in combination with grass-clover silage *ad libitum*.

		Diet			Parity			Breed			P-value		
Item	No. of obs.	LC	HC	SEM	Primiparous	Multiparous	SEM[1]	SR	SH	SEM[1]	Diet	Parity	Breed
Number of cows		13	13	–	12	14	–	14	12	–	–	–	–
EB, MJ NE/d	77	−16.8	−10.3	2.95	−13.7	−13.3	2.98	−14.0	−13.0	2.98	0.14	0.91	0.82
RFI, MJ NE/d	78	3.29	2.97	4.56	0.81	5.44	4.56	1.35	4.91	4.61	0.96	0.49	0.60
ECM/NE, kg/MJ	77	0.26	0.25	0.006	0.25	0.26	0.007	0.25	0.25	0.007	0.56	0.19	0.99
ECM/DMI, kg/kg	76	1.68	1.63	0.041	1.61	1.69	0.042	1.65	1.66	0.042	0.38	0.22	0.89
N-efficiency[2], g/kg	75	359	334	8.28	340	353	8.36	355	337	8.37	0.05	0.27	0.15
BCS[3]	68	3.41	3.43	0.079	3.54	3.30	0.080	3.60	3.25	0.081	0.87	0.05	0.01
BW, kg	78	660	619	18.7	608	672	18.8	631	648	18.9	0.14	0.03	0.53
BCS change, points/week	25	−0.04	−0.06	0.008	−0.03	−0.06	0.008	−0.04	−0.05	0.008	0.09	0.04	0.64
BW change, kg/week	26	−2.33	2.22	1.443	−0.56	0.44	1.450	0.09	−0.20	1.459	0.04	0.64	0.89

[1] SEM values are weighted averages to adjust for the imbalance of observations between the two treatment diets.
[2] Nitrogen efficiency = (Milk protein yield/6.38)/(CP intake/6.25).
[3] A 1–5 point system.

different feeding behaviour. The difference in concentrate intake between parities in the present study was because the heavier multiparous cows were offered more concentrate than primiparous cows with the aim to reach a more similar ratio of concentrate (kg) /BW (kg).

The higher levels of glucose, insulin and IGF-1, and lower levels of NEFA, in primiparous cows compared with multiparous cows indicates less mobilization of body tissue which was also reflected in that primiparous cows having a higher BCS than multiparous cows. Wathes et al. (2007) made similar findings and concluded that there is a major difference between primiparous and multiparous cows in how these metabolites influence milk yield and BCS. Higher levels of the growth-promoting insulin and IGF-1 in primiparous cows are not surprising as younger cattle have a higher growth rate and, although the growth rate declines with age, dairy cows continue to grow until at least their third lactation (Coffey et al., 2006). The higher levels of NEFA in multiparous cows could reflect a higher degree of body tissue mobilisation to support higher milk yield compared with primiparous cows. In line with their higher plasma NEFA levels, multiparous cows also lost more body condition. Intriguingly, multiparous cows were not observed to be in deeper negative EB. However, the EB values in the present study were estimated based on energy intake and requirements, and not measured in respiration chambers, which can influence the results (Erdmann et al., 2019). Smaller cows have a lower maintenance requirement per kg metabolic BW (VandeHaar et al., 2016), which might lead to an overestimation of EB in primiparous cows that are smaller than multiparous cows. Similarly, the estimated maintenance requirement could affect RFI. Others have found that multiparous cows have lower RFI than primiparous cows in early lactation (Connor et al., 2013). However, we found no differences between parities for the efficiency measures RFI and ECM/NE. For the latter, Oldenbroek (1989) did not find any parity effect on milk energy/net energy in feed in the first, second and third lactation in cows fed either roughage only or 50% concentrate on DM basis. We expected the primiparous cows to gain more weight, as they generally have a higher growth rate than multiparous cows (Coffey et al., 2006). The lack of difference we observed in BW change between parities might be explained by the study period being too short to detect differences, as BW change was only measured over four weeks. Another possibility is that milk production might be more prioritised than growth in early lactation primiparous cows.

4.3. Breed

In our study, SH cows had higher forage intake than SR cows, which resulted in also a higher total DMI, as there were no breed difference in concentrate intake. Others have also found that SH cows consume more in total than SR cows (Li et al., 2018; Andrée O'Hara et al., 2018). Li et al. (2018) attributed this to SH cows generally having higher BW than SR cows, which is likely related to larger digestion volume (Beecher et al., 2014). However, like Andrée O'Hara et al. (2018), we did not observe any difference in BW between breeds but we too observed that the SR cows had higher body condition than the SH cows. The higher BCS might have contributed to the lower DMI of SR cows, as it is widely accepted that cow BCS is negatively associated with DMI (Roche et al., 2008). Swedish Holstein cows have been bred more intensively for high milk yields, while SR breeding goals focus strongly on health traits along with milk yield (Mark, 2004). These genetic differences might contribute to the higher DMI and milk, fat and lactose yield in SH cows in the present study. Swedish Holstein cows generally have lower fat and protein concentrations in milk than SR cows (Växa Sverige, 2019) although in the present study only the protein content was lower in SH cows.

The lower concentration of IGF-1 in blood plasma of SH cows than of SR cows might be related to the lower BCS of SH cows, as others have found that leaner cows have lower levels of IGF-1 in the blood (Meikle et al., 2004; Gobikrushanth et al., 2018). Our results for glucose, BHB, NEFA and IGF-1 in relation to breed correspond with those of Andrée O'Hara et al. (2019) in a study on multiparous cows. We found no breed difference in concentrations of insulin in blood plasma between SH and SR cows. However, others have found that SH cows have lower concentrations of insulin than SR cows (Nyman et al., 2008; Andrée O'Hara et al., 2019) which could be a pure breed effect or possibly an effect of breed differences in BCS.

When studying primiparous cows, Ntallaris et al. (2017) found that SH cows had a less positive EB than SR cows. Others studying EB in multiparous cows have failed to detect a breed difference in EB between SR and SH (Andrée O'Hara et al., 2018; Karlsson et al., 2020). The lack of breed effect on EB in this study was also accompanied by lack of interaction between breed and parity.

5. Conclusions

We found no differences between the high- and low-concentrate diets for total DMI, ECM, and EB, and no differences in concentration of metabolic indicators in blood plasma or milk FA during the first six weeks of lactation in dairy cows offered high-digestibility grass-clover silage *ad libitum*. However, cows fed the LC diet produced less ECM in lactation week 6 and lost weight in comparison with cows fed the HC diet. There was no difference in EB between parities, but multiparous cows lost more BCS and had higher NEFA blood plasma concentrations, suggesting they used more adipose tissue to support milk production than primiparous cows. Cows of SH breed had a higher DMI and ECM yield than SR cows, but there were no breed differences in EB. The results indicate that both primiparous and multiparous cows of SR and SH breeds within the first six weeks of lactation can perform well on high-forage diets comprising high-digestibility grass-clover silage combined with low levels of byproduct-based concentrate.

CRediT authorship contribution statement

Johanna Karlsson: Formal analysis, Investigation, Data curation, Writing - original draft, Project administration. **Mikaela Lindberg:** Investigation, Writing - review & editing, Supervision. **Maria Åkerlind:** Writing - review & editing, Resources, Supervision. **Kjell Holtenius:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

None

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