ABSTRACT
The objective of this study was to characterize total-tract nutrient digestibility, energy balance, and N balance in the critical dietary and metabolic transitions of the lactation cycle. Twelve dairy cows were housed in tiestalls from 10 wk before to 16 wk after parturition. After 2 wk of adaptation to the facility and diet, digestibility of organic matter (OM), neutral detergent fiber (NDF), starch, and N were measured, and energy and N balances determined at weekly intervals by total collection of feces, urine, and milk over 48 h. Cows were individually fed ad libitum a grass silage- and corn silage-based total mixed ration during lactation and a corn silage- and barley straw-based total mixed ration during the dry period. Effects of stage of lactation were evaluated by clustering week in 5 groups: late lactation (wk −8 to −7), dry period (wk −6 to −1), and 3 early lactation periods (wk 1 to 5, wk 6 to 10, and wk 11 to 16). In lactation, apparent total-tract digestibility of OM, NDF, and starch was lowest in the first 5 wk of lactation. From wk 2 to 16 after parturition, apparent nutrient digestibility of all nutrients increased linearly, but with a negative quadratic component for dry matter, OM, and NDF, to levels comparable to those reported in last 2 wk of the previous lactation. However, differences in digestibility across lactation stage were moderate, illustrated by the difference between OM digestibility in late lactation (last 2 wk, 74.8%) and early lactation (first 5 wk, 72.5%). Cows were in negative energy balance for the first 8 wk after calving, and in negative N balance for the first 4 wk after calving. Based on energy and N balance, we predicted that 36.5 kg of body fat and 3.5 kg of body protein were gained in the last 8 wk before calving, and that 47.5 kg of body fat and 7.6 kg of body protein were mobilized in the first weeks of lactation. These predicted changes in body mass, both the gain before calving and loss after calving, were greater by 37% and 10%, respectively, than fluctuations in measured body weight (corrected for predicted gut fill and fetus weights). At wk 1 and 2 postpartum, body N loss corresponded to 25 and 29%, respectively, of total N excretion in milk, and body energy loss corresponded to 64% and 44%, respectively, of the energy exported to milk, illustrating the important contribution of N and energy from body stores to milk production in early lactation. Metabolic N efficiency, measured as total N output (milk and body) over digestible N input (from diet and body), averaged 54.4% in the last 2 wk of lactation, increased to 65.9% 2 wk after calving, and decreased linearly as lactation advanced to 61.9% by wk 16. Short (48 h) but weekly repetition of total collection of feces and urine appears to be a suitable approach to evaluate temporal changes in nutrient digestibility, energy balance, and N balance across lactation and the dry period.

Key words: nutrient balance, transition, periparturient, dry-off

INTRODUCTION
The ability of dairy cattle to adapt to the metabolic changes that occur during the transition period, often defined as the period from 3 wk before to 3 wk after parturition (e.g., Grummer, 1995; Drackley, 1999), has received considerable attention (e.g., reviews from Bell, 1995; Grummer, 1995; Drackley, 1999; Cardoso et al., 2020). As a result, nutritional strategies have been proposed that effectively reduce the incidence of metabolic diseases associated with the transition to lactation after calving (LeBlanc et al., 2006). In contrast, far fewer studies have attempted to describe the changes occurring during the transition period in terms of digestive competence. The sudden introduction of a highly fermentable diet at calving, concomitantly with the rapid increase of feed intake in this period, is a risk factor for ruminal acidosis (Penner et al., 2007). For this reason, aspects of rumen adaptation in terms of morphological and functional changes have been assessed during the periparturient period (Dieho et al., 2016a,b, 2017).
demonstrating important increases after calving in rumen papillae surface area and the fractional absorption rate of VFA. The transition to lactation is also characterized by changes in visceral mass (Gibb et al., 1992; Reynolds et al., 2004). However, it is not clear how total-tract nutrient digestibility is affected during this time, or how changes in digestibility may contribute to higher efficiencies of conversion of nutrients into milk in early lactation.

The process of dry-off has traditionally received limited attention. However, it is increasingly evident that it represents an equally relevant and challenging transition compared with calving. The abrupt cessation of milking combined with the corresponding change in diet leads to a sudden reduction in DMI, with a profound impact on metabolism (Odensten et al., 2005; Mezzetti et al., 2020). However, we are not aware of any published data reporting changes in nutrient digestibility and nutrient balance relative to dry-off in dairy cattle. Consequently, the objective of this study was to characterize digestibility of DM, OM, NDF, starch, and N, energy balance, N balance, and serum metabolites concentrations at weekly intervals from 8 wk before until 16 wk after parturition. The suitability of our total collection method to assess nutrient balance over time was evaluated against fluctuation of BW.

**MATERIALS AND METHODS**

This study was conducted between September 2019 and April 2020 at the Dairy Research Facility of Trouw Nutrition Research & Development (Kempenhof, Boxmeer, the Netherlands). All experimental procedures were approved by the Central Authority for Scientific Procedures on Animals (CCD) and conducted under the Dutch Act on Animal Experiments (AAE), which complies with European Directive 2010/63/EU.

**Animal Management**

Twelve Holstein Friesian dairy cows were used in this study. At the start of the study, 6 cows were in their first lactation and 6 cows were multiparous (Supplemental File S1, https://doi.org/10.6084/m9.figshare.21220703.v1). Approximately 10 wk before expected calving, cows were moved from a freestall barn to a tiestall barn with individual feed troughs and free access to water. The first 2 wk of tiestall housing were considered an adaptation to the diet and housing facility. Cows remained in the tiestall barn until the study ended at 16 wk after calving. For dry-off, cessation of twice-a-day milking occurred abruptly together with the changes in diet, and teat sealant (OrbeSeal, Zoetis) was applied on all cows. Dry-off occurred 6 wk before expected calving, resulting in an average dry period length of 42 d (range: 35 to 54 d; Supplemental File S1). Cows were moved to a straw pen at the first sign of calving. Within the first hour after calving, cows were individually offered 1 kg of a Ca-energy supplement (Farm-O-San Reviva, Trouw Nutrition) mixed in 20 L of water for voluntary consumption. After about 2 d in the straw pen, cows were returned to tiestall housing. None of the cows suffered from clinical diseases associated with calving. Unfortunately, 1 cow died 34 d after calving. The cause of death, diagnosed by necropsy by GD Animal Health (Deventer, the Netherlands), was an aneurysm of the cranial mesenteric artery. All cows were weighed once weekly after morning milking; BCS was assessed weekly by 2 independent and trained assessors using a 1 to 5 scale with increments of 0.25 (Edmonson et al., 1989), and the average of the 2 scores was used. Weekly individual cow data are accessible online (https://doi.org/10.6084/m9.figshare.20348583.v1).

**Diets and Feeding**

The compositions of the TMR formulated for this study are shown in Table 1. The respective TMR were offered ad libitum during the dry and lactation periods. To calculate daily TMR intake, orts were removed daily between 0930 and 1000 h and subtracted from the TMR offered the previous day. Immediately after removal of the orts, the fresh TMR was weighed and allocated into individual feed troughs. The mash compound feeds were manufactured by Research Diet Services B.V. Analyzed chemical composition of forages and concentrates and calculated composition for TMR are shown in Table 2.

**Sampling**

**Total Collection of Feces and Urine.** Feces and urine were collected over a 48-h period each week from 8 wk before expected calving until 16 wk after calving. Each collection period started at 1000 h on Monday and ended at 1000 h on Wednesday, coinciding with feeding. The configuration of the tiestall system was adjusted weekly for fecal collection by covering the manure gutter and separating the stalls. From 0500 to 0100 h, cows were under continuous supervision; after each defecation, feces were immediately collected from the floor and placed in buckets. Between 0100 and 0500 h, cows were left unsupervised, and accumulated feces on the floor were collected and placed in buckets at 0500 h. The buckets with the collected feces were weighed at 1000 h on Tuesday and Wednesday. The feces were thoroughly mixed and a sample of 1% (wt/wt) was taken at the time of weighing on Tuesday and Wednesday and immediately frozen at -18°C. Samples
collected at 1000 h on Wednesday were added to the 1000-h sample from Tuesday to obtain a single composite urine sample per cow per weekly collection period. The specific gravity of a vortexed urine sample was measured at each sampling time by weighing 1,000 mL of acidified urine with a calibrated pipette. Specific gravity was used to convert urine weight to urine volume.

**Feed, Milk, and Serum.** Grass silage, corn silage, barley straw, and compound feeds were sampled every other week while fed. Cows were milked twice daily at 0600 and 1700 h, and milk yield was recorded at each milking. Milk samples were collected on Monday and Tuesday evenings and Tuesday and Wednesday mornings. Samples were collected in tubes containing sodium azide and bronopol as preservatives and stored at 4°C. Both morning and evening samples were pooled by weighing each sample proportionally to the yield per milking. Blood samples were collected from the coccygeal vein in 9-mL tubes containing clot activator (Vacutest Serum; BD) every Monday at 1200 h. The tubes were centrifuged at 1,500 × g for 10 min at 20°C. The serum aliquots were then stored in 1.5-mL cryotubes at −18°C.

**Biopsies.** Although the data will not be presented here, biopsies of liver, muscle, and adipose tissue (the latter 2 collected from the longissimus dorsi) were collected 4 times on each cow: 9 d before and 6 d after dry-off, between 5 and 10 d before calving, and between 10 and 20 d after calving. Biopsies were performed on Wednesday afternoon following the 48-h collection period of urine and feces so that the biopsy procedure would have minimal effect on the next collection period.

**Chemical Analysis.** Feed samples were analyzed at Masterlab (Boxmeer, the Netherlands) for DM, gross energy, crude ash, crude fat, NDF, ADF, ADL, starch, and sugar. Fecal samples were analyzed at Masterlab for DM, crude ash, NDF, and starch. The DM content was determined by drying to a constant weight in a 103°C oven for 4 h (EC 152/2009; EC, 2009). Gross energy was analyzed using an adiabatic bomb calorimeter (C7000, IKA Werke GmbH and Co.). Crude ash was analyzed by incineration in a muffle furnace and expressed as glucose (71/250/EEC; EC, 1971). In addition, a dry aliquot of each feed sample was analyzed for moisture and minerals at the University of Nottingham.
Urine, milk, dry feed, and fresh feces were analyzed for N by combustion at Nutrilab (Giessen, the Netherlands) using the Dumas method (ISO 16634-1; ISO, 2008). For corn silage and grass silage, NH3 content was determined in fresh material, and the ammonia N concentration was added to the subsequent N concentration from the Dumas method in the dried samples to estimate total N content. For NH3 content determination, fresh silage samples were deproteinized by adding 10% (wt/vol) trichloroacetic acid and then centrifuged (10 min at 14,000 × g at room temperature). Indophenol blue was then formed by the Berthelot reaction with phenol and hypochlorite in an alkaline solution, which was determined spectroscopically at 623 nm.

Milk samples were analyzed for fat, CP, lactose, and urea (Qlip, Deventer, the Netherlands) using a Foss Milkoscan FT6000 (ISO, 2013), and for SCC using a Fossomatic FC 5000 (ISO, 2006). Serum samples were analyzed at the University of Nottingham (NUVetNA Laboratory, Nottingham, UK). Nonesterified fatty acids (NEFA), BHB, glucose, urea, and triglycerides were measured using Randox kits using RX IMOLA (Randox Laboratories Ltd.). Insulin was analyzed using the Merodia Bovine Insulin ELISA kit (Mercodia) with a Varioskan Flash (Thermo-Fisher Scientific).

Calculations

Week from Calving. Variability in gestation length meant that dry period duration ranged from 35 to 54 d (see Supplemental File S1, https://doi.org/10.6084/m9.figshare.21220703.v1). To present the data in the same figures, data near dry-off were adjusted. Weeks −8, −7, −6, and −5 from calving, as shown in all reported figures, correspond to actual wk −2, −1, +1, and +2 from dry-off, whereas wk −4 to 16 correspond to the actual week from calving.

Apparent Total-Tract Digestibility. Apparent total-tract digestibility of OM, NDF, starch, and N was calculated as the difference between average weekly intake and average daily total fecal excretion of each nutrient and expressed relative to average weekly intake (%).

Energy Balance. The following assumptions were made to calculate energy balance (EB). First, because the gross energy (GE) content of feces was not mea-
sured, total-tract energy digestibility (%) was predicted from apparent total-tract digestibility of OM (%), using the regression equation from Daniel et al. (2016): $y = -5.779 + 1.036x$, root mean square error (RMSE) = 0.94 ($R^2 = 0.99$). Digestible energy intake was then calculated by multiplying GE intake by predicted energy digestibility. Daily urinary energy excretion was predicted using urinary N excretion (Hoffmann et al., 1972), and energy losses in the form of methane were predicted using equations from Sauvant and Nozière (2016). Calculated ME intake was converted to NE\textsubscript{L} intake using the equation of INRA (2018): $\text{NE}_\text{L} (\text{MJ/d}) = k_s \times \text{ME} (\text{MJ/d})$, where the efficiency of ME utilization in milk ($k_s$) = 0.65 + 0.247 × ($Q$ − 0.63), and the metabolizability of energy ($Q$) = ME intake/GE intake. The associated NE\textsubscript{L} maintenance requirement was also derived from INRA (2018), where $\text{NE}_\text{L}$ maintenance (MJ/d) = 0.394 × BW$^{0.75}$, where BW$^{0.75}$ = metabolic BW. Energy accumulated in the gravid uterus was considered as follows: $\text{NE}_\text{L}$ gestation (MJ/d) = 7.1 × ($0.00072 \times 45 \times e^{(0.16 \times \text{Weeks in pregnancy})}$) (INRA, 2018). The NE\textsubscript{L} in milk (MJ/d) was calculated as 39.8 × milk fat yield (kg/d) + 23.9 × milk protein yield (kg/d) + 16.5 × milk lactose yield (kg/d). Finally, EB was calculated as follows: $\text{EB} (\text{MJ/d}) = \text{NEL intake} - \text{(NEL maintenance + NEL milk + NEL gestation)}/k_{\text{gt}}$, where $k_{\text{gt}}$ is the efficiency of ME utilization from or to body reserves and corresponds to $k_s + 0.15$ (INRA, 2018). In all equations where BW was the input, the individual average from the postcalving period (i.e., first 16 wk) was used.

**N Balance.** Nitrogen balance was calculated as the difference between total N intake and total N excretion in feces, urine, and milk over the 48-h total collection period. The amount of N predicted to be produced in the gravid uterus (INRA, 2018; $N_{\text{gestation}}$, g/d = 0.16 × ($0.07 \times 45 \times e^{(0.111 \times \text{Weeks in pregnancy})} \times 0.64$) was also subtracted from the N balance.

**Empty BW.** An estimate of gut fill was made to calculate empty BW from BW measured on the scale. An equation to predict the liquid weight within the reticulorumen (RRLiq, % of BW) was derived from a literature data set described in Sauvant and Nozière (2016) using NDF intake (NDFI, kg) and BW (kg) as predictors. The resulting equation (based on 517 treatment means; $R^2 = 0.94$, RMSE = 0.74) was

$$\text{RRLiq} (\% \text{ of BW}) = 12 + 3.78 \times \left[\frac{\text{NDFI}}{\text{BW}} \times 100\right] - 1.$$  

Gut fill was then calculated as in Martin and Sauvant (2003):

$$\text{Gut fill (kg)} = \frac{\left[(\text{RRLiq} \times \text{BW})/100\right] \times (100 - 11.4)/100}{69.6/100},$$

where 11.4% is the DM content of the reticulorumen and 69.6% is the weight of the reticulorumen contents relative to the weight of the whole digestive tract contents. Finally, empty BW was calculated by subtracting the predicted gut fill and an estimate of conceptus weight (Bell et al., 1995) from measured BW.

**Comparison of EB and N Balance With Empty BW**

Changes in body fat and fat-free mass were calculated using EB and N balance. First, a body fat-free mass balance (kg/d) was calculated as follows: (N balance, kg/d × 6.25)/0.215, where 21.5% is the assumed protein content of the fat-free mass (NRC, 2001). Then, body fat balance (kg/d) was calculated as follows: [EB, MJ/d – (N balance, g/d × 6.25 × 23.6)]/39.8, where 23.6 and 39.8 represent the energy content (in MJ) of 1 kg of protein and fat, respectively. Finally, weekly cumulative changes in body fat and fat-free mass from wk −8 before calving were calculated, and the sum was compared with the weekly change in calculated empty BW as a means to assess the consistency of the data.

**Statistical Analysis**

The data over the whole measurement period were compared by grouping weeks into 5 periods corresponding to the late lactation period (wk −8 and −7), the dry period (wk −6 to −1), and 3 periods of early lactation (wk 1 to 5, wk 6 to 10, and wk 11 to 16). Weekly cow data were analyzed with PROC MIXED of SAS 9.4 (SAS Institute Inc.) according to the following model:

$$Y_{ij} = \mu + W_i(P_j) + P_j + \varepsilon_{ij},$$

in which $Y_{ij}$ is the dependent variable, $\mu$ is the overall mean, and $\varepsilon_{ij}$ is the random residual. The model included the fixed effect of week relative to calving ($W_i$; −8 to 16) nested in periods ($P_j$, 1 to 5) and the fixed effect of $P_j$. The cow was considered the experimental unit, and $W$ was included as a repeated statement using the first-order autoregressive covariance matrix. For least squares means of EB and N balance, statistical differences from 0 are also reported in this article. Additionally, to evaluate the pattern of weekly changes in early lactation, regressions were made on a selection of variables (i.e., apparent nutrient digestibility, energy, and N efficiency) using the following models:
RESULTS AND DISCUSSION

The current study describes weekly evolution of nutrient digestibility, EB, N balance, and N efficiency from late lactation to 16 wk after calving. To our knowledge, such a description of digestive and metabolic processes over a broad and continuous period that includes both dry-off and calving is unique and novel information. Figures 1 and 2 illustrate several key adaptations that occur in cows during the transitions. In the last week before dry-off, cows were producing 24.2 kg of milk (1.19 kg/d of fat and 0.95 kg/d of protein, median values; Figure 1). Dry-off coincides with a change in diet and a decrease in DMI, from 19.8 kg/d during the last week of lactation to 14.3 kg/d during the first week of the dry period (a 25% decrease, Figure 1, median values). Although traditionally underestimated, the abrupt cessation of milking combined with a decrease in DMI represents a severe metabolic challenge for the cow, which can alter redox balance and cause inflammation with consequences for performance and health during lactation (Mezzetti et al., 2019, 2020). In contrast to the transition into the dry period, characterization of which is limited, calving and the start of lactation is the best studied event of the cow reproductive cycle (e.g., Drackley, 1999; Ingvartsen and Andersen, 2000). This is in part because it is the phase with the highest reported incidences of disease (Friggens et al., 2004). Metabolic flexibility is essential for copious milk production, and plasticity of body stores is illustrated by major changes in BCS during these critical transition phases from dry-off through calving (Friggens and Badsberg, 2007). The BCS and BW in the current study (Figure 2) align with the expected increase in body fatness before calving and subsequent mobilization of acquired energy stores in the following lactation to support, in this case, the production of 31.0 kg of milk, 1.73 kg/d of milk fat, 1.37 kg/d of milk protein, and 1.29 kg/d of milk lactose in the first week after calving (median values). The main novelty of the present data set lies in its description of the changes in nutrient digestibility and energy and N balance during these transition phases, made possible by the weekly total collection of feces and urine beginning in late lactation and following through until 16 wk into the subsequent lactation.

Apparent Digestibility

The data for apparent total-tract digestibility of DM, OM, NDF, N, and starch are presented in Tables 3 and 4 and illustrated in Figure 3. The measurements were derived from a short period (48 h) of total collection of feces. Such duration is uncommon for determination of digestibility and shorter than recommendations (e.g., Hristov et al., 2019). However, this limitation of short duration may be compensated for by the weekly repetition of the total collection, leading to a total of 24 periods of 48 h for each cow. Additionally, using 7 d of average intake instead of 2 d may have improved the accuracy of digestibility measurements in the current study. Overall, the standard errors of the means (SEM) of digestibility measurements were very low compared with literature data. For example, OM digestibility was 0.44, much lower than the median SEM of 1.00 reported in a large data set of literature (761 observations, Daniel et al., 2020). Across lactation phases (i.e., excluding the dry period where a different diet was fed), digestibility for all nutrients evaluated was lowest when cows were in the first 5 wk after calving. Average digestibilities for DM, OM, NDF, starch, and N between wk 2 and 5 were lower by 2.0, 2.3, 4.3, 0.6, and 2.3% (absolute), respectively, compared with values in late lactation. This lower digestibility occurred despite similar DMI, which is known to be negatively associated with digestibility (NRC, 2001; Sauvant and Nozière, 2016), between the 2 periods. In the first 10 wk after calving, the dynamics of DMI and nutrient digestibility on a week-by-week basis (Table 4) did not change according to this expected relationship. Indeed, whereas DMI increased from 17.8 kg/d (2.52% of BW) at wk 2 to an average of 24.3 kg/d at wk 10 (3.42% BW), digestibility of all nutrients analyzed also increased. Using the equation of Sauvant and Nozière (2016), developed using a large body of literature data describing the effect of feed intake on measured digestibility, an opposite change in digestibility would be predicted to occur based on the change in intake over this period. The significant increase in visceral mass that occurs in early lactation (Gibb et al., 1992; Reynolds et al., 2004) is the most likely ex-

\[ Y_{ij} = \mu + C_i + p1 \times (W_j - 2) + p2 \times (W_j - 2)^2 + \varepsilon_{ij} \]
Figure 1. (a) Dry matter intake, milk yield (kg/d), (b) milk component yields (g/d), and (c) milk component contents (g/kg) relative to calving (from wk −10 to wk 4, n = 12; from wk 5 to wk 15, n = 11; wk 16, n = 10). Boxes represent interquartile range, diamonds represent mean, whiskers include all values within ±1.5× interquartile range; values with an × are outside that range; lines connect boxes at their median.

Figure 2. (a) Body weight (kg), (b) BCS (1–5 scale; Edmonson et al., 1989), and (c) energy balance (MJ/d; see text for details of calculation) relative to calving (from wk −10 to wk 4, n = 12; from wk 5 to wk 15, n = 11; wk 16, n = 10). Boxes represent interquartile range, diamonds represent mean, whiskers include all values within ±1.5× interquartile range; values with an × are outside that range; lines connect boxes at their median.
planation for this apparent discrepancy, with nutrient digestion and absorption increasing as the digestive surface expands. However, the few studies evaluating digestibility in the first few weeks after calving report either no change in OM digestibility (Penner and Oba, 2009) or a decrease in DM digestibility (de Souza et al., 2019). Inconsistencies might be due to differences in the techniques used, as both of these studies used indigestible NDF as an internal marker to estimate apparent total-tract digestibility as opposed to the total collection method used in this study. Another study using the total collection method reported no statistical effect of week of lactation on energy digestibility from wk 1 to 8 (Sutter and Beever, 2000). However, when we applied regression to the reported data of Sutter and Beever (2000), we found a negative relationship between measured energy digestibility and week in milk \( y = 67.4 - 0.48x, R^2 = 0.74 \). In the same study, N digestibility was also negatively correlated with week in milk, decreasing from 64% at wk 1 to 59% at wk 8. Reasons for the discrepancies in nutrient digestibility in the first week of lactation among studies are not clear and warrant further investigation. Interestingly, when an estimation of true N digestibility was made by correcting fecal N excretion with a predicted fecal endogenous N, the relationship between N digestibility and week relative to calving was not significant (in contrast to apparent N digestibility).

As expected, the dietary differences between the lactation diet and the dry cow diet influenced nutrient digestibility. Nitrogen and starch digestibility increased \( (P \leq 0.01) \) by 5.6 and 1.2 percentage points, respectively, in the dry period compared with the lactation period (Figure 3), whereas OM and NDF digestibility decreased \( (P \leq 0.01) \) by 4.8 and 5.5 percentage points, respectively (Figure 3). During the dry period, soybean

### Table 3. Nutrient digestibility relative to calving in dairy cows

| Item                      | Late lactation | Dry period | Early lactation |
|---------------------------|----------------|------------|-----------------|
|                           | Wk −8 to −7    | Wk −6 to −1| Wk 1 to 5       |
| DMI, kg/d                 | 19.4b          | 13.7c      | 23.5c           |
| Water intake, L/d         | 69.9b          | 38.1d      | 80.3b           |
| Urine output, L/d         | 21.4b          | 11.6b      | 22.3b           |
| Fecal DM output, kg/d     | 5.2b           | 4.5b       | 6.4b            |
| Apparent digestibility, % |                |            |                 |
| DM                        | 73.4a          | 67.1c      | 71.4b           |
| OM                        | 74.8b          | 68.8c      | 72.5b           |
| NDF                       | 69.3b          | 63.5b      | 65.0b           |
| Starch                    | 97.7c          | 98.5c      | 97.1b           |
| N                         | 64.0b          | 68.1b      | 61.7b           |
| Estimated endogenous N, g/d| 75.5b         | 63.7b      | 84.8b           |
| Predicted true N digestibility, % | 78.1b | 90.8b | 76.8a |

**Means within a row with different superscript letters are significantly different \((P \leq 0.05)\).**

**Number of cows is 12 from wk −8 to wk 4, 11 from wk 5 to 15, and 10 for wk 16.**

**Data from wk −6 and wk 1 were not used because fecal excretion was still influenced by previous diet.**

**Predicted using equations from INRA (2018): [DMI × (5.7 + 0.074 × undigested OM)]/6.25 with undigested OM expressed in g/kg of DMI.**

**\(N_{\text{intake}} - (N_{\text{feces}} - \text{Estimated endogenous fecal } N) / N_{\text{intake}} \times 100.\)**

## Table 4. Linear and quadratic coefficients relating nutrient digestibility with week from calving (from wk 2 to 16) in dairy cows

| Nutrient digestibility | Intercept ± SE \(2\) | Linear coefficient | Quadratic coefficient | RMSE \(3\) |
|------------------------|------------------------|--------------------|-----------------------|------------|
|                        | Mean ± SE              | P-value            | Mean ± SE              | P-value    |          |
| Apparent digestibility, % |                       |                    |                       |            |         |
| DM                     | 70.7 ± 0.47            | 0.506 ± 0.1042     | <0.01                 | −0.029 ± 0.0072 | <0.01 | 1.53 |
| OM                     | 71.7 ± 0.48            | 0.571 ± 0.1013     | <0.01                 | −0.032 ± 0.0070 | <0.01 | 1.49 |
| NDF                    | 66.1 ± 0.77            | 0.510 ± 0.1390     | <0.01                 | −0.030 ± 0.0096 | <0.01 | 2.04 |
| Starch                 | 97.1 ± 0.15            | 0.035 ± 0.0102     | <0.01                 |          |        | 0.56 |
| N                      | 61.5 ± 0.60            | 0.096 ± 0.0420     | 0.02                  |          |        | 2.33 |
| True N digestibility, % | 76.6 ± 0.56            | 0.043 ± 0.0381     | 0.26                  |          |        | 2.11 |

**Models (linear vs. quadratic) were chosen based on lowest corrected Akaike’s information criterion. Wk 1 was not used because fecal excretion was still influenced by previous diet. Number of cows is 12 from wk 2 to wk 4, 11 from wk 5 to 15, and 10 for wk 16.**

**Week from calving was mean centered on 2 so that the intercept represents digestibility at wk 2 after calving.**

**Root mean square error.**
meal provided the majority of N intake (64% of total dietary N) whereas grass silage provided the majority of N intake during lactation (49% of dietary N). Therefore, solely based on feed table digestibility, we would expect a higher digestibility of N from the dry cow diet compared with the lactation diet as the digestibility of CP from soybean meal is 80%, whereas that of grass silage varies between approximately 54 and 70% (INRA, 2018). Similarly, barley straw provided most of the NDF fraction of the dry cow diet (52% of dietary NDF), whereas grass silage provided most of the NDF in the lactation ration (54% of dietary NDF), and corn silage contributed 46 and 39% of dietary NDF in the dry period and during lactation, respectively. Because NDF from barley straw is considered less digestible than NDF from grass silage (49% for barley straw vs. 57–80% for grass silage; INRA, 2018), the decline in NDF digestibility during the dry period reported here was expected.

**Energy Balance**

The calculation of EB relative to calving (Figure 2) was based on the measurement of OM digestibility, a key determinant of the NE\text{L} value. However, some assumptions had to be made to calculate EB, because energy losses in the form of methane and heat were not measured but predicted from published empirical equations (Sauvant and Nozière, 2016; INRA, 2018). The calculated EB was positive from late lactation until calving ($P < 0.01$) and negative from calving to wk 8 ($P < 0.01$ except in wk 7 and 8, when EB was not statistically different from 0; $P > 0.13$), mirroring changes in BW (Figure 2; discussed below). Cows returned linearly to a positive EB 9 wk after calving. At wk 1 and 2, 64 and 44%, respectively, of the energy exported to milk was derived from body reserves, underscoring the importance of tissue energy mobilization to support milk production during early lactation. A negative EB
was also observed for the first 8 wk after calving in the study by Sutter and Beever (2000), in which cows were housed in calorimeters. The predicted energy loss in the form of CH₄ between wk 2 (20.1 MJ/d) and wk 8 (25.7 MJ/d; change in loss: +5.6 MJ/d) in the current study agrees with the measurements of Sutter and Beever (2000; change in loss: +6.4 MJ/d). However, our prediction of energy loss in the form of heat did not agree with the measurements of Sutter and Beever (2000), where no change in heat production was observed from wk 1 to 8 after calving (mean = 126 MJ/d). In contrast, the equation used in this study (INRA, 2018), which accounts for both a fixed portion (i.e., maintenance) and a variable portion (conversion of ME to NEL, i.e., mainly in terms of DMI) of heat production, predicted an increase in heat production from 140 to 161 MJ/d between wk 1 to 5 and wk 6 to 10 (Table 5), when DMI increased from 19.4 to 23.5 kg/d. The change in the efficiency of conversion of ME (from intake and body reserves) to milk production (i.e., milk energy efficiency) was comparable in both studies between wk 2 and 8, decreasing from 70.3 to 67.1% in this study and decreasing from 62.7 to 58.3% in the study of Sutter and Beever (2000). Thus, a possible underestimation of heat production associated with maintenance in the first week of lactation could explain the differences in total heat production. Indeed, the increase in splanchnic tissue size after calving (Gibb et al., 1992; Reynolds et al., 2004) would be expected to cause additional energy demand.

Concentrations of NEFA, BHB, triglycerides, glucose, and insulin in serum relative to calving (Figure 4) followed the typical patterns reported for transition cows (e.g., Marcos et al., 1990; Ingvartsen and Andersen, 2000; Reynolds et al., 2003). Concentrations of metabolites and hormones in blood are not easy to interpret, as they reflect the outcome of secretions from both dietary and endogenous origins, as well as their removal by various tissues. Nonetheless, a considerable body of research has shed light on these processes and greatly improved our understanding of metabolic changes during the periparturient period. The sharp increase in serum NEFA concentration during the first week after calving reflects the increased mobilization of fatty acids from adipose tissue (Dunshea et al., 1988). Extraction of NEFA by the liver, mammary gland, and peripheral tissues is linearly proportional to blood concentration (Bell, 1979; Miller et al., 1991). A consequence of increased liver uptake of NEFA may be an increase in hepatic BHB secretion. The sharp rise in hepatic gluconeogenesis along with the increased blood flow that occurs in the first week after calving reflects the increased mobilization of fatty acids from adipose tissue (Dunshea et al., 1988). Extraction of NEFA by the liver, mammary gland, and peripheral tissues is linearly proportional to blood concentration (Bell, 1979; Miller et al., 1991). A consequence of increased liver uptake of NEFA may be an increase in hepatic BHB secretion. The sharp rise in hepatic gluconeogenesis along with the increased blood flow that occurs in the first week after calving (Larsen and Kristensen, 2013) may also pose an additional challenge to the complete oxidation of NEFA (Baird et al., 1968; White, 2015), resulting in further elevated circulating BHB concentrations. Interestingly, as reported by others (Kessel et al., 2008; Ghaffari et al., 2020), the maximal serum BHB concentration was not reached in the current study until 2 to 3 wk after the peak concentration of serum NEFA. This delay might be related to the expected lower alimentary BHB generated during ruminal absorption of butyrate (Reynolds, 2002; Kristensen and Harmon, 2004; Loncke et al., 2009) when postpartum DMI is still low and increasing gradually. In the current experiment, the contribution of BHB from dietary origin to blood levels is well illustrated by the sharp decrease (from 1.20 to 0.82 mM) in blood BHB immediately after dry-off following the DMI pattern, whereas NEFA only marginally decreases. This
agrees with previous reports where alimentary BHB accounted for about half of BHB blood entry rate (Heitmann et al., 1987). The uptake mechanism of BHB in both muscle (Balasse and Féry, 1989) and mammary gland (Miller et al., 1991) can become saturated with increasing BHB levels, which can further exacerbate increases in serum BHB concentration when secretion of BHB from the digestive tract and liver are already high in the weeks postpartum.

Circulating triglycerides and glucose are the main substrates for milk fat and lactose, respectively. As such, blood concentrations are strongly influenced by milk output, sharply increasing at dry-off when milk production stops (~2.3-fold and 1.2-fold for triglycerides and glucose, respectively) and decreasing as lactation resumes (55% for triglycerides, 6% and 24% for glucose in wk 1 and 2, respectively). Similarly, it is well established that glucose-dependent insulin secretion is blunted in early lactation, contributing to glucose sparing for milk synthesis (Sano et al., 1993). In agreement, in the current study, serum insulin concentrations followed a similar pattern to that of glucose in late lactation and during the dry period. A notable exception is the first week after calving, when insulin concentration sharply decreased, with a 67% reduction, whereas decrease in serum glucose concentration was only 6%.

**N Balance**

The calculated N balance and measured N excretion fluxes in this study are shown in Figure 5 and Table 6. Nitrogen balance was positive (although only significantly different from 0 at wk −7, −3, and 10) throughout the entire study except during the first 4 wk after calving (first 3 wk were significantly different from 0), when it ranged from −70 to −9 g/d, indicating intensive mobilization of body protein and use of endogenous N. As highlighted in recent reviews, accurate estimation of N balance is difficult, and published data on N balance are often unrealistically high (Hristov et al., 2019; Spanghero and Kowalski, 2021). In the current study, efforts were made to apply the best practices mentioned in these reviews to avoid N losses. The quality of the N balance was evaluated along with the estimated EB based on the change in BW. Figure 6 illustrates the change in body fat-free mass (i.e., body protein divided by 0.215; NRC, 2001) and body fat that was expected based on the reported weekly N balance and EB. These predicted changes in body mass, however—both the gain before calving and the loss after calving—were greater by 37% and 10%, respectively, than the fluctuation in measured BW (corrected for predicted gut fill and fetus weights). A total of 35.2 kg of body fat-free mass, corresponding to 7.6 kg of body protein, was expected to be lost between calving and wk 4, and this loss was partly (34%) replenished by wk 16 postpartum. Predicted losses of body fat continued until wk 11 to 14, reaching 47.5 kg of total fat loss. These losses are close to those in available data from the literature where body fat and protein changes have been measured in relation to DIM (Belyea et al., 1978; Martin and Ehle, 1986; Chilliard et al., 1991; McGuffey et al., 1991; Gibb et al., 1992; Andrew et al., 1994; Komaragiri and Erdman, 1997; Komaragiri et al., 1998; see Supplemental File S3, https://doi.org/10.6084/m9.figshare.21220703.v1). Reported losses of body fat and body protein from this literature data set were 49 kg [quartile (q)1 = 39 kg; q3 = 59 kg] and 14 kg (q1 = 5

![Figure 4](https://example.com/figure4.png)
kg; q3 = 21 kg), respectively. Overall, our data confirm the previously established importance of metabolic flexibility and body reserves in supporting the synthesis of milk fat, protein, and lactose in early lactation (Baumgard et al., 2017).

**N Efficiency**

Milk N efficiency, calculated as the ratio of milk N to N intake, varied widely from 28.1% (±SE, 2.0) in late lactation (wk −7) to 57.8% (±3.1%) in the first week after calving. The latter value is high compared with the average value from feeding trials with dairy cows in a large Northern European data set (27.7 ± 3.6%; Huhtanen and Hristov, 2009) and the theoretical maximum of 43% for a dairy cow consuming 24.1 kg of DM/d and producing 40 kg/d of fat- and protein-corrected milk with a true protein content of 31.5 g/kg (Dijkstra et al., 2013). However, this extreme peak in milk N efficiency was short-lived, with milk N efficiency declining to 49.5% (±2.0) and 43.2% (±1.6) in wk 2 and 3, respectively. Obviously, body N mobilization in the first 3 wk after calving (Figures 5 and 6) explains, to a large extent, these artificially inflated efficiencies. Indeed, accounting for N supply from endogenous sources (i.e., adding calculated body N mobilization as “intake”) in addition to total dietary N intake reduces milk N efficiency in the first week of lactation (50.2 ± 1.8, 43.2 ± 1.2, and 41.0 ± 1.3% for wk 1, 2, and 3, respectively). Figure 7 illustrates the change in metabolic N efficiency, calculated as the secretion of milk N plus accretion of body N relative to digestible N intake plus body N mobilization, and the change in digestive N efficiency, considered equal to measured apparent total-tract N digestibility. Changes in digestive N efficiency during lactation were moderate with a linear increase from 61.4 (±0.7) to 62.6 (±0.7)% from wk 2 to 16, and the highest value reported in late lactation (64.4 ± 0.7%, Figure 7). In contrast, metabolic N efficiency was lowest at the end of the previous lactation (54.6 ± 3.0%), highest 2 wk after calving (64.6 ± 1.6%, Table 3), and decreased linearly to 61.9 ± 1.5% by 16 wk of lactation (Table 3, Figure 7). These data make it clear that metabolic efficiency, not digestive efficiency, is the main reason for the overall decline in N efficiency observed as lactation progressed.

The change in serum urea-N concentration (Figure 8) during lactation was inversely related to the calculated metabolic N efficiency. Interestingly, serum urea-N concentration was higher in the first week after calving than in the 3 following weeks. Together with the elevated milk N efficiency calculated in the first week (57.8 ± 3.1%), this indicates that intensive N mobilization was also occurring during the first week after calving. Indeed, the N balance in the first week after calving was calculated at −61 g/d (±SE, 23). Mobilization of endogenous N from skeletal muscle is also supported by observations that the highest concentrations of 3-methylhistidine in plasma during lactation are reported to occur in the first 3 to 4 d after calving (Bell and Baum, 1997; van der Drift et al., 2012).

The metabolic efficiency of 19.5% for dairy cows during the dry period (Table 7) appears to be low compared with the metabolic efficiency observed in growing cattle [e.g., 43–69% for N intakes of 0.26–0.52 g of N/kg of BW in 18 treatments, based on values from Titgemeyer et al. (2012) and de Souza et al. (2021)]. Cows in the current study had an average daily N intake of 278 g during the dry period, where 189 g was digested. Of the digested N, 152 g was excreted in urine, leav-
ing 22 g for the gravid uterus and 16 g for accretion in the body. Based on these data, it is tempting to conclude that higher metabolic efficiency in the dry period can be achieved by feeding diets with lower CP content while maintaining health and productivity. Indeed, according to a recent meta-analysis, increasing MP supply above 800 g/d (compared with 1,180 g/d in the present study) for multiparous cows during the dry period has minimal effect on subsequent lactation performance (Husnain and Santos, 2019). The supply

Table 6. Nitrogen intake and partitioning relative to calving in dairy cows

| Item                              | Late lactation | Dry period | Early lactation |
|-----------------------------------|----------------|------------|-----------------|
|                                  | Wk −8 to −7    | Wk −6 to −1| Wk 1 to 5       | Wk 6 to 10      | Wk 11 to 16 | SEM  |
| N intake, g/d                    | 534b           | 278c       | 533b            | 648a            | 671a        | 17.7 |
| N output, g/d                    |                |            |                 |                 |             |      |
| Feces                            | 193b           | 95c        | 196b            | 243a            | 250a        | 6.1  |
| Urine                            | 154b           | 151b       | 130b            | 150b            | 159b        | 5.2  |
| Milk                             | 158b           | —          | 242b            | 244a            | 250b        | 9.1  |
| N balance, g/d                   | 16.2a          | 11.7a      | −32.6b          | 10.4a           | 9.5a        | 6.49 |
| Milk N efficiency, %             |                |            |                 |                 |             |      |
| Relative to N intake             | 29.1c          | —          | 43.5a           | 37.9b           | 37.5b       | 1.24 |
| Relative to N intake + body N    | 45.7           | —          | 70.9a           | 60.9b           | 60.1b       | 2.27 |
| N efficiency (body N accretion + milk N yield), % |                |            |                 |                 |             |      |
| Relative to N intake + body N    | 34.9b          | 13.4c      | 41.6a           | 39.9b           | 39.4a       | 1.01 |
| mobilization                     |                |            |                 |                 |             |      |
| Relative to N intake + body N    | 54.4b          | 19.5c      | 65.2a           | 63.5a           | 62.2a       | 1.53 |
| mobilization                     |                |            |                 |                 |             |      |
| Urinary N, %                     |                |            |                 |                 |             |      |
| Relative to N intake             | 45.6b          | 79.7a      | 34.8c           | 36.5b           | 37.5c       | 1.48 |
| Relative to N intake + body N    | 45.9b          | 80.6a      | 38.2c           | 37.2c           | 38.4c       | 1.53 |
| mobilization                     |                |            |                 |                 |             |      |

*Means within a row with different superscript letters are significantly different (P ≤ 0.05).
1Number of cows is 12 from wk −8 to wk 4, 11 from wk 5 to 15, and 10 for wk 16.
2Data from wk −6 and wk 1 not used because fecal excretion was still influenced by previous diet.
3Body N accretion was considered as positive N balance + N accrued into gravid uterus.
4Body N mobilization was considered when body N balance was negative.
of MP of 1,180 g/d in the current study was about 12.5% above recommended supply (940 g/d in NRC, 2001), further supporting the argument that N was fed in excess during the dry period.

**CONCLUSIONS**

In this study, weekly changes in nutrient digestibility, EB, N balance, and N efficiency were examined from late lactation until 16 wk after calving. Apparent digestibility of all nutrients increased linearly with stage of lactation, but with a negative quadratic component for DM, OM, and NDF. The energy and N balances calculated in this study were in line with the change in BW after calving, confirming the importance of mobilizing fat and protein to support milk production in early lactation. As lactation progresses, metabolic N efficiency decreases, whereas digestible N efficiency increases only moderately. It appears that dietary N was in excess of metabolic requirements during the dry period, resulting in low metabolic N efficiency and revealing that higher efficiency is possible during this period.

**Table 7.** Linear and quadratic coefficients relating N and energy partitioning with week from calving (from wk 2 to 16) in dairy cows

| Item                                              | Linear coefficient | Quadratic coefficient | RMSE³ |
|---------------------------------------------------|--------------------|-----------------------|-------|
| Milk N efficiency, %                              |                    |                       |       |
| Relative to N intake                               | 46.5 ± 1.22        | −2.064 ± 0.1937       | <0.01 |
| Relative to digestible N intake                    | 76.5 ± 2.29        | −3.748 ± 0.398        | <0.01 |
| N efficiency (body N accretion⁴ + milk N yield), % | 41.6 ± 0.69        | −0.192 ± 0.0431       | <0.01 |
| Relative to N intake + body N mobilization⁵       | 65.3 ± 1.08        | −0.234 ± 0.0532       | <0.01 |
| Milk energy efficiency,⁶ %                         | 37.9 ± 1.09        | −1.327 ± 0.1618       | <0.01 |
| Relative to GE intake                              | 55.5 ± 1.60        | −2.372 ± 0.2955       | <0.01 |

³Models (linear vs. quadratic) were chosen based on lowest corrected Akaike’s information criterion. Wk 1 was not used because fecal excretion was still influenced by previous diet. Number of cows is 12 from wk 2 to wk 4, 11 from wk 5 to 15, and 10 for wk 16.

⁴Week from calving was mean centered on 2 so that the intercept represents digestibility at wk 2 after calving.

⁵RMSE = root mean square error.

⁶Body N accretion was considered as positive N balance + N accrued into gravid uteruses.

⁷Body N mobilization was considered when body N balance was negative.

⁸GE = gross energy; DE = digestible energy.

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