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Digestive response of dairy cows fed diets combining fresh forage with a total mixed ration

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ABSTRACT

The objective of this experiment was to quantify the response of dairy cows fed a total mixed ration (TMR) to increasing access to high-quality temperate fresh forage with respect to energy intake, rumen fermentation, microbial protein flow, passage rate, nutrient digestion and utilization, and metabolic and endocrine profiles. Nine Holstein cows fed a TMR were assigned to the following treatments according to a 3 × 3 Latin square replicated 3 times with 20-d periods and sampling on the last 10 d of each period: 0 (T0), 4 (T4), or 8 (T8) h of daily access to fresh forage. The forage (*Lolium multiflorum*; 17.1% crude protein, 26.5% acid detergent fiber) was cut daily and offered ad libitum beginning at 0800 h, and a TMR (16.1% crude protein, 22.9% acid detergent fiber) was offered ad libitum during the remaining time. Energy intake and balance were higher in T0 than in T8, which was reflected in higher blood glucose and insulin concentrations in T0. Total volatile fatty acid concentrations in the rumen were higher in T0 and T4 than in T8, pH was lower in T4 than in T8, and ammonia-N was higher in T0 than in T8. No differences among treatments were detected in microbial protein flow to the duodenum, digestibility of nutrients, apparent efficiency of energy, or N utilization for milk production, but the total mean retention time of feed in the digestive tract was higher in T8 than in T0. It is concluded that more than 4 h of daily access to high-quality fresh forage in the diet of dairy cows fed a TMR reduced energy intake and balance but had no effects on nutrient digestion or utilization.

Key words: total mixed ration, fresh forage, rumen fermentation, digestibility

INTRODUCTION

Recently, interest has been renewed in the utilization of fresh forage (**FF**) for dairy cows. Where dairying relies on the sole use of a TMR for feeding dairy cows, this interest in FF may be justified when feed costs increase, as well as a greater volatility in the price of conventional feeds. Additionally, inclusion of FF in the diet of cows increases the proportion of certain milk components (e.g., rumenic and vaccenic FA) that may have nutritional benefits for human health (Elgersma et al., 2006). On the other hand, in countries where dairying relies on direct grazing of forage, utilization of FF in combination with a TMR may improve milk yields when compared with the more traditional supplementation of grazing cows with concentrates in the milking parlor or conserved forage on a feed pad (Bargo et al., 2002a; Wales et al., 2013).

Part of the observed differences in milk yield between these feeding systems may be related to differences in nutrient intake, which has been reported to be higher in TMR-fed cows than in grazing cows (Bargo et al., 2003). For example, we have recently reported that cows with 4 h of access to high-quality FF had similar DMI levels and milk yields as cows fed only with TMR, but more than 4 h of access reduced DMI and performance, although milk fat had higher content of beneficial fatty acids (Mendoza et al., 2016). Although animal performance is also explained by how nutrients are digested in the gastrointestinal tract and how they are used in different tissues, the effect of feeding diets that combine a TMR and FF on the digestion and metabolism of nutrients in dairy cows remains largely unknown. In an in vitro study, Vibart et al. (2010) reported no differences in rumen pH or NH_3-N concentrations, but observed linear increases in total diet digestibility with a greater proportion of fresh annual ryegrass in a TMR-based diet, whereas Soder et al. (2013) reported reduced pH, NH_3-N concentration and apparent NDF digestibility, and true OM digestibility in fermentors fed only a TMR compared with only fresh orchardgrass. However, considerable differences exist in VFA concentrations and

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nutrient digestion data obtained by rumen simulation techniques or in vivo trials (Hristov et al., 2012). For example, Bargo et al. (2002b) reported no differences in the rumen pH or NH_3-N concentration but lower NDF digestibility when dairy cows were fed a TMR compared with TMR plus a grass-based FF (Bargo et al., 2002a). Santana et al. (2011, 2012) reported a higher rumen pH in beef heifers fed a legume-based FF compared with only TMR or a TMR plus 6 h of access to the FF, but no differences were detected in DM digestibility. These discrepancies among experiments may be related to the particular experimental approach chosen in each study (in vitro or in vivo), the nutritional quality of the TMR and FF used, the type of FF, or the proportion of FF in the diet, but the relative contribution of each factor is unknown.

The design of optimal feeding strategies requires a better understanding of the digestive and metabolic responses of cows when offered diets that combine a TMR and FF. However, to date there is a paucity of published information on key aspects that affect in vivo nutrient digestion such as the dynamics of rumen fermentation, digesta passage rate, and microbial protein flow to the duodenum, as well as on associated metabolic indices of energy and protein status. Therefore, to gain insight on the formulation of diets that combine FF and TMR and maximize nutrient availability to the animal, the objective of this experiment was to determine the changes in energy intake, rumen fermentation, nutrient digestion and utilization, and metabolic and endocrine profiles of TMR-fed lactating cows with varying degrees of access to high-quality FF.

MATERIALS AND METHODS

Animals, Treatments, and Experimental Design

The experiment complied with regulations set by the Bioethics Committee of the Veterinary Faculty (Universidad de la República, Uruguay). Nine multiparous Holstein cows fitted with permanent rumen catheters, and with a milk yield record during the previous 305 d of lactation of 7,079 kg (SD = 1,226), were selected from the herd at Experimental Station of the Veterinary Faculty (Universidad de la República, Uruguay) in San José, Uruguay (34°40′ S, 56°32′ W). Cows were blocked into 3 squares balanced for BW, previous milk yield, DIM, and parity, and within each square were randomly assigned to treatment sequences according to a replicated 3 × 3 Latin square design. At the start of period 1, cows had an average BW of 572 kg (SD = 76), 100 DIM (SD = 25), and a parity of 4.3 (SD = 1.2). Each period lasted 20 d and consisted of 10 d for adaptation followed by 10 d of data and sample collection. The treatments evaluated were 0 h of access to FF plus 24 h of access to a TMR (**T0**), 4 h of access to FF plus 20 h of access to a TMR (**T4**), or 8 h of access to FF plus 16 h of access to a TMR (**T8**).

Cows were housed in individual tie stalls (2.0 × 1.3 m) with ad libitum access to water and were milked at 0700 and 1800 h. A pastureland of Italian ryegrass (*Lolium multiflorum*; var. INIA Bakarat) was seeded (15 kg per ha) on March 3, 2011, and was fertilized with 27 kg of N per ha and 69 kg of P per ha as diammonium phosphate and was used throughout the experimental period. The pastureland was divided into 3 paddocks with 1 paddock used during each period. Average herbage mass for the 3 periods was 2,413 ± 552 kg of DM per ha, with a height between 20 and 25 cm; all forage used was in a vegetative stage. Herbage was harvested daily at 0700 h with a mower, leaving a residual height of 10 cm. The FF was immediately collected, stored under a roof, and offered unchopped in individual feed troughs from 0800 to 1200 h, or from 0800 to 1600 h, to T4 and T8 cows, respectively. To ensure that amount of feed was not limiting at any time, the feed trough was observed every 30 min, and if necessary, more feed was added. Cows in T4 and T8 had access to the TMR from 1200 to 0700 h, and from 1700 to 0700 h, respectively, which was delivered as described above for the FF. Cows in T0 had ad libitum access to the TMR all day. Every day at 0800 h, which will herein be referred to as h 0, orts from the previous 24 h were removed from the feed trough, and new feed was offered.

Feed Analysis

Samples of TMR and FF were taken at 0800, 1200, and 1600 h on d 13 to 20 of each period and composited to obtain one sample per day, whereas approximately 20% of feed orts were sampled from each cow. Particle size distributions of the TMR were assessed using the modified Penn State Particle Size Separator (Kononoff et al., 2003). All samples were kept frozen at −20°C until analysis. Feed samples were dried in a forced-air oven at 60°C and ground to pass through a 1-mm Wiley mill screen (Arthur H. Thomas Co., Philadelphia, PA). Feed samples were analyzed for DM, ash, total N, and ether extract (AOAC, 1990; methods 934.01, 942.05, 955.04, and 920.39, respectively); NDF using heat-stable α-amylase and sodium sulfite; ADF and ADL (Van Soest et al., 1991), expressed exclusive of residual ash; and NDIN and ADIN (Licitra et al., 1996). Organic matter was determined as the difference between DM and ash content. The concentration of NFC was estimated as 100 − (% NDF + % CP + % ether extract + % ash) (NRC, 2001). The concentration of NE_L was calculated from chemical composition analyses, actual

Table 1. Ingredients and mean nutrient composition (SD in parentheses) of TMR and fresh forage (% DM, unless otherwise indicated)

Item	TMR	Fresh forage
Ingredients of TMR		
Corn silage	45.2	—
Ground corn grain	31.6	—
Solvent-extracted soybean meal	21.3	—
Sodium bicarbonate	0.60	—
Dicalcium phosphate	0.40	—
Urea	0.30	—
Calcium carbonate	0.20	—
Salt	0.20	—
Magnesium oxide	0.20	—
Vitamin and mineral premix[1]	0.04	—
Mycotoxin adsorbent	0.04	—
Nutrient composition		
DM, % of as fed	35.8 (2.9)	15.3 (0.7)
OM	92.0 (0.3)	90.1 (0.2)
NDF	40.3 (3.3)	47.1 (2.6)
ADF	22.9 (0.8)	26.5 (2.2)
ADL	2.7 (0.3)	4.4 (0.7)
NFC	34.0 (3.0)	23.7 (2.1)
Ether extract	1.8 (0.3)	2.3 (0.1)
CP	16.1 (0.3)	17.1 (0.9)
NDIN	0.55 (0.07)	1.32 (0.07)
ADIN	0.19 (0.02)	0.23 (0.04)
NE_L, Mcal/kg of DM	1.55 (0.04)	1.46 (0.01)
Particle size distribution, % as fed		
>19 mm	2.8 (0.9)	—
8 to 19 mm	50.5 (0.3)	—
1.8 to 8 mm	40.4 (1.5)	—
<1.8 mm	6.3 (0.3)	—

[1]Provided (per kg of DM): 0.85 g of Cu, 2.6 g of Zn, 0.9 g of Se, 1.0 g of Mn, 23 mg of I, 3 mg of Co, 63,700 IU of vitamin A, 12,700 IU of vitamin D, and 250 IU of vitamin E.

DMI, and individual characteristics of cows during each period according to NRC (2001; Table 1).

Energy Balance and Energy Efficiency

Daily energy balance (**EB**) was estimated between d 13 and 20 of each period as EB (Mcal of NE_L/d) = energy intake (Mcal of NE_L/d) – [maintenance requirement (Mcal of NE_L/d) + lactation requirement (Mcal of NE_L/d)]. Energy intake was calculated as DMI × NE_L concentration in feeds. Ingestion of DM was recorded between d 13 and 20 of each period by weighing the amount of feed offered and refused each day, as described in detail by Mendoza et al. (2016). Energy requirements were calculated according to NRC (2001). Briefly, maintenance requirement was calculated as $0.08 \times BW^{0.75}$. Body weight was measured with a digital scale at the beginning of the experiment and at the end of each period, and the average for each period was used in the calculation. The requirement for lactation was calculated as milk yield × (0.0929 × fat % + 0.0547 × CP % + 0.0395 × lactose %), using the average milk composition for each period, which was determined as described by Mendoza et al. (2016). Requirements for pregnancy and growth were not considered because the cows were not gestating and were in at least their third lactation. Apparent efficiency of energy utilization for milk production was calculated as milk NE_L yield/NE_L intake.

Nutrient Digestion

Apparent total-tract nutrient digestibility as described by Huhtanen et al. (1994) using indigestible NDF (**iNDF**) as an internal marker. On d 12 and 13 of each period, 2 spot fecal samples were collected from all cows at 0200 and 1400 h (± approximately 4 h relative to the beginning of the feeding bout). Approximately 200 g of feces was collected directly from the rectum for each sample and dried in a forced-air oven at 60°C for 72 h. Samples were ground to pass through a 1-mm screen, and 1 composite sample per cow per period was obtained by mixing equal DM amounts from each subsample. Fecal composite samples were analyzed for DM, ash, NDF, ADF, and total N as described previously. Fecal composite samples, as well as TMR and FF samples, were also analyzed for iNDF. Briefly, dried samples of feces, TMR, and FF (both offered and orts) were ground to pass through a 2-mm screen, and 6-g samples were weighed into 22 × 10.5 cm nylon bags (Ankom Technology Corporation, Macedon, NY) with a pore size of 50 μm and a sample size to surface area ratio of 13 mg/cm^2 and were incubated for 288 consecutive hours in the rumen of 2 nonlactating Holstein cows fed a diet consisting of (DM basis) *Setaria italica* hay (60%), high-moisture corn grain (25%), soybean meal (13%), and a mineral and vitamin mix (2%). Following incubation, bags were rinsed with tap water for 15 min and dried in a forced-air oven at 60°C for 72 h, and the residues were analyzed for NDF as previously described. Fecal output was estimated for each animal by dividing iNDF daily intake by the iNDF concentration in feces. Apparent total-tract digestibility coefficients for DM, OM, NDF, ADF, and total N were calculated as {[ingestion (g/d) – fecal output (g/d)]/ingestion (g/d)} × 100. Ingestion of OM, NDF, ADF, and total N were calculated as described by Mendoza et al. (2016).

Passage Rate

Passage rate of large particles was estimated using Cr-mordanted NDF. The Cr-mordanted NDF was prepared from alfalfa hay according to Udén et al. (1980), resulting in a material with 1.99% of Cr (DM basis). Experimental diets did not contain supplemental Cr. On d 14 of each period at 0800 h, 150 g of Cr-mor-

danted NDF was orally administered in a single dose to each cow, and fecal samples were collected directly from the rectum at 0, 6, 9, 12, 24, 30, 34, 38, 42, 48, 60, 72, 96, 120, and 144 h after dosing with the marker. Fecal samples were dried in a forced-air oven at 60°C, ground to pass through a 1-mm screen and stored for analysis. Fecal Cr concentrations were determined using atomic absorption spectrometry (Perkin Elmer 3300, Perkin Elmer, Wellesley, MA) according to the method of Williams et al. (1962). Individual fecal Cr excretion curves were fitted to the multi-compartmental model proposed by Dhanoa et al. (1985):

$$Y = A \times \exp(-k_1 \times T) \times \exp\left\{-(n-2)\exp\left[-(k_2 - k_1)T\right]\right\},$$

where Y (mg/kg of DM) is the fecal marker concentration at time t (h); T = t – TT, where TT is the transit time or time delay (h) between marker administration and its first appearance in feces; k_1 (h^{-1}) is the passage rate from reticulorumen; k_2 (h^{-1}) is the passage rate from cecum-proximal colon; A is a scale parameter dependent on k_1; and n is the number of compartments in the model (2 in this case, the reticulorumen and the cecum-proximal colon). Mean retention times in the reticulorumen (**RMRT**) and the cecum-proximal colon (**CMRT**) were calculated as $1/k_1$ and $1/k_2$, respectively. Total mean retention time (**TMRT**) was calculated as TMRT = RMRT + CMRT + TT (Colucci et al., 1990).

Rumen Fermentation

On d 19 of each period, samples of ruminal fluid were taken hourly for 24 consecutive hours. Ruminal fluid was pressed through 2 layers of cheesecloth and pH was immediately measured using a digital pH meter (EW-05991–36, Cole Parmer, Vernon Hills, IL). A 10-mL sample of ruminal fluid was preserved with 0.2 mL of 6.6 *M* H_2SO_4 for NH_3-N analysis, and another 0.5-mL sample was preserved with 0.5 mL of 0.1 *M* $HClO_4$ for VFA analysis; both were stored at −20°C until analysis. For NH_3-N determination, samples were thawed at room temperature and analyzed by direct distillation using sodium tetraborate and titration with 0.05 *M* HCl (Aguerre et al., 2013). For VFA determination, only samples taken at h 0, 2, 4, 6, 8, 10, and 12 were analyzed. Samples were thawed at room temperature, centrifuged (10,000 × *g* at 4°C for 15 min) and analyzed using HPLC (Dionex Ultimate 3000, Sunnyvale, CA) as described by Adams et al. (1984), using an Acclaim Rezex Organic Acid H^+ (8%) and a 7.8 × 300 mm column and set at 210 nm. Concentrations of acetic, propionic, and butyric acid were reported in concentration units and as molar proportions; total VFA concentration was the sum of acetic, propionic, and butyric concentrations.

Microbial N Flow and N Utilization

On d 12 and 13 of each period, 2 spot urine samples were collected from all cows at 0200 and 1400 h (approximately 4 h before and after the beginning of the feeding bout). A 15-mL sample of fresh urine was acidified with 60 mL of 0.072 *N* H_2SO_4 and stored at −20°C (Broderick et al., 2009). Urine samples were later thawed at room temperature, filtered through Whatman no. 1 filter paper, and analyzed for purine derivatives (**PD**; i.e., allantoin and uric acid) using HPLC (Dionex Ultimate 3000) as described by Balcells et al. (1992), using an Acclaim, C18, 5 μm, 4.5 × 250 mm column and set at 205 nm. Urine samples were also analyzed for creatinine with a commercial kit (picric acid; Wiener Laboratorios, Rosario, Argentina) and a Vitalab Selectra 2 Autoanalyser (Vital Scientific, Dieren, the Netherlands). Intraassay CV was less than 10%. The level of PD excretion (mmol/d) was calculated as the ratio of the concentrations (mmol/L) of PD:creatinine in the spot sample times the expected creatinine excretion (mmol/d), which was estimated assuming a daily creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Flow of microbial N (g/d) to the duodenum was determined as described by Chen and Gomes (1992), except that a 0.134 ratio of purine N/microbial N was used (Valadares et al., 1999). Efficiency of N use for microbial N synthesis was calculated as (microbial N flow/total N intake) × 100. Efficiency of utilization of feed N for milk production was calculated as (milk N output/N intake) × 100. Milk N secretion (milk protein/6.38) was calculated from milk yield and milk protein concentration. Composite samples of urine per cow per period were taken and analyzed for N, as described earlier, to calculate urine N excretion.

Metabolic and Endocrine Profile

On d 10 of each period, individual blood samples were collected by jugular venipuncture at h 0, 2, 4, 6, 8, and 10 into tubes without anticoagulants. Tubes were left at room temperature for 2 h, placed in a refrigerator at 4°C for 1 additional h, centrifuged (3,000 × *g* for 20 min at 20°C), and serum was then separated and stored at −20°C until analyzed for insulin and urea. Another blood sample was collected in tubes containing Na_2-EDTA, aprotinin (500 kIU/mL of blood; Laboratorio Rivero, Buenos Aires, Argentina) was immediately added and gently mixed, and tubes were held on ice until centrifugation (3,000 × *g* for 20 min at 20°C). Plasma was harvested and stored at −20°C

until analyzed for glucagon. An additional sample was collected into a tube with sodium fluoride and Na_2-EDTA (Wiener Laboratorios), which was immediately centrifuged (3,000 × *g* for 20 min at 20°C), and plasma was separated and stored at −20°C for analysis of glucose. Concentrations of serum urea and plasma glucose were determined by spectrophotometry (Unico S1200, United Products & Instruments Inc., Dayton, NJ) using commercial kits (urea:urease/salicylate, BioSystems S.A., Barcelona, Spain; glucose: glucose oxidase/peroxidase, BioSystems S.A.). Intraassay CV was less than 10% for both analyses. Serum insulin concentrations were determined by an immunoradiometric assay (Diasource Immuno Assays, Nivelles, Belgium), and intraassay CV was 6.8 and 9.5% for low (28.2 μIU/mL) and high (103.4 μIU/mL) controls, respectively. Plasma glucagon concentrations were determined by a sequential RIA (Siemens Healthcare Diagnostics Inc., Los Angeles, CA), and intraassay CV was 8.5 and 12.9% for low (66.6 pg/mL) and high (235.4 pg/mL) controls, respectively.

Statistical Analysis

All data were analyzed using SAS software version 9.1 (SAS Institute Inc., Cary, NC). Energy intake and NE_L milk yield were averaged per period before data analysis, and together with NE_L balance, nutrient digestibility, microbial N flow, N utilization, and passage rate were analyzed using the PROC MIXED procedure with the following model:

$$Y_{ijkl} = \mu + S_i + C_j(S_i) + P_k + T_l + e_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the random effect of the square (i = 1 to 3), $C_j(S_i)$ is the random effect of cows nested within the square (j = 1 to 3), P_k is the random effect of period (k = 1 to 3), T_l is the fixed effect of treatment (l = T0, T4, or T8), and e_{ijkl} is the residual error.

Rumen pH, N-NH_3, and VFA, and blood traits were analyzed using the PROC MIXED procedure with the following model:

$$Y_{ijklm} = \mu + S_i + C_j(S_i) + P_k + T_l + H_m + T_l \times H_m + e_{ijklm},$$

where Y_{ijklm} is the dependent variable, μ is the overall mean, S_i is the random effect of the square (i = 1 to 3), $C_j(S_i)$ is the random effect of cows nested in the square (j = 1 to 3), P_k is the random effect of period (k = 1 to 3), T_l is the fixed effect of treatment (l = T0, T4, or T8), H_m is the fixed effect of the hour of measurement (m = 0, 1, 2,..., 23 for pH and NH_3-N; m = 0, 2, 4, 6, 8 10, 12 for VFA; m = 0, 2, 4, 6, 8, 10 for blood traits), $T_l \times H_m$ is the fixed effect of the interaction between treatment and hour of measurement, and e_{ijklm} is the residual error. Period × cow interaction within a square was the subject of repeated measurements, and AR(1) was the covariance structure chosen (Littell et al., 1998). Treatment × period effect was tested in both models, but it was not significant and therefore was removed. Residuals were tested for a normal distribution using the PROC UNIVARIATE statement. Means were compared with a Tukey test, significant differences were declared at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Energy output in milk was 7% higher in T0 and T4 than in T8 and correlated well with the higher energy intake of those animals (Table 2). As a result, EB tended to be lower in T8 than in T0 or T4 (Table 2). Previous studies had reported an increase in milk energy output as a result of a higher DM or energy intake in cows fed a TMR compared with a TMR plus FF (Bargo et al., 2002a; Vibart et al., 2008). Treatments had no effect on the apparent efficiency of energy use for milk production, which is an interesting result because it suggests that high-quality FF can be included in the diet of high-producing dairy cows without negatively affecting this trait. In fact, other researchers have reported higher feed efficiencies (DMI/FCM) in cows fed a TMR and with access to pasture than fed an all-TMR diet (Vibart et al., 2008). However, it should be noted that cows had 100 DIM at the beginning of our experiment, and this may have caused a portion of the energy intake to not be used for milk production but to be directed to body reserves (Yan et al., 2006), which may partially explain the lack of response of treatments on the apparent efficiency of energy use for milk production.

The rate of passage from the reticulorumen (k_1) or cecum-proximal colon (k_2), and the mean retention times in these organs were not affected by the treatments, but TMRT was higher in T8 than in T0 cows (Table 2). The higher TMRT in T8 is consistent with the lower intake observed in these animals. In addition, the increase in TMRT in T8 may be related to the fact that FF was offered to the animals without chopping. Boudon et al. (2006) reported that offering unchopped FF under indoor conditions resulted in a reduced rate of comminution of feed particles, which in turn could restrict the outflow of particles from the rumen. This could have resulted in a longer RMRT, which was not significantly different but numerically longer in T8 than T0. Because digestion is a time-dependent process,

it was expected that a higher TMRT might result in greater nutrient digestion along the digestive tract, particularly for fiber (Allen and Mertens, 1988). However, in the present experiment, nutrient digestibility was not affected by the treatments (Table 2). Others also did not observe differences in DM digestibility between a 100% TMR diet and a combined TMR and FF diet (Bargo et al., 2002a), or between a 100% TMR diet and a 100% FF diet (Santana et al., 2012), but Bargo et al. (2002a) detected a lower NDF digestibility in the 100% TMR diet. In cattle, NDF digestion occurs principally in the rumen, accounting for approximately 95% of total NDF digestion (Huhtanen et al., 2010), and any reduction of digestion in this organ could compromise digestion in the entire digestive tract. Although suboptimal ruminal conditions for cellulolytic bacteria may reduce the rate and extent of ruminal fiber digestion, in our study, pH values were always above the minimum level that optimizes the growth of rumen microbiota (Hoover, 1986), and hence it appears that they did not limit the digestion of this fraction in any treatment (Figure 1).

Average rumen pH was lower in T4 than in T8, probably because the former group had a higher OM intake, which increased the supply of fermentable substrates and led to a higher total VFA production (Table 3). No treatment effect was found on ruminal acetic and propionic acid proportions, but butyric acid proportion was higher in T4 and T8 than in T0 (Table 3), perhaps because of a higher sugar content in the FF. On the contrary, Bargo et al. (2002b) and Santana et al. (2012) did not observe an effect of diet (TMR or TMR plus FF) on total VFA concentration. Pérez-Ruchel et al. (2014) also reported no differences of different TMR to FF ratios on total VFA concentrations in growing lambs, but observed a higher molar proportion of propionic acid as the proportion of TMR increased in the diet. Differences in the chemical composition of the basal TMR (e.g., NFC concentrations), between species, or both, may explain the discrepancies between our results and those of Pérez-Ruchel et al. (2014). For total and each VFA concentrations, no interactions between treatment and hour were detected in our study, which is consistent with the lack of interaction for ru-

Table 2. Energy intake, energy balance, apparent efficiency of energy utilization for milk production, digestibility of nutrients, and passage rate of dairy cows fed a TMR with or without access to fresh forage

	Treatment[1]				
Item	T0	T4	T8	SEM	*P*-value
Diet composition					
OM, % of DM	93.5[a]	92.9[b]	92.6[b]	0.39	<0.01
NDF, % of DM	39.6[b]	40.6[a]	40.8[a]	2.66	<0.01
ADF, % of DM	22.8[b]	23.1[ab]	23.5[a]	0.53	0.03
NFC, % of DM	35.1[a]	33.6[b]	33.1[c]	2.50	<0.01
CP, % of DM	17.2	16.9	16.8	0.16	0.09
NE_L, Mcal/kg of DM	1.60[a]	1.58[b]	1.57[b]	0.03	<0.01
Milk NE_L yield, Mcal/d	25.9[a]	25.9[a]	24.2[b]	0.53	<0.01
NE_L intake, Mcal/d					
Fresh forage	0[a]	4.2[b]	5.5[c]	0.69	<0.01
TMR	39.1[a]	36.1[a]	29.9[b]	2.86	<0.01
Total	39.1[a]	40.4[a]	35.4[b]	1.76	0.04
NE_L balance, Mcal/d	4.0	4.2	2.0	0.87	0.10
NE_L milk/NE_L intake	0.68	0.64	0.69	0.028	0.41
Digestibility					
DM	58.2	57.4	64.9	5.57	0.27
OM	63.4	62.1	67.6	4.46	0.42
N	55.1	56.7	62.7	8.45	0.11
NDF	53.6	54.3	59.6	6.30	0.56
Passage rate[2]					
k_1, h^{-1}	0.063	0.061	0.043	0.0081	0.19
k_2, h^{-1}	0.261	0.159	0.184	0.0386	0.22
RMRT, h	17.3	16.6	24.0	2.91	0.13
CMRT, h	4.0	6.7	6.1	1.07	0.17
TT, h	10.1[b]	12.1[a]	9.1[b]	1.74	0.02
TMRT, h	31.4[b]	35.4[ab]	39.1[a]	3.66	0.05

[a–c]Within a row, means with different superscripts are different ($P \leq 0.05$).

[1]T0 = 24 h of access to TMR; T4 = 4 h of access to fresh forage plus 20 h of access to TMR; T8 = 8 h of access to fresh forage plus 16 h of access to TMR.

[2]k_1 is the passage rate from the reticulorumen; k_2 is the passage rate from cecum-proximal colon; RMRT = mean retention time in the reticulorumen; CMRT = mean retention time in the cecum-proximal colon; TT = transit time; TMRT = total mean retention time.

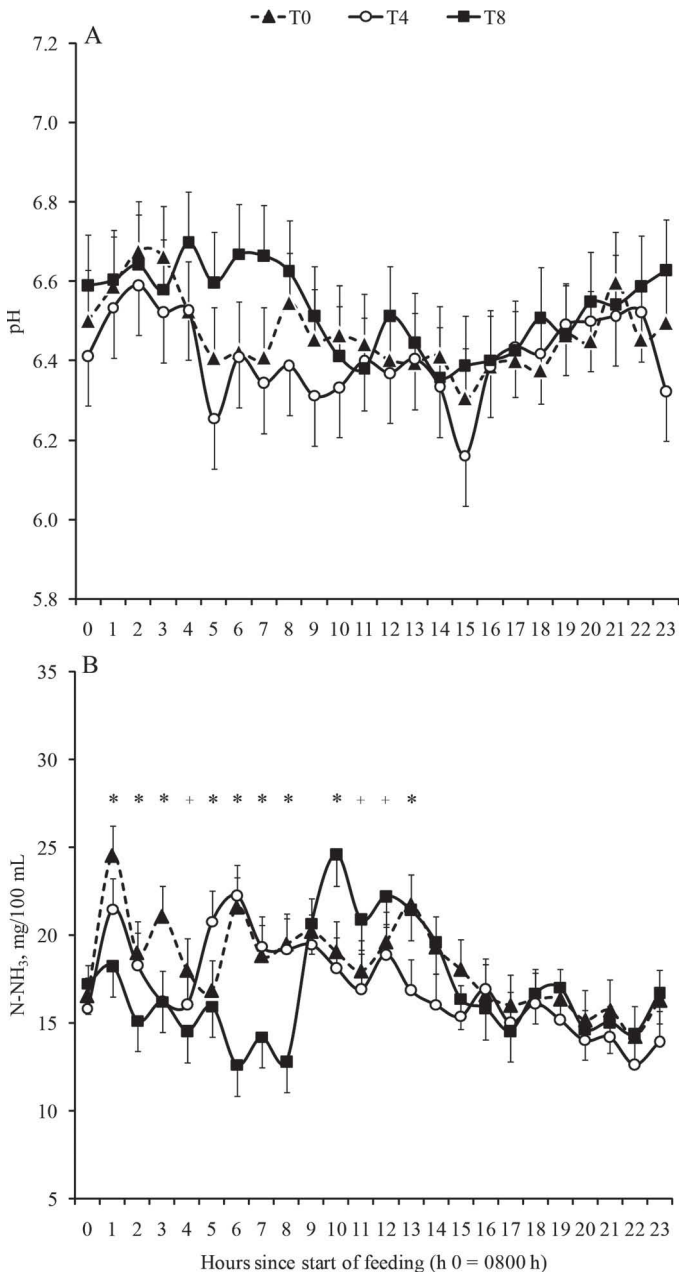

Figure 1. Ruminal pH (A) and NH_3-N concentrations (B) of dairy cows fed a TMR with or without access to fresh forage. Asterisks or crosses at each hour indicate at least one difference among the treatments, $P \leq 0.05$ or $0.05 < P \leq 0.10$, respectively. T0 = 24 h of access to TMR; T4 = 4 h of access to fresh forage plus 20 h of access to TMR; T8 = 8 h of access to fresh forage plus 16 h of access to TMR. Error bars represent SEM.

men pH, but all increased or tended to increase over time, reflecting the postprandial increase in the supply of fermentable substrates (Table 3). Average total VFA concentration remained constant between h 0 and h 6 and then increased from 107.4 to 133.6 m*M* at h 10. Acetic acid concentration rose from 62.2 to 74.2 m*M* from h 0 to 8 and then remained constant, whereas propionic acid concentration rose from 23.7 to 32.3 m*M* from h 0 to 10, and butyric acid concentration rose from 15.1 to 23.2 m*M* from h 0 to 10.

In every treatment, average and hourly ruminal concentrations of NH_3-N were above the reported levels needed to optimize microbial growth (Reynal and Broderick, 2005), but an interaction between treatment and hour was detected for this variable (Table 3). Between h 1 and 8, NH_3-N concentrations were lower in T8 than in the other treatments, and between h 10 and 14 they were or tended to be higher in T8 than in T4 (Figure 1). This pattern of NH_3-N concentrations reflects the distinct eating behavior during the first 8 h after the beginning of the feeding bout, where T8 cows had a very low intake of FF (Mendoza et al., 2016) and probably a lower N intake during that period than compared with the other treatments. This situation was reversed when animals had access to TMR after h 8, probably because T8 cows subsequently displayed a high DMI rate.

Urinary concentrations of allantoin and uric acid, urinary excretion of purine derivatives, intestinal flow of microbial N, and efficiency of N use for microbial N synthesis were not affected by the treatments (Table 4). Our results are inconsistent with the higher energy and N intake observed in T0 and T4 compared with T8 cows, which, according to Clark et al. (1992), should have provided more substrate for ruminal microbial protein synthesis resulting in a higher microbial N flow in those treatments. Additionally, feeding a TMR should lead to a more synchronized ruminal availability of energy and N sources than including FF in the diet because of the higher protein:energy ratio of the latter (117 vs. 104 g of CP/Mcal of ME). In turn, it can be conjectured that a higher efficiency of N use for microbial N synthesis could be achieved by using a TMR rather than a combination of TMR plus FF, but no differences were observed in this variable among treatments (Table 4). Using the allantoin/creatinine ratio, Bargo et al. (2002a) also failed to detect differences between TMR and TMR plus FF diets with regard to microbial N flow, whereas Santana et al. (2012) reported that feeding beef heifers with an all-FF diet compared with an all-TMR diet reduced microbial N flow by 35%. In our study, T8 cows consumed 13% less NFC and 9% less N than T0 cows (Mendoza et al., 2016), whereas in the study of Bargo et al. (2002a) cows fed the TMR plus FF diet consumed an estimated 18% less NSC and 7% more N than TMR-fed cows. However, in the study of Santana et al. (2012) FF-fed heifers consumed 64% less NFC than TMR-fed heifers, suggesting that differences among studies may be related to the different amounts or proportions of energy and N substrates supplied to

Table 3. Ruminal pH, VFA, and ammonia-N concentrations of dairy cows fed a TMR with or without access to fresh forage

Item	Treatment[1]			SEM	*P*-value		
	T0	T4	T8		Treatment	Hour	Treatment × hour
pH							
Mean	6.47[ab]	6.41[b]	6.53[a]	0.105	0.01	<0.01	0.29
Minimum	6.08	5.92	6.16	0.100	0.17	—	—
Maximum	6.85	6.81	6.86	0.079	0.80	—	—
Range	0.77	0.89	0.70	0.073	0.23	—	—
VFA,[2] m*M*							
Acetic acid	73.8[a]	74.5[a]	64.9[b]	3.30	0.02	0.10	0.71
Propionic acid	26.5[a]	27.8[a]	23.0[b]	1.67	0.03	<0.01	0.33
Butyric acid	17.7[b]	21.0[a]	17.6[b]	1.34	0.02	<0.01	0.55
Total	117.9[a]	123.2[a]	105.6[b]	5.82	0.02	0.01	0.51
VFA, mol/100 mol							
Acetic acid	63.0	60.7	61.9	0.89	0.15	<0.01	0.49
Propionic acid	22.6	22.7	21.9	0.78	0.72	<0.01	0.81
Butyric acid	14.3[b]	16.7[a]	16.2[a]	0.64	0.02	0.02	0.65
Acetic:propionic acid	2.92	2.80	3.00	0.160	0.62	<0.01	0.85
(Acetic+butyric acid):propionic acid	3.57	3.55	3.79	0.191	0.58	<0.01	0.88
NH_3-N[2], mg/100 mL							
Mean	18.3[a]	17.1[b]	17.0[b]	1.12	0.03	<0.01	<0.01
Minimum	10.7	10.9	10.9	1.13	0.94	—	—
Maximum	28.7	26.6	26.2	2.92	0.427	—	—
Range	17.9	15.6	15.3	3.28	0.356	—	—

[a,b]Within a row, means with different superscripts are different ($P \leq 0.05$).

[1]T0 = 24 h of access to TMR; T4 = 4 h of access to fresh forage plus 20 h of access to TMR; T8 = 8 h of access to fresh forage plus 16 h of access to TMR.

[2]Ammonia-N.

the rumen microorganisms. Also, insufficient sensitivity of the spot sampling technique for comparing treatments when differences in microbial protein flow are small cannot be ruled out (Bargo et al., 2002a). Treatments had no effect on apparent N efficiency for milk production (Table 5). Although the contribution of FF to the total energy intake in T8 was low (<16%), this is a remarkable result because it has been held that this trait is inherently low in FF-fed cows (Hoekstra et al., 2007) and lower than in TMR-fed cows (Kolver and Muller, 1998). Urinary N excretion was not affected by the treatments, but fecal N excretion was higher in T0 and T4 compared with T8 (Table 5), a result that could be explained by the higher N intake observed in T0 and T4. Metabolic fecal CP is excreted at a rate of 30 g/kg of DMI (NRC, 2001); thus, the higher DMI in T0 and T4 compared with T8 reported elsewhere (Mendoza et al., 2016) could also partially explain the differences in fecal N excretion among treatments. It is also noteworthy that although manure N excretion was higher in T0 and T4 compared with T8, when expressed as a percentage of N intake or per gram of N excreted in milk, no differences among treatments were found (Table 5).

Table 4. Microbial N flow in dairy cows fed a TMR with or without access to fresh forage

Item	Treatment[1]			SEM	*P*-value
	T0	T4	T8		
Creatinine, m*M*	5.2	5.6	5.7	0.60	0.60
Allantoin, m*M*	20.5	19.6	18.9	1.72	0.45
Uric acid, m*M*	4.4	4.1	4.5	3.78	0.28
PD,[2] m*M*	24.9	23.8	23.4	2.02	0.53
PD:creatinine	4.85	4.41	4.40	0.598	0.51
PD, mmol/d	538.8	487.5	477.9	56.38	0.50
Microbial N flow, g/d	367.5	329.9	322.6	41.84	0.50
Efficiency of N use for microbial N synthesis, %	54.6	47.5	52.6	8.23	0.25

[1]T0 = 24 h of access to TMR; T4 = 4 h of access to fresh forage plus 20 h of access to TMR; T8 = 8 h of access to fresh forage plus 16 h of access to TMR.

[2]Purine derivatives.

Table 5. Nitrogen utilization of dairy cows fed a TMR with or without access to fresh forage

Item	Treatment[1]			SEM	*P*-value
	T0	T4	T8		
N intake, g/d	671[a]	694[a]	611[b]	38.9	0.05
Urinary N excretion					
g/d	164	161	172	17.1	0.70
% of N intake	24.6	23.1	28.2	1.91	0.12
Fecal N excretion					
g/d	303[a]	324[a]	228[b]	37.6	0.03
% of N intake	43.8	46.4	39.8	8.45	0.36
Manure N excretion					
g/d	467[a]	486[a]	400[b]	79.2	0.02
% of N intake	68.3	69.6	69.9	9.29	0.86
g/g milk N	2.7	2.7	2.4	0.36	0.32
Milk N excretion					
g/d	173[a]	181[a]	166[b]	6.1	0.02
% of N intake	26.0	26.0	27.2	0.84	0.22

[a,b]Within a row, means with different superscripts are different ($P \leq 0.05$).

[1]T0 = 24 h of access to TMR; T4 = 4 h of access to fresh forage plus 20 h of access to TMR; T8 = 8 h of access to fresh forage plus 16 h of access to TMR.

Concentration of blood glucose was higher in T0 than in T8 (Table 6), which may reflect higher hepatic gluconeogenesis as a result of a higher production of propionic acid in the rumen, a greater quantity of undigested starch reaching the duodenum because of their higher TMR intake, or both. The higher insulin concentration in T0 cows appears to be a response to the higher glycemia of those animals (Table 6) and could be the result of increased secretion stimulated by VFA such as propionic acid, as already reviewed by Harmon (1992) in ruminants. Collectively, these results are in agreement with the higher EB in T0 compared with T8 cows. An hourly effect was detected for insulin (Table 6), where insulin increased from 22.0 to 30.9 μIU/mL from h 0 to 10 following the beginning of the first meal (Figure 2), reflecting an increased ruminal VFA production and a greater supply of glucogenic precursors (Brockman, 1978). Average blood urea concentrations were not affected by treatments (Table 6), which is consistent with the lack of effect of treatments on MUN (Mendoza et al., 2016). Our results are similar to those reported by Bargo et al. (2002a), who found no differences in blood urea in dairy cows fed only a TMR or a TMR plus FF. However, an interaction between treatment and hour was detected (Table 6), where T8 had a lower blood urea concentration than T0 at h 2 ($P < 0.07$) and h 4 ($P < 0.01$) and a higher concentration than T4 at h 8 ($P < 0.05$; Figure 2). Although it was not measured throughout the day, this pattern mimics the change in ruminal NH_3-N for 12 h following the beginning of the first meal and may reflect the changes in N ingestion of T8 cows, as previously explained.

Overall, the results suggest that differences in milk production reported in the literature between cows fed a TMR with or without FF, including those observed in this experiment (Mendoza et al., 2016), are primarily explained by differences in the intake of energy and other nutrients rather than by changes in nutrient digestion or utilization. This is consistent with the work of Kolver and Muller (1998), who estimated that more than 60% of the difference in milk yield between cows fed only a TMR or only FF was attributable to differences in DMI, and specifically energy intake. Additional research is needed to identify management options that allow an increase in the proportion of high-quality FF in the diet of TMR-fed cows without depressing energy intake.

CONCLUSIONS

In this study, cows with 4 h of access to high-quality FF had an energy intake and balance similar to cows fed only a TMR. However, 8 h of access reduced the energy intake and balance, with no differences in nutrient digestion or utilization. This suggests that eventual differences in milk production between cows fed a TMR with or without access to FF would be explained by

Table 6. Blood metabolite and hormone concentrations of dairy cows fed a TMR with or without access to fresh forage

Item	Treatment[1]			SEM	*P*-value		
	T0	T4	T8		Treatment	Hour	Treatment × hour
Glucose, m*M*	4.34[a]	4.24[ab]	4.12[b]	0.168	0.02	0.20	0.49
Urea, m*M*	6.72	6.57	6.23	0.329	0.17	0.29	0.01
Insulin, μIU/mL	28.2[a]	24.5[ab]	23.2[b]	2.25	0.02	<0.01	0.83
Glucagon, pg/mL	105.9	106.4	104.3	13.34	0.94	0.87	0.50
Insulin:glucagon	0.31	0.26	0.24	0.058	0.11	0.47	0.92

[a,b]Within a row, means with different superscripts are different ($P \leq 0.05$).

[1]T0 = 24 h of access to TMR; T4 = 4 h of access to fresh forage plus 20 h of access to TMR; T8 = 8 h of access to fresh forage plus 16 h of access to TMR.

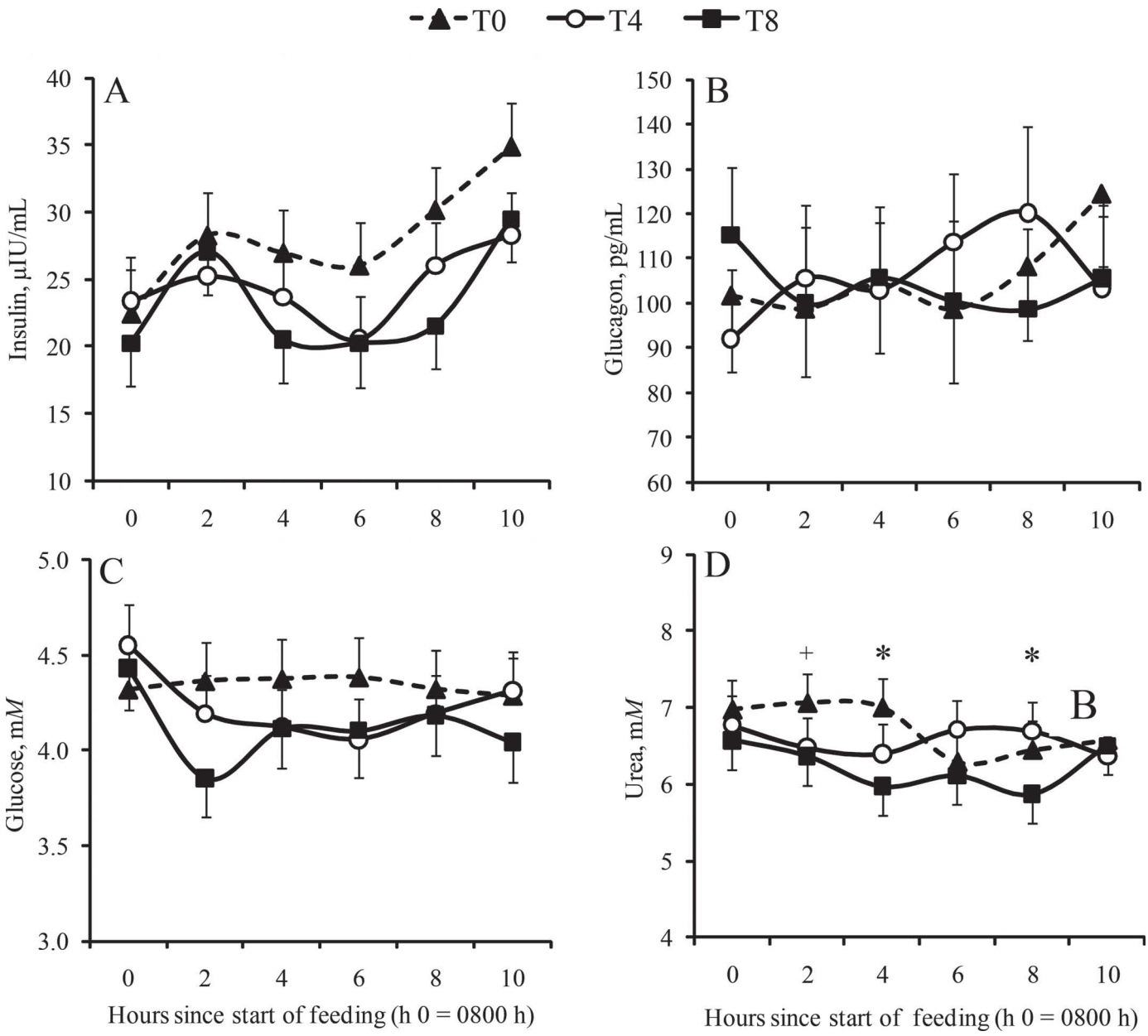

Figure 2. Blood insulin (A), glucagon (B), glucose (C), and urea (D) concentrations of dairy cows fed a TMR with or without access to fresh forage. Asterisks or crosses at each hour indicate at least one difference among the treatments, $P \leq 0.05$ or $0.05 < P \leq 0.10$, respectively. T0 = 24 h of access to TMR; T4 = 4 h of access to fresh forage plus 20 h of access to TMR; T8 = 8 h of access to fresh forage plus 16 h of access to TMR. Error bars represent SEM.

differences in intake rather than by changes in nutrient digestion or utilization.

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