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INTERPRETIVE SUMMARY

Total mixed rations (TMR) based on dry hay are widely used in the Parmigiano Reggiano production area of Italy. In this study, mid-lactation cows were offered access to TMR for 24 or 19 h/d with or without additional free choice hay. Feed efficiency was improved with the restricted feed access, and most rumen parameters were similar. However, milk production, ruminal function, and pH stability were improved when TMR and long hay were available continuously.

Effects of ad libitum or restricted access to total mixed ration with supplemental long hay on production, intake, and rumination

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ABSTRACT

Limiting feeding time has been a concept used in growing and non-lactating ruminant animals with good success, especially in improving feed efficiency while maintaining normal rumen function and fiber digestibility. This study evaluated the physiological and productive responses of cows fed a total mixed ration (TMR) available for 24 or 19 h/d with or without access to additional long hay. Eight multiparous Holstein cows were used in a replicated 4×4 Latin square design. Rations were formulated to mimic TMR used in the Parmigiano Reggiano cheese production area of Italy, consisting of all dry and non-fermented components. Intakes were reduced slightly in cows with restricted TMR access and without supplemental hay. Rumen characteristics, as well as production of milk and components, were similar for all groups. The results show that once cows adapted to diet changes, there were few differences in dry matter intake when offering TMR continuously or with limited access. In addition, the presence of long hay during the TMR restriction offered the best conditions for ruminal function and pH stability yet no effects observed on body weight change. A Latin square with 21-d periods could have a carry-over effect on energy storage and mobilization of fat reserves might be able to mask negative energy balance during restriction. This feeding system could be used as a strategy to manage feed availability according to cow production and metabolic condition, in order to maximize the use of nutrient resources, reducing the cost of milk production and improving the cows' welfare and health.

Key words: limit feeding, lactating dairy cow, rumination

INTRODUCTION

Total mixed rations (TMR) with dry hay as the only forage source are widely used in the Parmigiano Reggiano production area of Italy. Typically, little to no water is added to the TMR to avoid the risk of fermentation in the manger. In this situation, particle size of the feed must be reduced to prevent feed sorting, and therefore physically effective NDF (peNDF) is generally below what is commonly considered acceptable (Fustini et al., 2016). This may led to a reduction of rumination time and saliva secretion thereby leading to SARA (Khafipour et al., 2009). Italian hays are often of low quality and reduced nutrient value due to the climatic conditions (Palmonari et al., 2016), which often necessitates an increased amount of concentrate in the diet to satisfy the energy requirements of lactating cows.

Adequate forage particle size in the ration is therefore critical to maintain rumen function and milk components. Furthermore, peNDF promotes chewing and salivary secretion, maintaining rumen conditions for VFA absorption, which improves pH stability in the rumen (Mertens, 1977, 1997). In a recent study, Kmicikewycz and Heinrichs (2014) concluded that supplementing rations with long hay favored the recovery of animals with SARA in early and mid-lactation. Moreover, inclusion of long hay in low proportions, particularly in diets based on finely chopped silages, has been shown to improve rumination time (Beauchemin et al., 1994).

Dairy cows may also be able to select in favor of longer particles if rumen pH and rumination levels are low. Maulfair et al. (2013) saw that during SARA cows were able to change their eating preferences in favor of diets with more long forage and lower contents of fermentable starch. Keunen et al. (2002) also showed that lactating cows with induced SARA increased their preference for long alfalfa hay compared to alfalfa pellets. It has been suggested that under conditions of reduced ruminal pH if given an option, cows will choose feeds with more buffering capacity (DeVries et al., 2008).

Limit feeding has been used in ruminant animals with good success, especially in improving feed efficiency while maintaining normal rumen function and digestibility (Owens et al., 1995). Limit feeding often improves digestibility (Zanton and Heinrichs, 2008), primarily through reducing the rate of passage of material from the reticulo-rumen of animals that are fed less (Bhatti et al., 2008). However, few trials based on this concept have been conducted with cows in mid lactation.

The purpose of this experiment was to evaluate the physiological and productive response in cows fed a dry, finely chopped TMR administered for a 24 or 19 h/d, with or without additional free choice long hay.

MATERIALS AND METHODS

This study was conducted at the University of Bologna (Italy), and all experimental procedures involving animals were approved by the University of Bologna Animal Care and Use Committee.

Experimental Design and Data Collection

Eight multiparous Holstein cows (avg. BW 629 ± 49 kg) were blocked by parity (2.25 ± 0.46), milk production (44.9 ± 2.5 kg/d), and DIM (99 ± 49) and used in a replicated 4 × 4 Latin square design study with 21-d periods (14 d adaptation and 7 d collection). Rations were formulated to mimic TMR used in the Parmigiano Reggiano cheese production area of Italy and consisted of all dry and non-fermented components. The basal diet was the same for all 4 treatments with or without access to additional long grass hay, which was the same hay used in the TMR (Table 1). Diets were offered in individual feed mangers divided in 2 equal parts, one for the TMR and the other for the long grass hay. Cows were offered ad libitum intake of TMR for 24 or 19 h/d; TMR (approximately 1.10× expected intake) was fed once a day at 2000 h (Zago Mixer; Padova, IT). The study was conducted during the months of September and December, and feeding was done at a time to encourage intake in the evening hours. Animals were housed in a naturally ventilated tie-stall barn and milked twice a day (0800 and 1930 h) in a double-5 herringbone milking parlor. Treatments were identified by the length of TMR access as 24 h or 19 h, with (F+) or without (F-) ad libitum long grass hay.

From d 15 to 21 of each period, we collected data and took samples. Body weight was measured daily (Afiweight scale, Afikim, Israel), and DMI was determined by recording feed offered and refused for each cow. Samples of diets and orts were collected daily and a portion of each sample was dried in a forced-air oven at 105°C for 24 h for DM determination. Milk yield was recorded daily (Afimilk Information Management System; Afikim, Israel) during d 15 to 21 of each period. Milk samples from 2 consecutive milkings for each cow were collected on d 17 and 18 of each period, preserved (Bronolab-W II Liquid Preservative; D & F Control Systems, Inc., Dublin, CA), and analyzed for fat, true protein, and lactose by infrared spectroscopy procedures (Associazione Provinciale Allevatori Bologna; Foss 4000, Foss Technology). Feed efficiency (kg/kg) was calculated as milk/DMI, 3.5% FCM/DMI, and solids-corrected milk/DMI during the experimental week.

Rumen Characteristics

Cows were monitored for rumination activity from d 15 to 20 of each period using the Hi-Tag rumination monitoring system (SCR Engineers Ltd., Netanya, Israel). Data Flow software analyzed rumination time with a resolution of 2 h (Schirmann et al., 2009) and calculated the rumination time during the last 24 h. To continuously monitor rumen pH, all cows received an indwelling wireless pH-transmitting unit (SmaXtec Animal Care Sales GmbH, Graz, Austria), which has been validated with rumen-cannulated dairy cows (Klevenhusen et al., 2014). These units (3.5 cm i.d., 12 cm long, and weighing 210 g) were manually inserted into the reticulorumen via the esophagus on d 14 of the first period. Prior to this, the units were calibrated following the company's instruction protocol. The units measured pH and temperature every 10 min and transmitted the data in real time to a basestation using the ISM band (433 MHz). Both data of pH and temperature were collected using an analog-to-digital converter and stored in an external memory chip. Data of pH from d 15 to 21of each period were analyzed as daily mean pH and time (min/d) below specific cut-off points (5.5 and 5.8). Because wireless sensors were located in the ventral reticulorumen (Gasteiner et al., 2009) and few differences were observed between pH measured by wireless units and in the ventral rumen sac (Klevenhusen et al., 2014), the term "reticuloruminal pH" will be used.

On day 5 and 6 of experimental week, rumen fluid was collected via esophageal probe at 12h (0800) and 24h (2000) after feeding. Volatile fatty acid (**VFA**) concentrations were determined by gas chromatography (Goetsch and Galyean, 1983), while ammonia was evaluated via commercial kit (Urea/BUN – color, BioSystems S.A. Barcelona, Spain) according to the producer procedure.

Chemical Analysis

Individual feed ingredients were collected weekly and dried in a forced-air oven at 55°C for 48 h for DM. During the experimental week of each period, diets were sampled daily and analyzed for chemical composition according the following methods: CP, amylase-treated, ash-corrected NDF (aNDFom), and ADF according to Mertens (2002) and AOAC 973.18. Starch was determined according to AOAC 996.11 and ether extract according to AOAC 920.390020. Forage and diet composite samples were used to determine particle size distribution on an as-fed basis using the Penn State Particle Separator (Lammers et al., 1996), physical effectiveness factor (pef) using a RoTap Separator (W.S. Tyler, Mentor, OH). Diet peNDF was calculated as the product of the total diet NDF content and its pef (Mertens, 1997). In vitro digestibility (24, 48, and 240 h) of aNDFom of forage composite and diet composite samples (1-mm grind; Wiley mill; Arthur H. Thomas, Philadelphia, PA) were determined using an in vitro fermentation (Tilly and Terry, 1963) in buffered media containing ruminal fluid (Goering and Van Soest, 1970). Ingredients and diets also were analyzed for in vitro aNDFom digestibility at 24 h and 240 h according to the procedure described by Palmonari et al. (2017). Briefly, in vitro aNDFom digestibility at 24 h and 240 h (**IVNDFD24h** and **IVNDFD240h**) was performed using the Tilley and Terry modified technique (Tilley and Terry, 1963; Van Soest et al., 1991).

Statistical Analysis

Data for DMI, milk yield and composition, feed efficiency, microbial protein supply, and BW, and were analyzed as a replicated Latin square design with model effects for diet, period, and replicate using the MIXED procedure of SAS (version 9.1, Statistical Analysis Systems Institute Inc., Cary, NC) using cow within replicate as a random effect. Repeated measurements on performance data (i.e. DMI, milk yield, etc.) were reduced to period means for each cow before statistical analysis. Data for ruminal pH, NH_3-N, and VFA were analyzed with repeated measures using the MIXED procedure of SAS. The model included effects of diet, period, time, and the interaction of diet and time, with cow as a random effect. Least squares means were separated using PDIFF option with Tukey's adjusted *P*-values when a significant *F*-test ($P \leq 0.05$) was detected.

RESULTS AND DISCUSSION

The TMR fed was adequate in terms of nutrient composition (NRC, 2001) but smaller in particle size (peNDF = 14.14 ± 1.30%) than what is often recommended (Heinrichs, 2013, Kmicikewycz et al., 2015; Table 1). This level of dietary particle size minimized the risk of cows sorting (Fustini et al., 2016). When cows were offered TMR 24 vs 19 h/d, TMR DMI increased (25.98 vs 23.49 kg, SEM = 1.66, $P = 0.04$, Table 2). Supplemental hay intake was similar between diets. Consequently, total DMI tended to be greater for cows with 24-h TMR access (26.21 vs 23.85 kg, SEM = 1.70, $P = 0.06$, Table 2) compared to cows offered TMR 19 h/d. Diets including hay tended to increase total NDF intake (8.26 vs 7.55 kg/d, SEM = 0.54, $P = 0.07$, Table 2). When hay was offered in addition to TMR, cows consumed relatively little hay (< 1 kg/d) and tended to consume more hay when TMR was offered for 19 vs 24 h/d. However, this hay intake was not equal to the reduction in TMR intake, likely due to feed palatability or preference for the TMR over the hay alone. A change in eating behavior may have occurred and may fall into the category of slug feeding once cows had adapted to this feeding (19 vs 24 h) change (Fustini et al., 2016). According to other authors (Albright, 1993; Maulfair et al., 2013), animals modified their eating behavior after adaptation to a different feeding regimen. It was thought that when dairy cows had a limited amount of TMR, it would result in faster eating rates, which could lead to substantial diurnal changes in VFA production and increase the risk of SARA (Van Soest, 1994). Previous work (Kmicikewycz and Heinrichs, 2014) demonstrated that long hay availability with no TMR offered could decrease risk of SARA in lactating dairy cows. This hay availability should also limit the chance of a higher amount of feed being consumed in the first meal after TMR delivery the following day. This condition is often called "slug feeding," and is characterized by a long period of time in which cows remain out of feed, after which they rapidly consume an amount of fermentable carbohydrate able to induce a drop in ruminal pH

(Krause and Oetzel, 2006). Cows with TMR available for 19 h/d consumed 37.1% of their hay intake in the 5 h after TMR was removed, while cows with TMR continuously available had 14.4% of their hay intake in the same period of time. Due to the quality of this hay, cows that had it available had higher intakes of NDF and unavailable NDF intake. Results of fiber digestibility (total tract digestibility > 75% in all the treatments; Table 4) appeared to be comparable to values obtained in other experiments and, confirm digestibility shown by Fustini et. al. (2017), given the quality of forage used in the dry diets of the Parmigiano Reggiano cheese production area of Italy (Palmonari et al., 2016) (total tract digestibility higher than 75% in all the treatments; Table 3).

There were no differences in milk production between the 24 and 19 h feed groups; however, there was a tendency for increased milk yield by cows that consumed supplemental forage (38.03 vs 35.95 kg, for F+ and F- respectively, SEM = 1.63, P = 0.09, Table 4). Cows with TMR access for 19 h/d had greater feed efficiency (Table 3) on both a milk volume (milk kg/DMI kg, P = 0.03) and component (ECM kg/DMI kg, P = 0.05) basis compared to the 24 h group. This improved efficiency is likely a result of the design of the study with relatively short periods where body reserves could be used to support production. However BW of cows did not change over the study. There were no differences in milk protein, fat, and urea content; however, somatic cells tended to be higher for 19 h/d TMR diets, in particular when hay was not offered (Table 3). Lactose tended to be higher in milk from cows with 24-h TMR access, likely because of the lower SCC (Table 3).

Rumination times were much higher than values considered as a minimum threshold to ensure rumen functionality (> 390 min/d; Zebeli et al., 2007, 2009). An interaction was observed between TMR availability and provision of supplemental hay for rumination time (Table 5). Cows with 19 h of TMR access ruminated less when hay was offered (P < 0.01). In contrast, hay had no effect on rumination time in cows with 24-h TMR access. Rumination time, average reticuloruminal pH values, and time when pH was below pH 5.8 and 5.5 are shown in Table 5. The results obtained are reflective of the high fiber diets used in this study. Time in which the pH fell below 5.5, the minimum critical threshold, was negligible. Average pH values were lower (*P* < .05) in the diets where long hay was offered, but for no obvious reason. Overall pH of cows was not affected by diet treatment. This was likely a result of the cows' preference for TMR and their ability to somewhat compensate for total TMR DMI once adapted to the restricted hours of availability (Maulfair et al., 2013) while the group having no access to feed for 5 h ate less..

There were no differences regarding average ammonia and ruminal VFA levels, as shown in Table 6. At 12 h after feeding (0800 h) there was a tendency for lower ammonia for the cows with 19-h TMR access. This could be explained by the lower DMI for the restricted diets that release a higher amount of fermentable carbohydrates allowing bacteria to consume more nitrogen during the part of the day when feed was available. Conversely, when looking the values obtained 24 h after feed distribution (2000 h), acetate, propionate, and total VFA tended to be lower in cows with 19 h of TMR access, and butyrate was significantly lower. This condition is likely due to the absence of new feed that could have reduced VFA production in the rumen. All other rumen parameters measured were similar. Restricting TMR comprised of dry forages while offering additional long hay appears to be consistent with previous research using silage-based diets (Kmicikewycz and Heinrichs, 2014). In the same research, once cows adapted to the restricted feeding period, the lack of hay made little change to their feed intake and digestion.

CONCLUSIONS

The results obtained in this study show that once cows adapted to diet changes, there were few differences when offering TMR continuously 24 h/d or limited to 19 h/d. In addition, the presence of long hay during the TMR restriction offered the best conditions for ruminal function and pH stability and tended to improve milk production. It was noted that a restriction of TMR availability for 5 h/d can potentially increase feed efficiency, as has been shown in other classes of ruminants. These results need to be confirmed by longer duration experiments using mid-lactation dairy cows. Due to the design of the experiment, milk production results need to be considered with caution. A Latin square with 21-d periods could have a carry-over effect on energy storage and mobilization of fat reserves might be able to mask negative energy balance during restriction. However, in our experiment we did not seen any BW variation. This feeding system could be used as a strategy to manage feed availability according to cow production and metabolic condition, in order to maximize the use of nutrient resources, reducing the cost of milk production and improving the cows' welfare and health.

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Table 1. Composition of the TMR diet and the hay (mean ± SD)

Item	TMR[1]	Hay
DM, %	87.66 ± 0.72	88.54 ± 2.93
Ether extract, % DM	2.53 ± 0.38	1.64 ± 0.20
Ash, % DM	9.76 ± 0.37	8.93 ± 0.63
aNDFom[2], % DM	31.44 ± 5.45	58.46 ± 3.45
ADF, % DM	20.77 ± 4.18	42.61 ± 1.62
ADL, % DM	2.94 ± 0.64	6.62 ± 0.80
IVNDFD[3] 24h, % aNDFom	77.33 ± 6.95	67.02 ± 3.21
d-NDF 24h, % aNDFom	45.51 ± 3.44	44.74 ± 5.27
$uNDF_{240}$[4], % DM	6.40 ± 1.98	17.82 ± 5.40
Starch, % DM	23.55 ± 4.78	1.98 ± 2.12
Sugar, % DM	6.96 ± 0.56	4.79 ± 0.66
CP, % DM	14.28 ± 0.90	8.76 ± 1.10
Soluble protein, % DM	3.91 ± 0.44	3.55 ± 0.37
NDIP[5], % DM	3.00 ± 0.28	4.92 ± 0.65
ADIP[6], % DM	1.01 ± 0.07	1.55 ± 0.15
PSPS[7]		
19 mm %	0.39 ± 0.79	
8 mm %	15.18 ± 5.10	
4 mm %	18.73 ± 1.92	
Bottom %	65.69 ± 5.60	
Ro-Tap % >1.18 mm	46.24 ± 3.90	
peNDF[8] % DM	14.14 ± 1.30	

[1]TMR Ingredients: 34.5% grass hay, 19.2% steam flaked corn, 3.9% cane-beet molasses blend, and 42.4% grain mix [29.6% wheat bran, 29.4% sorghum grain, 21.6% soybean meal, 14.7% flaked soybean, 2.2% calcium carbonate, 1% sodium chloride, 0.4% magnesium oxide, 0.9% sodium bentonite, and 0.3% vitamin and mineral premix (provided 40,000 IU vitamin A, 4,000 IU vitamin D3, 30 mg vitamin E 92% α-tocopherol, 5 mg vitamin B1, 3 mg vitamin B2, 1.5 mg vitamin B6, 0.06 mg vitamin B12, 5 mg vitamin K, 5 mg vitamin H1 (para-aminobenzoic acid), 150 mg vitamin PP (niacin), 50 mg choline chloride, 100 mg Fe, 1 mg Co, 5 mg I, 120 mg Mn, 10 mg Cu, and 130 mg Zn)].
[2]Amylase- and sodium sulfite-treated NDF with ash correction.
[3]In vitro NDF digestibility, in % aNDFom.
[4]Unavailable NDF estimated via 240-h in vitro fermentation.
[5]Neutral detergent insoluble protein (Neutral detergent insoluble nitrogen*6.25).
[6]Acid detergent insoluble protein (Acid detergent insoluble nitrogen*6.25).
[7]Penn State Particle Separator, % sample retained on each sieve (Lammers et al., 1996).
[8]Physically effective NDF (aNDFom*pef), calculated using the Ro-Tap system (Mertens, 1997).

Table 2. Effect of feed restriction and supplemental hay[1] on DMI and fiber fraction intake

Item	Feed availability				SEM	*P* value	
	24 h		19 h				
	F+	F-	F+	F-		24 vs 19	F+ vs F-
TMR DMI, kg	27.36	24.60	23.27	23.71	1.66	0.04	0.31
Hay DMI, kg	0.55	-	0.70	-	0.09	0.09	-
DMI, kg	27.85	24.56	24.05	23.65	1.70	0.06	0.13
BW, kg	637.93	637.86	646.56	631.67	20.99	0.76	0.08
aNDFom[2], kg/d	8.79	7.66	7.73	7.44	0.54	0.10	0.07
aNDFom[2], % BW	9.39	9.48	9.31	9.51	0.34	0.83	0.24
uNDF[3], kg/d	1.89	1.60	1.70	1.57	0.12	0.17	0.02
uNDF[3], % BW	0.30	0.26	0.26	0.25	0.02	0.25	0.19

[1]F+ = supplemental hay; F- = no supplemental hay.

[2]Amylase- and sodium sulfite-treated NDF with ash correction.

[3]Unavailable NDF estimated via 240-h in vitro fermentation.

Table 3. Effect of feed restriction and supplemental hay[1] on fecal fiber content and total tract fiber digestibility

Item	Feed availability				SEM	*P* value	
	24 h		19 h				
	F+	F-	F+	F-		24 vs 19	F+ vs F-
Fecal aNDFom[2],% DM	60.42	60.15	60.14	60.38	0.48	0.92	0.95
Fecal uNDFom[3], % DM	32.21	31.99	32.48	31.69	0.83	0.97	0.21
Fecal pdNDF[4], % DM	28.20	28.17	27.66	28.69	1.03	0.98	0.34
aNDFom TTD[5], %	59.64	60.21	59.54	59.76	1.08	0.58	0.42
pdNDF TTD[5], %	75.90	76.30	76.10	75.69	1.37	0.76	0.99

[1]F+ = supplemental hay; F- = no supplemental hay.
[2]Amylase- and sodium sulfite-treated NDF with ash correction.
[3]Unavailable NDF estimated via 240-h in vitro fermentation.
[4]Potentially digestible NDF.
[5]Total tract fiber digestibility.

Table 4. Effect of feed restriction and supplemental hay[1] on milk production and quality

Item	Feed availability				SEM	P value	
	24 h		19 h				
	F+	F-	F+	F-		24 vs 19	F+ vs F-
Milk, kg/d	37.87	36.56	38.19	35.33	1.63	0.71	0.09
ECM, kg/d	40.26	38.23	39.13	36.84	1.33	0.13	0.01
Milk kg/DMI kg	1.40	1.54	1.91	1.64	0.18	0.03	0.64
ECM kg/DMI kg	1.48	1.60	1.92	1.68	0.17	0.05	0.63
Fat, %	3.82	3.62	3.58	3.60	0.18	0.31	0.46
Fat yield, kg/d	1.27	1.32	1.35	1.33	0.16	0.71	0.91
Protein, %	3.38	3.38	3.42	3.31	0.11	0.74	0.34
Protein yield, kg/d	1.13	1.26	1.28	1.20	0.10	0.65	0.83
Lactose, %	4.88	4.88	4.80	4.79	0.05	0.04	0.94
Lactose yield, kg/d	1.65	1.83	1.86	1.75	0.15	0.68	0.85
Urea, %	14.68	15.42	15.30	15.53	1.36	0.72	0.65
SCC, log cfu/mL	2.39	3.49	3.38	4.00	0.80	0.07	0.04
BW variation, kg	0.52	-3.48	17.63	17.47	9.60	0.08	0.85

[1]F+ = supplemental hay; F- = no supplemental hay.

Table 5. Effect of feed restriction and supplemental hay[1] on rumination time and reticuloruminal pH

Item	Feed availability				SEM	*P* value	
	24 h		19 h				
	F+	F-	F+	F-		24vs19	F+ vs F-
Rumination, min/d	424.98[a]	443.10[a]	446.26[a]	412.22[b]	30.49	0.62	0.41
Daily average pH	6.10	6.11	6.09	6.12	0.09	0.88	0.04
Time pH < 5.8, min/d	18.79	19.06	22.02	18.44	11.78	0.32	0.21
Time pH < 5.5, min/d	0.79	0.25	0.10	1.74	0.84	0.51	0.36

[1]F+ = supplemental hay; F- = no supplemental hay.

[ab] Means with different superscripts differ at $P < 0.05$. (Significant interaction between effects of feed availability and provision of supplemental hay.)

Table 6. Effect of feed restriction and supplemental hay[1] on ruminal VFA and ammonia content

Item	Feed availability				SEM	*P* value	
	24 h		19 h				
	F+	F-	F+	F-		24 vs 19	F+ vs F-
Average, mmol/L							
NH_3	3.95	4.00	3.33	3.56	0.49	0.24	0.75
Acetic	45.46	50.76	47.54	42.95	3.19	0.37	0.91
Propionic	18.94	21.92	20.26	18.29	1.86	0.48	0.76
Butyric	9.14	9.61	9.48	8.98	0.82	0.49	0.76
Isobutyric	0.47	0.56	0.49	0.45	0.08	0.83	0.98
Valerianic	0.91	1.07	1.06	0.95	0.09	0.88	0.84
Isovalerianic	0.92	1.02	1.10	0.98	0.13	0.50	0.96
Total VFA	75.84	84.94	79.93	72.60	5.14	0.45	0.87
12 h after feeding, mmol/L							
NH_3	4.23	4.70	3.54	3.24	0.57	0.07	0.88
Acetic	39.77	48.38	47.03	42.78	4.37	0.86	0.63
Propionic	15.11	19.63	20.13	17.71	2.09	0.46	0.62
Butyric	7.77	8.96	9.95	9.22	1.11	0.24	0.82
Isobutyric	0.42	0.61	0.45	0.40	0.09	0.34	0.42
Valerianic	0.79	0.99	1.05	0.95	0.12	0.37	0.70
Isovalerianic	0.76	1.03	0.97	0.96	0.15	0.56	0.30
Total VFA	64.62	79.59	79.59	72.03	7.18	0.62	0.62
24 h after feeding, mmol/L							
NH_3	3.66	3.31	3.11	3.87	0.51	0.99	0.68
Acetic	51.17	53.17	47.99	42.61	3.86	0.10	0.69
Propionic	22.76	24.20	20.42	19.07	2.45	0.10	0.96
Butyric	10.53	10.28	8.97	8.40	0.82	0.03	0.56
Isobutyric	0.53	0.51	0.53	0.48	0.08	0.81	0.59
Valerianic	1.04	1.14	1.07	0.92	0.11	0.44	0.89
Isovalerianic	1.07	1.01	1.21	0.92	0.14	0.79	0.16
Total VFA	87.08	90.30	80.22	72.75	6.35	0.09	0.77

[1]F+ = supplemental hay; F- = no supplemental hay.