

## PAPER

# Effects of feeding frequency and environmental conditions on dry matter intake, milk yield and behaviour of dairy cows milked in conventional or automatic milking systems

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## Abstract

The aim of the study was to investigate the effect of feeding frequency on milk production, dry matter intake (DMI) and cow behaviour on two dairy farms with conventional and automatic milking systems (AMS) in different environmental conditions. Cows on two farms were monitored. On the first farm, 96 primiparous cows were milked in a herringbone parlor while on the second a group of nearly 50 cows were milked in two AMS with a forced traffic. On each farm, treatments consisted of two different frequencies of total mixed ration (TMR) delivery (2 *vs* 3 on the conventional farm; 1 *vs* 2 on the AMS farm) replicated in two different periods of the year with THI of 72.6 and 60.7, respectively. The behaviour of the cows was monitored by continuous video recording. Statistical analysis was performed separately for the two farms. Increasing the frequency of TMR deliveries did not result in any variation in DMI but significantly improved milk yield on both farms. The increase in feeding frequency at the bunk in the AMS farm mitigated the negative effect of hot conditions on production with a 7.6% increase in milk yield. Feeding frequency did not influence cow behaviour on either farm. Hot conditions showed a depressive effect on DMI (nearly 8% on both farms) compared with thermoneutral conditions but caused a reduction in milk yield (an average 17%) only on the farm with multiparous high-producing cows milked automatically. In the hot period, cows on both farms showed a reduction in daily lying time and an increase in daily standing time.

## Introduction

Hot and humid environmental conditions tend to reduce dry matter intake (DMI) and milk yield in lactating dairy cows (West, 2003; West *et al.*, 2003). According to Kadzere *et al.* (2002), high-producing dairy cows may enter heat stress much earlier than their lower-producing counterparts due to high metabolic heat increment. Armstrong (1994) identified temperature and relative humidity to be among the most important variables to quantify heat stress and used the temperature-humidity index (THI) to combine them. He reported an adverse effect on cows when the value of THI rises over 72. Heat stress in lactating dairy cows reduces DMI, rumen digestion (due to reduced blood flow in the digestive tract), rumen pH and secretion of saliva (Abeni, 2009). Hot weather conditions also modify cow behaviour. In particular, during heat stress, cows are less active, seek shade and wind, and increase their respiratory rate (West, 2003). A close negative relationship was found between THI and the duration of lying time during the day (Zähner *et al.*, 2004) with a consequent increase in claw horn lesions frequently reported in late summer (Cook *et al.*, 2007).

To prevent the negative effects of heat stress, some authors recommend, among other solutions, increasing the number of feed deliveries during the hot season in order to assure the availability of fresh Total Mixed Ration (TMR) and motivating cows to eat (Gottardo *et al.*, 2005). Shabi *et al.* (1999) reported that increased feeding frequency, from two to four meals a day, reduced the diurnal variation in ruminal pH, and enhanced DMI and total tract digestibility of organic matter and crude protein. As a consequence, Shabi *et al.* (1999) reported that milk fat, protein and lactose contents were raised but milk yield did not vary. An experiment conducted with ruminally fistulated heifers showed more stable ruminal conditions during daytime when heifers were fed twice daily instead of only once (Robles *et al.*, 2007). In lactating cows fed once or four times a day (Nocek and Braund, 1985), feeding frequency had no significant influence on DMI or milk yield. However, cows fed four times a day showed a consistent weekly tendency to be lower in DMI and higher in milk yield than those fed once daily. According to Gottardo *et al.* (2005), cows receiving feed in two daily distributions during the summer season increased DMI (+9.0%) and milk yield (+15.0%) compared with animals fed once a day.

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The daily number of feed deliveries can affect cow behaviour. In particular, DeVries *et al.* (2005) showed that the increased frequency in feeding delivery enhanced feeding time, without making any changes to total daily lying time. On the contrary, Phillips and Rind (2001) reported that feeding frequency can interfere with the possibility of the animal resting; frequently fed (4 times/d) cows had less circadian variation in sleeping and lying ruminating, with a lower milk yield compared with cows fed once a day. However, Robles *et al.* (2007) did not observe any effect of feeding frequency on feeding behaviour of fistulated heifers.

On farms that operate an Automatic Milking System (AMS) the type of cow traffic (free or forced) seems to have no effect on the duration of time spent in the feeding area (Munksgaard *et al.*, 2011). In the AMS farms equipped with forced traffic systems, increasing the frequency of TMR deliveries may modify milking frequencies attracting cows to the feeding area via the milking unit. This is particularly intriguing considering the general interest in enhancing the number of visits to the milking robot to increase milk yield. According to Melin *et al.* (2005), the motivation to eat is a better incentive in attracting the cows to the milking unit than the motivation to be milked. Very few studies investigated

the relationship between the number of TMR deliveries at bunk and behaviour of cows milked in a forced traffic AMS. Oostra *et al.* (2005) reported that the daily number of visits to the AMS was not affected by the change in feeding frequency at the bunk. In forced traffic AMS milking frequency and access to feed bunk can be affected by environmental conditions. Speroni *et al.* (2006) observed that milking frequency decreased during the hot season especially in primiparous cows; this resulted in a reduced number of visits to the feeding area and probably in fewer meals.

The objective of this study was to evaluate the effects of feeding frequency on milk production, DMI and cow behaviour on two farms equipped with conventional and automatic milking systems over two time periods characterized by different THI.

## Materials and methods

### Housing system and animals

The study was carried out on two dairy farms located in Lombardy (Italy) where animals were kept in loose housing conditions with cubicles. On the conventional farm, cows were milked in a herringbone milking parlor twice daily at 5:00 a.m. and 5:00 p.m. The barn is oriented NW-SE; the monitored group of 96 primiparous cows (Table 1) was housed on the NE side of the barn equipped with 100 cubicles (2 rows) with mattresses covered with chopped straw. The manger, positioned centrally in the barn, had 90 feeding spaces. The barn was equipped with a cooling system consisting of 8 axial fans (Ø 138 cm; 0.75 kW; 37,500 m<sup>3</sup>/h), 4 placed above the feeding area and 4 above the rest area. In the rest area, each fan was equipped with 2 high-pressure nozzles which were thermostatically controlled and which were activated once air temperature reached 30°C.

On the AMS farm, 95 cows were milked in two milking units (VMS, DeLaval, Tumba, Sweden). A forced traffic system was applied so that the animals were forced to pass through the AMS units before they could reach the feed troughs. Cows had access to both AMS units 24 h/d (except for a total of 1 h/d dedicated to the cleaning of the milking system). Cows were granted milking permission after 6 h from the previous milking unless a milking failure occurred; in this case, cows would be milked again immediately. Cows with more than 12 h since last milking were fetched and forced to visit the AMS. The barn is oriented

NE-SW and the studied group of nearly 50 primiparous and multiparous cows (Table 1) was housed on the NE side equipped with 61 cubicles (4 rows) with mattresses covered with sawdust. The manger, located on the SE side of the barn, had 39 feeding spaces. The barn was equipped with a cooling system, consisting of 2 axial fans (Ø 153 cm; 1.5 kW; 55,800 m<sup>3</sup>/h) placed above the rest area with a tilt angle of 30 degrees and without sprinklers and misters.

### Environmental monitoring

For each farm, 2 data loggers were used to measure the air temperature and relative humidity (HOBO U12 Temp/RH/Light/External Data Logger, Onset Computer Corporation, Bourne, MA, USA). These were placed in the barn at a height of 2 m above the floor and the time interval of recording was set at 15 min. THI was calculated by the following equation:

$$THI = Tdb + 0.36 \times Tdp + 41.2$$

where Tdb is the dry bulb temperature in °C and Tdp is the dew point temperature in °C as reported by Yousef (1985).

### Feeding frequency, cow rations and milk recordings

Feeding treatments on both farms consisted of two different frequencies of TMR distributions replicated in two different periods of the year. Each experimental period (replicated 4 times, 2 times for different feeding frequencies and 2 times for the period effect) lasted 15 days: 7 days for adaptation and 8 days for measurements, recorded separately on each farm. On the conventional farm, the study was conducted in the hot (June) and thermoneutral (October) periods, and feeding frequencies were tested two (7:00 a.m. and 5:00 p.m.) or three (8:00 a.m., 11:00 a.m. and 5:00 p.m.) times a day. On the AMS farm, the experiment was carried out in the thermoneutral (April) and in the hot (July) periods, and the feeding frequencies were tested once (9:00 a.m.) or twice (9:00 a.m. and 6:00 p.m.) a day. On the AMS, farm cows received a TMR at the feed bunk and different individual amounts of feed concentrate at the AMS during milking,

depending on milk yield.

Dry matter intake of the whole group of monitored cows on each farm was recorded every day during the 8 measurement days of each experimental period (32 observations for each farm) by weighing TMR and Orts. On the same days, daily samples of TMR and Orts were taken for DM content and chemical analysis (NIR System 5000, FOSS) as reported in Table 2. Individual milk production was automatically recorded at each milking on both farms.

### Behavioural recordings

Behavioural activities of the cows were monitored by continuous video recording. The video surveillance system consisted of four IR day/night weatherproof varifocal cameras with 42 infrared LEDs for night vision (420SS-EC5, Vigital Technology Ltd., Sheung Wan, Hong Kong, PRC). The four cameras were placed at a height of 5 m above the pen floor. An analysis of the recorded video data evaluated the number of cows engaged in different behavioural activities (*i.e.* feeding, lying, standing). *Standing* was considered to be an upright posture (*i.e.* motionless or walking), while *lying* included only those cows observed in total lateral or sternal recumbency within the confines of a stall (Overton *et al.*, 2002). *Feeding* was defined as actively ingesting feed or water, or standing within 0.6 m of the feed bunk and oriented towards the feed (Overton *et al.*, 2002). Behavioural activities were analyzed at scan intervals of 60 min (Mattachini *et al.*, 2011) and the following cow behavioural indices were calculated: cow lying index (CLI), cow standing index (CSI) and cow feeding index (CFI). Indices were obtained by counting the cows engaged in each activity divided by the total number of cows in the barn. The entire behavioural observation period covered the days of trial (8 d) for each feeding treatment in each period for a total of 32 d for each farm.

### Statistical analysis

Data collected during the experiments (32 observations for each farm) were analyzed by ANCOVA using a generalized linear model (proc GLM; SAS, 2001a) for testing the effects of feeding frequency in each period of experi-

**Table 1. Characteristics of the cows monitored on the two dairy farms (mean ± SD).**

	Conventional	Automatic milking
Cows monitored, n	96±0	47.7±1.2
Lactation, n	1	1.83±0.03
Days in milk, d	214±9.37	193±17.8
Milking frequency, n/d	2	2.48±0.65

ment, separately for each farm. Average milk yield was computed for each experimental day from the daily individual milk production of the tested groups (32 observations for each farm). Average days in milk (DIM) were computed separately for each experimental day (32 observations for each farm) and used as a covariate; however, its effect was never statistically significant. Principal Component Analysis (PCA, proc PRINCOMP; SAS, 2001b) was used to study the relationships among several quantitative variables (milk yield, total DMI, dairy efficiency, milking frequency, THI, CSI, CLI, CFI).

## Results and discussion

Table 1 shows the main characteristics of the groups of cows monitored on the two farms. Average lactation figures differed between the two farms: on the conventional farm, the monitored group was only made up of primiparous cows whereas in the AMS group there were also multiparous cows. Milking frequency of monitored cows on the AMS farm was an average 2.48 per day, similar to results reported by other authors for AMS (Bach *et al.*, 2009; Wagner-Storch and Palmer, 2003). On both farms, lactating cow rations were based on maize silage and maize grain (Table 2). On the AMS farm, the average percentage of concentrate feed ingested was higher than on the conventional farm because of the amount of concentrate distributed at AMS during milking in addition to that included in TMR (on average  $3.22 \pm 0.32$  kg of DM for the group of monitored cows). On the AMS farm, the average DMI of the whole experiment was lower than on the conventional farm ( $19.9 \pm 1.81$  vs  $22.1 \pm 1.28$  kg/d) but the average milk yield was higher ( $30.0 \pm 3.05$  vs  $27.1 \pm 0.66$ ). This could be due to the higher energy content of the AMS farm ration (Table 2). The low milk production of primiparous cows on the conventional farm could be referred both to the number of parity and to the lower energy content of the diet.

## Frequency of feed deliveries

Changes in frequency of TMR deliveries had no significant effect on DMI on both farms (Tables 3 and 4) in agreement with results reported by other authors (DeVries *et al.*, 2005; Nocek and Braund, 1985; Robles *et al.*, 2007). On the contrary, feeding frequency significantly affected milk yield on both farms with production rising as the number of daily feed deliveries increased (+2.1% and +4.5% on the conventional and on the AMS farm, respective-

**Table 2. Ingredients and average chemical composition of monitored cows rations on the two farms.**

Composition as fed, %	Conventional	Automatic milking
Maize, silage	59.4±0.10	51.7±3.4
Maize, grain	15.3±0.01	10.4±1.2
Lucerne, hay	9.4±0.13	3.9±0.4
Straw	0.6±0.01	
Grass, hay		2.8±1.8
Beet pulp, dried		2.3±0.1
Concentrate feed in TMR	15.3°±0.03	12.9 <sup>a</sup> ±1.2
Concentrate feed at AMS		16.0 <sup>b</sup> ±1.1
Chemical composition of TMR		
DM, %	56.6±0.7	55.8±2.2
OM, % DM	92.4±0.4	92.7±0.3
CP, % DM	14.7±0.6	13.8±1.7
EE, % DM	3.1±0.7	2.8±0.6
NDF, % DM	37±0.7	34.8±2.3
ADF, % DM	22±0.7	21.7±1.0
ADL, % DM	4.2±0.1	4.4±0.3
Starch, % DM	22.5±0.8	24.2±1.9
NEI, MJ/kg DM	6.85±0.14	7.07±0.25

TMR, total mixed ration; AMS, automatic milking systems; °concentrate (on DM), CP 31%, EE 5.2%, CF 10%, Ash 21%; <sup>a</sup>concentrate (on DM), CP 30%, EE 3.5%, CF 9.5%, Ash 11%; <sup>b</sup>concentrate AMS (on DM), CP 17%, EE 6%, CF 11.5%, Ash 8.5%, NEI 8.32 MJ/kg. DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignine.

**Table 3. Effect of period and feeding frequency on milk yield, dry matter intake and behavioural indices on the conventional farm (least squares means, n=32).**

Period	Thermoneutral		Hot		SEM	Feeding frequency	Period	F*P
Daily feeding frequency	2	3	2	3				
THI	58.4	57.8	71.9	74.3	0.647	0.163	<0.001	0.019
DMI, kg/d	23.3	22.5	20.3	21.8	0.352	0.271	<0.001	0.004
Milk yield, kg/d	26.9	27.1	26.8	27.7	0.226	0.012	0.250	0.191
Dairy efficiency	1.14	1.21	1.31	1.28	0.018	0.372	<0.001	0.016
Behavioural indices								
CLI	0.62	0.61	0.54	0.52	0.015	0.394	<0.001	0.751
CSI	0.14	0.14	0.21	0.23	0.074	0.374	<0.001	0.051
CFI	0.24	0.25	0.25	0.25	0.011	0.560	0.382	0.334

THI, temperature-humidity index; DMI, dry matter intake; dairy efficiency, milk yield (kg/d)/DMI (kg/d); CLI, cow lying index; CSI, cow standing index; CFI, cow feeding index.

**Table 4. Effect of period and feeding frequency on milk yield, dry matter intake and behavioural indices on the automatic milking systems farm (least squares means, n=32).**

Period	Thermoneutral		Hot		SEM	Feeding frequency	Period	F*P
Daily feeding frequency	1	2	1	2				
THI	60.3	66.4	73.3	71.4	0.575	0.001	<0.001	<0.001
DMI, kg/d	20.5	20.8	18.5	19.4	0.675	0.322	0.009	0.615
Milk yield, kg/d	32.5	33.1	26.3	28.2	0.316	<0.001	<0.001	0.043
Dairy efficiency	1.59	1.61	1.42	1.46	0.055	0.515	0.004	0.830
Milking frequency, n/d	2.53	2.48	2.39	2.35	0.040	0.265	0.002	0.910
Behavioural indices								
CLI	0.54	0.5	0.45	0.46	0.018	0.432	0.002	0.171
CSI	0.22	0.22	0.28	0.26	0.011	0.303	<0.001	0.275
CFI	0.18	0.19	0.17	0.18	0.005	0.052	0.240	0.998

THI, temperature-humidity index; DMI, dry matter intake; DMI calculated as TMR intake plus concentrate feed at AMS; dairy efficiency, milk yield (kg/d)/DMI (kg/d); CLI, cow lying index; CSI, cow standing index; CFI, cow feeding index.



ly). Similarly, Nocek and Braund (1985) reported a tendency towards higher milk yield but lower DMI in lactating cows fed four times a day instead of once.

The positive effect of higher feeding frequencies on milk yield on both farms could be related to more stable ruminal conditions and higher total tract digestibility, as suggested by other authors (Shabi *et al.*, 1999; Robles *et al.*, 2007). However, Shabi *et al.* (1999) observed a positive effect of increased feed delivery frequency on milk composition but not on milk yield. It is important to underline that on the AMS farm there was a significant interaction between feeding frequency and the period of the year: the increased feeding frequency, from once to twice a day, mitigated the negative effect of hot conditions on milk production with an increment of approximately 2 kg/d (+7.6%) of milk produced during the hot period. Gottardo *et al.* (2005), in a study of 30 conventional dairy farms, observed that cows receiving their feed in two daily distributions during the summer season showed an increase in both DMI (+9.0%) and milk yield (+15.0%) compared with animals fed once a day. Frequency of feed distributions did not have any effect on milking frequency on the AMS farm although this could have been expected with a forced traffic system; increasing TMR deliveries could attract cows at the feed bunk through the milking robot. Our results are in agreement with those of Oostra *et al.* (2005) who found that the daily number of visits to the AMS was not affected by the change in feeding frequency at the bunk for

cows milked in a forced traffic AMS. Behavioural indices did not show any influence of feeding treatments on either farm. The result obtained for CLI is partially in agreement with findings of DeVries *et al.* (2005) who observed that as frequency of feed delivery increased cows spent more time feeding without changing total daily lying time. But in our experiments, increase in daily feed deliveries did not modify the number of cows at the feed bunk during the day (CFI). CFI stability is consistent with DMI values. The number of feed deliveries had no effect on DMI values.

### Period of the year

Average THI registered on the two farms was significantly higher during the hot period compared with the thermoneutral period (an average  $72.6 \pm 2.02$  vs  $60.7 \pm 3.8$ ;  $P < 0.001$ ). During the hot period, average THI was slightly above 72, the threshold identified for the onset of heat stress (Armstrong, 1994). The total number of days characterized by average THI of over 72 during the trials conducted in the hot period was 12 out of 16 (minimum 63.9 and maximum 80.3 of total measurements) for the conventional farm and 9 out of 16 (minimum 64.0 and maximum 80.1 of total measurements) for the AMS farm.

On the conventional farm, DMI was 8.1% lower in the hot period compared with the thermoneutral period ( $P < 0.001$ ). This agrees with findings reported by other authors (Kadzere *et al.*, 2002; West *et al.*, 2003). Milk yield was not affected by environmental conditions on the conventional farm (Table 3). Consequently,

dairy efficiency, i.e. milk (kg/d)/DMI (kg/d), was slightly higher in the hot period than in the thermoneutral period. On the AMS farm, both DMI and milk yield were significantly affected by the environmental conditions (Table 4) with lower values in the hot period compared with the thermoneutral period. In the hot period, DMI was 8.3% lower while there was a 17.0% reduction in milk yield. Consequently, dairy efficiency was lower in the hot period than in the thermoneutral period. Speroni *et al.* (2006) found a 10% reduction in milk production in the hot season compared to the thermoneutral period for a group of multiparous cows milked automatically.

The different response to hot conditions in terms of milk production on the two farms could be partly explained by the different production level, different costs of thermoregulation and different metabolic conditions for primiparous and multiparous cows on the two farms, according to West (2003) and Kadzere *et al.* (2002). In fact, as reported by Kadzere *et al.* (2002), high-producing dairy cows may enter heat stress much earlier than their lower-producing counterparts due to high metabolic heat increment. Furthermore, the effects of hot conditions seem to be greater in multiparous cows than in primiparous ones; Holter *et al.* (1997) reported a 22% reduction in DMI for multiparous cows and 6% for primiparous cows during heat stress. On the AMS farm, milking frequency was significantly lower in the hot period than in the thermoneutral period ( $2.37$  vs  $2.50$ ;  $P < 0.01$ ) and this could help explain the decrease in milk produc-

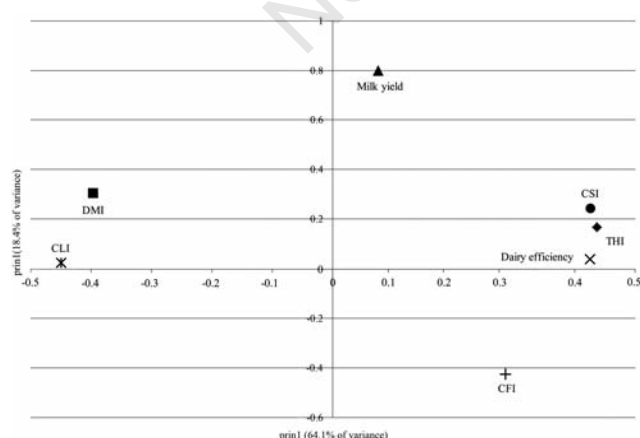


Figure 1. Principal component analysis for variables on the conventional farm (THI, temperature humidity index; CLI, cow lying index; CSI, cow standing index; CFI, cow feeding index).

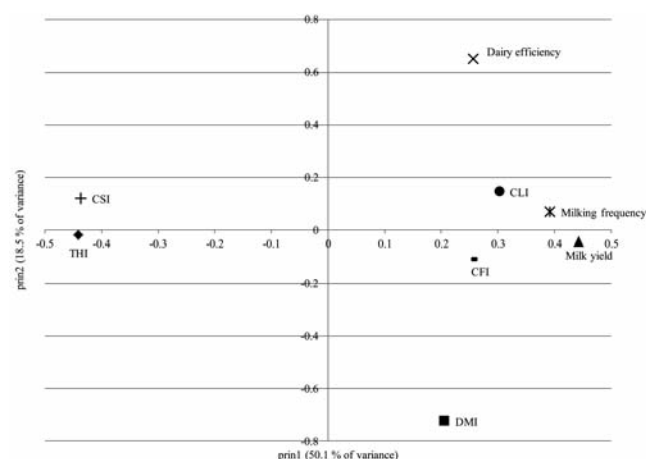


Figure 2. Principal component analysis for variables on the AMS farm (DMI calculated as TMR intake plus concentrate feed at AMS; THI, temperature humidity index; CLI, cow lying index; CSI, cow standing index; CFI, cow feeding index).

tion. Similarly, Speroni *et al.* (2006), comparing conventional and AMS milking, reported that there was a greater reduction in milk yield during the hot season for cows milked with AMS than in the milking parlor and that milking frequency in AMS was significantly reduced during spring-summer compared with autumn-winter. On both farms, environmental conditions affected lying and standing indices (CLI and CSI) but not the feeding index (CFI). In particular, CLI was significantly lower during the hot period compared with the thermoneutral period on both farms. These results are in agreement with those of Zähler *et al.* (2004) who observed a decrease in the duration of lying as THI increased. As expected, CSI was higher during the hot period compared with the thermoneutral period on both farms. Similarly, Cook *et al.* (2007) observed a greater time spent standing in the alley and in the stall during the hot season. This is due to the fact that lactating cows have great difficulty in dissipating heat in hot, humid conditions (West, 2003). This is why they try to cool off by standing. The reduction in time spent lying exposes the animals to the risk of developing claw horn lesion as shown by Cook *et al.* (2007) who reported an increase in lameness in late summer associated with the greater total amount of time spent standing per day. Despite the significant reduction in DMI during the hot period on both farms, feed bunk attendance (CFI) was not affected.

### Principal component analysis

Two principal component analyses evaluated the relationships among average farm values (Figures 1 and 2). For both farms, the following variables were significant: DMI, milk yield, dairy efficiency, milking frequency, THI, CLI, CSI and CFI. On the conventional farm, the first dimension described 64.1% of the total variation (Figure 1) and the second dimension described 18.4%. The analysis confirms the negative effects of increasing THI on ingestion and lying behaviour, and the positive relationship between THI and standing time. In fact, DMI and CLI were clustered in the same space and had a positive correlation. In contrast, they had a negative correlation with CSI, THI and dairy efficiency. On the automatic milking farm, the first dimension described 50.1% of the total variation (Figure 2) and the second dimension described 18.5%. Also in this case, THI shows a positive relationship with standing behaviour and a negative relation with lying time. Furthermore, the analysis highlights the strong relationship among milking frequency, milk yield and lying behaviour.

### Conclusions

Increasing daily TMR deliveries did not result in any variation in cow activities and DMI, but did improve milk yield on both farms. In particular, for multiparous cows, increasing feeding frequency at the bunk can represent a helpful strategy to reduce the negative effects of moderate heat stress on milk production, as shown in our experiment on the AMS farm.

In hot, humid conditions, cows of both farms showed a reduction in daily lying time and an increase in time spent standing in comparison with thermoneutral conditions. Time at the feed bunk did not vary with the environmental conditions or with the different feeding frequencies. Hot conditions showed a depressive effect on DMI of lactating cows on both farms but caused a reduction in milk yield only on the farm with multiparous high-producing cows milked automatically. This is consistent with the decrease in milking frequency at the robot during the hot period. These results could suggest that cows milked in an AMS are more exposed to heat stress than cows milked conventionally, especially if they are high-producing multiparous cows.

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