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Effects of changing milking and feeding times on the behaviour, body temperature, respiration rate and milk production of dairy cows on pasture

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ABSTRACT

Management strategies to reduce heat stress are needed, especially when there is no or little shade in pasturebased dairy systems. We investigated management practices used to reduce heat load in summer: milking later when it is cooler, feeding later, and milking only in the morning. Fifteen groups (n = 4 pregnant Friesiancross cows/group) were managed on pasture and milked at 0700 h followed by a new pasture allocation including silage and one of five afternoon/evening treatments (n = 3 groups/treatment): 1) Late milking (1935 h)/early feed (1630 h), 2) Late milking (1935 h)/late feed (2015 h), 3) Early milking (1550 h)/early feed (1630 h, control), 4) Early milking (1550 h)/late feed (2015 h), 5) Once-a-day milking (OAD): cows were milked only in the morning and provided feed at 1630 h. Lying, grazing and ruminating were recorded using validated accelerometers over 25 d (mean temperature: 19 °C, range: 5-32 °C). Body temperature (BT) was recorded using vaginal temperature loggers and respiration rate (RR) was recorded manually. Individual milk production and water intake (group level) were recorded daily. Data were analysed using linear mixed models with group as the experimental unit (n = 3/treatment). There was no evidence of an overall treatment effect at the 5% significance level for grazing, ruminating, lying time, BT or RR, however, the diurnal pattern varied among treatments (grazing, ruminating, lying, BT: P < 0.001). Typically, cows spent more time grazing after fresh pasture was provided followed by rumination, however, this may have resulted in an observed numerical reduction in lying time for cows with delayed milking and/or feeding. Control cows had numerically the highest RR in the afternoon and OAD cows and cows with delayed feeding the lowest. Water intake tended to differ between treatments (P = 0.060); cows milked early in the afternoon consumed most water. Cows with delayed milking had the greatest peak in BT in the afternoon/evening, whereas cows with delayed milking and feeding, and OAD cows had the lowest BT in the afternoon/evening (P < 0.001). Milk production declined over time, which is normal in summer, however, this decline was numerically lowest for cows with delayed milking and feeding. In summary, OAD cows may have experienced reduced heat load. Delaying milking and feeding and milking OAD reduced the afternoon peak in BT that is associated with walking into milking. Modifying milking and feeding times can be used to change diurnal patterns of behaviour but more information is needed to understand what this means for cow welfare.

1. Introduction

Warm weather conditions in summer can lead to heat stress in dairy cattle with severe production and welfare problems as a consequence (Stull et al., 2008; Vitali et al., 2015). It has been estimated that heat stress is costing the US dairy industry between US\$897 and 1506 mil/year in production losses depending on the degree of heat abatement (St-Pierre et al., 2003). Cows are diurnally active and produce a greater amount of heat during the day compared to the night (Veissier

et al., 2017). Lactating dairy cattle are sensitive to heat stress due to considerable internal heat production associated with milk production (Kadzere et al., 2002; Collier et al., 2012) and rumination processes after a feeding event (Ando et al., 1997). Heat stress is a major issue in tropical and subtropical regions, however, cows in countries with more modest air temperatures, such as New Zealand, which has a mix of temperate and subtropical climates, are also experiencing heat stress conditions (Kendall et al., 2006; Bryant et al., 2007; Schütz et al., 2010). Dairy cattle respond to heat load by seeking shade (Tucker et al., 2008;

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Schütz et al., 2010), increased water intake, time around water and respiration rate (RR) (Muller et al., 1994; Schütz et al., 2010), and reduced feed intake and milk production (Armstrong, 1994; Blackshaw and Blackshaw, 1994; Ominski et al., 2002).

New Zealand has a pasture-based dairy industry where dairy cattle are managed predominantly outdoors and produce on average 25-40 L of milk/d on a grass-based diet (Fonterra, 2023). Whereas this management system has many welfare advantages, such as providing grazing opportunities and freedom of movement, it also means that cows are exposed to warm weather conditions in summer which can lead to heat stress and impaired welfare and production losses (Bryant et al., 2007; Schütz et al., 2010). Shade is a highly valued resource to cows (Schütz et al., 2010), however, it is not often provided. New Zealand has a rotational grazing system and large herds (average herd size is approximately 400 cows) are frequently moving among many paddocks. In addition, it is not uncommon for cows in New Zealand to walk long distances to get to and from milking in the afternoon (Tucker et al., 2005), which is typically the warmest part of the day. High ambient temperatures in combination with heat production associated with walking, can lead to increased body temperature (BT) and RR (Schütz et al., 2011). The vaginal BT peaks at afternoon milking for New Zealand dairy cattle in summer and remains elevated for several hours after milking, compared to cows only milked in the morning (Kendall et al., 2008). Cooling cows using overhead water is efficient in reducing the internal heat load, e.g., by reducing BT and RR (Schütz et al., 2011; Chen et al., 2015, 2016). Cooling cows with water at the milking parlour before afternoon milking, with or without shade, is more efficient in reducing heat load of dairy cattle by reducing the BT and RR, compared to shade alone or no cooling (Kendall et al., 2007). However, cooling cows before afternoon milking only provides relief from the heat at that point and does not provide relief from the heat in the pasture.

There is research showing that management strategies, other than shade and water cooling, can help in reducing heat load in dairy cattle. For example, milking once-a-day (OAD) in the morning rather than two times daily reduces the circadian BT likely due to changes in metabolic heat production and heat generated by physical activity (Kendall et al., 2007). In 2020/21, approximately 8% of New Zealand herds were milked OAD the whole season, whereas up to 42% of herds were milked OAD for parts of the season (Edwards, 2021). There is also some indication that changing milking times can influence heat load experienced by cattle. In an un-replicated study in Australia, cows that were milked 2 h later than conventional milking times produced more milk compared to cows milked earlier in the day, whereas wetting the cows with water 30 min prior to milking had no effect (Wieland et al., 1998). Management strategies have been investigated to alter the peak/pattern of BT of feedlot beef cattle, for example by altering feed consumption (Holt et al., 1999; Mader et al., 2002), feeding time (Brosh et al., 1998; Davis et al., 2003) and dietary energy concentration (Mader et al., 1999) in warm weather. However, changing the frequency and timing of milking and timing of feeding to reduce heat load have received little attention in the dairy industry.

Therefore, the aim of this study was to investigate the efficiency of different management practices to decrease heat load, i.e., by reducing milking frequency (from twice to once per day) or delaying feeding and milking times to later in the evening when it is cooler. A secondary aim was to investigate heat production in association with feeding and rumination, and while walking to the milking parlour in the afternoon. We predicted that cows milked OAD and cows milked and fed later in the day would show fewer heat load responses.

2. Materials and methods

2.1. Animals and study design

The study was undertaken at the DairyNZ Lye Farm, Hamilton, New Zealand (3776'S 17537'E) during January and February 2020 (Southern

Hemisphere summer). All procedures involving animals were approved by the Ruakura Animal Ethics Committee under the New Zealand Animal Welfare Act 1999 (Application no. 14844). Sixty lactating, pregnant Friesian and Friesian-cross dairy cows were divided, at random, into 3 replicates of 20 cows, each replicate was further divided, at random, into 5 treatment groups consisting of 4 cows per group (n = 3 groups per treatment). All 15 groups were tested simultaneously. Each treatment group was approximately equal with respect to age, body weight, somatic cell count (SCC) and expected calving date. At the start of the study, the average body weight was 520 kg (SD = 48.0), BCS was 4.1 (on a 10-point scale, Roche et al., 2004, SD = 0.29), SCC was 34 (SD = 31.0), and time until calving was 193 d (SD = 10.7). The cows were habituated to their groups and treatments for 1 wk before measurements commenced and received Coopers Blaze® fly repellent pour-on to reduce irritation from flies and help with RR measurements. Data were gathered for a period of 25 d.

Each replicate, consisting of 5 treatment groups, was grazed in one paddock (120 m x 80 m) which was divided into 5 equal pasture areas using electrical fencing. The areas were further divided into breaks of pasture to be provided twice per day. In total, the cows were offered 20 kg dry matter (DM)/cow/day, consisting of 50% pasture and 50% pasture silage – all of the silage was provided in the morning, whereas pasture was provided twice daily. Each group was provided freely available filtered bore drinking water in 200 L troughs. Zinc chloride was supplied by a dosatron at the rate of 10 g/cow/day for facial eczema prevention and bloatenz plus for bloat control at a rate of 5 ml/cow /day, which is common practice for dairy farms in the region at this time of year. The 5 treatment groups within a replicate were always in the same paddock to ensure similar pasture quality and similar walking distances to the milking parlour. The location of treatment groups in the paddock was randomised and changed each time a new paddock was utilised, though for practical purposes to move cows in and out from the pastures, groups with the same milking time were positioned side by side. Cows were milked through a 30-bail rotary platform and using a GEA milking system (GEA, Hamilton, New Zealand). Replicates and treatment groups were identified using different coloured collars and paint applied across each cow's shoulders. Animals were sorted into replicates at the parlour and into treatment groups at the paddock gate. The cows had no access to any shade in the paddocks, and there was no cooling provided at milking.

The amount of pasture offered (to enable a 50% pasture diet) and the width of the break were estimated daily by measuring the compressed pasture height using a rising plate meter following a W pattern over the whole paddock. A plate reading was obtained every 2nd step and over a break at least 100 sampling points were obtained. Samples of pasture from each paddock were gathered at the same time and along with samples of the pasture silage were analysed for DM and energy content and composition. The samples were obtained in the morning and were refrigerated and processed straight after collection. Pasture samples (every 10 steps) were collected using "hand shears" and the pasture was cut at grazing height (30–40 mm), with each sample about 15 cm long. Following oven drying at 60 °C, the average DM content for the pasture was 33.8% (SD = 0.98), and the DM for the pasture silage was 35.7% (SD = 0.36). The Metabolizable Energy (MJ/kgDM) of the pasture was estimated data using NIR calibrations. For the pasture it was calculated as an average of 9.2 MJ/kgDM (SD = 0.29) and for the pasture silage an average of 10.6 MJ/kgDM (SD = 0.38). The pasture nutritional composition was as follows: Ash: 8.3 g/100gDM (SEM = 0.05), Crude Protein: 13.9 g/100gDM (SEM = 0.43), Neutral Detergent Fibre: 49.0 g/ 100gDM (SEM = 0.77), Acid Detergent Fibre: 27.7 g/100gDM (SEM = 0.77) 0.43), Fat: 2.7 g/100 gDM (SEM = 0.08), Starch: 9.4 g/100 gDM (SEM = 0.43), Organic Matter Digestibility: 68 g/100gDM (SEM = 0.97). The pasture silage nutritional composition was as follows: Ash: 9.6 g/ 100gDM (SEM = 0.38), Crude Protein: 15.5 g/100gDM (SEM = 0.55), Neutral Detergent Fibre: 46.4 g/100gDM (SEM = 0.76), Acid Detergent Fibre: 34.2 g/100 gDM (SEM = 0.81), Fat: 3.1 g/100 gDM (SEM = 0.20),

Starch: 6.8 g/100gDM (SEM = 1.19), Digestibility: 66.1 g/100gDM (SEM = 1.20), pH: 4.3 (SEM = 0.06), NH₄N: 187.9 mg/100gDM (SEM = 10.66). The average walking distance from paddocks to the milking parlour was 702 m, with a range between 520 and 1065 m.

2.2. Treatments

All treatment groups were milked at 0700 h in the morning. On return to the paddock the cows were immediately offered 66% of their daily feed allowance (a total of 20 kg DM/cow/day) consisting of all their silage and part of their pasture allocation. The remaining pasture allocation was offered in the afternoon/evening depending on treatment. The amount of feed offered to each group of cows remained constant, however, the time at which the feed allocation was offered in the afternoon/evening and time the cows were milked in the afternoon/evening formed the treatments for this study. Cows were free to utilise previous pasture areas, e.g., they were never back fenced. There were 5 treatments:

- Delayed milking: cows were milked at 1935 h, and provided feed at 1630 h. This treatment was predicted to reduce the heat load generated while walking in the warm part of the day, however, it was predicted that heat production in association with grazing and rumination would still occur.
- Delayed milking and feeding: cows were milked at 1935 h, and provided feed at 2015 h upon return. This treatment was predicted to reduce the heat load generated while walking and the heat load associated with grazing and ruminating in the warm part of the day.
- 3. Control (conventional): cows were milked at 1550 h, and provided feed at 1630 h upon return. This treatment represents conventional milking and feeding times. Cows have to walk, graze and ruminate in the warm part of the day.
- 4. Delayed feeding: cows were milked at 1550 h, and provided feed at 2015 h, several hours after return. This treatment was predicted to reduce the heat load associated with grazing and ruminating in peak heat of the day. Cows still have to walk into milking in the warm part of the day.
- 5. OAD (once-a-day) milking: cows were milked in the morning only, and provided feed at 1630 h. This treatment was predicted to reduce metabolic heat by reducing milk yield, and remove the heat generated by walking to milking in the warm part of the day.

Cows that were milked either in the afternoon or evening were held as one group in a small unshaded yard $(1.5-2 \text{ m}^2/\text{cow})$ for 1 h before afternoon/evening milking (at either 1550 h or 1935 h) to simulate normal times that cows can spend waiting before being milked.

2.3. Lying behaviour

Lying behaviour was recorded continuously using Onset Pendant G data loggers (64k, Onset Computer Corporation, Bourne, MA, USA) set to record the y and z-axes at a 30-s interval. The data loggers were placed in a durable fabric pouch and attached on the lateral side of the hind leg above the metatarsophalangeal joint. The pouch was held in position by Velcro patches, one sewn to the pouch, the other glued (Kamar Adhesive, Kamar Products Inc. Zionsville, IN, USA) to the leg of the cow. The pouch was further held in place by a strap around the leg of the cow. The data were downloaded using HOBOware Pro software (Onset Computer Corporation) and daily summaries of lying behaviour (total lying time and bout information (number of bouts and bout duration) calculated using raw data in Excel, correcting for single events as suggested by Ledgerwood et al. (2010). If a lying bout lasted over midnight, it was allocated to the hour before midnight (i.e., we calculated number of bouts starting in each day). However, if this was the case, the duration of lying bouts split across two days was allocated as the time in each of those days.

2.4. Grazing and rumination

To monitor daily eating and ruminating time, electronic ear tags (CowManager SensOor, Agis Automatisering BV, Harmelen, the Netherlands; validated in grazing cattle by Pereira et al., 2018) were attached to the existing EID ear tag of each cow while restrained in a crush. These devices automatically downloaded data to a server through readers installed near the paddocks. The data output from CowManager were analysed.

2.5. Water intake and water temperature

Voluntary water intake was measured daily on a group level, volume consumed being measured through Zenner RNK-RP-N water meters (MICO TeRapa, Hamilton, New Zealand). The water meter was read after the morning milking as the cows were given their morning feed allocation. The water temperature was measured every 10 min using iButtons (Embedded Data Systems, DS1922L, resolution: $0.0625~^{\circ}$ C, accuracy: $0.5~^{\circ}$ C, Lawrenceburg, KY) placed at the bottom of the water trough in weighted plastic bags.

2.6. Respiration rate and panting behaviour

Respiration rate and panting behaviour were assessed on pasture 3 times per day, two or three times per week, each cow being monitored once in each half hour period. Sessions were 1030–1230 h, 1340–1640 h, and 1720-1950 h in the pasture. Cows that were removed for the afternoon/evening milking were monitored for 1 h in the pasture before being walked to the milking parlour. While the cows were waiting to be milked for 1 h, the RR and panting behaviour data were collected twice. On their return to the pasture, they were held at the gate while RR and panting behaviour were again measured, twice consecutively. After this, the cows were then returned to their pasture areas and fed according to treatment. Respiration rate was defined as the time it took for a cow to complete 10 full breaths, this was then converted to breaths/minute. Respiration rate was not recorded when the cows were moving, grazing, or grooming. The panting behaviour was measured after the completion of a successful RR recording; the cow was observed for 10 s and 3 panting characteristics were recorded as being present or absent during this period. These panting characteristics were visible drool: clear droplets on nose or drool falling from mouth/nose, open mouth breathing: space between the lips is visible, tongue outside the mouth: tongue tip (or more) crosses the edge of the bottom lip (without touching any body parts). There were very few observations of open mouth breathing and tongue outside the mouth, hence, these results are not presented.

Inter-observer reliability was undertaken for RR and panting behaviour. The agreement between the 13 trained observers and a gold standard for RR was measured using the Pearson correlation coefficient function in Excel and was r=0.989, on average. Agreement between 11 observers and a gold standard for panting behaviour was measured using % agreement and ranged between 90% and 98%; it was lowest for assessing if drool was present or not.

2.7. Body temperature

Vaginal BT was recorded every 10 min by Star Oddi DST Centi-T temperature loggers (Star-Oddi, Gardabaer, Iceland) which were attached to a shortened cow CIDR (Zoetis, New Zealand) with 2 layers of heat shrink and inserted vaginally. A regime of 1 week in 1 week out was used, meaning that during every logger exchange session, one cow from each treatment group received a logger and one cow had a logger removed, thus 2 cows in each group always had a logger and that the data collected overlapped. This was done to minimise irritation caused by the loggers, and also to ensure that there were always some cows with data loggers inserted during the whole study period and therefore no gaps in data collection. The BT data were analysed as the daily average,

minimum, maximum and amplitude (maximum-minimum).

2.8. Milk production

Individual milk yield was recorded at each milking. A milk sample was taken from each milking and combined for daily analysis of composition and SCC conducted by Livestock Improvement Corporation Ltd. (LIC, Hamilton, New Zealand, data not presented).

2.9. Environmental measures

Air temperature (°C), black globe temperature (BGT, °C), solar radiation (W/m²), relative humidity (%), rainfall (mm), and wind speed (m/s) were recorded at 10-min intervals using 2 portable weather stations (Fan-Aspirated Vantage $Pro2^{TM}$ Plus Stations, model 6163, Davis Instruments Hayward, CA, USA). The weather stations were located in a paddock adjacent to the observation paddocks, in an unsheltered and non-shaded area. The temperature-humidity index (THI, Igono et al., 1992) and heat load index (HLI, Gaughan et al., 2008) were used as follows:

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$$

HLI = IF [BGT > 25,
$$8.62 + (0.38 \times RH) + (1.55 \times BGT) + exp(2.4 - WS) - 0.5 \times WS, 10.66 + (0.28 \times RH) + (1.3 \times BGT) - WS]$$

Where T = temperature (°C), RH = relative humidity (%), BGT = black globe temperature (°C) and WS = wind speed (m/s). Average temperature during the duration of the trial period was 19 °C (range: 5-32 °C) and average humidity was 75% (range: 30-99%).

2.10. Statistical analysis

Treatment effects on milk production for the whole period of the study were analysed as a randomised block analysis of variance with replicates as blocks. Residual plots were examined for consistency with the assumptions of constant variance and approximate normality. Only one variable, number of lying bouts, required transformation; the square root transformation was used.

Data were summarised as hourly averages for all cows in each treatment by replicate group over all days of the study. Treatment effects on the diurnal pattern for grazing, ruminating, RR and BT were analysed using a repeated measures analysis using REML (also called linear mixed model) with random smoothing splines and including random effects for replicates and cow treatment groups within replicates. The random splines were used to model the overall pattern over time and how the pattern varied with treatment, replicates and treatment groups within replicates, this latter term generating the repeated measures correlation structure. Treatment differences in the diurnal pattern were evaluated using a chi squared (Chisq) test on the difference in the deviance between the full model and that with the treatment by time spline dropped. Evidence of treatment differences was set at the 5% significance level, however, we are presenting and discussing differences at the 10% significance level as these differences may be biologically relevant (Greenland et al., 2016; Ganesh and Cave, 2018). Means for each replicate, treatment and hour for RR and proportion of cows showing drool were calculated. These data were used to examine the relationship between drool and RR using a repeated measures analysis using random smoothing splines including random effects for replicates and treatment groups. All statistical analyses were conducted using the statistical package Genstat, version 17 (VSN International, Hemel Hempstead, UK).

3. Results

3.1. Weather conditions

The ambient air temperature, average THI and HLI are presented in Fig. 1a and b. On average, the ambient air temperature was 19.6 $^{\circ}$ C (daily range: 15–24 $^{\circ}$ C), and the maximum air temperature was 27.2 $^{\circ}$ C (daily range: 23–30 $^{\circ}$ C). Average THI was 65.3 (daily range: 58–70) and average HLI was 71.1 (daily range: 62–77).

3.2. Lying behaviour

Lying times for the different treatment groups were, on average, for Control: $8.9 \, h/d$, Delayed feeding: $8.5 \, h/d$, Delayed milking: $8.1 \, h/d$, Delayed milking and feeding: $8.5 \, h/d$, and OAD: $9.1 \, h/d$ (SED = 0.32, $F_{4,8} = 2.98$, P = 0.088); cows milked OAD spent the most time lying down and the cows that had delayed milking spent the least amount of time lying down. There was no evidence that the number of lying bouts nor the bout duration differed between treatments ($P \ge 0.394$). On average, the number of lying bouts were for Control: $6.4 \, \text{no./d}$, Delayed feeding: $7.4 \, \text{no./d}$, Delayed milking: $6.0 \, \text{no./d}$, Delayed milking and feeding: $7.1 \, \text{no./d}$, and OAD: $6.9 \, \text{no./d}$ (SED = 0.76). The average bout length was for Control: $87 \, \text{min}$, Delayed feeding: $78 \, \text{min}$, Delayed milking: $86 \, \text{min}$, Delayed milking and feeding: $77 \, \text{min}$, and OAD: $86 \, \text{min}$ (SED = 6.23).

The pattern of lying time (% of time lying per hour) across the day varied significantly among treatments (Chisq = 114.3 (1df), P < 0.001, Fig. 2a); cows that had delayed feeding and milking, and delayed feeding only were lying down more during the afternoon/early evening than the other treatments. Treatments that had delayed feeding or milking, or both, also spent less time lying down during the nighttime (Fig. 2a).

3.3. Grazing and rumination

The average grazing time was for Control: 8.5 h/d, Delayed feeding: 8.0 h/d, Delayed milking: 8.1 h/d, Delayed milking and feeding: 7.9 h/d, and for OAD: 8.6 h/d (SED = 0.24, $F_{4,8} = 3.0$, P = 0.087, Fig. 2b). The average rumination time was for Control: 7.8 h/d, Delayed feeding: 7.9 h/d, Delayed milking: 7.6 h/d, Delayed milking and feeding: 7.7 h/d, and for OAD: 7.8 h/d (SED = 0.29, $F_{4,8} = 0.33$, P = 0.850, Fig. 2c). The pattern of grazing (Chisq = 273.0 (1df), P < 0.001, Fig. 2b) and ruminating (Chisq = 96.8 (1df,) P < 0.001, Fig. 2c) varied significantly among treatments. All cows had a peak in grazing time after provision of fresh pasture followed by rumination.

3.4. Water intake and water temperature

The average voluntary (trough) water intake was for Control: 89 L/cow/d, Delayed feeding: 89 L/cow/d, Delayed milking: 83 L/cow/d, Delayed milking and feeding: 85 L/cow/d, and for OAD: 86 L/cow/d (SED = 1.9, $F_{4,8} = 3.56$, P = 0.060); the water intake was greatest for the 2 treatments being milked in the afternoon. The average water temperature for all troughs was 22.0 °C (SD = 2.9).

3.5. Respiration rate and panting behaviour

There was no evidence of an overall treatment difference in RR (P=0.742) or proportion of cows with drool present (P=0.667). The average RR was for Control: 66 breaths/min, Delayed feeding: 60 breaths/min, Delayed milking: 61 breaths/min, Delayed milking and feeding: 62 breaths/min, and for OAD: 59 breaths/min (SED = 6.0). The RR pattern during the day did not differ significantly (Chisq = 0.41 (1df), P=0.261) between the treatments, nor was there any significant difference on average ($F_{4,8}=0.34$, P=0.841, Fig. 3). Control cows had numerically the highest RR in the afternoon and OAD cows and cows

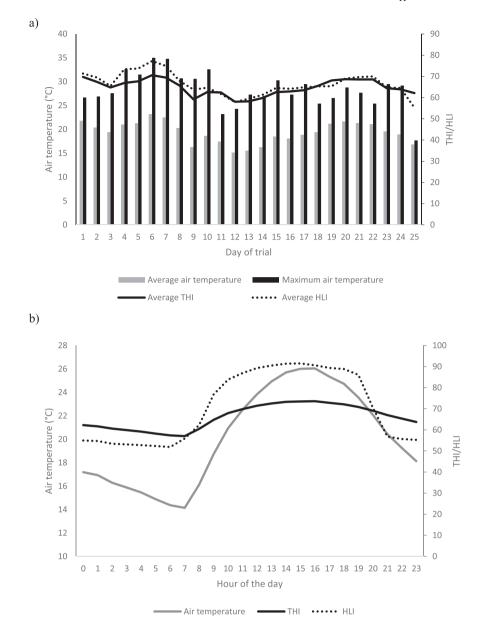


Fig. 1. a-b. a) Average ambient and maximum air temperature and average temperature-humidity-index (THI) and heat load index (HLI) during the trial, and b) diurnal pattern of average air temperature, THI and HLI.

with delayed feeding the lowest (Fig. 3). The average proportion of cows that had drool present was for Control: 60.1%, Delayed feeding: 60.8%, Delayed milking: 59.5%, Delayed milking and feeding: 60.7%, and for OAD: 53.7% (SED = 5.4). There was a clear relationship between RR and drool rate (Fig. 4). There was no treatment difference in the shape of the relationship (Chisq = 1.05 (1df), P = 0.156), however, the treatments differed significantly in the linear components (slope) of the relationship (F_{4,59} = 3.08, P = 0.023); the steepest slope was for OAD and it was significantly steeper than the control and delayed feeding treatments but not significantly steeper than the two other treatments with delayed milking (Fig. 4).

3.6. Body temperature

There was no evidence of an overall treatment effect of any BT measures (average, minimum, maximum or amplitude (maximum – minimum), $P \ge 0.128$, Table 1). The pattern of average BT across the day varied significantly between treatments (P < 0.001); the cows with delayed milking had the greatest peak in BT in the afternoon/evening,

whereas the cows that had delayed milking and feeding, and the OAD cows had the lowest BT in the afternoon/evening (Fig. 5).

3.7. Milk production

The milk production (milk weight) declined over time for all treatment groups, however, when compared to baseline, there was a significant difference in the change in milk production among the treatment groups ($F_{4,8}=4.63,\ P=0.031$); the cows in the OAD treatment decreased their production more compared to the other treatment groups and compared to baseline (Fig. 6). Numerically, cows with delayed milking and feeding had the smallest decrease in milk production when compared to baseline.

4. Discussion

This study aimed to investigate whether heat load in dairy cattle could be reduced in summer by changing management practices, i.e., by reducing milking frequency, or delaying feeding and milking times to

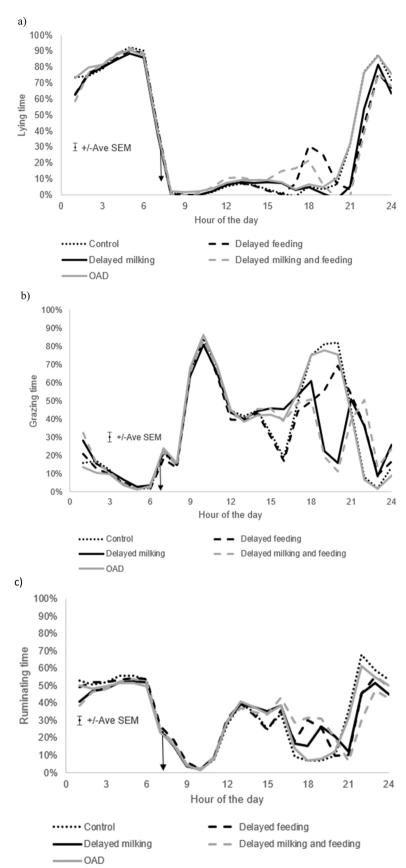


Fig. 2. a-c. Average (\pm SEM) lying time (a), grazing time (b) and ruminating time (c), expressed as percentage of time per hour, of dairy cattle (n = 3 groups/treatment, 4 cows/group) with different milking and feeding times during 25 d in summer. All groups were milked and fed in the morning (morning milking indicated by the arrow) in addition to the afternoon/evening milking/feeding. OAD cows (once-aday) were only milked in the morning however fed at 1630 h. Cows in the Control treatment were milked at 1550 h and fed at 2015 h, Delayed milking were fed at 1630 h and milked at 1935 h, and Delayed milking and feeding were milked at 1935 h and fed at 2015 h.

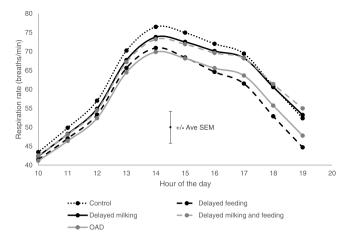


Fig. 3. Average (\pm SEM) respiration rate of dairy cattle (n = 3 groups/treatment, 4 cows/group) with different milking and feeding times during 25 d in summer. All groups were milked and fed in the morning in addition to the afternoon/evening milking/feeding. OAD cows (once-a-day) were only milked in the morning however fed at 1630 h. Cows in the Control treatment were milked at 1550 h and fed at 1630 h, Delayed feeding were milked at 1550 h and fed at 2015 h, Delayed milking were fed at 1630 h and milked at 1935 h, and Delayed milking and feeding were milked at 1935 h and fed at 2015 h.

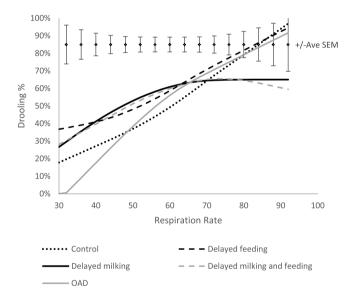


Fig. 4. Relationship (\pm SEM) between respiration rate (breaths/min) and percentage of cows that had drool present. Cows had different milking and feeding times (n = 3 groups/treatment, 4 cows/group) during 25 d in summer. All groups were milked and fed in the morning in addition to the afternoon/evening milking/feeding. OAD cows (once-a-day) were only milked in the morning however fed at 1630 h. Cows in the Control treatment were milked at 1550 h and fed at 1630 h, Delayed feeding were milked at 1550 h and fed at 2015 h, Delayed milking were fed at 1630 h and milked at 1935 h, and Delayed milking and feeding were milked at 1935 h and fed at 2015 h.

later in the evening when it is cooler. We also aimed to explore the heat production associated with feeding and rumination, and while walking to the milking parlour in warm weather. The study found evidence of only a few overall treatment differences significant at the 10% level, however, there were often treatment differences in diurnal patterns significant at the 5% level. In general, cows in the different treatments were grazing after a fresh area of pasture was provided followed by rumination. Consistent with our prediction, cows milked OAD or had delayed milking and feeding to later in the evening had lower BT in the afternoon/evening. In addition, there were a number of other numeric

Table 1Body temperature (°C) of dairy cattle (n = 3 groups/treatment, 4 cows/group) with different milking and feeding times during 25 d in summer. Two cows per group were monitored at any given time. All groups were milked and fed in the morning in addition to the afternoon/evening milking/feeding.

Treatment	Average	Minimum	Maximum	Amplitude
Delayed milking ^a	38.56	37.85	39.30	1.45
Delayed milking and feeding ^b	38.50	37.90	39.09	1.19
Control ^c	38.53	37.90	39.22	1.32
Delayed feeding ^d	38.50	37.87	39.23	1.36
OAD ^e	38.45	37.91	38.97	1.07
SED^f	0.048	0.048	0.118	0.145
P-value	0.318	0.786	0.128	0.168

- ^a Milking times: 1935 h, Feeding time: 1630 h
- ^b Milking times: 1935 h, Feeding time: 2015 h
- ^c Milking times: 1550 h, Feeding time: 1630 h
- ^d Milking times: 1550 h, Feeding time: 2015 h
- ^e Once-a-day: Milked in the morning only, Feeding time: 1630 h
- ^f Standard Error of the Difference

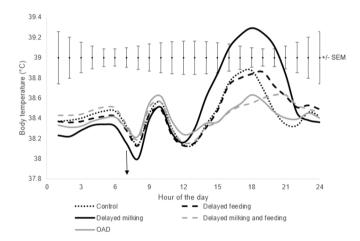


Fig. 5. Average (\pm SEM) diurnal body temperature (°C) of dairy cattle (n = 3 groups/treatment, 4 cows/group) during 25 d in summer. The cows had different milking and feeding times in summer. All groups were milked and fed in the morning (morning milking indicated by the arrow) in addition to the afternoon/evening milking/feeding. OAD cows (once-a-day) were only milked in the morning however fed at 1630 h. Cows in the Control treatment were milked at 1550 h and fed at 1630 h, Delayed feeding were milked at 1550 h and fed at 2015 h, Delayed milking were fed at 1630 h and milked at 1935 h, and Delayed milking and feeding were milked at 2015 h.

differences that were consistent with our predictions. For example, RR was highest for all treatments in the afternoon, control cows had the highest RR and OAD cows the lowest. Cows that walked into milking in the early afternoon (control and delayed feeding) consumed the most water. Lastly, milk production decreased over time for all treatments, but the decline was greatest (and significant at the 5% level) for OAD cows and numerically lowest for the cows that had delayed milking and feeding.

Cows managed on pasture spend between 6 and 13 h per 24-h period grazing and between 4 and 11 h ruminating (Kilgour, 2012). The feeding time is split into a number of smaller meals occurring throughout the day, with the largest meals occurring in the early morning and late afternoon. The grazing and rumination times for the different treatments were within the range for what is considered normal for cows on pasture according to Kilgour (2012). Greatest feeding activity typically occurs after feed is delivered in a total mixed ration (TMR) system (King et al., 2016) and this was also true in the current study where cows in the different treatments in general spent more time grazing following provision of new pasture, followed by rumination. Consequently, the cows that were milked and/or fed later in

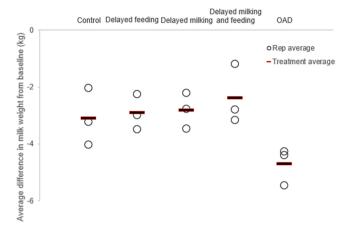


Fig. 6. Average difference in milk weight from baseline of dairy cattle (n = 3 groups/treatment, 4 cows/group) with different milking and feeding times during 25 d in summer. All groups were milked and fed in the morning in addition to the afternoon/evening milking/feeding treatment. OAD cows (oncea-day) were only milked in the morning however fed at 1630 h. Cows in the Control treatment were milked at 1550 h and fed at 1630 h, Delayed feeding were milked at 1550 h and fed at 2015 h, Delayed milking were fed at 1630 h and milked at 1935 h, and Delayed milking and feeding were milked at 1935 h and fed at 2015 h. The circles represent the different replicate group (rep) averages and the line the treatment averages.

the evening spent less time lying down during this time of the day. Some of the treatment groups (delayed feeding and delayed milking and feeding) instead spent more time lying in the afternoon when there was no fresh feed on offer. Cows in the current study spent, on average, 8.5 h/24 h lying down, which is within the range of other studies of well-fed lactating dairy cattle on pasture in New Zealand (8.3-10.1 h/24 h, Kendall et al., 2006; Tucker et al., 2007, 2008; Fisher et al., 2008; Schütz et al., 2013, 2020). Grazing time and lying time is in general negatively correlated in dairy cattle managed on pasture (Tucker et al., 2021), however, in our study the cows with delayed feeding and/or milking spent numerically less time grazing as well as less time lying down, and it is uncertain what this means in terms of welfare. Cows in general spend less time lying down in warm weather (Tucker et al., 2008; Schütz et al., 2010), which is typically in the afternoon in summer, and if cows have to spend evening and nighttime hours engaged in activities other than lying down, this may impact negatively on their health and welfare long term. The cows that were only milked OAD in the morning spent numerically the most time lying down. This is likely due to these cows having more time available because they did not have to go into afternoon milking. Indeed, at peak lactation, dairy cattle milked twice daily spent 1.5 h less lying down compared to cows milked once per day (Tucker et al., 2007). The difference between OAD cows and the other groups milked twice daily was not as great in the current study, and we speculate that this could be due to the current study being undertaken in late lactation where milk production declines and therefore also feeding requirements. Overall, the above-mentioned results show that the diurnal patterns of grazing, ruminating and lying down behaviour can be altered by changing feeding and milking times, however, we encourage more research into what this means in terms of welfare.

Typically, cows in New Zealand have the greatest peak in BT in the afternoon when they walk into milking in the afternoon (Kendall et al., 2008). Cows that had delayed milking had the greatest peak in BT in the afternoon/evening, whereas the cows with delayed milking and feeding, and the OAD cows had the smallest peak in BT in the afternoon/evening. The cows that had delayed milking were given a fresh pasture break before going into milking, and these cows were grazing, then walking, then grazing again, which is a likely explanation to the peak in BT. The results, however, demonstrate that the peak in BT in the afternoon in

summer can be reduced by changing milking frequency and delaying milking and feeding. Some of these findings were reflected in the RR throughout the day albeit not significant at the 5% significance level; OAD cows and cows with delayed feeding had the numerically lowest increase in RR throughout the day. It has been demonstrated by others that feeding in the late afternoon rather in the morning is efficient in reducing heat production in beef cattle (Brosh et al., 1998; Davis et al., 2003). Ominski et al. (2002) fed a TMR to lactating mature cows either at 0830 h or 2030 h, however time of feeding had no statistically significant effect on vaginal temperature, RR, DM intake, water intake, or milk yield in that study. In an un-replicated study by Wieland et al. (1998) milking at 1700 h rather than 1500 h improved milk production compared with cows milked earlier with or without access to 30 min sprinklers. Others have demonstrated that milking frequency influences the circadian BT pattern, particularly in the late afternoon and evening at both peak and mid-lactation, with cows milked OAD having lower mean BT than cows milked twice daily (Kendall et al., 2008). These authors suggested that the differences were likely due to differences in metabolic activity and heat production associated with milk production and locomotor activity. Even though not statistically significant, the cows milked OAD had the lowest mean BT in the current study.

Even though there was no evidence of a treatment difference in the proportion of cows that had drool present, there was a relationship between RR and presence of drool. We encourage further analysis of using presence of drool as a farmer friendly indicator of heat stress. At RR greater than 60 breaths per minute, more than half of the cows in all treatments were observed having drool present.

Cows will drink more water in warm weather and with increased physical activity (Golher et al., 2021) and the higher water intake (numerical difference only) in cows that had to walk to the milking parlour in peak heat (control cows and cows that had delayed feeding only) is likely due to the need to cool down and replenish water lost by evaporation (RR, drooling and sweating). These 2 treatments had similar BT profiles, thus, the BT peak is likely associated with walking to and from milking in the afternoon for those 2 treatments rather than feeding activities. Cows with delayed feeding however had numerically lower RR than control cows, which indicates that grazing is also associated with increased RR – cows with delayed feeding instead spent more time lying down in the afternoon, which partly could explain the lower RR.

The milk production decreased for all treatments throughout the study. A decline in milk production is normal for this time of year in New Zealand which has seasonal calving in winter. The decline was largest for OAD cows, which makes sense since cows milked OAD produce 20% less milk (Stelwagen, 2001). Numerically, cows that had delayed milking and feeding had the lowest decline in milk production, which indicates that this possibly may be a useful strategy to decrease heat load and the loss in production in summer.

There were few overall treatment differences at the 5% significance level in our study. This could in part to be due to limited days with ambient conditions which would thermally challenge the cows. It has been demonstrated, however, in other studies carried out in the same region that cows start to use shade at HLI around 65 (Schütz et al., 2010). In addition, cows in New Zealand reduced their milk production at a 3-d average of THI of 64 (equivalent to maximum air temperature of 20 °C and 40% humidity, Bryant et al., 2007). Most of the observation days in the current study were warmer than these thresholds (14 d out of 25 had THI > 64 using daily mean air temperature and humidity, and 15 d out of 25 had HLI > 65), therefore, we think that there were enough warm days to affect the cows.

5. Conclusions

Daily patterns of lying and grazing behaviour can be modified by changing milking and feeding times. Milking OAD may reduce heat load in dairy cattle. Delaying milking and feeding and milking OAD reduced the peak in BT seen in the afternoon in association with walking into

milking.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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