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Effect of altering milking interval when milking 3 times in 2 days on milk and component yields in pasture-based dairy systems

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ABSTRACT

Dairy farmers face challenges attracting and retaining staff, partly due to the difficulty meeting the desires of the modern workforce. These include flexible work hours and regular time off. The task of milking fundamentally affects the ability of dairy farmers to meet these desires. Milking contributes to a large proportion of the hours spent working on dairy farms. The number of milkings (milking frequency) and their timing (milking interval) within a day influence the number of hours spent milking and what time in the day they occur. Milking 3 times in 2 d (3-in-2) reduces the amount of time spent milking compared with milking twice a day (TAD), without reducing milk yield as much as milking once a day (OAD). However, long intervals between 3-in-2 milkings can still lead to a long workday if farmers are expected to work between milkings. The objective of this study was to determine the effect of milking interval within a 3-in-2 milking frequency on milk yield and composition at 2 stages of lactation and compare these with OAD and TAD milking. Cows (n = 200) were milked in 5 groups of 40 at 3 intervals of 3-in-2: 8–20–20 h, 10–19–19 h, and 12–18–18 h, along with 24 h (OAD), and 10 and 14 h (TAD), for 6 wk at early lactation (mean 24 d in milk ± 7 d, SD) and again at mid lactation (mean 136 d in milk ± 18 d). Milk yields were recorded at each milking and milk samples collected weekly to determine composition. At both early and mid lactation there were no significant differences in milk, fat, protein, or lactose yields between the three 3-in-2 intervals. Cows milked 3-in-2 produced 8% less milk than cows milked TAD and 14% more than cows milked OAD, with smaller differences observed at mid lactation between TAD and 3-in-2. For a 3-in-2 milking frequency, a shorter milking interval can be implemented on the days when cows are milked twice. This may allow farmers to shorten the working day when using 3-in-2, without compromising milk or component yields.

Key words: milking frequency, milking interval, milk composition, pastoral

INTRODUCTION

Dairy farmers are finding it increasingly challenging to recruit and retain staff (Mugera and Bitsch, 2005; Eastwood et al., 2018). Dairy industry roles often entail working more than 40 h a week, with these hours falling outside the standard 9 a.m. to 5 p.m. workday. Milking twice a day (**TAD**), which is typical in pasture-based systems, is one of the reasons for the long working days (Edwards et al., 2020). The average time spent milking on pasture-based dairy farms in New Zealand is 19 h/wk per person and is affected by several factors, including the parlor type and the herd size (Edwards et al., 2020). This statistic does not include the additional time spent herding the cows to and from the paddock and cleaning the parlor after milking. Dairy farming also requires early starts so that cows have sufficient time between milkings to rest, graze, and replenish their milk supply. The combination of long milkings and allowing sufficient time between milkings can create long working days, especially when staff are expected to complete tasks between milkings. As the modern workforce increasingly values aspects of their work life such as more flexible hours and vacation time (Jones, 2017), the long hours and rigid milking times associated with dairy farming roles are regarded as unattractive (Teagasc, 2018).

To increase workplace attractiveness, some pasture-based dairy farmers have reduced their milking frequency from TAD to once per day (**OAD**), which reduces the time spent milking. However, milking OAD can result in losses of milk, component yields, and profitability (Edwards, 2019, 2020). Even though the time spent milking is reduced after switching to OAD, working hours per person may not decrease as farmers attempt to maintain their profitability by also reducing the number of labor units on farm (Bewsell et al.,

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2008). Milking more frequently than TAD in pasture-based dairy systems is not common as the profit from the additional milk does not cover the cost of additional milkings (Culotta and Schmidt, 1988). Profitability is the key driver for TAD milking remaining the most common milking frequency in pasture-based systems.

Several studies have investigated the relationship between milk production and milking interval as a means of exploring options outside of TAD and OAD milking. Researchers have found that milk secretion is linear up to ~16 h after milking (Elliott et al., 1960; Wheelock et al., 1966; Davis et al., 1998). This relationship suggests a milking frequency of 3 times in 2 d (**3-in-2**) is a possibility without compromising milk production. In this scenario, cows are milked twice on d 1 and once on d 2 in a repeating pattern (Eldridge and Clark, 1978). However, a 16–16–16 h interval, when milkings are evenly spread over the 2 d, requires an early start and a late finish on the day where cows are milked twice, resulting in a long working day. For example, if milking 1 occurred at 5 a.m., milking 2 would start at 9 p.m. Consequently, variations of the 16–16–16 h interval have been adopted, while maintaining the same 3-in-2 milking frequency.

Few studies have investigated the relationship between 3-in-2 milking and milk production. One study compared a 10–19–19 h interval with TAD and reported a reduction in milk yield of 18% in early lactation (wk 4), and a 11% yield reduction at wk 20 in mid lactation (Eldridge and Clark, 1978). Another study used an 11–18.5–18.5 h interval over an entire lactation and reported a reduction in milk yield of 8% and milk fat of 6% (Woolford et al., 1985). More recently, a comparison of TAD to a 12–18–18 h interval reported an 11% reduction in milk yield, 8% reduction in protein yield, and 12% reduction in lactose yield for the proportion of the lactation that 3-in-2 was used (Edwards et al., 2022a). No effect on fat yield was detected. There is the potential for the 48-h period of 3-in-2 to be divided into many different milking intervals, including reducing the d 1 milking interval to reduce the length of the working day and thus, increase its appeal to the modern workforce. Consequently, there is a need to explore the relationship between 3-in-2 milking intervals and milk composition and yield.

Milk component yields differ throughout a cow's lactation. The effect of OAD relative to TAD milking on milk components at different stages of lactation has been researched (Davis et al., 1999; Rémond and Pomiès, 2005; Phyn et al., 2010). However, there has been no research exploring the effect of differing 3-in-2 intervals at multiple stages of lactation on milk composition. The 2 studies that reported the effect of stage of lactation on milk composition under one interval of a 3-in-2 milking schedule had differing results. The first compared 3-in-2 with TAD and found no significant difference in fat and protein yields at wk 3 of lactation; however, there was a difference at wk 12 (Rémond and Boit, 1997). The second, a comparison of 3-in-2 with TAD over a whole lactation, found no effect of stage of lactation on the reduction of milk component yields (Edwards et al., 2022a). Consequently, when exploring the effect of milking interval on milk components in pasture-based systems the stage of lactation should be considered.

The objective of this study was to determine the effect of milking interval within a 3-in-2 milking frequency on milk yield and composition at 2 stages of lactation and compare these with OAD and TAD milking. This study provides information for farmers to make informed decisions about shortening the milking interval on the day cows are milked twice when using a 3-in-2 milking frequency.

MATERIALS AND METHODS

Experimental Site and Design

The study ran over 2 separate 6-wk periods, between September 11 and October 22, 2020 (early lactation), and January 15 and February 25, 2021 (mid lactation), at the Ashley Dene Research and Development Station (43°38′48″S 172°20′44″E; 35 m above sea level), Canterbury, New Zealand, under the authority of the Lincoln University Animal Ethics Committee (application 2020–12).

Before each experimental period, all cows were milked TAD. Two weeks before each experimental period all available cows were milk sampled at a single morning and afternoon milking, and a BCS recorded using a 10-point scale (Roche et al., 2009). These data were used to block cows at early lactation and mid lactation, respectively, on DIM (24 d ± 6.7; 136 d ± 17.5), combined fat and protein yield (1.67 kg/cow ± 0.29; 1.93 kg/cow ± 0.26), SCC (arithmetic mean, 113,000 cells/mL ± 221,000; 66,500 cells/mL ± 112,000), BCS (4.4 ± 0.28; 4.4 ± 0.19), parity (20% primiparous; fourth lactation average), breed (Friesian cross), and genetic merit (125 ± 50 breeding worth; 157 ± 107 production worth). No significant differences were detected between any blocking factors and the resulting treatment groups in postallocation analysis. To detect a 4 to 6% difference in milk yield with 80% power and 5% significance, 40 cows were required per group (n = 200).

Cows were drafted into their treatment groups 10 d before the data collection period. This allowed for social groups to form before the data were collected, and 1 wk before each experimental period (wk 0) another milk

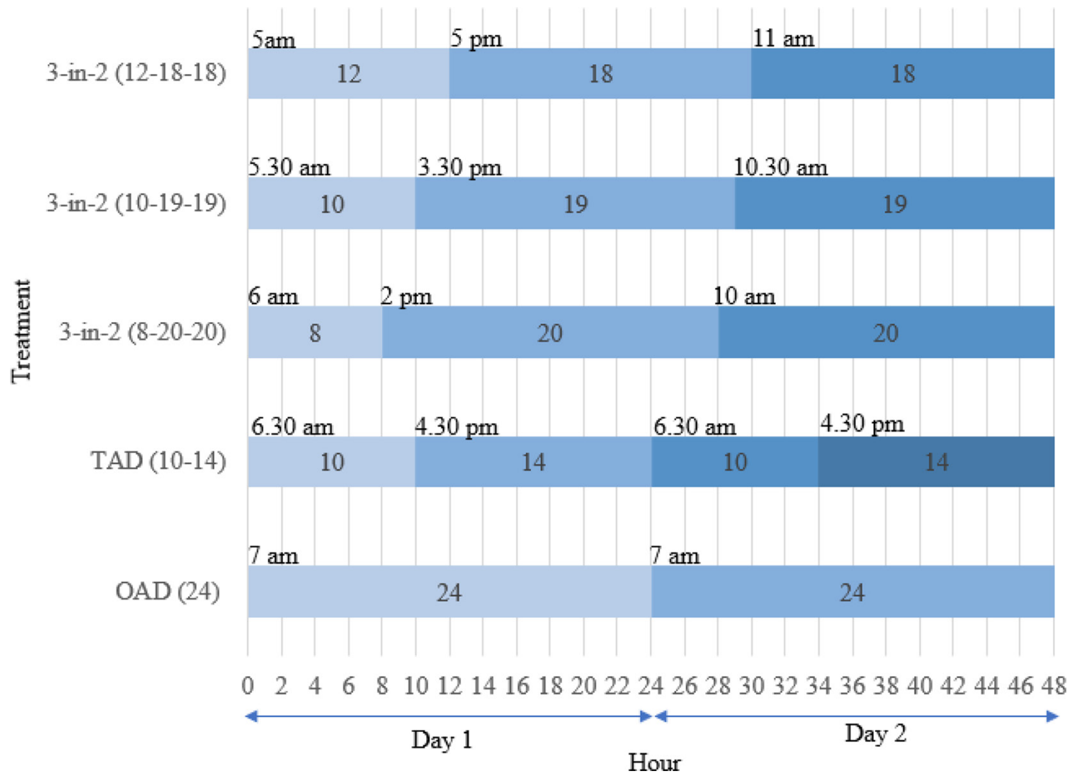

Figure 1. The milking intervals and milking times over 48 h by treatment group (n = 40 cows), including once a day (OAD) with a 24-h interval, twice a day (TAD) with an interval of 10–14 h, and 3 treatments of 3 times in 2 d (3-in-2) milking including intervals of 8–20–20 h, 10–19–19 h, and 12–18–18 h.

sample was collected for milk composition, to provide a covariate. Following the early-lactation experimental period, cows rejoined the main research herd and were milked TAD. There was a smaller pool of available animals to select from for the mid-lactation period, resulting in 90 cows from the early-lactation period being reused, who were equally distributed among treatments. For these animals, previous treatment was used as an additional blocking factor. At early lactation, 9 cows were replaced due to mastitis. At mid lactation 6 cows were replaced: 4 for lameness, one for mastitis, and one for other health reasons. Each of the 5 groups were allocated a milking interval treatment (Figure 1). All cows were milked through a 54-stall rotary dairy parlor (Waikato Milking Systems, Hamilton, New Zealand), with automatic cup removers, which used a threshold of greater than 18 s between the filling of the milk meter, which was equivalent to a flow rate of 630 mL/min.

The experimental area soil types include Balmoral, Lowcliffe, and Lismore, which are stony, well to moderately drained sandy to silt loams (Webb and Bennett, 1986). The 75.5-ha experimental area was subdivided into 3.75- and 4.5-ha paddocks. The paddocks consisted of established perennial ryegrass and white clover mix (**RGWC**; 38.25 ha), diverse pastures containing RGWC plus Italian ryegrass, red clover, and plantain (24.75 ha), and new pastures (autumn sown) containing Italian ryegrass and white clover (7.5 ha). An additional 25 ha (5 paddocks) were used in the mid-lactation period, of which 15 ha were diverse and 10 ha RGWC. Rainfall was supplemented with center pivot irrigation for all paddocks during both experimental periods. Effluent was applied through the pivot weekly and following grazing, and urea was applied at a rate of 50 kg of N/ha directly after each grazing event.

The average temperature during the early-lactation trial was 11.5°C, with the average daily maximum temperature being 17.7°C and daily minimum 6.3°C. At mid lactation the average temperature was 16.8°C, with the daily average maximum being 22.8°C and minimum 11.5°C. A total of 20 mm of rain fell over the early-lactation period, and 39 mm fell at mid lactation.

Experimental Area Management

Groups were rotationally grazed around the experimental area. Pasture mass of all paddocks was measured weekly using a rising plate meter (**RPM**, Jenquip, Fielding, New Zealand), and paddocks ranked from highest to lowest mass to determine grazing order.

Target allocation for early lactation was 17 kg of DM/cow per day above a postgrazing height of 4 compressed centimeters. This was estimated using the assumptions of a mature liveweight of 450 kg and combined target fat and protein yield of 2.2 kg/cow per day and walking 2 km/d over flat terrain and maintaining liveweight with pasture at 12 MJ of ME/kg of DM (Nicol and Brookes, 2007). Milk production, for the calculation of DMI, at early lactation was estimated from farm records from previous years instead of the covariate milk samples as milk production was still increasing. For the mid-lactation experimental period, cows were declining in milk production as DIM increased past peak milk production, therefore target DM allocation was determined using the covariate milk component yields and reduced, compared with early-lactation DM allocation, to 16 kg DM/cow per day.

Pasture DM allocation was kept consistent between groups by adjusting the grazing area to ensure there was no effect of feeding during the experimental period or between treatments. When paddocks exceeded target pregrazing height by more than 20%, a proportion of the paddock was fenced off to maintain target allocation. If postgrazing target height was not met, other cows present on the farm grazed the paddock for up to 2 d after experimental cows had finished to achieve as close to the postgrazing target as possible. When there was insufficient pasture to meet target DM allocation, pasture was supplemented with pasture silage fed out to the respective treatment group.

Paddocks were subdivided into 5 subpaddocks and each treatment group randomly allocated to one of the 5 subpaddocks. These subpaddocks were split again to provide grazing for 2 d. All groups grazed the same paddock on the same days for ease of management and consistency of diet. Cows received a fresh pasture allocation after each milking at early lactation and the size of the allocation was proportional to the milking interval for all groups. At mid lactation each subpaddock was divided into 2 allocations (63:37 split). Groups spent 30 h in the first allocation and then the remaining 18 h with both allocations. All groups were given their second allocation after the mid-morning milking of the groups milked 3-in-2. Pasture allocation was changed between periods to allow greater access to water troughs over the warmer summer months during mid lactation. Back fencing was not used during either period.

Groups were randomly allocated to subpaddocks to ensure group to group interaction did not bias results. A random number was assigned to each group for each paddock and the value of the random numbers dictated the subpaddock the group was assigned, with subpaddock 1 being the closest to the parlor and subpaddock 5, the furthest away.

Animal Measurements

Milk weight was recorded by in-line milk meters (AfiMilk, Kibbutz Afikim, Israel) at each milking. Subsamples of milk were collected weekly. The OAD group was sampled once at the morning milking, and the TAD group was sampled at both morning and following afternoon milking. The 3-in-2 groups were sampled at 3 consecutive milkings: morning, afternoon, and mid-morning milking the next day. Individual subsamples were sent to MilkTestNZ (Hamilton, New Zealand) for analysis of composition (protein, fat, lactose, milk urea, and SCC) by CombiFoss (Foss Electric, Hillerød, Denmark). Milk components were reported in % mass/volume so component yields were calculated by converting milk weight to volume by dividing by 1.03. Body condition was scored during the covariate period and scored again in wk 6 of each experimental period.

Dry matter intake per cow was estimated by back calculation from animal measures (Nicol and Brookes, 2007), where DMI = (MJ ME maintenance + MJ ME lactation + MJ ME liveweight gain or loss + MJ ME walking)/MJ ME/kg DM of feed; MJ ME maintenance was calculated using (0.43/{[(MJ ME/kg DM of feed/18.4) × 0.35] + 0.503}) × liveweight$^{0.7}$; MJ ME lactation was calculated by [(0.376 × fat %) + (0.209 × protein %) + 0.948]/{[(MJ ME/kg DM of feed/18.4) × 0.35] + 0.42} × milk volume; MJ ME walking (flat) = average distance to paddock (km) × number of milkings per day × 2 MJ ME/km. The average distance to paddocks was calculated from each paddock and averaged over a week. Liveweight per cow was a treatment group average over each 6-wk experimental period. Given the short duration of the experiment liveweight change was not analyzed. Energy requirements for pregnancy were not included because cows were not pregnant at early lactation and were not subsequently mated before the mid-lactation trial due to a *Mycoplasma bovis* infection and planned depopulation at the end of the season. The effect of *Mycoplasma bovis* infection on the early-lactation period is unknown; however, it is prevalent in commercial herds globally. Cows that tested positive for *Mycoplasma bovis* were removed before the start of the mid-lactation experimental period.

Pasture Measurements

Pre- and postgrazing pasture height was measured using an RPM for each subpaddock. Calibration cuts were harvested every 2 wk to convert compressed pasture height recorded by the RPM to pasture mass. For the calibration cuts a total of 30 quadrats every second week were harvested, 3 quadrats (25 × 25 cm) per treatment group for pre- and postgrazing. Quadrats

Table 1. Average pasture and silage chemical and botanical composition, presented as a percentage of DM at early and mid lactation[1]

Parameter mean	Early lactation		Mid lactation	
	Mean	SEM[2]	Mean	SEM[2]
Silage				
DM (%)	53.2	1.69	56.3	NA[3]
OM (g/kg DM)	91.9	0.95	92.9	1.05
ME (MJ/kg DM)	10.8	0.16	11.9	NA[3]
Protein (g/kg DM)	14.3	1.38	8.5	NA[3]
Pasture				
DM (%)	23.4	2.87	23.2	2.34
ME (MJ/kg DM)	12.4	0.26	11.7	0.40
Protein (g/kg DM)	18.1	2.26	14.4	2.04
ADF (g/kg DM)	21.4	1.46	24.6	1.36
NDF (g/kg DM)	40.5	3.29	42.1	4.20
Water-soluble carbohydrates (g/kg DM)	24.9	4.10	22.2	3.27
DM digestibility (%)	81.5	2.02	75.4	2.71
Pasture botanical				
Perennial ryegrass leaf (%)	67	21.7	54	13.5
Perennial ryegrass seed head (%)	0	0	11	8.6
Italian ryegrass (%)	13	22.5	0	1.5
Other grass (%)	0	0.3	0	0.2
White clover (%)	8	6.6	17	6.9
Red clover (%)	0	0.0	6	12.2
Plantain leaf (%)	3	5.8	5	9.0
Plantain seed head (%)	0	0.0	5	9.1
Weed (%)	2	3.2	3	2.7
Dead (%)	7	4.3	5	4.6

[1]No differences were detected between treatments ($P > 0.05$), so grand means are reported.

[2]Average SEM.

[3]Due the small amount of silage fed, only one sample was taken.

were harvested to ground level from random areas in each allocation (avoiding dung patches). The compressed height was measured by 2 adjacent readings of RPM in the quadrat area before harvesting. Samples were washed, and any root material was removed, then oven-dried to a constant weight at 60°C. Linear regression between the RPM height and the oven-dried yield (kg DM/ha) was used to derive a calibration equation, which was used to convert pre- and postgrazing pasture mass RPM heights to kg DM/ha. Pre- and postgrazing pasture mass values were used to estimate apparent pasture DMI (kg DM/cow) using the pasture disappearance method (Macoon et al., 2003). When pasture silage was offered, in addition to grazed pasture, the amount of silage was added to estimate total DMI.

Pasture DM samples and samples for botanical compositions were collected weekly in dry conditions. These were cut to grazing height, approximately 50 g fresh weight for each group before grazing. Each subpaddock was walked in a W shape and a handful of pasture plucked every 10 steps. The sample was then refrigerated until processing. Pluck samples within each treatment were combined, mixed and then subsampled for botanical composition and nutritive value. Silage samples for DM and nutritive values were taken as grab samples from 5 points along the line of silage offered to each group. The samples were mixed and a 50-g wet weight sample was oven-dried to a constant weight at 60°C.

Samples for analyzing nutritive value were kept in the freezer until processing. Samples were then freeze-dried and a subsample ground and assessed with near-infrared spectroscopy (**NIRS**; FOSS NIRSystem 5000, Foss Electric, Hillerød, Denmark) using calibration equations derived from similar pasture types. The ME concentration (MJ/kg DM) was estimated using the equation ME = dry OM digestibility × 0.16. Pasture composition between treatments did not show any significant differences (Table 1); however, there were differences between the early- and mid-lactation experimental periods. Silage samples were analyzed by NIRS as described for pasture using a separate calibration equation for silages.

The samples for botanical content were separated into the components: perennial ryegrass, Italian ryegrass, ryegrass seed head, other grasses, white clover, red clover, plantain leaf, plantain seed head, weed, and dead material from a 30-g fresh weight sample and oven-dried at 60°C until they were a constant weight. Dry weights were recorded, and the percentage of herbage types calculated (Table 1), with no significant differences ($P < 0.05$) observed between treatment groups in early or mid lactation.

Data Analysis

The cow was the observational unit and treatment group the experimental unit, with repeated measures through time (n = 6 wk per experimental period). Milk data were analyzed using linear mixed model approach to repeated measures ANOVA (Proc Mixed, SAS 9.4, SAS Institute Inc.). Data from both experimental periods were analyzed together. The model included treatment, period, week and all their interactions as fixed effects, breed and parity (1, 2, 3–4, and 5+) as fixed blocking factors, season calving day (number of days from June 1) and wk 0 data as covariates, and cow as random effect. A covariance pattern structured by both period and week (unstructured across periods and compound symmetry across weeks), recognizing that weeks are repeated within period, was chosen. The ANOVA was followed by pairwise comparisons of treatments within week and period using Tukey adjustment to account for multiple comparisons. Results for the main effect of treatment are presented separately for each period. To achieve representative average daily yields, regardless of milking frequency, raw data were processed as follows: individual cow milk yields for each milking (morning, afternoon, and mid morning) were averaged by week and multiplied by the individual milking composition for the respective week to achieve weekly average milk and component yields for each milking. Note for the 3-in-2 groups, weeks were alternatively defined as 6- or 8-d periods to include a balanced set of morning, afternoon, and mid-morning milkings in each week. Yields for each milking within week were then added to get weekly average 24-h (OAD and TAD) or 48-h (3-in-2) yields. The 48-h yields for the 3-in-2 groups were divided by 2, resulting in weekly average 24-h yields comparable to those from the OAD and TAD groups. Weighted average component concentrations were obtained from dividing 24-h component yields by 24-h milk yields. Data were removed from the analysis if cows were milked in the wrong group or yield was unexpectedly low, for example when milk ejection was affected by estrus. This amounted to <0.01% of all data in both periods. Data were log-transformed for SCC, which did not exhibit a normal distribution. Significance was declared if $P \leq 0.05$.

Dry matter intake and pasture measures were analyzed using one-way ANOVA (Genstat, 21st edition, VSN International, Hemel Hempstead, UK) by treatment. Experimental periods were analyzed separately. Data were removed when values were not biologically likely, for example, when DMI was above 20 kg/cow per day or below 7 kg/cow per day.

RESULTS

Early Lactation

Milk, fat, protein, and lactose yields (per cow per day), as well as milk urea (**MU**) and $\log_{10}$ SCC were not significantly different between 3-in-2 milking intervals at early lactation (Table 2). Protein and fat percentage also did not significantly differ between 3-in-2 treatments. However, there was a small significant decrease (−0.5%; $P < 0.001$) in lactose percent associated with longer 3-in-2 intervals.

Milk yield was affected by milking frequency. Cows milked 3-in-2 produced 8% less milk than cows milked TAD and 14% more than cows milked OAD ($P < 0.05$, Table 2). Milk, protein, and lactose yields and MU concentration decreased ($P < 0.001$) with decreasing milking frequency. Fat concentration increased with decreasing milking frequency; resulting in no significant differences in fat yield. $\log_{10}$ SCC was significantly lower for cows milked 3-in-2 and TAD ($P < 0.01$) compared with OAD. Body condition score did not significantly change during the trial or differ between treatments. The average time taken for milking (minutes/cow per day) was significantly less for the OAD group (−2 min; $P < 0.001$) compared with the 3-in-2 groups, and conversely greater for the TAD (+1.75 min; $P < 0.001$).

Given the significant treatment × week interaction (Table 2), milk and component yields, including fat, protein, and lactose are also presented by week (Figure 2). Milk, protein, and lactose yields for all treatment groups followed a similar trend over the experimental period, initially increasing and then declining from wk 2. The significant treatment × week interaction for fat, protein, and lactose yields was likely the result of the 8–20–20 and 10–19–19 treatments in the 3-in-2 groups from wk 3 onward (Figure 2). Fat yield did not exhibit any clear trends between treatment groups over the experimental period.

Mid Lactation

Milk production was less in mid lactation than in early lactation but the effect of 3-in-2 milking interval on milk and component yields were similar to early lactation. Milk and component yields did not significantly differ between 3-in-2 intervals (Table 2), the exception being MU, where the 8–20–20 treatment had a lower value (−4.1 mg/dL) than the other 3-in-2 intervals. Component percentages also showed the same trend as early lactation.

Table 2. A comparison of both experimental periods, early lactation (mean 24 DIM) and mid lactation (136 DIM) LSM for milk yield and composition, and milking time by milking frequency and interval treatment[1]

		Treatment[2]						Probability[4]				
Parameter mean	Period	TAD	12–18–18	10–19–19	8–20–20	OAD	SED[3]	Trt within period	Trt	Period × Trt	Trt × Wk	Period × Trt × Wk
Milk yield (kg/cow per d)	Early lactation	22.91[a]	21.46[b]	20.86[b]	20.75[b]	18.12[c]	0.364	<0.001	<0.001	<0.001	<0.001	<0.001
	Mid lactation	19.16[a]	18.89[ab]	18.40[b]	18.58[b]	16.65[c]	0.208	<0.001				
Fat yield (kg/cow per d)	Early lactation	1.06	1.13	1.11	1.12	1.13	0.032	0.108	0.515	0.002	0.004	0.190
	Mid lactation	0.93[a]	0.91[abc]	0.92[ab]	0.88[bc]	0.86[c]	0.018	<0.001				
Protein yield (kg/cow per d)	Early lactation	0.85[a]	0.80[b]	0.77[b]	0.76[b]	0.69[c]	0.013	<0.001	<0.001	<0.001	<0.001	<0.001
	Mid lactation	0.76[a]	0.74[ab]	0.72[b]	0.74[ab]	0.67[c]	0.008	<0.001				
Lactose yield (kg/cow per d)	Early lactation	1.14[a]	1.06[b]	1.03[b]	1.02[b]	0.88[c]	0.019	<0.001	<0.001	<0.001	<0.001	<0.001
	Mid lactation	0.94[a]	0.91[ab]	0.89[b]	0.92[ab]	0.81[c]	0.011	<0.001				
Fat (%)	Early lactation	4.75[c]	5.37[b]	5.41[b]	5.60[b]	6.28[a]	0.118	<0.001	<0.001	<0.001	0.009	0.316
	Mid lactation	5.19[b]	5.14[b]	5.27[b]	5.07[b]	5.56[a]	0.084	<0.001				
Protein (%)	Early lactation	3.84[ab]	3.84[ab]	3.79[b]	3.70[b]	3.90[a]	0.031	0.007	<0.001	0.261	<0.001	<0.001
	Mid lactation	4.09[b]	4.09[b]	4.07[b]	4.13[b]	4.22[a]	0.028	<0.001				
Lactose (%)	Early lactation	5.16[a]	5.11[b]	5.10[bc]	5.06[cd]	5.02[d]	0.019	<0.001	<0.001	<0.001	<0.001	<0.001
	Mid lactation	5.04[ab]	4.99[bc]	4.98[c]	5.08[a]	4.99[bc]	0.018	<0.001				
Milk urea (mg/dL)	Early lactation	22.43[a]	17.56[b]	17.34[b]	17.36[b]	14.99[c]	0.553	<0.001	<0.001	<0.001	<0.001	<0.001
	Mid lactation	24.41[a]	22.35[b]	22.39[b]	18.29[d]	19.81[c]	0.509	<0.001				
Log_{10} SCC (cells/mL)	Early lactation	4.47[b]	4.50[b]	4.50[b]	4.49[b]	4.70[a]	0.063	<0.001	0.026	0.048	<0.001	0.130
	Mid lactation	4.65	4.67	4.67	4.72	4.69	0.062	0.852				
Time milking (min/cow per d)	Early lactation	10.94[a]	9.29[b]	9.16[b]	9.13[b]	7.19[c]	0.215	<0.001	<0.001	<0.001	0.264	<0.001
	Mid lactation	9.40[a]	8.41[b]	8.62[b]	8.23[b]	6.77[c]	0.197	<0.001				

[a–d]Mean values in the same row with different superscripts differ ($P < 0.05$) for the interaction between milking interval treatments (Trt).

[1]Percentage values are % component mass/sample milk volume.

[2]Treatments include once a day (OAD); twice a day (TAD); and 3 intervals of milking 3 times in 2 d: 8–20–20 h; 10–19–19 h; and 12–18–18 h (n = 40 cows/treatment) over the experimental period (n = 6 wk) as per cow per day values.

[3]Maximum standard error of the difference.

[4]Probability of treatment effects: Trt within period = effect of milking interval treatment with experimental period (early or mid lactation); Trt = effect of milking interval treatment; Period × Trt = interaction between experimental period (early or mid lactation) and milking interval treatment; Trt × Wk = interaction between milking interval treatment and week of the experimental period; Period × Trt × Wk = interaction between interaction between experimental period (early or mid lactation), milking interval treatment and week of the experimental period.

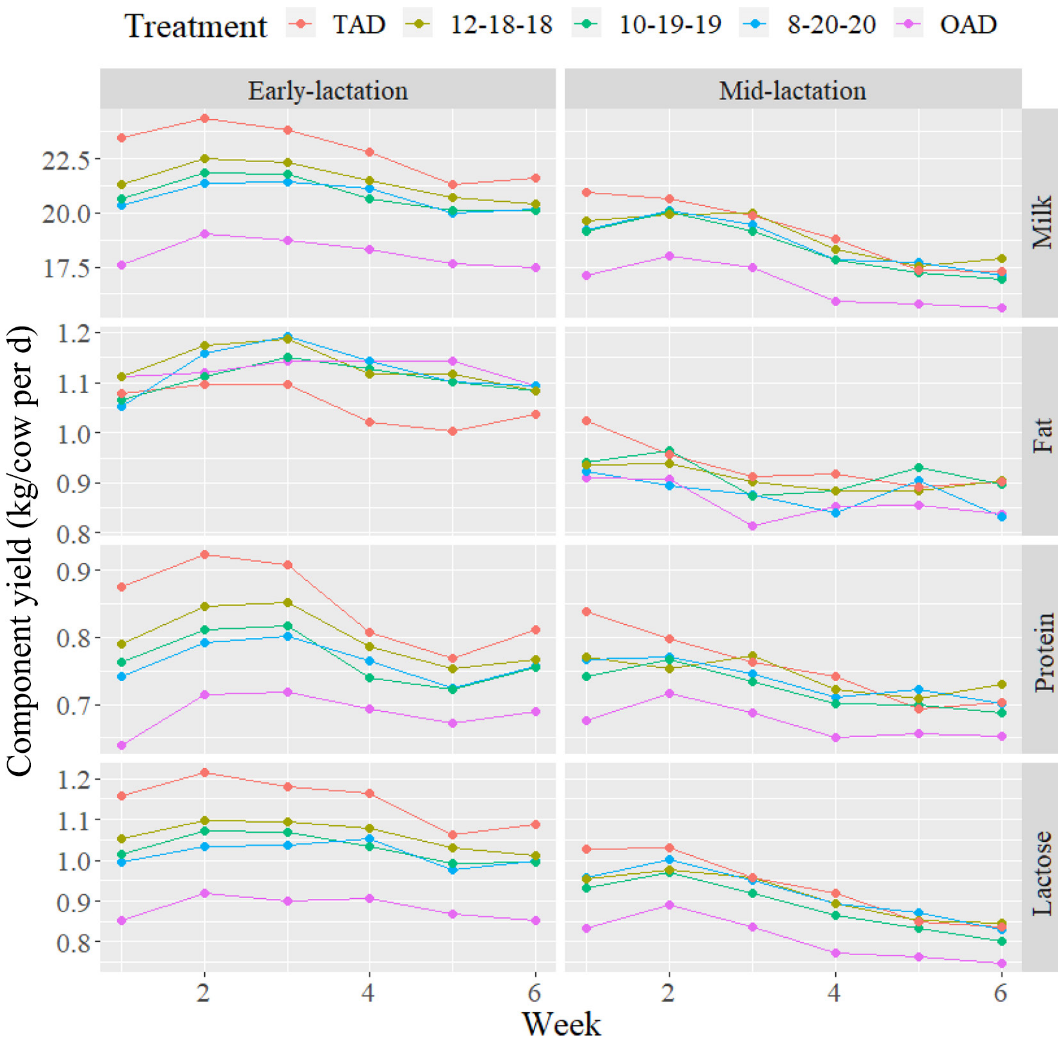

Figure 2. The LSM of milk and component yields, including fat, lactose, and protein in kilograms per cow per day, presented by each experimental period (n = 6 wk) at early (24 DIM) or mid lactation (136 DIM) and milking interval treatment group, including once a day (OAD) with a 24-h interval, twice a day (TAD) with an interval of 10–14 h, and 3 treatments of 3 times in 2 d milking including intervals of 8–20–20 h, 10–19–19 h, and 12–18–18 h.

Milk and component yields for cows milked 3-in-2 were greater than those milked OAD ($P < 0.001$, Table 2) and similar to cows milked TAD. The interval of 12–18–18 was not significantly different to TAD for milk and component yields, with small numerical and statistical differences when comparing the shorter intervals of 8–20–20 to TAD. Fat yield differed between milking frequencies at mid lactation, in contrast to early lactation. It was intermediate for 3-in-2, the highest for TAD and lowest for the OAD treatment. Protein and lactose yield only significantly differed between the TAD and OAD treatments. This differed from early lactation, where protein and lactose yields increased with increasing milking frequency. Milk yield followed the same trend as fat yield; however, there was no significant difference between the 12–18–18 interval and TAD ($P > 0.05$). As at early lactation, MU increased with increasing milking frequency. There were no differences in $\log_{10}$ SCC between treatments. Body condition score did not significantly change during the trial or differ between treatments. As at early lactation, the average time taken for milking (minutes/cow per day) was significantly less for the OAD group compared with the 3-in-2 groups, and conversely greater for the TAD; however, numerically the differences were smaller.

Given the significant treatment × week interaction (Table 2), milk and component yields, including fat, protein, and lactose are also presented by week (Figure 2). Milk, protein, and lactose yields for all groups, excluding TAD, increased to wk 2 and then declined over the experimental period. Fat, protein, and lactose yields all declined at a greater rate for TAD than other

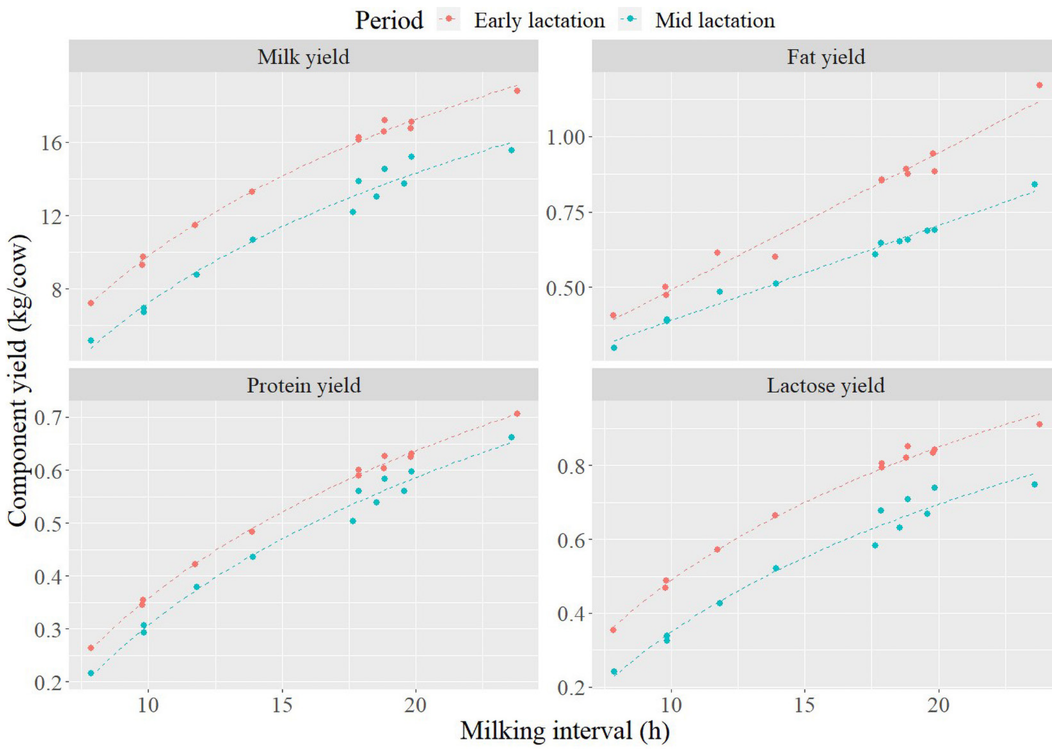

Figure 3. The mean values for milk and component yields, including fat, lactose, and protein in kilograms per cow for each milking interval for early-lactation (24 DIM) and mid-lactation (136 DIM) experimental periods (n = 6 wk). The dashed line indicates the general trend of the data.

milking intervals (Figure 2). Similar to early lactation, fat yield did not exhibit any clear trends between treatment groups over the experimental period.

Milk and Component Yield by Milking Interval

The relationship between milking interval and yield appeared to differ by milk component (Figure 3). Fat exhibited a linear relationship at both early and mid lactation, indicating the rate of fat accumulation was not affected by interval; however, the gradient of the lines differed, indicating different rates of accumulation. Conversely, milk, protein, and lactose yield exhibited a curvilinear relationship with the rate of accumulation decreasing with increasing milking interval. A retarding effect of consecutive long intervals can be observed for 18-, 19-, and 20-h intervals, particularly in mid lactation. In these instances, the lower yield corresponded to the morning milking.

Dietary Measures

Pasture mass offered, area grazed, and apparent DMI did not significantly differ between treatments at either stage of lactation when calculated using the disappearance method (Table 3). Back-calculated energy, and therefore, calculated DM, intake differed in early lactation due to significant differences being detected in milk component yields, with DMI of cows milked OAD being 6% lower than for cows milked TAD ($P < 0.05$, Table 3).

DISCUSSION

The primary objective of this study was to assess the effect of different milking intervals within a 3-in-2 milking frequency on milk and component yields, and secondly, to compare these with the more common milking schedules of OAD or TAD milking. Milk, fat, protein, or lactose yields were not significantly affected by 3-in-2 interval in early or mid lactation.

A significant difference in protein and lactose yields between 3-in-2 treatment groups was expected due to biological mechanisms that decrease yields with increasing milking interval; however, our results did not support this (Table 2). Milk secretion has been shown to be linear to 16 h (Elliott et al., 1960; Wheelock et al., 1966; Davis et al., 1998), beyond which time it is hypothesized there are multiple mechanisms that decrease the mammary gland's production of protein and lactose. First, protein, and lactose may be inhibited by the feedback inhibitor of lactation (**FIL**) glycoprotein,

Table 3. A comparison of pasture and grazing measurements, and DMI values (pasture and silage; kg DM/cow per day) as means by milking frequency and interval: once a day (OAD); twice a day (TAD); and 3 intervals of milking 3 times in 2 d: 8–20–20 h, 10–19–19 h, and 12–18–18 h (n = 40 cows) over the experimental period (n = 6 weeks) by stage of lactation

	Treatment						
Parameter mean	TAD	12–18–18	10–19–19	8–20–20	OAD	SED	*P*-value[1]
Early lactation							
Pregrazing pasture height (cm)	8.8	8.8	8.9	8.6	8.8	0.55	NS
Postgrazing pasture height (cm)	4.8	4.9	4.9	4.9	4.9	0.30	NS
Area grazed per day (ha)	0.4	0.4	0.4	0.4	0.4	0.01	NS
Pasture DMI (kg DM/cow per d) (disappearance method)	14.7	14.6	14.3	13.9	14.2	1.07	NS
Silage DMI (kg DM/cow per d)	0.7	0.7	0.6	0.9	0.6	0.10	NS
DMI (back calculation)	14.8[a]	14.8[a]	14.5[ab]	14.4[ab]	13.9[b]	0.21	0.002
Mid lactation							
Pregrazing pasture height (cm)	9.1	8.9	8.9	9.1	8.8	0.31	NS
Postgrazing pasture height (cm)	4.9	4.7	4.6	4.7	4.9	0.49	NS
Area grazed per day (ha)	0.4	0.4	0.4	0.4	0.4	0.02	NS
Pasture DMI (kg DM/cow per d) (disappearance method)	18.1	18.4	18.7	19.0	16.8	0.95	NS
Silage DMI (kg DM/cow per d)	0.2	0.2	0.2	0.2	0.2	0	NS
DMI (back calculation)	15.5	15.0	15.1	14.9	14.2	0.55	NS

[a,b]Mean values in the same row with different superscripts differ ($P < 0.05$) for the interaction between milking interval treatments.

[1]Significance declared at $P < 0.05$.

which reduces secretory cell activity (Wilde et al., 1995). More recently it has been suggested that permeability of tight junctions between secretory cells may increase with increasing milking interval and mammary pressure, reducing the flow of ions and solute to mammary epithelial cells. This would decrease the concentration of lactose and protein in milk, and act in conjunction with the effect of FIL (Stelwagen et al., 1997; Delamaire and Guinard-Flament, 2006). Downregulation of lactose and protein genes in the mammary gland has also been reported with longer milking intervals (Grala et al., 2011). Due to these biological mechanisms, it would be logical to expect significant reductions associated with extended milking intervals (for example 18 vs. 19 vs. 20 h) for both protein and lactose yields. Although our results comparing 3-in-2 treatment groups numerically support these hypotheses, particularly in early lactation, the differences were not significant. A post hoc analysis revealed that treatment groups of 40 cows provided sufficient power to detect differences of approximately 1 kg of milk yield at early lactation and 0.6 kg at mid lactation. Milk component yields had substantially less variance and therefore greater sensitivity, allowing for detection of less than 0.1 kg for each component at both stages of lactation, implying any underlying biological differences were small. Consequently, from our results, we conclude that differences in milk and component yields between 3-in-2 milking intervals would be difficult to detect in a commercial context.

As a secondary objective, 3-in-2 treatment groups were compared with the milking frequencies of OAD and TAD. Fat yield was not affected by milking frequency in early lactation, which is supported by previous studies (Elliott et al., 1960; Edwards et al., 2022a) where fat synthesis rate was shown to be linear over 24 h. This is also illustrated through our results in Figure 3. However, at mid lactation, fat yield showed a significant difference between milking frequencies, which is supported by different studies (Rémond and Boit, 1997; Clark et al., 2006), implying that fat yield may be affected by milking frequency at the later stages of lactation. Fat percentage increases with decreasing volume (Wheelock et al., 1966), as observed in the OAD treatment. However fat synthesis may have a maximum rate, as it is uncommon to record fat percentages over 6% (DairyNZ and Livestock Improvement Corp., 2021). Therefore, when milk yield decreases below a certain volume, usually at a later stage of lactation, the fat percentage may not increase at a sufficient rate relative to the decrease in milk yield. Additionally, downregulation of milk fat genes has been reported in cows milked once a day (Grala et al., 2011). This is shown in Figure 3, where the mid lactation relationship between fat yield and milking interval, differed to early lactation. It is also possible that greater lipolysis from adipose tissue is occurring for cows milked more frequently (Grala et al., 2014), which would have affected the availability of milk fat precursors and therefore milk fat yields between milking frequencies. Additionally, the number of animals on the farm available to select from for the mid lactation experimental period had been reduced, which gave a smaller range around the mean of fat yield (SED 0.015 vs. 0.032 in early lactation). This provided more sensitivity for declaring significance between treatments at mid lactation compared with early lactation. These explanations remain hypotheses, but our data supports that fat yield may be affected by milking frequency by stage of lactation.

Milk yields at early lactation were greater for TAD and less for OAD when compared with 3-in-2 groups. Protein and lactose yields had similar trends to milk yield. Protein and lactose yields decreasing with reduced milking frequency is supported by literature (Elliott et al., 1960; Roginski et al., 2003). However, at mid lactation protein and lactose yields were not as affected by milking interval, as they did not differ between TAD and 2 of the 3-in-2 intervals. Milk yield at mid lactation was also not significantly different between TAD and the 12–18–18 h interval of 3-in-2. This is clearly illustrated in Figure 2. The milk yield, lactose, and protein results are likely to be connected. Milk yield was declining at the time of the mid-lactation period, compared with early lactation where it increased. Therefore, it could be expected that, in later lactation, there would be fewer negative effects on milk yield from reaching udder and alveolar capacity from longer milking intervals. This would then reduce the potential for milk yield differences between milking frequencies. As discussed earlier, the mechanisms that decrease protein and lactose yield are positively correlated with increased milk yield. Therefore, at mid lactation, it could then be expected that protein and lactose were not inhibited as significantly, resulting in the lack of significant difference between TAD and 3-in-2 intervals. However, this conflicts with the most recent comparison between 3-in-2 and TAD, which reported a consistent difference in milk, lactose, and protein yields across the entire lactation (i.e., stage of lactation had not effect on protein and lactose yields; Edwards et al., 2022a). More investigation is warranted into the relationship between milk and component yields, milking interval and stage of lactation.

Milk urea concentration was generally greater with increasing milking frequency at both stages of lactation. There is relatively little literature comparing milking frequency and MU; however, shorter milking intervals have been associated with higher MU (Nielsen et al., 2005). As MU concentration is highly correlated with dietary protein, and therefore N intake, the likely explanation for the relationship between MU and milking intervals is related to the timing of feeding and therefore N intake, relative to the timing of milk sampling (Gustafsson and Palmquist, 1993). For example, MU yield was greater for the TAD group, as their pasture allocation and therefore N intake was closer to the time of milk sampling. This explanation may account for the difference in MU between treatment groups observed in early lactation when feed allocation was determined by milking times, and there was a greater time difference between milk sampling and peak grazing for groups with longer milking intervals. However, at mid lactation all treatment groups were offered fresh feed at the same time. Nevertheless, the timing of milk sampling would have differed for each treatment group relative to pasture allocation which could still account for differences in MU between milking intervals.

Pastoral dairy farmers have reported difficulty attracting and retaining quality staff (Eastwood et al., 2018). Current workforces require conditions with desirable attributes including flexible hours week and regular time off (Jones, 2017), which can be difficult to achieve on dairy farms due to long and structured milking hours. Decreasing milking frequency from TAD allows for a decrease in time spent milking, greater flexibility in when milking occurs, and greater time in the paddock for the cows, but any changes must be weighed up against any undesirable outcomes. For example, a decrease in milk yield, particularly seen with OAD milking (Edwards, 2019), can potentially lead to a decrease in profit if costs are not sufficiently reduced (Edwards, 2020). To offset the decrease in profit, some farmers reduce labor and wages (Bewsell et al., 2008), which may erode the benefits of a decrease in milking frequency for people working on the farm. It has also been noted in previous studies that despite more paddock time, cows that are milked OAD are less active (Hall et al., 2023), the reasons for this are unclear; however, cow behavior should also be taken into consideration when making changes to milking frequency.

Milking 3-in-2 is an intermediate option between OAD and TAD in both potential production and time spent milking (Edwards et al., 2022b), and therefore, may offer a greater opportunity to maintain profitability on some farms. However, as with OAD, it is not a given that 3-in-2 will be beneficial for all people on farm, particularly if a milking interval is chosen that results in a longer workday on d 1, when cows are milked twice. This research has demonstrated that any differences in milk and component yields between 3-in-2 intervals are small. Consequently, when applying these results in a commercial setting, it is likely that there will be greater contributing factors to changes in milk yield and components between seasons, outside of the 3-in-2 interval chosen. These factors could include climatic conditions, pasture quality and quantity; and should be taken into consideration when assessing any milk production changes that seemingly come from a change in milking interval or frequency. Accordingly, farmers using 3-in-2 can choose a shorter milking interval on day one without compromising on milk production or have the flexibility to select a milking interval that suits best the individual needs of the people on farm. Decreasing working hours and day length, through a shortened

3-in-2 milking interval, may have additional benefits such as less fatigue (Park et al., 2001), fewer mistakes (Landrigan et al., 2004), fewer injuries (Dembe et al., 2005), and improved well-being (Rajgopal, 2010). All of these are aspects that will benefit the modern dairy farmer and farming businesses. Additionally, there may be benefits for cows that are milked on an 8-h versus a 12-h interval (Hall et al., 2023), due to milking times not coinciding with natural peaks in grazing behavior. Therefore, if the expression of natural behavior is of importance to the farmer, a shorter 3-in-2 milking interval on the day cows are milked twice may have further benefits.

CONCLUSIONS

We detected no significant differences in milk yield and milk components between 3-in-2 milking intervals during early or mid lactation. Consequently, the results of this study suggest that farmers using 3-in-2 could choose a shorter milking interval on the day with 2 milkings. Or alternatively, have the flexibility to select a milking interval that suits best their needs without significantly compromising milk yield. When comparing 3-in-2 to TAD a stage of lactation effect was seen, where milk and component yields (other than fat) were not as affected at mid lactation. Therefore suggesting 3-in-2 offers a more viable milking frequency for those concerned about milk production from mid lactation onwards. Farmers can utilize this knowledge to evaluate which milking frequency and 3-in-2 milking interval is best suited to their needs.

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