Uneven milking intervals are adequate to achieve the benefits of increased milking frequency in early lactation. *By Hanling et al., page XXXX.* Increasing milking frequency, the number of milkings per day, enhances milk production. Milking cows four times daily as opposed to twice for 3 weeks postpartum elevates milk production in that timeframe and, to a lesser extent, through the remainder of lactation. Milking interval defines the time between milkings; prolonged milking intervals decrease milk production. This study aimed to change the time between milkings for four-times daily milking frequency in early lactation to further enhance milk yield. There was no difference in the improved milk yield carry-over effect through 300 DIM between cows milked on even or uneven milking intervals for 20 days in early lactation.

RUNNING HEAD: EFFECT OF MILKING INTERVAL ON MILKING FREQUENCY

Uneven milking intervals are adequate to achieve the benefits of increased milking frequency in early lactation

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ABSTRACT

Increasing milking frequency (MF) increases milk yield (MY) and farm profit, and optimal milking intervals (MI) prevent milk production decline. The objective of this experiment was to compare the MY effect of even and uneven 4X MI in early lactation under increased MF. Fourteen multiparous and 6 primiparous cows were milked using unilateral frequent milking (UFM) with right udder halves milked 4X and left udder halves milked 2X for 20 d in early lactation starting on d 5 postpartum. Ten (7 multiparous and 3 primiparous) cows were allocated evenly based on parity and assigned to either the even or uneven MI groups distinguished by 9:3:9:3 h or 6:6:6:6 h intervals. The left and right udder halves were milked at 01:00 and 13:00. The right udder glands were additionally milked at 04:00 and 16:00 for the uneven MI group and 07:00 and 19:00 for the even MI group. Milk from each udder half was weighed and sampled for components on the final day of treatment and at 60, 120, 180, 240, and 300 DIM. The overall effect of 4X milking on the right udder halves was a 5.96 ± 0.70 kg/d increase in MY on d 21 of UFM compared to the 2X udder halves. This elevated MY continued through 300 DIM averaging 1.56 ± 0.70 kg/d. Increased MF in early lactation increased the udder half difference in total yield throughout a 300 d lactation by 508 kg for milk, 25 kg for milk fat, and 15 kg for milk protein. Increased MF in early lactation increased milk component yields, but there were no differences between MI groups. The lack of treatment difference may be beneficial to farmers. The ability to achieve the same increased MY effect with uneven MI may optimize labor efficiency because early lactation cows could be milked at the beginning and end of milking sessions. Farmers would not have to add additional milking sessions to achieve the enhanced MY response regardless of normal milking session length.

Key words: Increased milking frequency, milking interval, unilateral frequent milking

INTRODUCTION

Increasing milking frequency **(MF)**, or the number of times a cow is milked per day, is a management practice used to increase milk yield **(MY)**. Erdman and Varner (1995) observed that milking cows 3 times per day **(3X)** as opposed to 2 **(2X)** caused an 18% increase in production. This increased yield can be seen at any point during lactation. When MF is increased in early lactation alone, MY is improved during the increased MF time frame and continues to be elevated into remaining lactation after cows are returned to typical farm practice MF (Wall et al., 2007a). Milkings are typically doubled for the first 3 wk of lactation. For instance, cows would be milked four times daily **(4X)** as opposed to 2X. An increase in MY was immediately apparent, and reached its maximum on d 21 (Wall et al., 2013, Wright et al., 2013b). During increased MF, cows milked 4X yielded close to 5 kg/d more than 2X cows in early lactation (Wall et al., 2013). Many studies utilized unilateral frequent milking **(UFM)** to observe effects of increased MF. This method involves milking udder halves or quarters at different frequencies and reduced variation by maintaining a control milking frequency within each cow. Wright et al. (2013b) used UFM and observed that 4X glands maintain an increased yield of 0.8 kg/d through d 270 of lactation compared to 2X glands after the 4X milking treatment.

Equally spaced milking intervals **(MI)** are another management practice to maximize dairy production. Milk yield decreases with increased MI. Dairies milking 2X can use 12 h intervals to minimize time between milkings, avoiding prolonged MI. In an early MI study cows were milked in early lactation at either 4, 8, 12, 16, or 20 h intervals with no difference in milk secretion rate up to 12 hours. However, there was a marked reduction at 16 h and 20 h (Schmidt, 1960). Even slight changes in MI such as 10 and 14 h intervals can impact cows’ production. Ouweltjes (1998) found that though the cows made more milk after the 14 h interval, their milk production rate was lower indicating that the mammary glands were less productive with longer MI. Conversely, Penry et al. (2018) found that a 2.4 h decrease in MI did not increase milk production rate in an automatic milking system setting. It is unknown whether small changes in MI affect the increased MY effect of early lactation increased MF. A practical management practice for 4X MF is to milk the early lactation cows at the beginning and end of a milking session, milking the rest of the herd in between. This can lead to an uneven MI for the 4X cows depending on milking session duration. Since extended MI can lead to decreased milk production, reducing the time between milkings may increase MY. Milking every 6 h, on an even MI, minimizes the time between milkings and may enhance the effect of early lactation increased MF.

The objective of this study was to maximize the increased MY effects of early lactation increased MF by comparing the MY of cows milked unilaterally on either an even or uneven MI. We hypothesized that cows milked on even MI would have an increased MY compared to cows milked on an uneven MI.

MATERIALS AND METHODS

Animals and Experimental Design

All animal procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee (17-001). Cows were housed at Virginia Tech's Kentland Farm. Twenty Holstein cows calving between March and April 2018 with even milk yield from both udder halves were used in this experiment. To ensure even udder half milk production, the left and right halves were milked using a Surge RX quarter milker into two separate buckets (Nasco, Fort Atkinson, WI) and weighed at 5 DIM. Animals were added to the experiment starting at 5 DIM if their udder half milk weights had less than a 0.75 kg difference at a 6 h MI and were free of clinical mastitis. After the initial measurement, every other multiparous and primiparous cow was randomly assigned to one of two groups, the even MI group or the uneven MI group with the subsequent cow or heifer assigned to the opposite group until there were 10 matched pairs of multiparous and primiparous animals on each treatment. This experiment began with 3 primiparous and 7 multiparous animals per group. One multiparous cow from the even MI group was removed from the study after 60 DIM due to chronic subclinical mastitis that affected her production, and her data were not included in statistical analyses. Two multiparous cows in the uneven MI group died after 180 DIM; one was euthanized due to chronic lameness and the other died of peritonitis. The data collected from these cows were included in the statistical analyses up to the point of death.

All cows were milked using unilateral frequent milking (UFM) for 20 d in early lactation (5-25 DIM); the left udder half was milked 2X and the right was milked 4X. The right udder half of the even MI group was milked at a 6:6:6:6 h interval (0100, 0700, 1300, and 1900). The uneven MI group was milked at a 9:3:9:3 h interval (0100, 0400, 1300, and 1600). Both udder halves were milked at 0100 and 1300 for all cows, and only the right halves were milked at the additional time points by plugging the left teat cups.

Milk samples and weights from individual udder halves were collected on d 20 of treatment. Sampling continued every 60 d starting at 60 DIM until 300 DIM. On sampling days, the cows were milked using a Surge RX quarter milker. Milk from the left and right udder halves was collected into separate buckets for sampling and weight measurements. Milk samples were tested for milk fat and protein using mid-infrared spectroscopy (FOSS Milkoscan FT+; FOSS

North America) and somatic cell count using flow cytometry (FOSS Fossomatic FC; FOSS North America) by United DHIA (Fairlawn, VA).

Statistical Analysis

Statistical analysis for milk, protein, and fat yields as well as SCC were performed with Statistical Analysis Software (SAS, version 9.4) using the GLIMMIX procedure. Data for udder half difference were calculated by subtracting 2X yields from 4X yields for individual cows. Treatment (df = 1), parity (df = 1), treatment by parity interaction (df = 5), day (repeated, df = 5), treatment by day interaction (df = 5), and day by parity interaction were fixed effects while cow nested within treatment by parity (df = 9) was random. Treatment was defined as the even or uneven MI group. Sampling days on 25, 60, 120, 180, 240, and 300 DIM signified day. Parity was defined as either primiparous or multiparous. Least squares means (LSM) were estimated for fixed effects. Udder half differences existed if there was a significant difference from 0 on a sampling day. Cumulative yield through each sampling day area under the curves was calculated using the trapezoidal rule to estimate the total udder half milk, fat, and protein yields. The LSM treatment by day interaction sliced by day was used to detect differences in yields between treatment groups on each sampling day. Significance was declared at $P < 0.05$ and a tendency at $P < 0.10$.

RESULTS

We analyzed the difference in milk and component yields by subtracting 2X from 4X yields. Four-times daily milked (4X) udder halves produced 29% more milk and significantly more fat and protein on the final day of 4X milking across both treatment groups (Figure 1). The increased milk yield due to 4X treatment was highest on d 20 of 4X milking at 5.96 ± 0.70 kg/d for all cows ($P < 0.001$; Figure 2). On d 20, 4X udder halves produced 4.78 ± 1.02 kg and 7.31 ± 0.99 kg more milk than the 2X halves for the even and uneven MI groups. The increased MY carry-over effect in 4X udder halves averaged 2.14 ± 1.10 kg/d for the even MI group and 1.26 ± 0.56 kg/d for the uneven group through 300 DIM. The average increased milk yield carry-over effect for all cows was significant at 1.56 ± 0.70 kg/d ($P = 0.03$; Figure 2B). However, the only significant difference between treatment groups was d 20 of 4X milking. Both groups had a significant increase in MY due to 4X milking on the final day of treatment (Figure 1A). There was no difference in udder half milk yields between the even and uneven MI groups throughout the remainder of sampling, thus, there was no difference in the increased MY carry-over effect between the treatment groups (Figure 2A).

Similarly, fat and protein yields were elevated in 4X milked glands both during the milking treatment period and when cows were returned to 2X milking. Right udder halves produced 312 ± 34 g/d more fat than the left on d 20 of treatment ($P < 0.001$; Figure 1B); these glands continued to produce 74 ± 34 g/d more fat throughout the remainder of lactation when the whole udder was milked 2X ($P < 0.03$). Glands milked 4X in early lactation produced 182 ± 22 g/d more protein than 2X glands at d 20 of treatment ($P < 0.0001$; Figure 1B), and when returned to 2X milking, the right udder halves continued to make 48 ± 22 g/d more protein than left udder halves ($P < 0.03$). However, there were no differences in udder half yields between the even and uneven MI groups throughout the study for total milk yield or components.

There was a significant effect of day for milk, fat, and protein yields ($P < 0.001$) largely due to the significant increase in yields at d 20. Increased fat yield in 4X glands was significant on all sampling days excluding 120 and 300 DIM and increased protein yield from 4X glands was significant on all days excluding days 60 and 120 (not shown). Lactose yield and SCC were not affected by treatment, parity, or sampling day. There was no significant difference between MI groups for any milk components. Cumulative udder half difference through 300 DIM was significant for milk, fat, and protein yields showing a 9% (P = 0.02), 12% (P = 0.01), and 9% (P = 0.02) increase in yields, respectively (Table 1).

Parity significantly influenced the increased MY effect, but there was no interaction between treatment and parity. Primiparous cows generally did not consistently respond to MF treatment (P = 0.10) unlike multiparous cows (P = 0.0006; Figure 3). Multiparous cows in both MI groups had increased yields of milk, fat, and protein. This was only true of primiparous cows in the uneven MI group. Primiparous cows in the even MI group had no significant increases for milk or milk component yields in the 4X halves. This study only utilized 3 primiparous cows per treatment group and this might have limited the detection of treatment differences. Larger numbers of first lactation cows could be needed to confirm their lack of response to MF treatment.

DISCUSSION

Changing milking management is a local and abrupt method to manipulate MY. Erdman and Varner (1995) observed that increasing MF to 3X as opposed to 2X causes an 18% increase in production. This increased yield effect can be seen at any point during lactation. However, increasing MF in early lactation alone causes an increased milk yield carry-over effect that may be more labor cost effective than 3X milking throughout lactation. Decreasing MF from 2X to once daily milking **(1X)** caused a 28-38% decline in MY (Stelwagen and Knight, 1997). Once-daily milking interval is synonymous with a 24 h MI. Milk production rate significantly declines after a 16 h MI, which explains the decline in total milk production (Schmidt, 1960). However, milk production rate did not differ up to 12 h milking intervals, which is the typical desired MI for a dairy farm milking 2X. Incomplete milking also causes a MY decline. Udder halves with only 70% milk removal as opposed to 100% had 27% lower milk production rate possibly due to milk accumulation similar to prolonged MI (Kuehnl et al., 2019).

There was no significant difference in milk or milk component yields between treatment groups. The effects of MI on milk and milk component yields have been linked to tight junction (TJ) integrity especially at extended intervals (Stelwagen and Singh, 2014). Tight junctions are intercellular structures that facilitate cell-to-cell communication and form the milk-blood barrier that prevents paracellular transport between blood and milk (Stelwagen and Singh, 2014). Stelwagen et al. (1994) measured ion concentrations in milk and lactose concentrations in blood plasma of dairy goats to determine leakiness of TJ. Milk secretion rate started to decline when TJ began to degrade at 19 and 21 h post-milking respectively. Tight junctions were completely disrupted after 36 h, which is approximately the same MI that causes milk production to stop completely (Schmidt, 1960). In a similar study with dairy ewes, researchers showed that plasma lactose decreased slightly from 8 to 16 h then significantly increased from 16 to 24 h (Castillo et al., 2008). However, the ewes had more individual variation in MY due to increased MI than goats (Stelwagen et al., 1994, Castillo et al., 2008). In dairy cows, TJ begin degrading at 18 h post milking. Blood plasma lactose and sodium and potassium ion concentrations in milk rapidly increased from 18 to 30 h (Stelwagen et al., 2008). Tight junctions between mammary epithelial cells in the udder remain closed with up to 18 h MI (Stelwagen et al., 2008) and the lack of treatment difference between the even and uneven MI groups may be due to similar levels of udder TJ integrity for both treatments. The six-hour MI was not long enough to cause TJ opening or any detrimental effects due to lactose leakage.

The results of our experiment confirm that increased MF in early lactation increases MY both in the 4X treatment period and through the remainder of lactation when cows are returned to 2X. These findings are in agreement with many previous studies (Wall and McFadden, 2007a, b, Wright et al., 2013b). The increased milk yield of 4X udder halves for all cows was highest on d 20 of treatment with 5.96 ± 0.70 kg/d ($P < 0.001$); the 4X udder halves continued to make 1.56 ± 0.70 kg/d more milk than the left halves from 25-300 DIM ($P = 0.03$). The increased milk yield results of this study were greater than those of Wright et al. (2013a) who saw a 2.8 ± 0.3 kg/d increase in 4X gland milk yield on d 21 of their increased milking treatment and 0.8 ± 0.3 kg/d increased MY carry-over effect through d 270 after cows were returned to 2X milking. However, the increased MY data of the present study was comparable to that of Wall and McFadden (2007a) who found a 6.6 ± 0.8 kg/d increase in milk production in 4X milked udder halves compared to 2X on d 21 of the increased MF period. Udder halves milked 4X then continued to yield 2.7 ± 0.3 kg/d more milk than 2X glands through 180 DIM. Our study continued sampling through 300 DIM, and showed that the udder half difference in milk yield continued with ongoing lactation.

Our study detected increased milk fat and protein yields both during and after early lactation increased MF. Wright et al. (2013b) observed a significant increase in protein yield during 4X UFM but found a significant decrease in fat percentage in 4X glands due to an increase in total milk volume. Shields et al. (2011) found a positive difference between 4X and 2X UFM udder halves in more components including fat, protein, and lactose yields as well as fat percentage with a 21d treatment period without a significant difference in SCC in agreement with the present study.

Primiparous cows did not have a significantly increased MY response to early lactation increased MF, but this might have been limited by sample size for primiparous cows. The difference in MY between their 4X and 2X udder halves was not different from zero. This may be due to the ongoing development of primiparous cows' mammary glands throughout their first lactation. As stated previously, Wright et al. (2013a) detected a lower increased MY carry-over effect than the present study and that of Wall and McFadden (2007a); this could be due to parity differences. Wright et al. (2013a) used only primiparous cows whereas Wall and McFadden (2007b) used multiparous cows. Primiparous cows have lower milk production compared to multiparous due to fewer mammary cells and on-going growth.

Unilateral frequent milking has often been employed in MF studies in order to limit cow to cow variation in milk production by imposing MF treatments within the same cow. This facilitates the detection of local effects of MI on mammary glands because systemic effects, such as circulating hormones reach glands that are milked with and without increased milkings, but there can be concerns that systemic factors influence the milk production of less frequently milked udder halves. This could be driven by the effect of hormones released with milking despite milking only one udder half. Oxytocin causes milk letdown in both udder halves despite milk removal from only one half. The lack of milk removal after milk letdown could reduce MY similar to incomplete milking (Kuehnl et al., 2019). Milking also induces prolactin release (Koprowski and Tucker, 1973), which could influence MY in less frequently milked udder halves. Wall et al. (2006) reported on an examination of the role of prolactin and milking frequency finding that cows milked 2X and given prolactin in early lactation tended to produce more milk than cows milked 2X alone. In this way, prolactin release triggered by increased milkings might have influenced the 2X udder halves. In this case, the udder half differences measured in this study might underestimate the true effect of 4X milking because the less frequently milked udder half might have responded to the circulating prolactin released with additional milkings and resulted in increased milk yield.

It is important for the efficiency of milking management that the maximum benefit of increased MY due to early lactation increased MF can be achieved at even and uneven MI. As little as 3 h between milkings can still achieve the desired effects. Uneven MI may be more practical for farmers and reduce labor; early lactation cows can be milked at the beginning and end of milking sessions as opposed to alone at a separate milking time between normally scheduled milkings and still have increased production.

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Figure 1: A) Udder half milk yield (MY) difference (4X – 2X) of cows milked 4X on the right udder half and 2X on the left udder half for 20 d. B) Udder half milk fat and protein yield difference (4X – 2X) of cows milked 4X on the right udder half and 2X on the left udder half for 20 d. Grey bars indicate an even milking interval (6:6:6:6) of right udder halves (n=9) and black bars indicate an uneven milking interval (9:3:9:3) of right udder halves (n=10) during the 4X period. Left udder halves of both groups were milked on an even milking interval (12:12). Udder half production differences were determined by testing the difference from 0. * indicates a significant udder half difference from 0 at $P < 0.05$ and ** indicates a significant udder half difference from 0 at $P < 0.001$.

Figure 2: A) Udder half milk yield difference (4X- 2X) through 300 d lactation by milking interval group. Right udder halves were milk 4X for 20 d and 2X for the remainder of lactation. Left udder halves were milked 2X for all of lactation with 12:12 milking interval. The even milking interval group (circle markers with dashed line) were milked 6:6:6:6 on the right udder half (n=9) and the uneven milking interval group (square markers with solid line) were milked 9:3:9:3 on the right udder half (n=10) during the 4X milking period. * indicates a significant difference between treatment groups at the indicated time point $P < 0.05$. B) Udder half milk yield difference (4X-2X) through 300 d lactation for cows milked 4X in the right udder half for 20 d at the start of lactation and 2X for the remainder of lactation and 2X for all of lactation on the left udder half. Udder half production differences were determined by testing the difference from 0. ** indicates a significant udder half difference $P < 0.01$, * indicates a significant udder half difference at $P < 0.05$, and † indicates an udder half difference tendency at $P < 0.10$ at each time point.

Figure 3: A) Milk yield, B) fat yield, and C) protein yield udder half difference (4X- 2X) for primiparous and multiparous cows milked 4X on the right udder half for 20 d at the start of lactation and 2X for the remainder of lactation and 2X on the left udder half for all of lactation. Grey bars indicate an even milking interval (6:6:6:6) of right udder halves (n=9) and black bars indicate an uneven milking interval (9:3:9:3) of right udder halves (n=10) during the 4X period. Left udder halves of both groups were milked on an even milking interval (12:12). Udder half production differences were determined by testing the difference from 0. * indicates a significant udder half difference from 0 at $P < 0.05$ and ** indicates a significant udder half difference from 0 at $P < 0.001$.

Table 1: Area under the curve for enhanced milk and component yields through 300 DIM[1]

	Milking Frequency by Udder Half				
Component (kg)	**2X**	**4X**	**SEM**	**Difference**	**P**
Milk	5591	6099	190	508	0.01
Fat	217	242	12	25	0.02
Protein	171	186	6	15	0.01

[1]Cows milked 4X on the right udder half and 2X on the left udder half for 20 d starting at 5 DIM. At 25 DIM, right udder halves were milked 2X for the remainder of lactation.

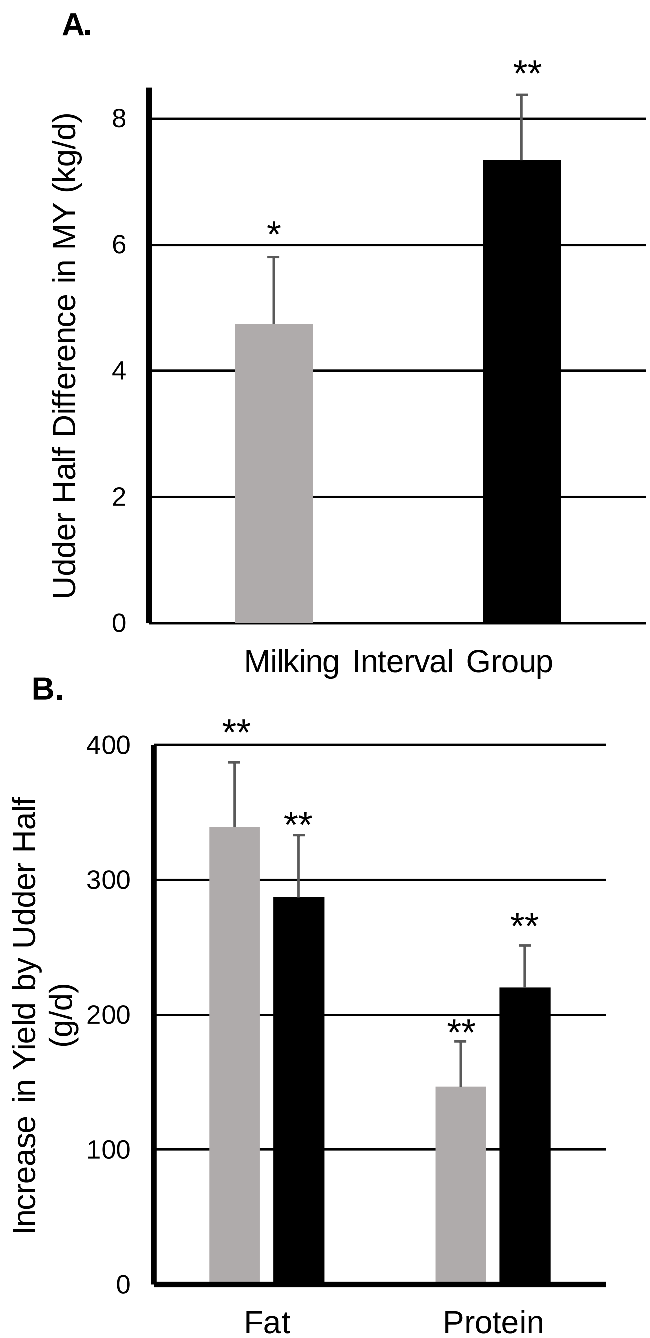
A.
**
*
8
6
4
2
0
Udder Half Difference in MY (kg/d)
Milking Interval Group
B.
**
**
**
**
400
300
200
100
0
Increase in Yield by Udder Half (g/d)
Fat
Protein

Figure 1.

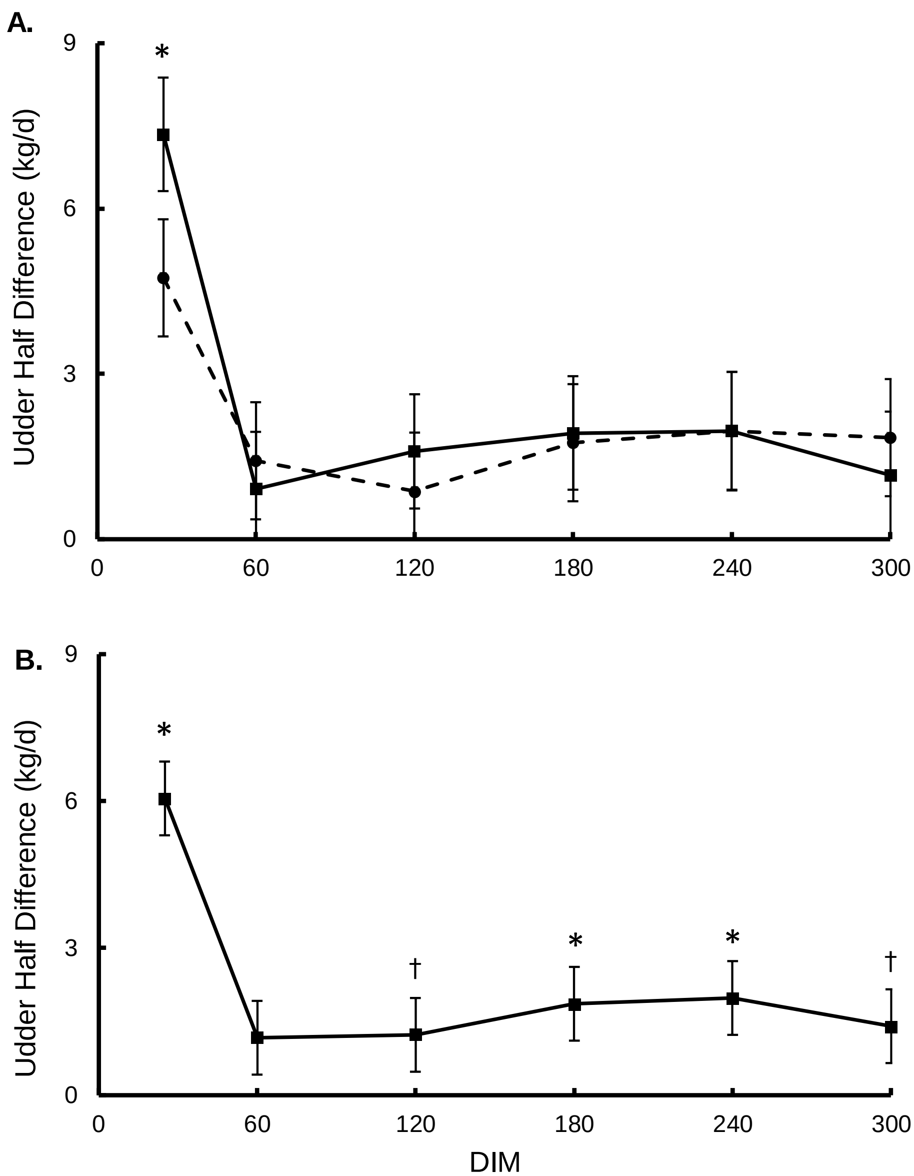
A.
Udder Half Difference (kg/d)
9
6
3
0
0
60
120
180
240
300
B.
Udder Half Difference (kg/d)
9
6
3
0
0
60
120
180
240
300
DIM

Figure 2.

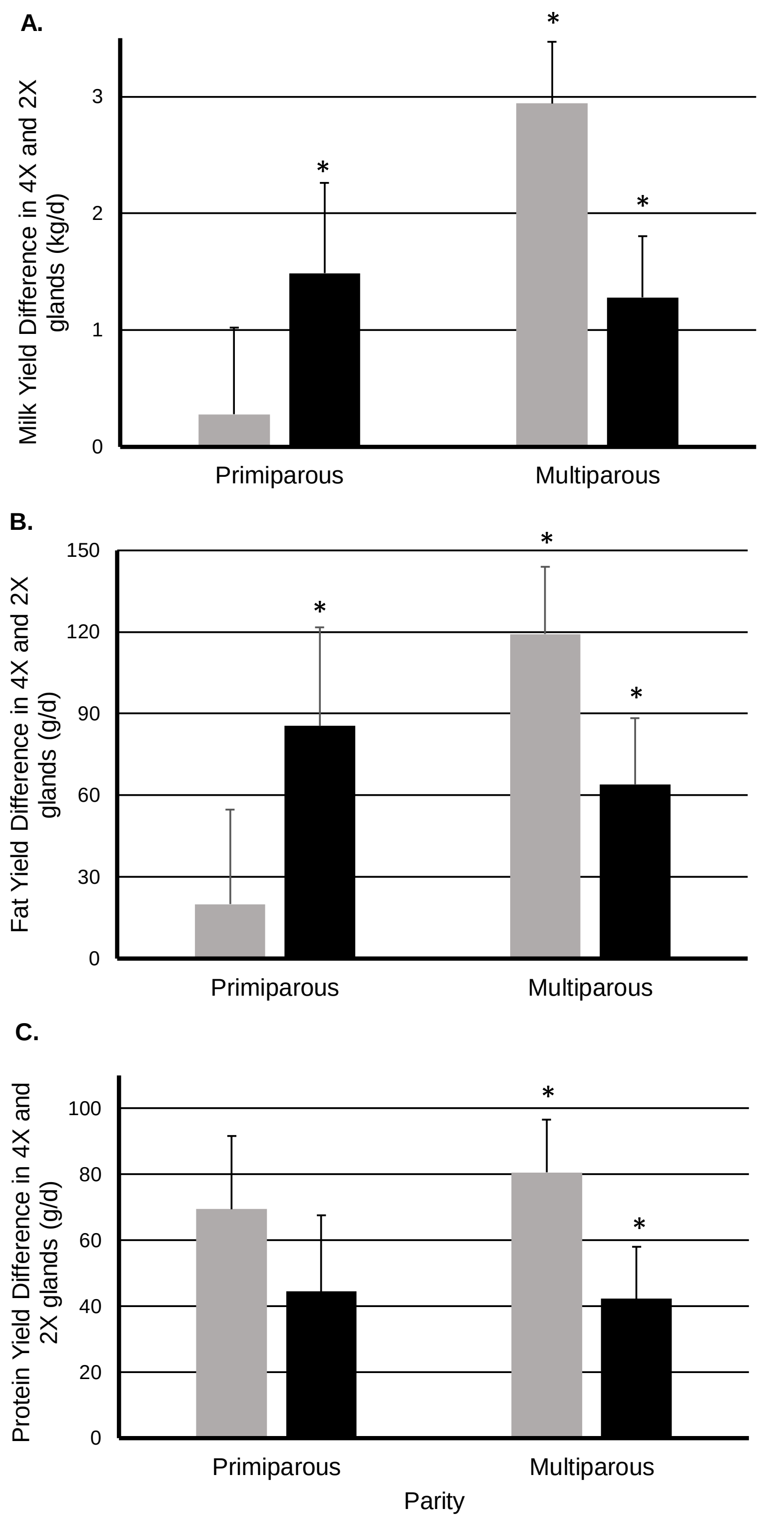
A.
Milk Yield Difference in 4X and 2X glands (kg/d)
0
1
2
3
*
*
*
Primiparous
Multiparous
B.
Fat Yield Difference in 4X and 2X glands (g/d)
0
30
60
90
120
150
*
*
*
Primiparous
Multiparous
C.
Protein Yield Difference in 4X and 2X glands (g/d)
0
20
40
60
80
100
*
*
Primiparous
Multiparous
Parity

Figure 3.