CHARACTERIZATION OF SENSOR AND NON-SENSOR COW, HERD MANAGEMENT, AND ENVIRONMENTAL DATA AND USE OF MACHINE LEARNING ALGORITHMS FOR PREDICTION OF PREGNANCY IN DAIRY CATTLE

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by

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ABSTRACT

The overarching objective of the research presented in this thesis was to characterize associations between cow, herd, and environmental data with insemination outcome, and develop machine learning algorithms (MLA) to predict the outcome of the first service (FS) after calving in lactating dairy cows. The specific objective of the study presented in Chapter I was to compare patterns of multiple cow behavioral, physiological, and performance parameters collected by automated sensors before insemination for cows that became pregnant or not at FS. A secondary objective was to explore associations between pregnancy outcome at FS with previous gestation and early lactation performance and events, and with environmental conditions before insemination. An observational retrospective cohort study was conducted using data collected at a commercial dairy farm. Daily values for milk yield, milk components percent and yield, rumination and eating activity, physical and walking activity, resting time and bouts, body temperature, milk conductivity, and body weight collected by wearable and non-wearable sensors from -14 to 56 d after calving for 932 primiparous and 2,070 multiparous cows with a FS pregnancy outcome were available for analysis. Daily data were summarized as the average of seven periods of 4 to 7 d long from -14 to 56 d after calving and from -27 to -11, -10 to -3, -2 to -1 d relative to timed AI for FS. The most notable differences observed for primiparous cows were greater milk yield, milk components yield, and fewer lying bouts per day for pregnant than non-pregnant cows. For the multiparous cow group, non-pregnant cows produced more milk and milk fat, had greater body temperature, more activity, more resting time, and had greater body weight changes after calving than pregnant cows. Associations of different strength and direction between FS outcome with previous gestation and previous and current lactation features, events, and performance for primiparous and multiparous cows were observed. Substantial variability

between parity groups for the direction and magnitude of differences between pregnant and non-pregnant cows warrants use of parity either as a model predictor, or the development of parity-specific models for predicting FS outcome of lactating dairy cows. Chapter II of this thesis presents the development and performance of multiple MLA for predicting FS outcome using data presented in Chapter I. Decision Trees, Support Vector Machine, Logistic Regression, and Extreme Gradient Boosting models were built and evaluated for primiparous and multiparous only and for both parities combined. Overall, we observed that these MLA trained with a combination of automated sensor cow behavioral, physiological and performance data, as well as herd outcomes and environmental data presented a wide range of performance. The best performing algorithms (i.e., most performance metrics values in the 90 to 95% range) were those for primiparous cows using Support Vector Machine and Logistic Regression models. Overall, the performance of MLA for multiparous cows was poor (i.e., all performance metrics <70%) considering the implications of predictions for practical application. In conclusion, different supervised MLA trained with a combination of cow parameters collected by automated wearable and non-wearable sensors, herd outcomes, and farm environmental conditions, presented large variation in performance despite using the same input data and the same algorithms. Large variation in algorithm performance due to parity suggested that different models might have to be developed for predicting FS outcome for primiparous and multiparous cows. Further research is needed to identify a combination of predictors, methods to summarize input data from predictors, and develop procedures to train MLA that yield the level of performance required for practical use of algorithms in commercial farms.

BIOGRAPHICAL SKETCH

German Enrique Granados Bustos was born in Bogota, Colombia on July 8, 1979. In February 2006 he graduated with a Bachelor of Science degree in Animal Science from the Universidad Nacional de Colombia. In September 2015 he joined Cornell University as a research intern and worked for six months in the Department of Animal Science under the supervision of Dr. Julio Giordano. In June 2017 he continued working for one year as a research scholar in Dr. Daryl Nydam's group at the Quality Milk Production Services. In this position German conducted laboratory and field work. In July 2018, German joined the Dairy Cattle Biology and Management Laboratory in the Department of Animal Science at Cornell University and began his Master of Science under the supervision of Dr. Julio Giordano. German's work focused on the characterization of sensor and non-sensor cow, herd management, and environmental data and use of machine learning algorithms for prediction of pregnancy in dairy cattle.

Liliana, Sara, & Emma.

The engine, fuel, and soul of all my projects.

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GENERAL INTRODUCTION

Dairy farms allocate resources for optimizing reproductive management of lactating dairy cows because herd reproductive performance influences farm profitability and sustainability through direct effects on milk production efficiency, the herd exit dynamics, availability of replacements, and reproductive program implementation cost (De Vries, 2006; Meadows et al., 2005; Giordano et al., 2011). Notably, recent improvements in herd management and technology implementation enhanced overall reproductive performance of lactating dairy cows through effects on reproductive outcomes such as days to insemination, service rate, and pregnancies per AI (**P/AI**) (Carvalho et al., 2018; Cardoso Consentini et al., 2021; Rial et al., 2022). These improvements have also generated new opportunities for maximizing dairy herd performance and profitability through targeted reproductive management (Giordano et al., 2022). Increasing the economic value of offspring and reducing replacement costs through use of sexed semen, beef semen, or embryo transfer (Wilson et al., 2005; DeJarnette et al., 2011), optimizing reproductive performance and profitability by manipulating timing of first service (Stangaferro et al., 2018), inseminating or not inseminating a cow based on expected profitability, and targeted use of hormonal therapies to increase fertility (Giordano et al., 2015; Wijma et al., 2016; Zolini et al., 2019), improve management (Bartolome et al., 2005; Wijma et al., 2017, 2018), or both (Perez et al., 2020) are examples of targeted reproductive management approaches. The suite of strategies available will likely expand as the dairy industry adapts to new market conditions, develops and adopts novel technologies, makes better use of data, and continues adjusting to increasing labor constraints.

Implementation of targeted reproductive management interventions for dairy cattle depends upon the ability to identify cows with different reproductive potential before key management interventions such as insemination, pregnancy testing, hormonal treatments, and culling are implemented. Therefore, an important new frontier in reproductive management decision-making is predicting the probability of pregnancy before insemination. To make accurate predictions, it is paramount to improve our understanding of intrinsic and extrinsic sources of variation of cow fertility and characterize associations and interactions between biological, management, and environmental factors with cow fertility. Characterizing the direction and strength of associations between potential predictors and fertility can help inform predictive models for identifying subgroups of cows with different reproductive potential. In this regard, a myriad of studies reported that cow-related factors and features such as age at calving, parity, experiencing adverse health conditions, milk volume and components yield, metabolic status, body condition score, body weight, and many others were positively or negatively associated with insemination outcomes (Rutten et al., 2016; Ghiasi et al., 2016; Caraviello et al., 2006). Moreover, external factors and conditions that directly or indirectly affect cows, such as management practices (Rensis et al., 2015; Vercouteren et al., 2015) and environmental conditions (López-Gatius., 2012; Djelailia et al., 2020; Wolfenson et al., 2020), have also been associated with insemination success.

Many sources of variation of cow fertility, and associations between predictors and insemination outcome are well-known and characterized (López-Gatius et al., 2012; Hempstalk et al., 2015; Rutten et al., 2016; Cockburn, 2020). Conversely, many others have not yet been described or have been poorly characterized because of previous limitations to generate and evaluate data for large numbers of cows under commercial farm conditions. This knowledge gap could be addressed by using novel sensor-based technologies that automatically collect and summarize cow behavioral, physiological, and performance parameters of cows. Sensor technologies enable data collection from more cows and their environment, in real time, at more frequent intervals, without cow manipulation, and at lower cost. Other sensor and non-sensor technologies used to monitor and record dairy herd management practices and environmental conditions are also readily available on commercial farms. These multiple technologies could be leveraged to improve our understanding of factors known to affect fertility of individual herds and cows, and to discover novel associations and interactions among cow, herd management, and environmental parameters with reproductive success.

Once associations between predictors and fertility are characterized, prediction models of cow fertility can be explored. Previous efforts focused on predicting fertility of individual AI services in lactating dairy cows with varying levels of success (Hempstalk et al., 2015; Rutten et al., 2016). These studies included a wide range of farm, herd, environmental, and cow level data. However, most of the cow level data included calving, health, and reproductive events, or aggregated production and reproductive performance data collected sparsely in previous lactations, or the lactation cycle of interest such as that obtained from dairy herd management software or monthly herd testing programs (Caraviello et al., 2006, Shahinfar et al., 2014). Conversely, no studies included data from behavioral, physiological, and performance parameters collected by automated wearable and non-wearable sensors for dairy cows. These sensor-generated parameters might offer additional predictive value as they might directly or indirectly reflect dynamic changes of dairy cow health, physiological, reproductive, and well-being status at key time points before insemination. Moreover, data collected through continuous monitoring of cow parameters might be more predictive as it enables capturing variation in behavioral and physiological data with time granularity ranging from minutes to days, and milk production data for individual milkings.

A key step in the development of prediction tools to inform decision-making for reproductive management is the dentification of modeling approaches capable of generating accurate predictions despite the complexity of the underlying data and associations between predictors and outcomes of interest. In this regard, predicting the outcome of individual inseminations using large, integrated dairy cow, herd, and environmental data might require more powerful analytical tools than traditional statistical methods. To this end, machine learning algorithms (**MLA**) might be an alternative to traditional statistical methods that rely on parametric functions because MLA are more effective at generating predictions using large, complex, and heterogeneous datasets (Cockburn, 2020). Moreover, MLA might uncover complex relationships in the data and can generate predictions despite missing observations for some timepoints for some predictors (Neethirajan, 2020).

Thus, the overarching objectives of the research presented herein were to characterize associations between cow, herd, and environmental data with the outcome of individual inseminations, and develop and evaluate MLA to predict the outcome of the first service (**FS**) after calving in lactating dairy cows. Specifically, the primary objective of the research presented in Chapter I was to compare the patterns of multiple cow behavioral, physiological, and performance parameters before insemination for cows that became pregnant or not at FS. A secondary objective was to evaluate the association between pregnancy outcome at FS with previous gestation and early lactation performance and events, as well as environmental conditions before insemination. Chapter II of this thesis presents the development and performance of multiple MLA trained for predicting FS outcome using all data collected for this study.

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CHAPTER I

ASSOCIATIONS BETWEEN SENSOR AND NON-SENSOR COW, HERD MANAGEMENT, AND ENVIRONMENTAL DATA WITH PREGNANCY OUTCOME AFTER FIRST SERVICE IN LACTATING DAIRY COWS

INTRODUCTION

A primary focus of most research with automated dairy cattle monitoring technologies has been the exploration of associations between dairy cow fertility and behavioral, physiological, and performance parameters monitored by sensors during the periestrus and estrus period (Reith et al., 2014; Schilkowsky et al., 2021; Giordano et al., 2022). As cows experience dramatic physical activity, rumination, eating behavior, and milk yield changes around estrus, monitoring these parameters has been used for exploring associations between estrus event features and the probability of pregnancy after insemination (Madureira et al., 2015; Cerri et al., 2021; Tippenhauer et al., 2021). Remarkably, less emphasis has been placed on exploring potential associations between dairy cow reproductive outcomes with patterns and features of behavioral, physiological and performance parameters that are known indicators of the health, metabolic status, and well-being of cows during the peripartal period and early lactation (Antanaitis et al., 2018; Stangaferro et al., 2019). As proxies of cow health, metabolic status, and well-being, the dynamics of sensor-monitored parameters before insemination might be associated with and be predictive of the outcome of artificial insemination (**AI**) services in lactating dairy cows (Giordano et al., 2022). The dynamics of these sensor-monitored parameters might also be associated with cow fertility because their patterns reflect the response of cows to herd management practices and environmental conditions (Bello et al., 2012; Cockburn, 2020;

Neethirajan, 2020). Therefore, further research is needed to identify and characterize in detail potential associations between sensor-generated data in the peripartum and early lactation period and outcomes of value for reproductive management decision-making. A better understanding of these associations might facilitate the design and implementation of targeted reproductive management strategies for dairy cattle.

Thus, the overarching goal of the research presented in this chapter was to characterize the association between multiple potential predictors of cow fertility and the outcome of first service (**FS**) in lactating dairy cows. Specifically, the primary objective was to compare the pattern of behavioral, physiological, and performance parameters collected by automated sensors for cows that became pregnant or not at FS. A secondary objective was to evaluate the association between pregnancy outcome at FS and previous gestation and early lactation performance and events, as well as environmental conditions before insemination.

EXPERIMENTAL PROCEDURES

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Cornell University.

Cows and General Management

This observational retrospective cohort study was conducted using data collected at a commercial dairy farm in Tompkins County, New York from January of 2018 to April of 2020. During the study period the average number of milking cows was 1,376 (range: 1,296 to 1,455) and the average number of dry cows was 207 (range: 149 to 262). Cows were housed in free-stall barns with concrete flooring and six or eight rows of stalls. Fans and sprinklers were present above the feeding lane. A TMR was delivered once daily, and cows had ad-libitum access to feed and water. Cows were milked in a 60-stall rotary parlor three times a day at ~8 h intervals.

Cows were moved from the far-off dry pen to a close-up pen approximately 10 d before calving. For cows in the close-up pen, farm personnel conducted visual observation every hour to identify cows with impeding signs of calving including increased activity, restlessness, tail raising, dripping milk, and the visualization of the amniotic sac. Cows with obvious signs of calving were moved to an adjacent bedded pack with straw bedding to facilitate calving and monitor calving progression. Calving event information was recorded by farm personnel into dairy herd management software (DairyComp305, ValleyAg software, Tulare, CA). After calving cows were moved to a milking cow pen where they remained for one day or three milkings. Thereafter, cows were moved to pens of primiparous or multiparous cows based on their lactation number.

After calving cows were monitored daily by trained personnel for detection of clinical health disorders. Visual observation and clinical examination of cows was conducted after milking with cows restrained in self-locking headgates or in a palpation rail. Clinical examination consisted of evaluation of general appearance and attitude, presence of fetal membranes and uterine content, feet appearance, abdominal auscultation, and manure appearance. Mastitis was evaluated at every milking by fore-stripping during milking. Cows with a putative case of clinical mastitis were moved to a hospital pen.

Reproductive Management and Study Eligibility Criteria

Cows received their first service by timed artificial insemination (**TAI**) after synchronization of ovulation with the Double-Ovsynch protocol (GnRH, 7 d later $PGF_{2\alpha}$, 3 d later GnRH, 7 d later $PGF_{2\alpha}$, 56 h later GnRH and 16 to 18 h TAI) as described in Souza et al. (2008) and modified by Wiltbank et al. (2015). Primiparous cows received TAI at 84 ± 3 DIM (n = 932; pregnancies per AI (**P/AI**) = 62.3%) whereas multiparous cows received TAI at 67 ± 3 (n = 1,823; P/AI = 48.7%) or 81 ± 3 DIM (n = 247; P/AI = 38.9%). Multiparous cows received TAI at 81 ± 3 DIM if had a record of retained placenta and metritis after calving. Pregnancy diagnosis (**PD**) was conducted at 32 ± 3 d after AI and re-confirmation of pregnancy at 63 ± 3 d after AI by transrectal ultrasonography (Easi-Scan, IMV imaging, Rochester, MN).

Criteria for inclusion in the study were the availability of a first service outcome after following the farm standard operating procedures for reproductive management and having data for at least half of the variables used to explore associations between predictors and the outcome of interest. During the study period a total of 4,239 calvings were recorded. Out of these calving events, 837 cows did not have a first service outcome because left the herd before first AI and 400 cows with a first service outcome did not meet the inclusion criteria for the study. Reasons for data exclusion for first service outcomes included lack of compliance with the synchronization of ovulation protocol for TAI (n = 79), AI at detected estrus (n = 104), and AI with no outcome (n = 217). Thus, the total number of cows with a first service outcome available for analysis was 3,002 with 932 from primiparous and 2,070 from multiparous cows. Because the period of eligibility for inclusion in the study was sufficient for some cows to have a first service outcome in more than one lactation, 1,135 cows provided data for more than one lactation.

Behavioral, Physiological and Performance Parameters Data Collection and Summarization

Upon entry to the close-up pen, cows received a neck-mounted (placed on the left side) 3D accelerometer (n = 2,016; Silent Herdsman, Afimilk Ltd., Kibbutz Afikim, Israel) and a reticulum-rumen bolus sensor tag (n = 1,081; Smaxtec, Graz, Austria). Neck-mounted sensors were removed at ~30 DIM or at herd removal due to sale or death. Behaviors monitored by the neck-mounted tag included eating and rumination activity reported hourly in arbitrary units (**AU**), single .csv files were received from the company, we added the units from the hourly values to get a daily total for eating and rumination. The reticulum-rumen bolus tag monitored reticulum-rumen temperature reported in degrees Celsius and physical activity reported in AU. Both temperature and physical activity were reported every 10 min; this information was retrieved in one .csv file per cow from the system API provided by the company.

After calving, all cows received a leg-mounted sensor tag (AfiAct II, Afimilk Ltd., Kibbutz Afikim, Israel) attached to the right rear leg. Behaviors monitored and recorded by this tag included walking activity (number of steps) and resting behavior. The latter was reported as rest bouts (number of lying bouts per day), and total resting time per day (minutes).

After calving, live weight in kilograms was recorded daily after each milking by a walk over scale (AfiWeight, Afimilk Ltd., Kibbutz Afikim, Israel). As more than one value per day may have been collected for some cows, a BW daily value is the average of all values captured in a day.

At every milking, an inline milk meter (Afimilk MPC, Afimilk Ltd., Kibbutz Afikim, Israel) and milk analyzer (Afilab, Afimilk Ltd., Kibbutz Afikim, Israel) recorded milk yield (g), milk components [fat (%), protein (%), lactose (%)], and milk conductivity (mmHo). Values for fat yield (kg), protein yield (kg), lactose yield (kg) were calculated by multiplying milk yield (kg) by milk components [fat (%), protein (%), lactose (%)]. All data from AFI systems was initially received from the company; afterwards, we exported the data in .dif files that were transformed in .xlsx files.

Data for behavioral, physiological, and performance parameters from -14to 56 d after calving were summarized in daily values regardless of the frequency of data collection by the different sensor systems [milk yield (accumulated), milk components % (average), milk components yield (accumulated), fat-to-protein ratio (average), total rumination and eating activity (count of AU), body temperature (average), milk conductivity (average), physical activity (count of AU), walking activity (count of steps), rest time (accumulated), and lying bouts (count of events), body weight (average)]. Thereafter, daily data were summarized as the average of the following time periods (n = 7) in relationship to calving: -14 to -8, -7 to -3, -2 to 2, 3 to 7, 8 to 14, 15 to 28, 29 to 56 d. Data were also collected and summarized in periods (n= 3) during synchronization of ovulation as for the period from -14 to 56 DIM as follows: -27 to -11, -10 to -3, -2 to -1 d before timed AI.

To reduce the potential influence of outliers on results, values above or below the mean ± 3 standard deviations for each parameter of interest were removed. The mean and standard deviation was calculated using the entire dataset for each parameter of interest. For reticulo-rumen temperature, all values below 37 ºC were removed. Summary statistics and the number of records that were capped for each parameter of interest are presented by parity group in Tables 1 and 2, respectively.

Table 1. Mean, standard deviation, minimum, maximum, number of outliers above and below the group mean, and total number of daily values for primiparous cows.

	Item						
Parameter[1]	Mean	SD	Min	Max	Below mean - 3 SD (n)	Above mean + 3 SD (n)	Total daily records (n)
Milk (kg)	33.1	9.06	5.38	65.5	0	495	47,457
Fat (%)	3.91	0.57	2.00	5.68	634	13	47,457
Fat (kg)	1.29	0.05	0.11	3.72	634	13	47,457
Protein (%)	3.00	0.24	2.17	3.73	30	109	47,457
Protein (kg)	0.99	0.02	0.12	2.44	30	109	47,457
Lactose (%)	4.70	0.20	3.86	5.58	577	2	47,457
Lactose (kg)	1.56	0.02	0.21	3.65	577	2	47,457
Conductivity (mmHo)	8.95	0.81	4.40	13.8	601	16	47,457
Walking (steps/h)	210	56.3	21	406	1,302	0	47,373
Rest Bout (#)	11.7	4.29	3.00	26.0	277	0	47,373
Rest time (min)	551	136	109	1,033	51	55	47,373
Weight (kg)	551	48.8	358	892	1	5	30,682
Activity (AU)	4.61	1.19	0.82	8.01	86	312	19,888
Eating (AU)	517	115	100	869	98	477	34,870
Rumination (AU)	385	111	23.3	784	91	462	34,870

[1]Data obtained from cow attached (activity, temperature, eating and rumination) and non-attached (milk production, components, and body weight) sensors.

Table 2. Mean, standard deviation, minimum, maximum, number of outliers above and below the group mean, and total number of daily values for multiparous cows.

	Item						
Parameter[1]	Mean	SD	Min	Max	Below mean - 3 SD (n)	Above mean + 3 SD (n)	Total daily records (n)
Milk (kg)	45.1	11.6	5.38	78.9	640	10	98,529
Fat (%)	3.93	0.61	2.00	5.68	44	1,029	98,529
Fat (kg)	1.77	0.07	0.11	4.48	44	1,029	98,529
Protein (%)	2.94	0.26	2.17	3.73	54	556	98,529
Protein (kg)	1.33	0.03	0.11	2.94	54	556	98,529
Lactose (%)	4.69	0.23	3.86	5.58	1,397	1	98,529
Lactose (kg)	2.12	0.03	0.21	4.40	1,397	1	98,529
Conductivity (mmHo)	9.12	1.14	4.40	13.8	3,888	52	98,529
Walking (steps/h)	180	54.3	1.00	406	1,649	0	108,341
Rest Bout (#)	9.39	3.64	1.00	26.0	206	0	108,341
Rest time (min)	579	157	109	1,033	131	440	108,341
Weight (kg)	677	72.3	408	892	0	295	52,227
Activity (AU)	4.42	1.11	0.81	8.01	183	1,108	51,675
Eating (AU)	476	120	100	869	280	1,457	64,926
Rumination (AU)	402	124	23.3	784	218	1,376	64,926

[1]Data obtained from cow attached (activity, temperature, eating and rumination) and non-attached (milk production, components, and body weight) sensors.

Environmental Data Collection

Ambient temperature and relative humidity inside and outside of freestall barns was recorded every 10 min using a HOBOnet Field Monitoring Systems (Onset Computer Corporation, Bourne, MA), this data was exported in .csv files from the company's website and averaged into daily values.

Based on these data temperature and humidity index (**THI**) was calculated using the formula described in (Mader, et al. 2006):

$$THI = (0.8 \text{ x } AT) + ((RH/100) \text{ x } (AT-14.4)) + 46.4$$

where AT is the ambient temperature (°C), and RH is the relative humidity (%).

Average daily THI values were estimated and organized by date. Thereafter, THI daily values were matched by date for each day a cow was included in the dataset for analysis.

Calving Features and Health Events Data Collection

All data for calving events, health events, and previous lactation or heifer period data were extracted from dairy herd management software (Dairy Comp 305, Valley Ag Software, Tulare, CA).

Calving event data collected for the lactation that provided the first service outcome included: date of calving, cow age at first calving (n = 2,762 had and 240 did not have a record), calving ease [1 = no assistance (n = 2,949); 2 = slight problem (n = 30); 3 = needed assistance (n = 7); 4 = considerable force (n = 12); 5 = extreme difficulty (n = 4)], number of calves born [singleton (n = 2,900); twins (n = 102)], calf sex [female (n = 1,723); male (n = 1,279)], and whether the calf born dead or alive [alive (n = 2,897); dead (n = 105)].

The occurrence and DIM at diagnosis of metritis (n = 338), retained placenta (n = 152), ketosis (n = 194), indigestion (n = 12), displaced abomasum (n = 94), mastitis (n = 213), lameness (n = 302), milk fever (n = 11), pneumonia (n = 15), and events of major physical injuries (n = 12) were collected. Information was collected only for events that occurred from 0 to 56 d after calving. Health disorders were grouped as uterine disease (i.e., cows with retained placenta, metritis, or both; n = 400), metabolic and digestive [i.e., cows with ketosis, displaced abomasum, indigestion, or more than one of these disorders (n = 256)]; and other [pneumonia, milk fever, and injuries; (n = 37)]. Cows with mastitis (n = 216) and lameness events (n = 321) were not grouped. Thereafter, a dichotomous variable (i.e., **DZ**; 0 = no disease, 1 = disease) was created to form groups of cows that had at least one (n = 956) versus no health disorders (n = 2,046) recorded.

Previous Lactation Production and Reproductive Performance Data

Reproductive outcomes retrieved were number of inseminations, days open (multiparous cows only), days dry (multiparous cows only), calving interval (multiparous cows only) and gestation length. Data were retrieved for the non-lactating period for primiparous cows and the lactation preceding the lactation from which the first service TAI outcome was included in the analysis for multiparous cows. Previous lactation (**PL**) whole lactation milk, fat, and protein yield, and milk yield adjusted to 305 d of lactation (**M305**) were collected for multiparous cows (n = 1,861).

Statistical Analysis

The study followed an observational retrospective cohort design with a convenience sample.

All data analyses were conducted separately for primiparous and multiparous cows, but the same variables were offered to models for primiparous and multiparous cows unless otherwise stated.

Continuous outcomes with repeated measurements including daily milk yield, milk components (fat, protein, lactose) yield and percent, fat-to-protein ratio, milk conductivity, walking activity, rest bouts, total resting time per day, and absolute BW were evaluated from calving until 56 d after calving with data grouped in periods of time before and after calving as described. Reticulum-rumen temperature, and physical activity, were evaluated from -14 until 56 d after calving. Eating and rumination activity, were evaluated from -14 until 28 d after calving. Normality of the data was evaluated for all variables using the Shapiro-Wilk statistic and graphical methods generated with PROC UNIVARIATE in SAS (SAS v9.4, SAS Institute, Cary, NC). No data transformations were necessary because all variables were normally distributed. Data for these outcomes were analyzed by ANOVA with repeated measurements using PROC MIXED of SAS. Models for each outcome of interest included FS outcome group (i.e., pregnant vs. non-pregnant), time (i.e., time periods in relationship to calving), and the pregnancy status group-by-time interaction as explanatory variables. Disease group (i.e., no disease vs. at least one health disorder from calving up to 56 d after calving) and season of calving (cold vs. warm) were forced in all models to control for the effect of season and health disorders. The cold season was defined as the period from September 21 to June 20 and the warm season from June 21 to September 20. For multiparous cows, the effect of parity (i.e., second vs. third and greater parity) was forced in all models. Cow within pregnancy group was included as a random effect in all models. Cow was the subject of repeated measurements, and all models were run using an autoregressive (AR-1) covariance structure. The ddfm option of PROC MIXED was used to improve the statistical performance of the t-test and F-test. The Least Significant Difference (**LSD**) post hoc mean separation test was used to determine differences between groups of means.

Similar analyses were conducted for variables available from -27 to -1 d after first service TAI. Available data were daily milk yield, milk components yield and percent, fat-to-protein ratio, milk conductivity, walking activity, rest bouts, total resting time per day, absolute BW, reticulum-rumen temperature, and physical activity.

Continuous outcomes with no repeated measurements including previous lactation production (total milk, total fat, and total protein yield, and milk projected to 305 d of lactation) and reproductive outcomes (number of inseminations, days open, days dry, calving interval and gestation length), and BW change after calving were analyzed by ANOVA using PROC MIXED of SAS. Data for BW consisted of percent change between 3 d after calving and the nadir for BW, percent change from the BW nadir to 56 d after calving, and total percent change from 3 to 56 d after calving. Models for each outcome of interest included group (e.g., pregnant vs. non-pregnant) as explanatory variable. Disease group and season of calving were forced to control for their effect on the outcome of interest. Parity group was forced for multiparous cow group models as described.

The association between BW change group after calving (i.e., loss, no change, or gain) for the three time periods evaluated, calving features (i.e., singleton vs. twin births, stillbirth vs. no stillbirth, and calf sex), and disease occurrence up to 56 d after calving (i.e., no disease or at least one health disorder) and P/AI to first service was analyzed using logistic regression with the GLIMMIX procedure of SAS fitting a binary distribution. Disease group (except in models to evaluate the effect of disease group) and season of calving (cold vs. warm) were forced in all models whereas parity was forced in models for multiparous cows.

All explanatory variables and their interactions were considered significant if $P \leq 0.05$, while $0.05 < P \leq 0.10$ was considered a tendency. All outcomes are presented as LSM ± SEM obtained with the LSMEANS option of the MIXED and GLIMMIX procedures of SAS.

RESULTS

Behavioral, Physiological and Performance Parameters for Primiparous Cows

Milk and component yields. Milk yield from 0 to 56 d after calving (Figure 1A) differed over time ($P < 0.001$), was greater ($P = 0.02$) for pregnant (26.2 ± 0.23 kg/d) than non-pregnant cows (25.4 ± 0.28 kg/d), but there was no group by time interaction ($P = 0.38$). In addition, there was an effect of season of calving ($P < 0.001$), whereby cows that calved during the cold season had greater milk yield (26.4 ± 0.20 kg/d) than cows that calved during the warm season (25.2 ± 0.32 kg/d). Cows with health disorders (25.1 ± 0.32 kg/d) recorded up to 56 d after calving had lesser ($P < 0.001$) milk yield than cows without health disorders (26.6 ± 0.20 kg/d).

Butterfat yield from 0 to 56 d after calving (Figure 1B) differed over time ($P < 0.001$), was greater ($P = 0.02$) for pregnant (1.08 ± 0.01 kg/d) than non-pregnant cows (1.05 ± 0.01 kg/d), but there was no group by time interaction ($P = 0.89$). In addition, there was an effect of season of calving ($P = 0.03$), whereby cows that calved during the cold season had greater butterfat yield (1.08 ± 0.01 kg/d) than cows that calved during the warm season (1.05 ± 0.01 kg/d). Cows with health disorders (1.05 ± 0.01 kg/d) recorded up to 56 d after calving tended to have lesser ($P = 0.06$) butterfat yield than cows without health disorders (1.08 ± 0.01kg/d).

Protein yield from 0 to 56 d after calving (Figure 1C) differed over time (*P* < 0.001), was greater (*P* = 0.04) for pregnant (0.78 ± 0.01 kg/d) than non-pregnant cows (0.76 ± 0.01 kg/d), but there was no group by time interaction (*P* = 0.60). In addition, there was an effect of season of calving (*P* < 0.001), whereby cows that calved during the cold season had greater protein yield (0.79 ± 0.01 kg/d) than cows that calved during the warm season (0.74 ± 0.01 kg/d). Cows with health disorders (0.74 ± 0.01 kg/d) recorded up to 56 d after calving had lesser (*P* < 0.001) protein yield than cows without health disorders (0.79 ± 0.01kg/d).

Lactose yield from 0 to 56 d after calving (Figure 1D) differed over time (*P* < 0.001), was greater (*P* = 0.03) for pregnant (1.23 ± 0.01 kg/d) than non-pregnant cows (1.20 ± 0.01 kg/d) but there was no group by time interaction (*P* = 0.58). In addition, there was an effect of season of calving (*P* = 0.04), whereby cows that calved during the cold season had greater lactose yield (1.23 ± 0.01 kg/d) than cows that calved during the warm season (1.20 ± 0.01 kg/d). Cows with health disorders (1.18 ± 0.01 kg/d) recorded up to 56 d after calving had lesser (*P* < 0.001) lactose yield than cows without health disorders (1.25 ± 0.01 kg/d).

Milk yield from -27 to -1 days before timed AI (Figure 2A) differed over time (*P* < 0.01), but there was no difference (*P* = 0.18) for pregnant (37.4 ± 0.32 kg/d) and non-pregnant cows (36.8 ± 0.39 kg/d), and there was no group by time interaction (*P* = 0.83). In addition, there was an effect of season of calving (*P* < 0.001), whereby cows that calved during the cold season had greater milk yield (38.2 ± 0.28 kg/d) than cows that calved during the warm season (36.1 ± 0.43 kg/d).

Butterfat yield from -27 to -1 days before TAI (Figure 2B) differed over time (*P* < 0.001), pregnant cows (1.38 ± 0.01 kg/d) tended (*P* = 0.10) to have greater butterfat yield than non-pregnant cows (1.36 ± 0.01 kg/d), but there was no group by time interaction (*P* = 0.31).

Protein yield from -27 to -1 days before TAI (Figure 2C) differed over time ($P < 0.01$), but there was no difference ($P = 0.24$) for pregnant (1.13 ± 0.01 kg/d) and non-pregnant cows (1.12 ± 0.01 kg/d), and there was no group by time interaction ($P = 0.89$). Cows with health disorders (1.11 ± 0.01 kg/d) recorded up to 56 d after calving tended to have lesser ($P = 0.08$) protein yield than cows without health disorders (1.14 ± 0.01 kg/d).

Lactose yield from -27 to -1 days before TAI (Figure 2D) tended to differ over time ($P = 0.07$). In addition, pregnant cows (1.75 ± 0.01 kg/d) tended to have greater lactose yield ($P = 0.08$) than non-pregnant cows (1.71 ± 0.02 kg/d) but there was no group by time interaction ($P = 0.78$). There was an effect of season of calving ($P < 0.001$), whereby cows that calved during the cold season had greater lactose yield (1.78 ± 0.01 kg/d) than cows that calved during the warm season (1.69 ± 0.02 kg/d).

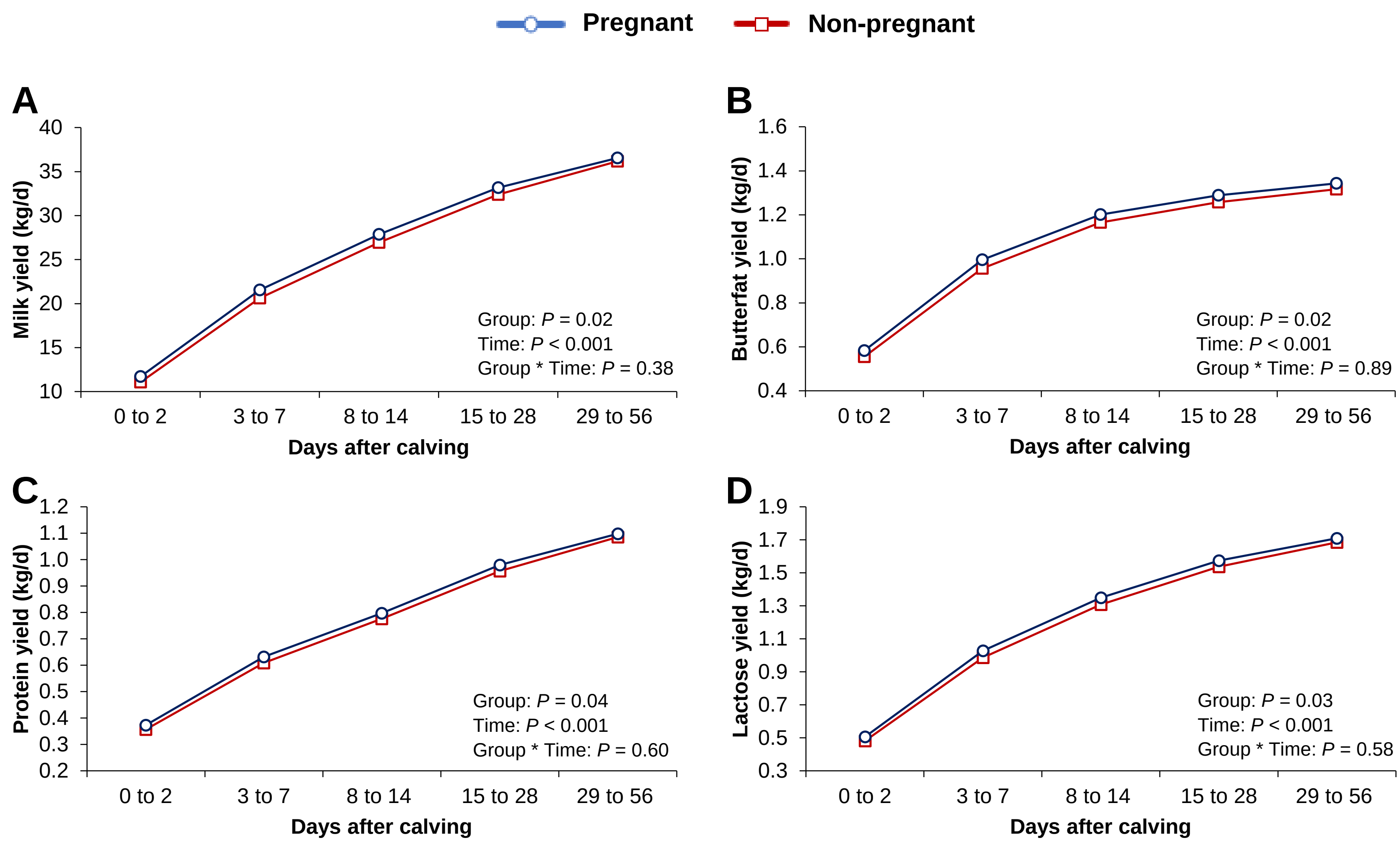

Figure 1. Milk (A), butterfat (B), protein (C), and lactose (D) yield from 0 to 56 d after calving for primiparous cows that were pregnant (n = 566) or non-pregnant (n = 330) after first service. Values are presented as LSM ± SEM.

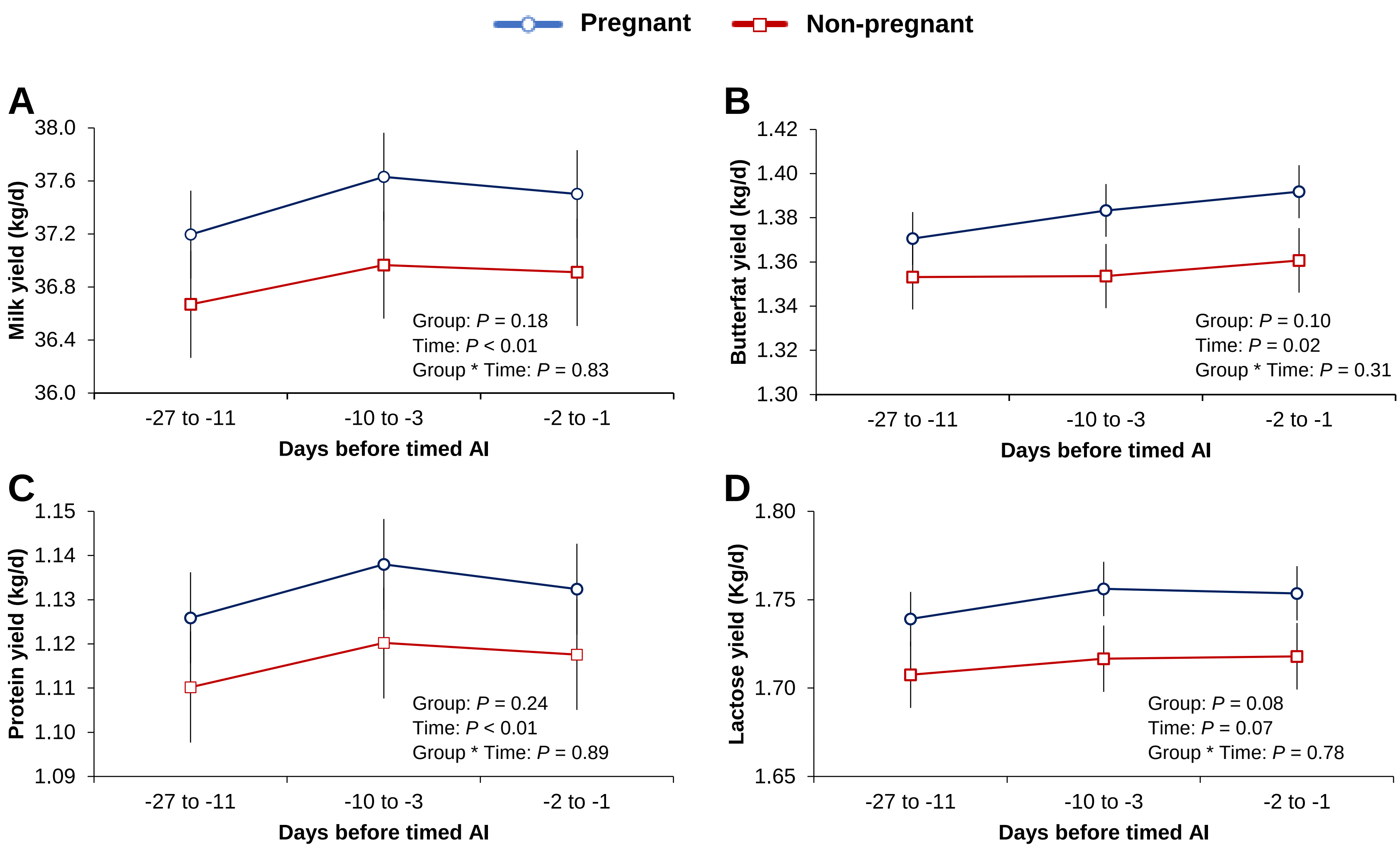

Figure 2. Milk (A), butterfat (B), protein (C), and lactose (D) yield from -27 to -1 d before TAI for primiparous cows that were pregnant (n = 566) or non-pregnant (n = 330) after first service. Values are presented as LSM ± SEM.

Milk component percentages and fat-to-protein ratio. Butterfat percent from 0 to 56 d after calving (Figure 3A) differed over time (*P* < 0.001) but there was no difference (*P* = 0.24) for pregnant (4.30 ± 0.02%) and non-pregnant cows (4.28 ± 0.02%), and there was no group by time interaction (*P* = 0.46). In addition, cows without health disorders (4.21 ± 0.01%) recorded up to 56 d after calving had lesser (*P* < 0.001) butterfat percent than cows with health disorders (4.36 ± 0.02%).

Protein percent from 0 to 56 d after calving (Figure 3B) differed over time (*P* < 0.001) but there was no difference (*P* = 0.46) for pregnant (3.00 ± 0.01%) and non-pregnant cows (3.00 ± 0.01%), and there was no group by time interaction (*P* = 0.48). In addition, there was an effect of season of calving (*P* < 0.01), whereby cows that calved during the cold season had greater protein percent (3.03 ± 0.01%) than cows that calved during the warm season (2.97 ± 0.01%).

Fat-to-protein ratio from 0 to 56 d after calving (Figure 3C) differed over time (*P* < 0.001) but there was no difference (*P* = 0.25) for pregnant (1.45 ± 0.01) and non-pregnant cows (1.43 ± 0.01), and there was no group by time interaction (*P* = 0.56). In addition, there was an effect of season of calving (*P* < 0.005), whereby cows that calved during the warm season had greater fat-to-protein ratio (1.46 ± 0.01) than cows that calved during the cold season (1.42 ± 0.01). Cows without health disorders (1.41 ± 0.01) recorded up to 56 d after calving had lesser (*P* < 0.001) fat-to-protein ratio than cows with health disorders (1.47 ± 0.01).

Lactose percent from 0 to 56 d after calving (Figure 3D) differed over time (*P* < 0.001) but there was no difference in (*P* = 0.66) for pregnant (4.66 ± 0.01%) and non-pregnant cows (4.66 ± 0.01%), and there was no group by time interaction (*P* = 0.37). In addition, there was an effect of season of calving (*P* < 0.001), whereby cows that calved during the warm season had greater lactose percent (4.69 ± 0.01%) than cows that calved during the cold season (4.62 ±

0.01%). Cows with health disorders (4.65 ± 0.01%) recorded up to 56 d after calving tended to have lesser (*P* = 0.09) lactose percent than cows without health disorders (4.67 ± 0.01%).

For butterfat percent from -27 to -1 days before TAI (Figure 4A) there was a tendency (*P* = 0.10) for the group by time interaction, an effect of time (*P* < 0.001) but there was no difference (*P* = 0.64) for pregnant (3.71 ± 0.02%) and non-pregnant cows (3.70 ± 0.02%). In addition, there was an effect of season of calving (*P* < 0.001), whereby cows that calved during the warm season had greater butterfat percent (3.83 ± 0.03%) than cows that calved during the cold season (3.59 ± 0.02). Cows without health disorders (3.67 ± 0.02%) recorded up to 56 d after calving had lesser (*P* < 0.05) butterfat percent than cows with health disorders (3.74 ± 0.03%).

Protein percent from -27 to -1 days before TAI (Figure 4B) differed over time (*P* = 0.02) but there was no difference (*P* = 0.64) for pregnant (3.03 ± 0.01%) and non-pregnant cows (3.04 ± 0.01%), and there was no group by time interaction (*P* = 0.26). In addition, there was an effect of season of calving (*P* < 0.001), whereby cows that calved during the warm season had greater protein percent (3.09 ± 0.01%) than cows that calved during the cold season (2.97 ± 0.01%). Cows with health disorders (3.02 ± 0.02%) recorded up to 56 d after calving tended to have less (*P* = 0.07) protein percent than cows without health disorders (3.05 ± 0.01%).

There was a group by time interaction (*P* = 0.05) for fat-to-protein ratio from -27 to - 1 d before TAI (Figure 4C). from -2 to -1 d before TAI Non-pregnant cows had lower fat-to-protein ratio (1.22 ± 0.01) than pregnant cows (1.24 ± 0.01). There was also an effect of time (*P* < 0.001) but no effect of group (*P* = 0.49; pregnant cows 1.22 ± 0.01 and non-pregnant cows 1.23 ± 0.01). In addition, there was an effect of season of calving (*P* < 0.01), whereby cows that calved during the warm season had greater fat-to-protein ratio (1.24 ± 0.01) than cows that calved during the cold season (1.21 ± 0.01). Cows with health disorders (1.24 ± 0.01) recorded up to 56 d after calving had greater ($P < 0.01$) fat-to-protein ratio than cows without health disorders (1.21 ± 0.01).

Lactose percent from -27 to -1 days before TAI (Figure 4D) differed over time ($P < 0.001$), was greater ($P = 0.03$) for pregnant (4.68 ± 0.01%) than non-pregnant cows (4.65 ± 0.01%), but there was no group by time interaction ($P = 0.34$). In addition, there was an effect of season of calving ($P < 0.01$), whereby cows that calved during the warm season had greater lactose percent (4.68 ± 0.01%) than cows that calved during the cold season (4.65 ± 0.01%).

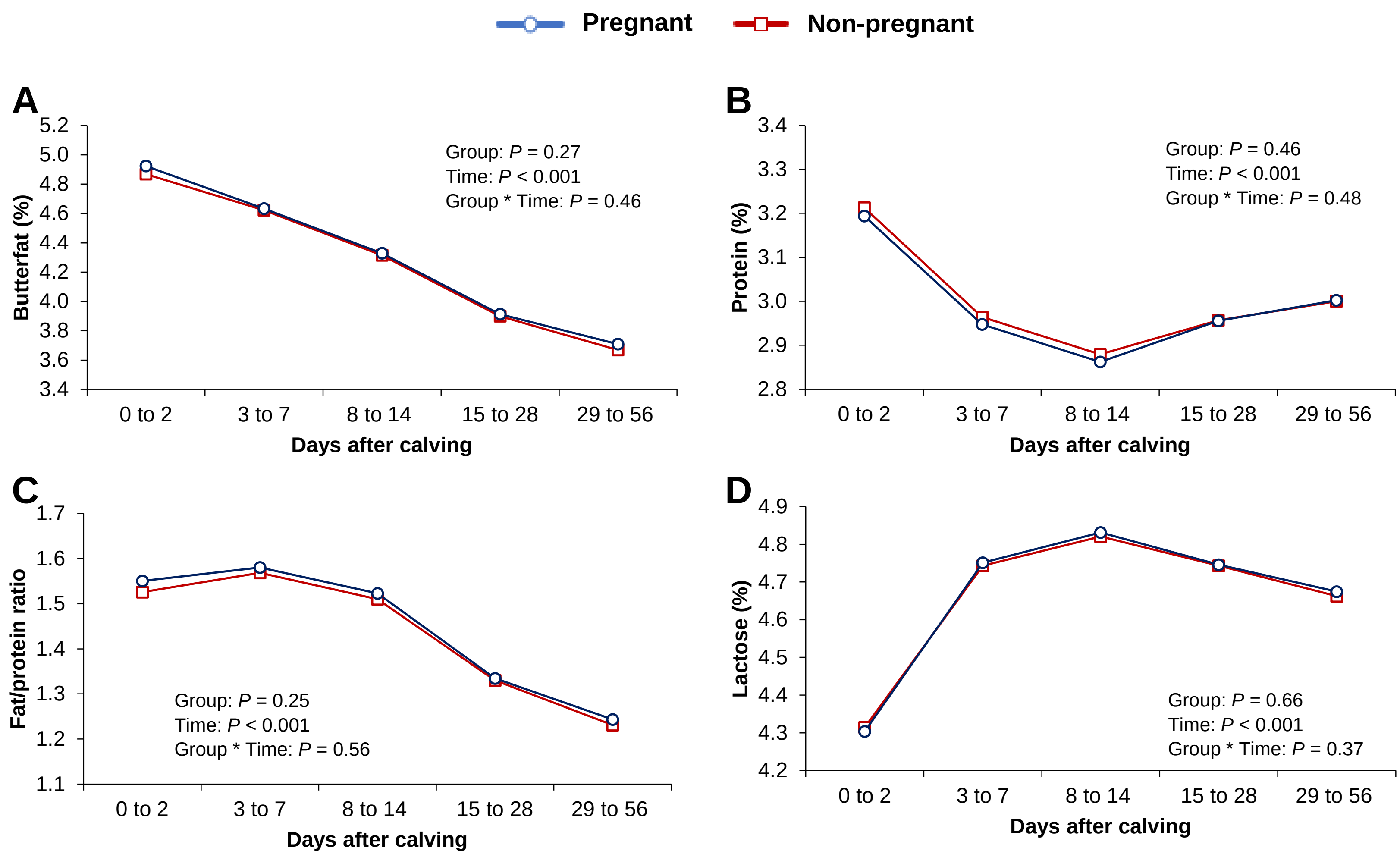

Figure 3. Butterfat percent (A), protein percent (B), fat-to-protein ratio (C), and lactose percent (D) from 0 to 56 d after calving for primiparous cows that were pregnant (n = 566) or non-pregnant (n = 330) after first service. Values are presented as LSM ± SEM.

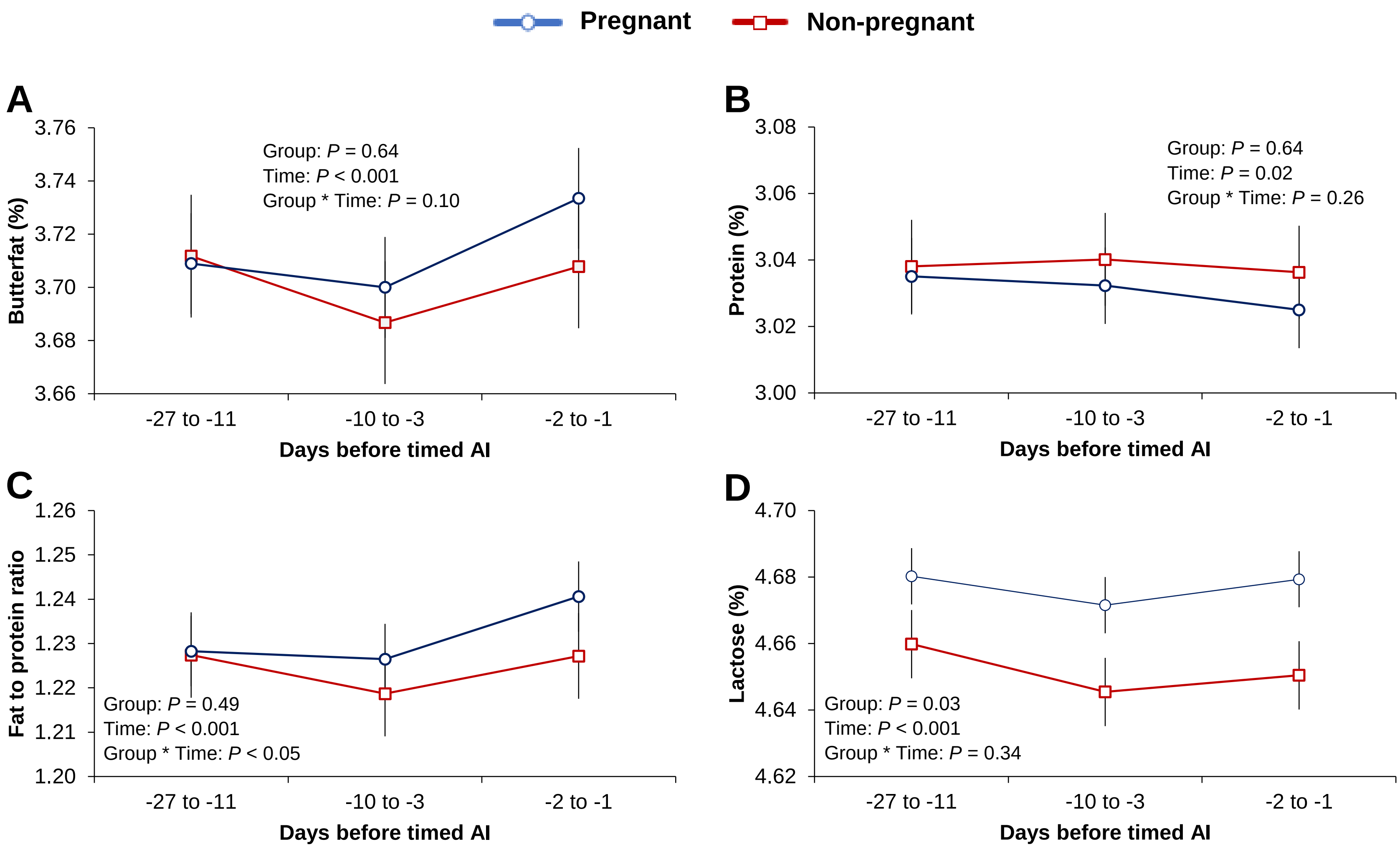

Figure 4. Butterfat percent (A), protein percent (B), fat-to-protein ratio (C), and lactose percent (D) from -27 to -1 d before TAI for primiparous cows that were pregnant (n = 566) or non-pregnant (n = 330) after first service. Values are presented as LSM ± SEM.

Rumination activity. Rumination activity from -14 to 28 d after calving (Figure 5A) differed over time ($P < 0.001$) but there was no difference ($P = 0.37$) for pregnant (358 ± 4 AU) and non-pregnant cows (353 ± 5 AU), and there was no group by time interaction ($P = 0.52$). In addition, there was an effect of season of calving ($P < 0.001$), whereby cows that calved during the cold season had greater rumination activity (368 ± 4 AU) than cows that calved during the warm season (342 ± 5 AU).

Eating activity. Eating activity from -14 to 28 d after calving (Figure 5B) differed over time ($P < 0.001$) but there was no difference ($P = 0.95$) between pregnant (509 ± 4AU) and non-pregnant cows (509 ± 5 AU), and there was no group by time interaction ($P = 0.84$). In addition, there was an effect of season of calving ($P < 0.05$), whereby cows that calved during the warm season had greater eating activity (516 ± 6 AU) than cows that calved during the cold season (503 ± 4 AU). Cows with health disorders (487 ± 6 AU) recorded up to 56 d after calving had lesser ($P < 0.001$) eating activity than cows without health disorders (531 ± 4 AU).

Body temperature. Reticulo-rumen temperature from -14 to 56 d after calving (Figure 5C) differed by time ($P < 0.001$). In addition, pregnant cows (39.7 ± 0.01 °C) tended to have greater temperature ($P < 0.10$) than non-pregnant cows (39.6 ± 0.01 °C), but there was no group by time interaction ($P = 0.92$). There was an effect of season of calving ($P = 0.02$), whereby cows that calved during the warm season had greater temperature (39.7 ± 0.01 °C) than cows that calved during the cold season (39.6 ± 0.01 °C). Reticulo-rumen temperature from -27 to -1 days before TAI (Figure 6A) differed by time ($P < 0.001$), but there was no difference ($P = 0.54$) between pregnant (39.4 ± 0.01 °C) and non-pregnant cows (39.4 ± 0.01 °C), and there was no group by time interaction (*P* = 0.19). Cows that calved during the cold season tended (*P* = 0.09), to have greater temperature (39.4 ± 0.01 °C) than cows that calved during the warm season (39.3 ± 0.01 °C).

Milk conductivity. Milk conductivity from 0 to 56 d after calving (Figure 5D) differed over time (*P* < 0.001) but there was no difference (*P* = 0.23) for pregnant (9.14 ± 0.03 mmHo) and non-pregnant cows (9.09 ± 0.04 mmHo), and there was no group by time interaction (*P* = 0.63). In addition, there was an effect of season of calving (*P* < 0.001), whereby cows that calved during the warm season had greater milk conductivity (9.22 ± 0.05 mmHo) than cows that calved during the cold season (9.01 ± 0.03 mmHo). There was a group by time interaction (*P* < 0.001) for milk conductivity from -27 to - 1 d before TAI (Figure 6B) because from -27 to -11 d before TAI non-pregnant cows had lower conductivity than pregnant cows. Conversely, from day -2 to -1 d before TAI pregnant cows had lower conductivity than non-pregnant cows. There was also an effect of time (*P* < 0.001) but no effect of group (*P* = 0.85; pregnant cows 9.07 ± 0.03 mmHo and or non-pregnant cows 9.08 ± 0.03 mmHo). In addition, there was an effect of season of calving (*P* < 0.01), whereby cows that calved during the cold season had greater conductivity (9.13 ± 0.03 mmHo) than cows that calved during the warm season (9.03 ± 0.04 mmHo).

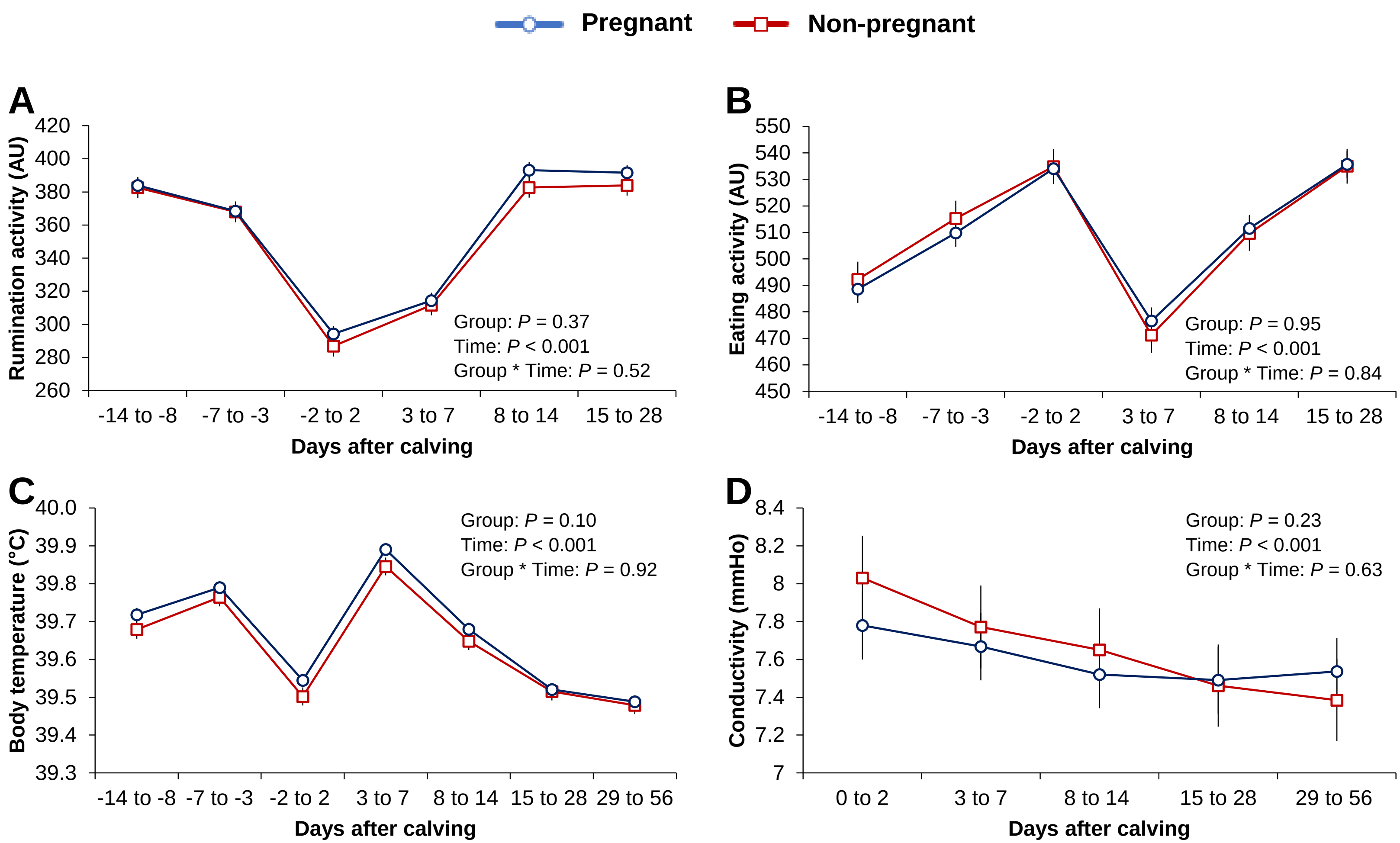

Figure 5. Rumination activity (A), eating activity (B), body temperature (C), and milk conductivity (D) from 0 to 56 d after calving for primiparous cows that were pregnant or not after first service. For rumination and eating activity data was available from 451 pregnant and 245 non-pregnant cows, for body temperature from 168 pregnant and 84 non-pregnant cows and for milk conductivity from 566 pregnant and 330 non-pregnant cows. Values are presented as LSM ± SEM

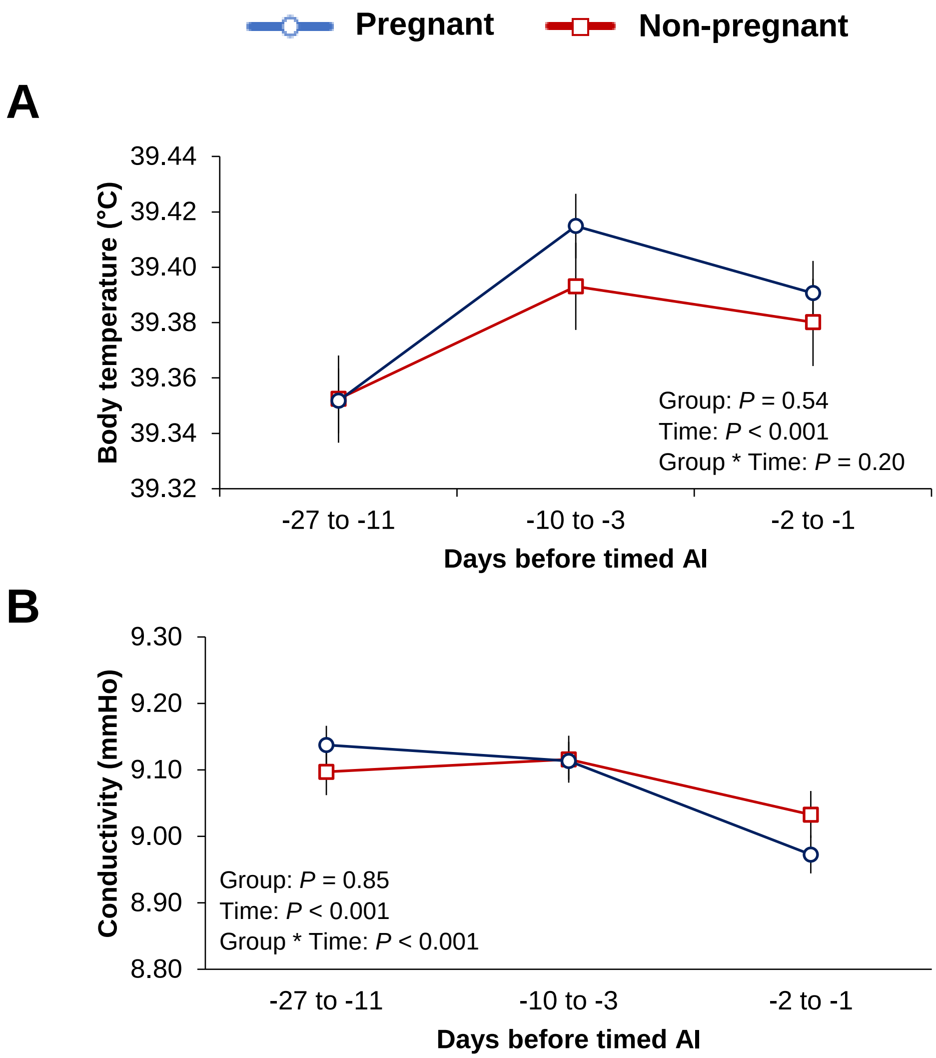

Figure 6. Body temperature (A) and milk conductivity (B) from -27 to -1 d before TAI for primiparous cows that were pregnant or not after first service. For body temperature data was available from 168 pregnant and 84 non-pregnant cows and for milk conductivity data was available from 566 pregnant and 330 non-pregnant cows. Values are presented as LSM ± SEM

Physical activity. Physical activity as determined by the reticulum-ruminal bolus from -14 to 56 d after calving (Figure 7A) differed over time (P < 0.001) but there was no difference (P = 0.95) between pregnant (4.66 ± 0.07 AU) and non-pregnant cows (4.67 ± 0.09 AU), and there was no group by time interaction (P = 0.15). There was a group by time interaction (P = 0.01) for physical activity from -27 to - 11 d before TAI (Figure 8A) because pregnant cows (4.30 ± 0.05 AU) tended to have lower activity than non-pregnant (4.46 ± 0.07 AU) cows. There was also an effect of time (P = 0.03) but no effect of group (P = 0.26); pregnant cows (4.37 ± 0.05 AU) and or non-pregnant cows (4.46 ± 0.07 AU). Cows with health disorders (4.34 ± 0.07 AU) recorded up to 56 d after calving tended to have lesser (P = 0.07) physical activity than cows without health disorders (4.49 ± 0.05 AU).

Walking activity. From 0 to 56 d after calving (Figure 7B), walking activity differed over time (P < 0.001) but there was no difference (P = 0.39) between pregnant (214 ± 1.95 steps/h) and non-pregnant cows (211 ± 2.37 steps/h), and there was no group by time interaction (P = 0.47). In addition, there was an effect of season of calving (P < 0.005), whereby cows that calved during the warm season had greater walking activity (217 ± 2.73 steps/h) than cows that calved during the cold season (208 ± 1.67 steps/h). Cows with health disorders (206 ± 2.72 steps/h) recorded up to 56 d after calving had lesser (P < 0.001) walking activity than cows without health disorders (219 ± 1.69 steps/h). There was a group by time interaction (P < 0.001) for walking activity from -27 to -1 d before TAI (Figure 8B) because from -27 to -11 d before TAI, pregnant (190 ± 1.93 steps/h) cows had less walking activity than non-pregnant cows (193 ± 2.39 steps/h). Conversely, from d -10 to -3 d before TAI non-pregnant (191 ± 2.39 steps/h) cows had less walking activity than pregnant (193 ± 1.96 steps/h) cows. There was no effect of time (P = 0.74), and no effect of group (*P* = 0.98) [pregnant cows (192 ± 1.88 steps/h) and or non-pregnant cows (192 ± 2.29 steps/h)].

Resting behavior. Total resting time per day from 0 to 56 d after calving (Figure 7C) differed over time (*P* < 0.001); however, there was no difference (*P* = 0.39) between pregnant (541 ± 4.74 min/d) and non-pregnant cows (546 ± 5.76 min/d), and there was no group by time interaction (*P* = 0.85). In addition, there was an effect of season of calving (*P* = 0.05), whereby cows that calved during the cold season had greater resting time per day (551 ± 4.07 min/d) than cows that calved during the warm season (537 ± 6.63 min/d). Total resting time per day from -27 to -1 d before TAI (Figure 8C) differed over time (*P* < 0.001) but there was no difference (*P* = 0.97) between pregnant (627 ± 4.35 min/d) and non-pregnant cows (627 ± 5.29 min/d), and there was no group by time interaction (*P* = 0.78). In addition, there was an effect of season of calving (*P* < 0.001), whereby cows that calved during the cold season had greater resting time per day (650 ± 5.90 min/d) than cows that calved during the warm season (604 ± 3.90 min/d).

The number of lying bouts per day from 0 to 56 d after calving (Figure 7D) differed (*P* < 0.001) over time, was greater (*P* = 0.04) for non-pregnant (11.9 ± 0.20 bouts/d) than pregnant cows (11.4 ± 0.16 bouts/d), but there was no group by time interaction (*P* = 0.72). For the number of lying bouts from -27 to -1 days before TAI (Figure 8D), there was a tendency (*P* = 0.07) for a group by time interaction, and an effect of time (*P* < 0.001) but there was no difference (*P* = 0.18) for pregnant (10.20 ± 0.13 bouts/day) and non-pregnant cows (10.43 ± 0.16 bouts/day).

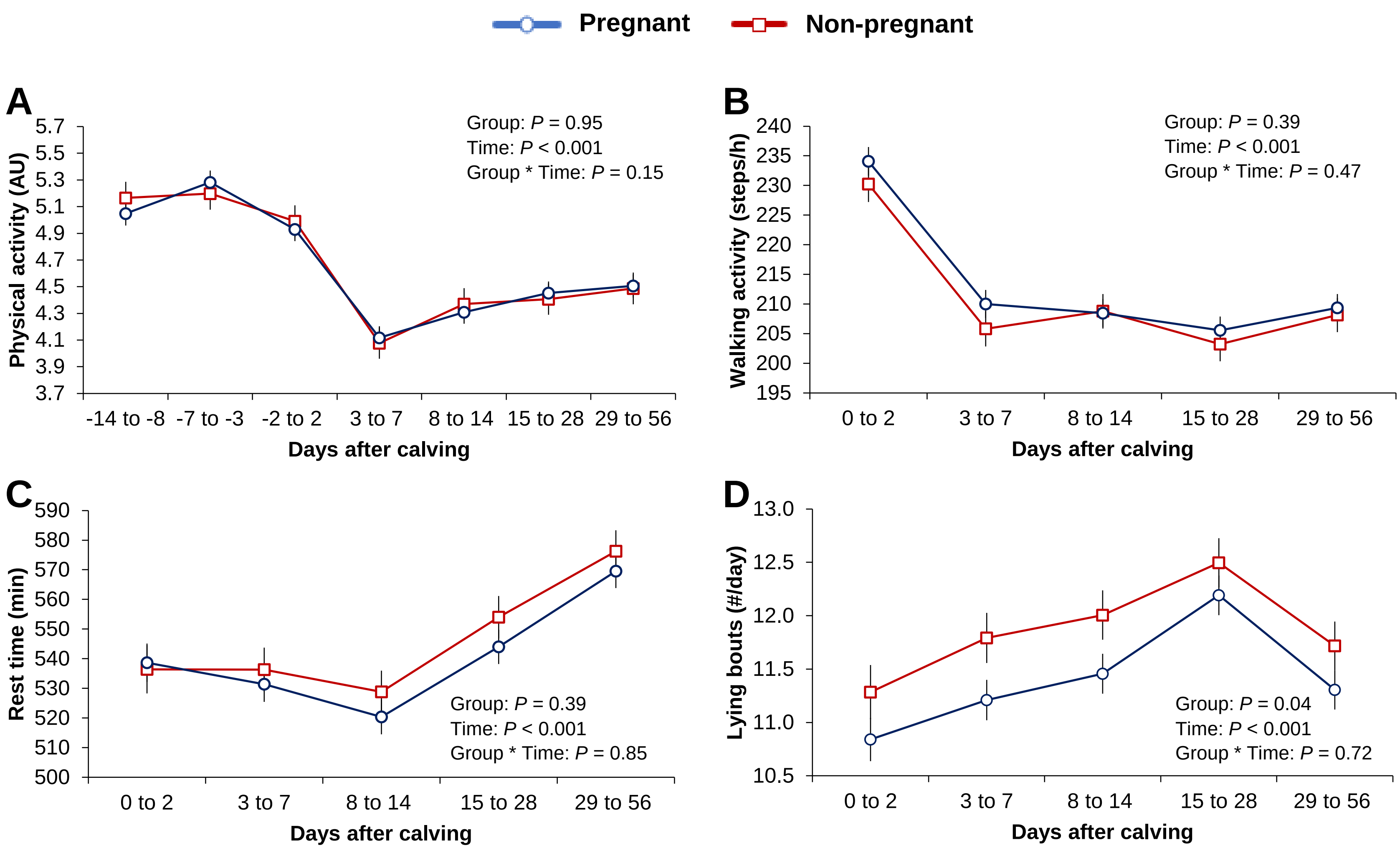

Figure 7. Physical activity (A), activity (B), rest time (C), and lying bouts (D) from 0 to 56 d after calving for primiparous cows that were pregnant or not after first service. For physical activity data was available from 168 pregnant and 84 non-pregnant cows, for activity, rest time and lying bouts data was available from 566 pregnant and 329 non-pregnant cows. Values are presented as LSM ± SEM

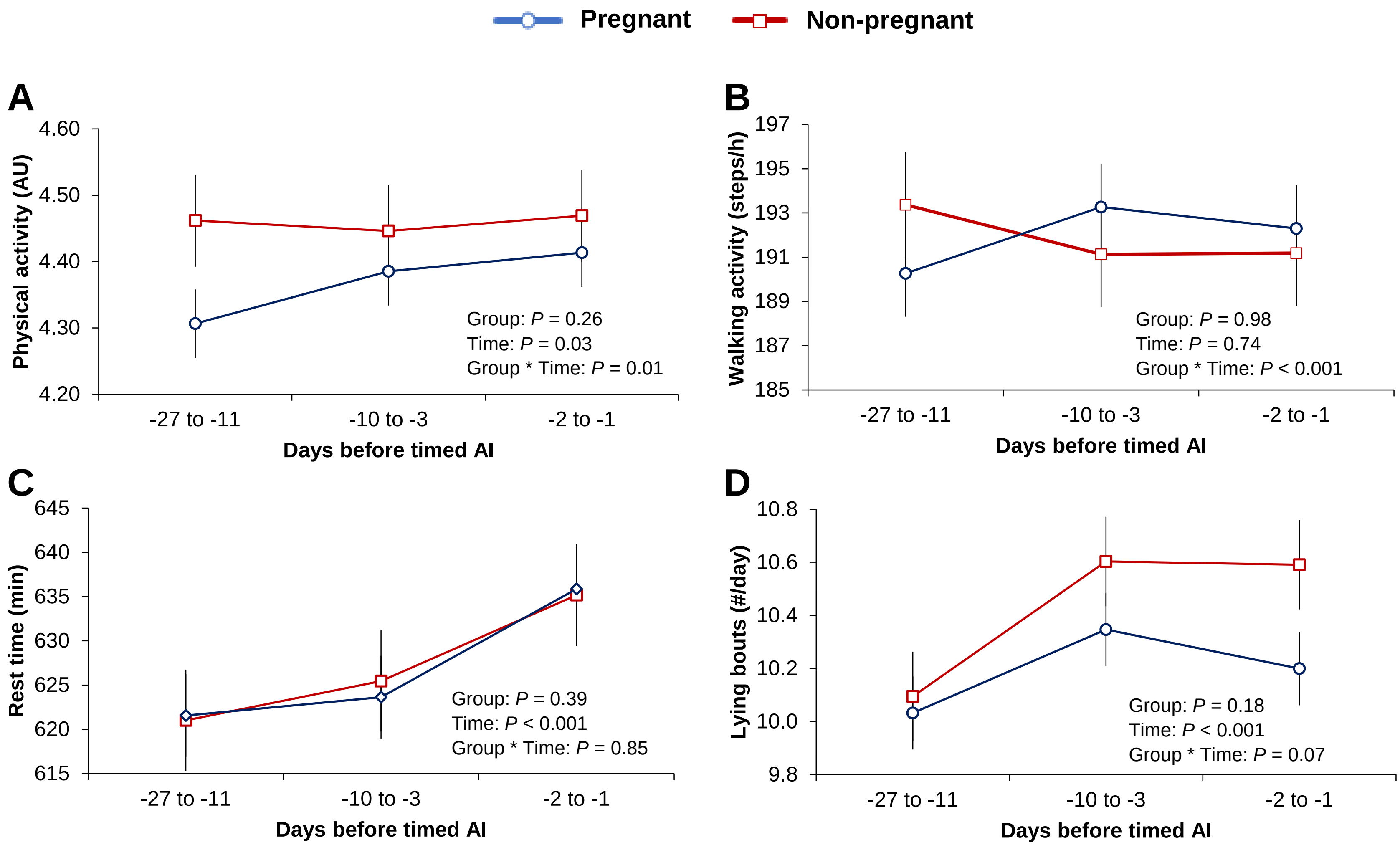

Figure 8. Physical activity (A), walking activity (B), rest time (C), and lying bouts (D) from -27 to -1 d before TAI for primiparous cows that were pregnant or not after first service. For physical activity data was available from 168 pregnant and 84 non-pregnant cows, for activity, rest time and lying bouts data was available from 566 pregnant and 329 non-pregnant cows. Values are presented as LSM ± SEM

Behavioral, Physiological and Performance Parameters for Multiparous Cows

Milk and component yields. Milk yield from 0 to 56 d after calving (Figure 9A) differed over time (*P* < 0.001) but there was no difference (*P* = 0.21) for pregnant (36.3 ± 0.21 kg/d) and non-pregnant cows (36.7 ± 0.20 kg/d), and there was no group by time interaction (*P* = 0.30). There was an effect of season of calving (*P* < 0.001), whereby cows that calved during the cold season had greater milk yield (37.2 ± 0.16 kg/d) than cows that calved during the warm season (35.8 ± 0.26 kg/d). Cows with health disorders (34.9 ± 0.23 kg/d) recorded up to 56 d after calving had lesser (*P* < 0.001) milk yield than cows without health disorders (38.0 ± 0.18 kg/d). Cows with ≥3 lactations had greater (*P* < 0.001) milk yield (37.2 ± 0.2 kg/d) than cows with 2 lactations (35.7 ± 0.2 kg/d).

Butterfat yield from 0 to 56 d after calving (Figure 9B) differed over time (*P* < 0.001) but there was no difference (*P* = 0.22) for pregnant (1.49 ± 0.01 kg/d) and non-pregnant cows (1.50 ± 0.01 kg/d), and there was no group by time interaction (*P* = 0.76). There was an effect of season of calving (*P* < 0.001), whereby cows that calved during the cold season had greater butterfat yield (1.52 ± 0.01 kg/d) than cows that calved during the warm season (1.47 ± 0.01 kg/d). Cows with health disorders (1.45 ± 0.01 kg/d) recorded up to 56 d after calving had lesser (*P* < 0.001) butterfat yield than cows without health disorders (1.54 ± 0.01 kg/d). Cows with ≥3 lactations had greater (*P* < 0.001) butterfat yield (1.54 ± 0.01 kg/d) than cows with 2 lactations (1.45 ± 0.01 kg/d).

Protein yield from 0 to 56 d after calving (Figure 9C) differed over time (*P* < 0.001) but there was no difference (*P* = 0.89) for pregnant cows (1.06 ± 0.01 kg/d) and non-pregnant cows (1.07 ± 0.01 kg/d), and there was no group by time interaction (*P* = 0.15). In addition, there was an effect of season of calving (*P* < 0.001), whereby cows that calved during the cold season had greater protein yield (1.09 ± 0.01 kg/d) than cows that calved during the warm season (1.04 ± 0.01 kg/d). Cows with health disorders (1.01 ± 0.01 kg/d) recorded up to 56 d after calving had lesser (P < 0.001) protein yield than cows without health disorders (1.12 ± 0.01 kg/d). Cows with ≥3 lactations had greater ($P < 0.001$) protein yield (1.09 ± 0.01 kg/d) than cows with 2 lactations (1.04 ± 0.01 kg/d).

Lactose yield from 0 to 56 d after calving (Figure 9D) differed over time ($P < 0.001$) but there was no difference ($P = 0.68$) for pregnant (1.72 ± 0.01 kg/d) and non-pregnant cows (1.73 ± 0.01 kg/d), and there was no group by time interaction ($P = 0.11$). In addition, there was an effect of season of calving ($P < 0.001$), whereby cows that calved during the cold season had greater lactose yield (1.75 ± 0.01 kg/d) than cows that calved during the warm season (1.70 ± 0.01 kg/d). Cows with health disorders (1.65 ± 0.01 kg/d) recorded up to 56 d after calving had lesser ($P < 0.001$) lactose yield than cows without health disorders (1.79 ± 0.01 kg/d). Cows with ≥3 lactations had greater ($P < 0.001$) lactose yield (1.76 ± 0.01 kg/d) than cows with 2 lactations (1.69 ± 0.01 kg/d).

Milk yield from -27 to -1 days before TAI (Figure 10A) differed over time ($P < 0.001$), was greater ($P = 0.04$) for non-pregnant (49.6 ± 0.29 kg/d) than pregnant cows (48.9 ± 0.30 kg/d), but there was no group by time interaction ($P = 0.12$). There was an effect of season of calving ($P < 0.05$), whereby cows that calved during the cold season had greater milk yield (49.7 ± 0.23 kg/d) than cows that calved during the warm season (48.8 ± 0.38 kg/d). Cows with health disorders (48.7 ± 0.34 kg/d) recorded up to 56 d after calving had lesser ($P < 0.005$) milk yield than cows without health disorders (49.8 ± 0.26 kg/d). Cows with ≥3 lactations had greater ($P < 0.001$) milk yield (50.9 ± 0.29 kg/d) than cows with 2 lactations (47.5 ± 0.20 kg/d).

Butterfat yield from -27 to -1 days before TAI (Figure 10B) differed over time (*P* < 0.001), was greater (*P* = 0.04) for non-pregnant (1.77 ± 0.01 kg/d) than pregnant cows (1.74 ± 0.01 kg/d), but there was no group by time interaction (*P* = 0.90). There was an effect of season of calving (*P* < 0.005), whereby cows that calved during the warm season had greater butterfat yield (1.78 ± 0.01 kg/d) than cows that calved during the cold season (1.72 ± 0.01 kg/d). Cows with health disorders (1.74 ± 0.01 kg/d) recorded up to 56 d after calving tended to have lesser (*P* = 0.09) butterfat yield than cows without health disorders (1.76 ± 0.01 kg/d). Cows with ≥3 lactations had greater (*P* < 0.001) butterfat yield (1.83 ± 0.01 kg/d) than cows with 2 lactations (1.67 ± 0.01 kg/d).

For protein yield from -27 to -1 days before TAI (Figure 10C) there was a tendency (*P* = 0.08) for the group by time interaction, and effect of time (*P* < 0.001) but there was no difference (*P* = 0.31) for pregnant (1.48 ± 0.01 kg/d) and non-pregnant cows (1.50 ± 0.01 kg/d). Cows with health disorders (1.47 ± 0.01 kg/d) recorded up to 56 d after calving had lesser (P < 0.001) protein yield than cows without health disorders (1.51 ± 0.01 kg/d). Cows with ≥3 lactations had greater (*P* < 0.001) protein yield (1.55 ± 0.01 kg/d) than cows with 2 lactations (1.1.43 ± 0.01 kg/d).

Lactose yield from -27 to -1 days before TAI (Figure 10D) differed over time (*P* < 0.001), non-pregnant cows (2.27 ± 0.01 kg/d) tended (*P* = 0.08) to have greater lactose yield than pregnant cows (2.24 ± 0.01 kg/d), but there was no group by time interaction (*P* = 0.32). In addition, cows with health disorders (2.23 ± 0.02 kg/d) recorded up to 56 d after calving had lesser (*P* < 0.005) lactose yield than cows without health disorders (2.28 ± 0.01 kg/d). Cows with ≥3 lactations had greater (*P* < 0.001) lactose yield (2.33 ± 0.01 kg/d) than cows with 2 lactations (2.18 ± 0.01 kg/d).

Milk component percentages and fat-to-protein ratio. There was a group by time interaction (*P* = 0.05) for butterfat percent from 0 to 56 d after calving (Figure 11A). Pregnant cows (4.16 ± 0.01%) had lesser butterfat percent from 0 to 2 d after calving than non-pregnant cows (4.24 ± 0.01%). There was also a tendency (*P* = 0.09) for the effect of group because butterfat percent was greater for non-pregnant (4.19 ± 0.01%) than pregnant cows (4.16 ± 0.01%). Cows with health disorders (4.23 ± 0.23%) recorded up to 56 d after calving had greater (*P* < 0.001) butterfat percent than cows without health disorders (4.12 ± 0.01%). Cows with ≥3 lactations tended to have greater (*P* = 0.07) butterfat percent (4.19 ± 0.01%) than cows with 2 lactations (4.16 ± 0.01%).

There was a group by time interaction (*P* < 0.005) for protein percent from 0 to 56 d after calving (Figure 11B) because pregnant cows had greater protein percent than non-pregnant cows starting at 8 to 14 d after calving. There was also an effect of time (*P* < 0.001) but there was no effect of group (*P* = 0.14; pregnant 2.98 ± 0.01 kg/d, non-pregnant cows 2.96 ± 0.01 kg/d). There was an effect of season of calving (*P* < 0.001), whereby cows that calved during the cold season had greater protein percent (3.00 ± 0.01%) than cows that calved during the warm season (2.95 ± 0.01%). Cows with health disorders (2.95 ± 0.01%) recorded up to 56 d after calving had lesser (*P* < 0.001) protein percent than cows without health disorders (3.00 ± 0.01%). Cows with ≥3 lactations had greater (*P* = 0.01) protein percent (2.98 ± 0.01%) than cows with 2 lactations (2.96 ± 0.01%).

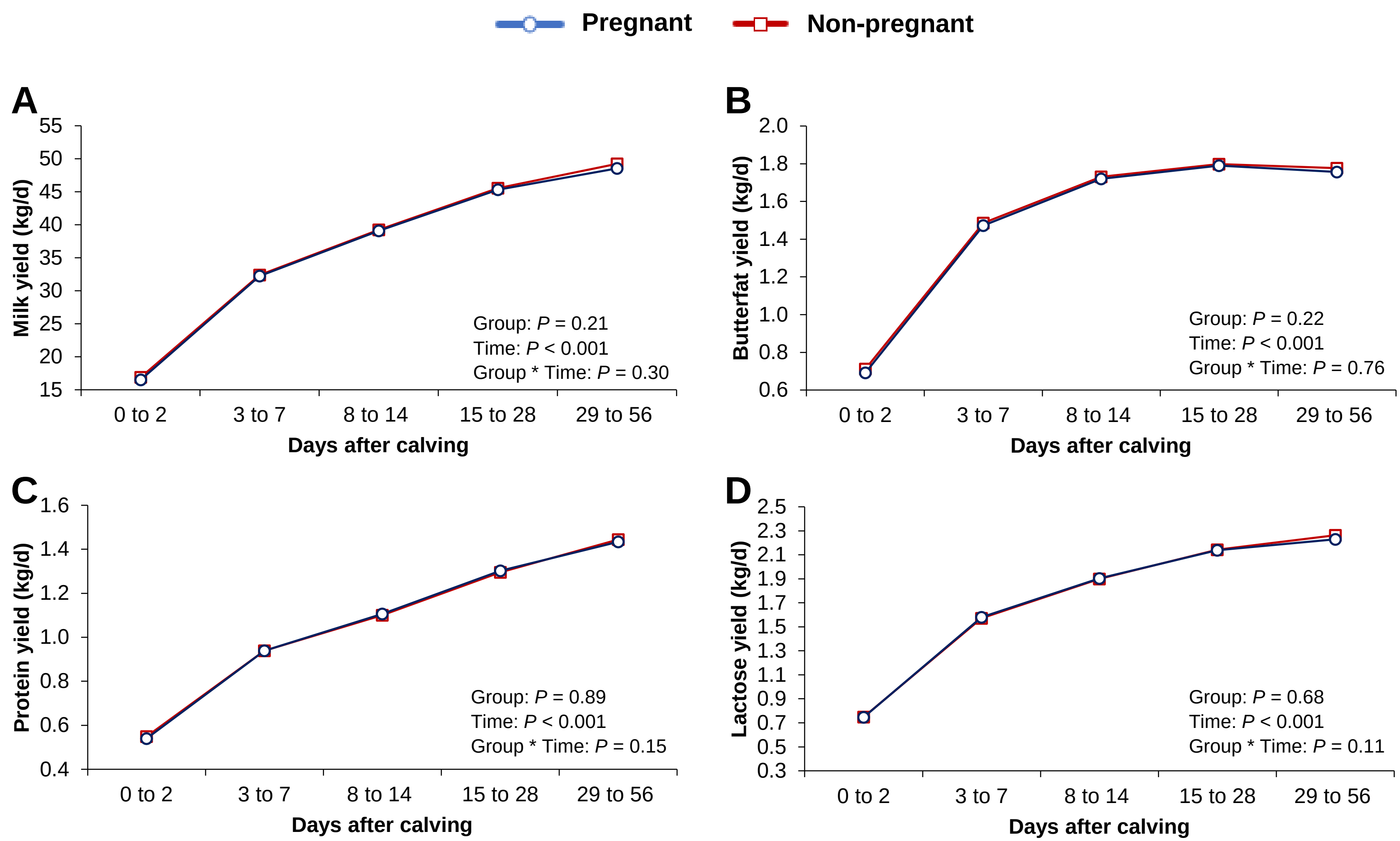

Figure 9. Milk (A), butterfat (B), protein (C), and lactose (D) yield from 0 to 56 d after calving for multiparous cows that were pregnant (n = 902) or non-pregnant (n = 959) after first service. Values are presented as LSM ± SEM

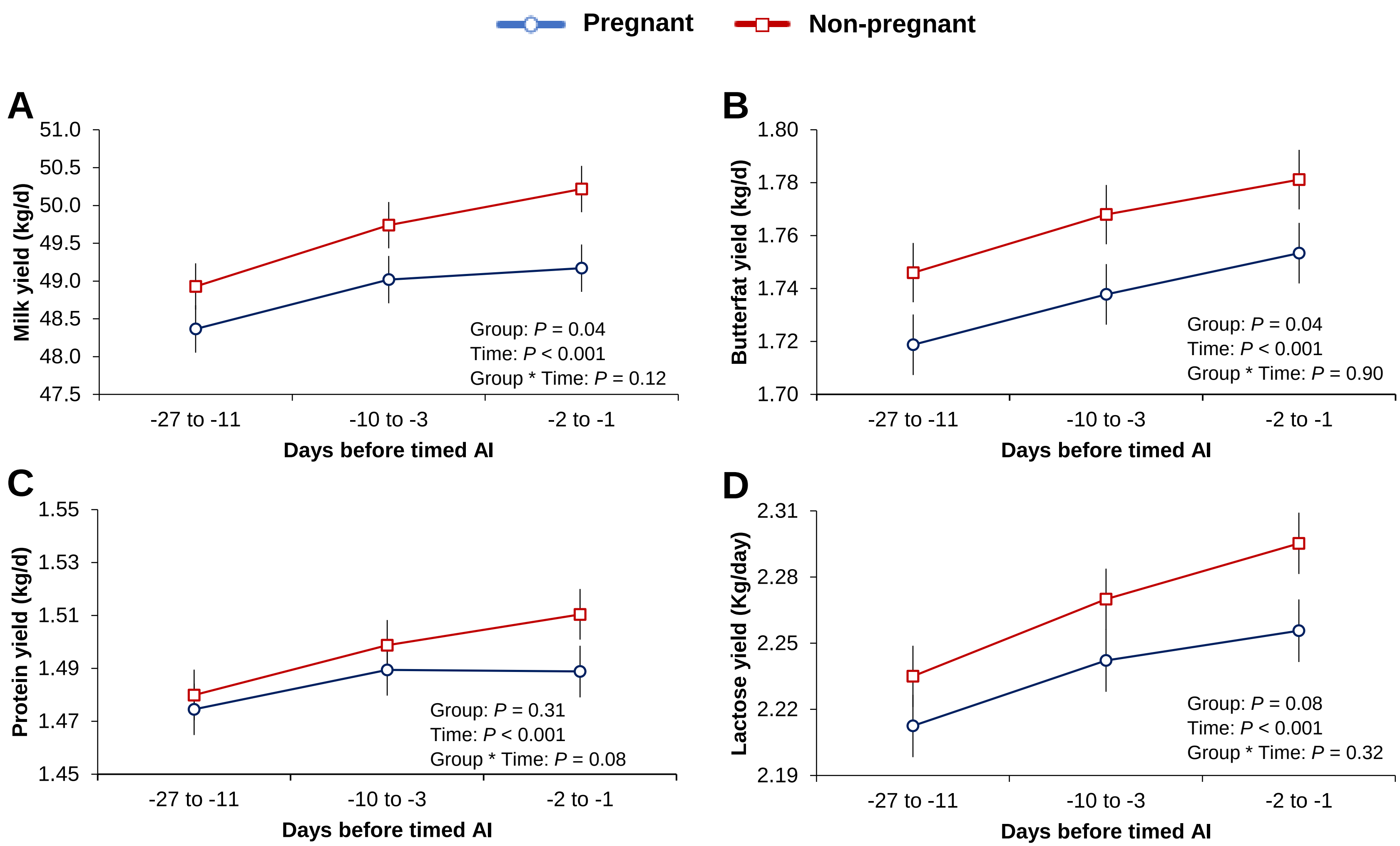

Figure 10. Milk (A), butterfat (B), protein (C), and lactose (D) yield from -27 to -1 d before TAI for multiparous cows that were pregnant (n = 902) or non-pregnant (n = 959) after first service. Values are presented as LSM ± SEM

Fat-to-protein ratio from 0 to 56 d after calving (Figure 11C) differed over time ($P <$ 0.001), was greater ($P = 0.04$) for non-pregnant (1.43 ± 0.01) than pregnant cows (1.41 ± 0.01) but there was no group by time interaction ($P = 0.73$). In addition, there was an effect of season of calving ($P = 0.02$), whereby cows that calved during the warm season had greater fat-to-protein ratio (1.43 ± 0.01) than cows that calved during the cold season (1.41 ± 0.01). Cows with health disorders (1.45 ± 0.01 kg/d) recorded up to 56 d after calving had greater ($P < 0.001$) fat-to-protein ratio than cows without health disorders (1.39 ± 0.01).

There was a group by time interaction ($P < 0.001$) for lactose percent from 0 to 56 d after calving (Figure 11D) because pregnant cows had greater lactose percent than non-pregnant cows up to 8 to 14 d after calving. There was also an effect of group whereby lactose percent from 0 to 56 d after calving was greater ($P < 0.01$) for pregnant (4.72 ± 0.01%) than non-pregnant cows (4.70 ± 0.01%), and there was an effect of time ($P < 0.001$). Cows that calved during the warm season tended ($P = 0.08$) to have greater lactose percent (4.72 ± 0.01%) than cows that calved during the cold season (4.70 ± 0.01%). Cows with health disorders (4.70 ± 0.01%) recorded up to 56 d after calving had lesser ($P = 0.01$) lactose percent than cows without health disorders (4.72 ± 0.01%). Cows with ≥3 lactations had lesser ($P = 0.03$) lactose percent (4.70 ± 0.01%) than cows with 2 lactations (4.72 ± 0.01%).

There was a group by time interaction ($P < 0.003$) for butterfat percent from -27 to -1 days before TAI (Figure 12A). From -27 to -11 d before TAI pregnant (3.57 ± 0.01%) cows had lower butterfat percent than non-pregnant cows (3.58 ± 0.01%). Conversely, from day -2 to -1 d before TAI non-pregnant (3.56 ± 0.01%) cows had lower butterfat percent than pregnant (3.58 ± 0.01%) cows. There was also an effect of time ($P = 0.01$) but there was no difference ($P = 0.98$) for pregnant (3.57 ± 0.01%) or non-pregnant cows (3.57 ± 0.01%). There was an effect of season of calving ($P < 0.001$), whereby cows that calved during the warm season had greater butterfat percent (3.66 ± 0.02%) than cows that calved during the cold season (3.48 ± 0.01%). Cows with ≥3 lactations had greater ($P < 0.001$) butterfat percent (3.61 ± 0.02%) than cows with 2 lactations (3.53 ± 0.02%).

There was a group by time interaction ($P = 0.04$) for protein percent from -27 to -1 days before TAI (Figure 12B) because non-pregnant had less protein percent than pregnant cows from -27 to -11 and -10 to -3 d before TAI. There was also an effect of time ($P < 0.001$) and pregnant cows (3.04 ± 0.01%) tended ($P = 0.08$) to have greater protein percent than non-pregnant cows (3.02 ± 0.01%). There was an effect of season of calving ($P < 0.001$), whereby cows that calved during the warm season had greater protein percent (3.06 ± 0.01%) than cows that calved during the cold season (3.01 ± 0.01%). Cows with health disorders (3.02 ± 0.01%) recorded up to 56 d after calving tended ($P = 0.09$) to have lesser protein percent than cows without health disorders (3.04 ± 0.01%). Cows with ≥3 lactations had greater ($P < 0.005$) protein percent (3.05 ± 0.01%) than cows with 2 lactations (3.01 ± 0.01%).

There was a group by time interaction ($P < 0.001$) for fat-to-protein ratio (Figure 12C) because non-pregnant cows had greater fat-to-protein ratio than pregnant cows from -27 to -11 and -10 to -3 d before TAI. There was also an effect of time ($P < 0.001$) but there was no effect of group ($P = 0.20$; pregnant 1.18 ± 0.01, non-pregnant cows 1.19 ± 0.01). There was an effect of season of calving ($P < 0.001$), whereby cows that calved during the warm season had greater fat-to-protein ratio (1.20 ± 0.01) than cows that calved during the cold season (1.16 ± 0.01). Cows with health disorders (1.20 ± 0.01) recorded up to 56 d after calving tended to have greater ($P = 0.09$) fat-to-protein ratio than cows without health disorders (1.17 ± 0.01). Cows with ≥3 lactations tended to have greater (*P* = 0.10) fat-to-protein ratio (1.19 ± 0.01) than cows with 2 lactations (1.18 ± 0.01).

There was a group by time interaction (*P* = 0.03) for lactose percent (Figure 12D) because from -27 to -11 d before TAI non-pregnant cows had lesser lactose percent than pregnant cows. There was an effect of time (*P* < 0.001) but no overall difference (*P* = 0.19) for pregnant (4.58 ± 0.01%) and non-pregnant cows (4.57 ± 0.01%). Cows that calved during the warm season had greater (*P* = 0.04) lactose percent (4.59 ± 0.01%) than cows that calved during the cold season (4.57 ± 0.01%). Cows with ≥3 lactations had lesser (*P* < 0.01) lactose percent (4.56 ± 0.01%) than cows with 2 lactations (4.59 ± 0.01%).

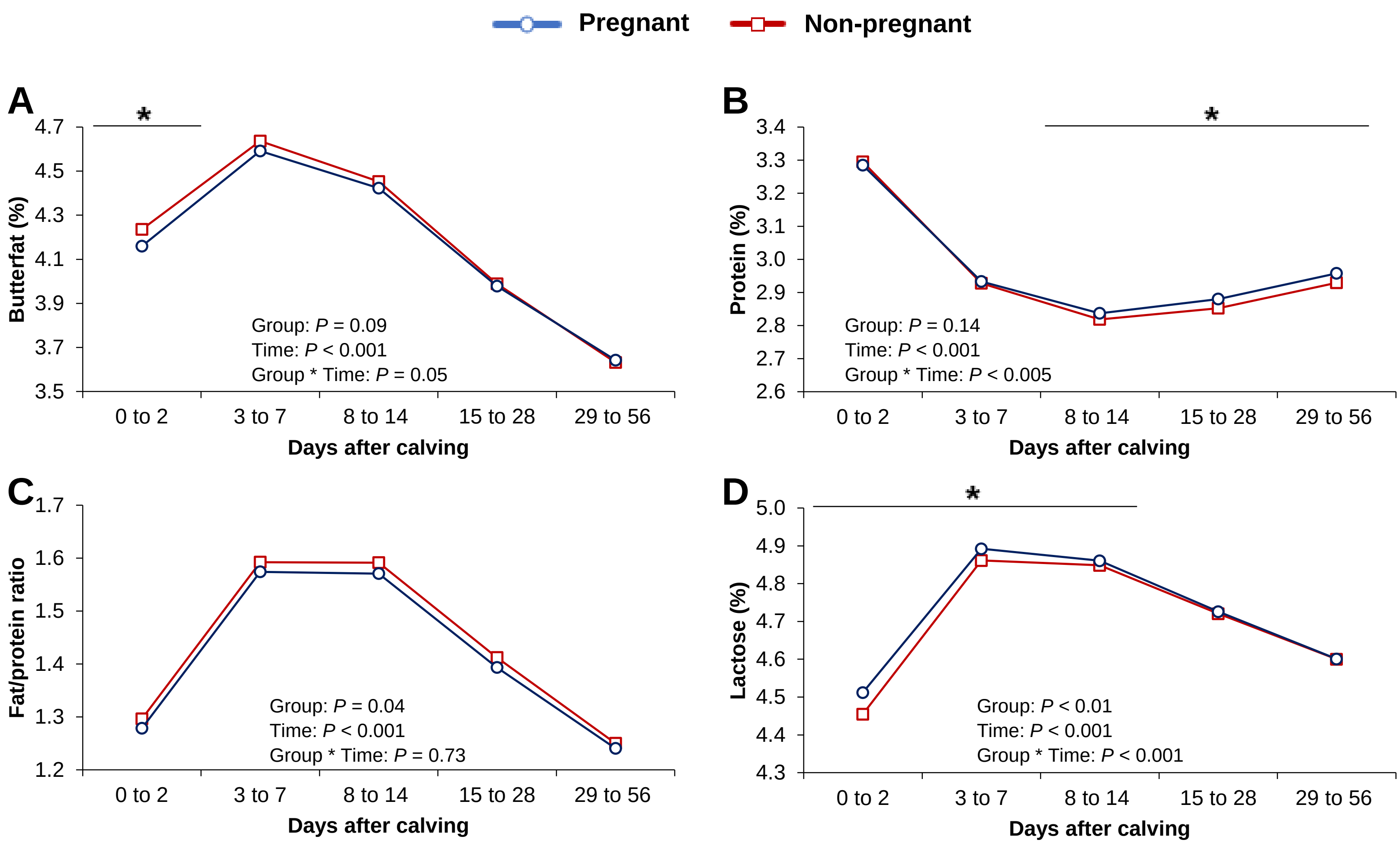

Figure 11. Butterfat percent (A), protein percent (B), fat-to-protein ratio (C), and lactose percent (D) in milk from 0 to 56 d after calving for multiparous cows that were pregnant (n = 902) or non-pregnant (n = 959) after first service. Within time points, an asterisk (*) represents differences ($P \leq 0.05$) for pairwise comparisons between pregnant and non-pregnant. Values are presented as LSM ± SEM

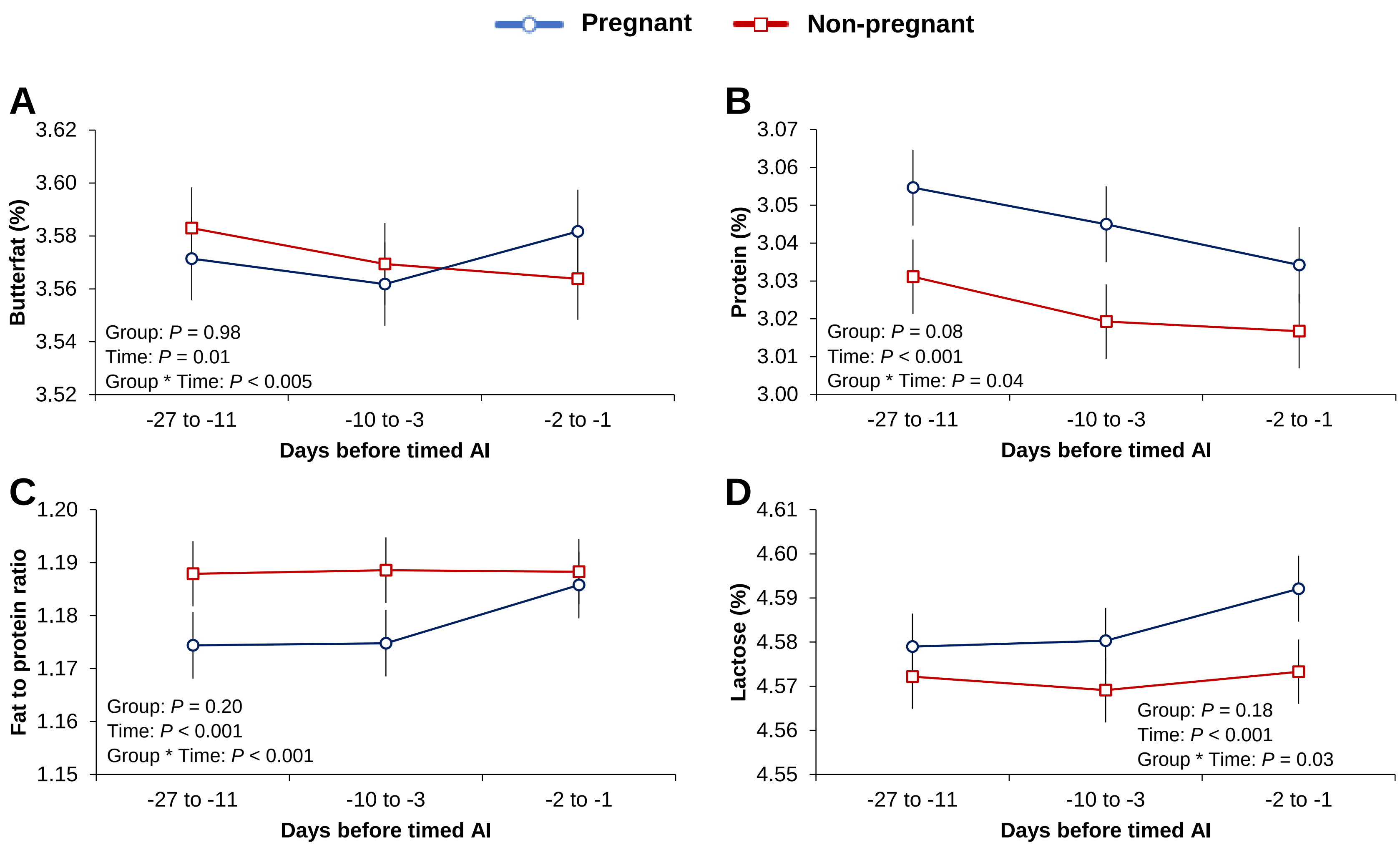

Figure 12. Butterfat percent (A), protein percent (B), fat-to-protein ratio (C), and lactose percent (D) in milk from -27 to -1 d before TAI for multiparous cows that were pregnant (n = 902) or non-pregnant (n = 959) after first service. Values are presented as LSM ± SEM

Rumination activity. For rumination activity from -14 to 28 d after calving (Figure 13A) there was a tendency (*P* = 0.06) for a group by time interaction, and effect of time (*P* < 0.001) but there was no difference (*P* = 0.14) between pregnant (358 ± 3.33 AU) and non-pregnant cows (361 ± 3.42 AU). In addition, there was an effect of season of calving (*P* < 0.001), whereby cows that calved during the cold season had greater rumination activity (382 ± 2.77 AU) than cows that calved during the warm season (338 ± 4.04 AU). Cows with health disorders (340 ± 3.85 AU) recorded up to 56 d after calving had lesser (*P* < 0.001) rumination activity than cows without health disorders (380 ± 2.90AU).

Eating activity. There was a group by time interaction (*P* = 0.01) for eating activity from 0 to 56 d after calving (Figure 13B). Pregnant cows (455 ± 4.73 AU) had lesser eating activity than non-pregnant cows (456 ± 4.62 AU) at -7 to -3 d before calving, whereas the opposite was observed after calving because pregnant cows had greater eating activity (458 ± 4.68 AU) than non-pregnant cows (446 ± 4.55 AU) at 3 to 7 d after calving. There was an effect of time (*P* < 0.001) but there was no overall effect of group (*P* = 0.81; pregnant cows 461 ± 3.67 AU and non-pregnant cows 459 ± 3.57 AU). Cows with health disorders (436 ± 4.14 AU) recorded up to 56 d after calving had greater (*P* < 0.001) eating activity than cows without health disorders (483 ± 3.11 AU). Cows with 2 lactations had greater (*P* < 0.001) eating activity (471 ± 3.07 AU) than cows with ≥3 lactations (448 ± 3.47 AU).

Body temperature. There was a group by time interaction (*P* < 0.001) for body temperature from 0 to 56 d after calving (Figure 13C). From -14 to -3 d before calving, non-pregnant cows had lower body temperature than pregnant cows. Conversely, from day 3 to 7 up to 15 to 28 d after calving, non-pregnant cows had greater body temperature than pregnant cows. There was also an effect of time ($P < 0.001$) but no effect of group ($P = 0.12$; pregnant cows 39.65 ± 0.01 °C and or non-pregnant cows 39.63 ± 0.01 °C). In addition, there was an effect of season of calving ($P < 0.001$), whereby cows that calved during the warm season had greater body temperature (39.69 ± 0.01 °C) than cows that calved during the cold season (39.58 ± 0.01 °C). Cows with health disorders (39.66 ± 0.01 °C) recorded up to 56 d after calving had greater ($P < 0.001$) body temperature than cows without health disorders (39.62 ± 0.01 °C). Cows with 2 lactations had greater ($P < 0.001$) body temperature (39.70 ± 0.01 °C) than cows with ≥3 lactations (39.58 ± 0.01 °C). Reticulo-rumen temperature from -27 to -1 days before TAI (Figure 14A) differed over time ($P < 0.001$) and was greater ($P < 0.001$) for non-pregnant (39.66 ± 0.01 °C) than pregnant cows (39.32 ± 0.01 °C) but there was no group by time interaction ($P = 0.85$). In addition, there was an effect of season of calving ($P = 0.02$), whereby cows that calved during the warm season had greater temperature (39.36 ± 0.01 °C) than cows that calved during the cold season (39.33 ± 0.01 °C). Cows with 2 lactations had greater ($P < 0.001$) temperature (39.37 ± 0.01 °C) than cows with ≥3 lactations (39.32 ± 0.01 °C).

Milk conductivity. Milk conductivity from 0 to 56 d after calving (Figure 13D) differed by time ($P < 0.001$) but there was no difference ($P = 0.24$) for pregnant (9.21 ± 0.04 mmHo) and non-pregnant cows (9.15 ± 0.04 mmHo), and no group by time interaction ($P = 0.77$). In addition, there was an effect of season of calving ($P < 0.001$), whereby cows that calved during the warm season had greater conductivity (9.33 mmHo) than cows that calved during the cold season (9.01 mmHo). Milk conductivity from -27 to -1 days before TAI (Figure 14B) differed over time ($P < 0.001$), was greater ($P < 0.01$) for non-pregnant (9.49 ± 0.02 mmHo) than pregnant cows (9.41 ±

0.02 mmHo) but there was no group by time interaction (P = 0.17). In addition, there was an effect of season of calving (P < 0.01), whereby cows that calved during the cold season had greater milk conductivity (9.49 ± 0.02 mmHo) than cows that calved during the warm season (9.41 ± 0.03 mmHo). Cows with 2 lactations tended to have lesser (P = 0.06) milk conductivity (9.42 ± 0.02 mmHo) than cows with ≥3 lactations (9.48 ± 0.02 mmHo).

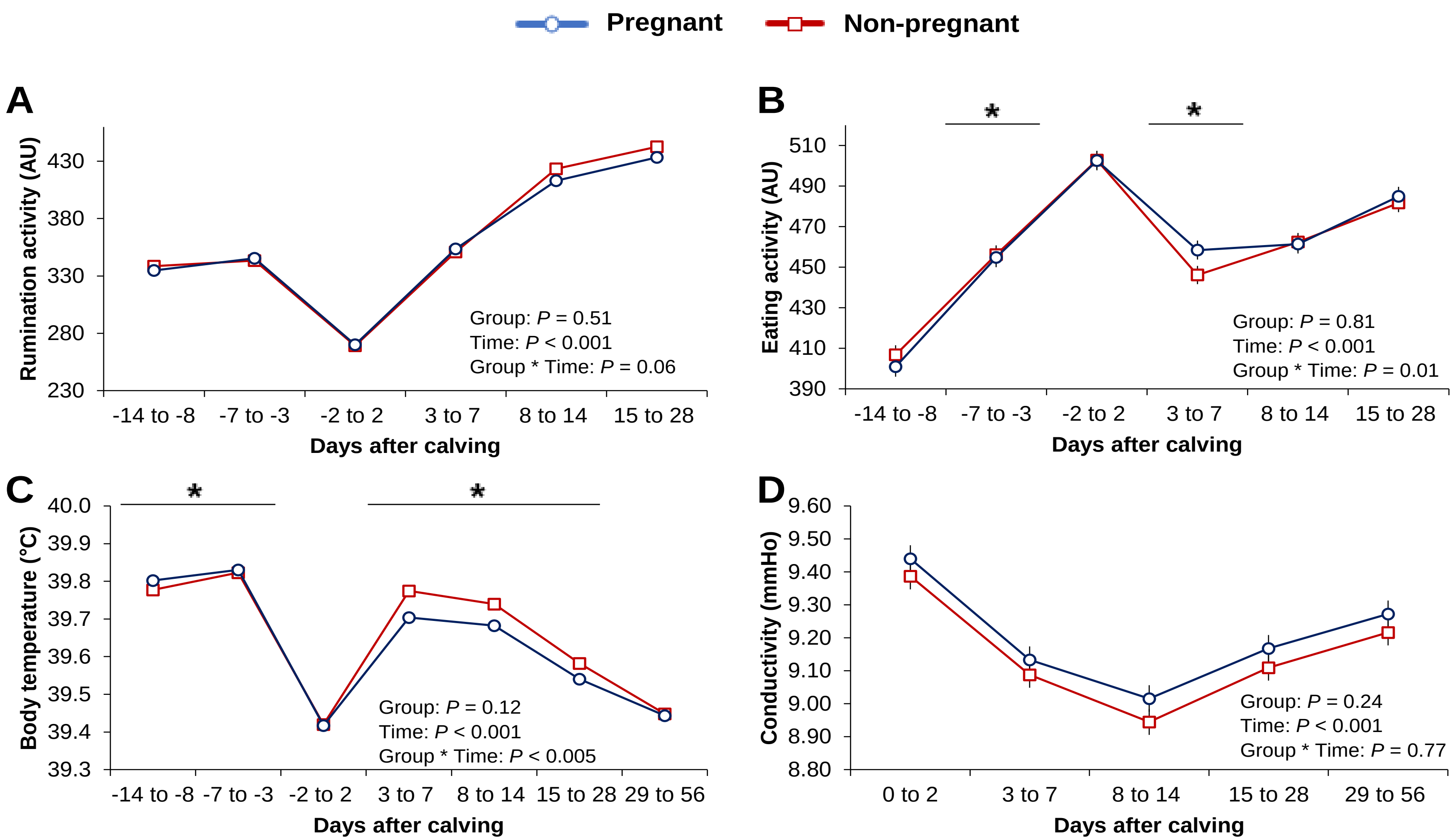

Figure 13. Rumination activity (A), eating activity (B), body temperature (C), and milk conductivity (D) from 0 to 56 d after calving for multiparous cows that were pregnant or not after first service. For rumination and eating activity data was available from 832 pregnant and 892 non-pregnant cows, for body temperature from 391 pregnant and 358 non-pregnant cows and for milk conductivity from 902 pregnant and 959 non-pregnant cows. Within time points, an asterisk (*) represents differences ($P \leq 0.05$) for pairwise comparisons between pregnant and non-pregnant. Values are presented as LSM ± SEM

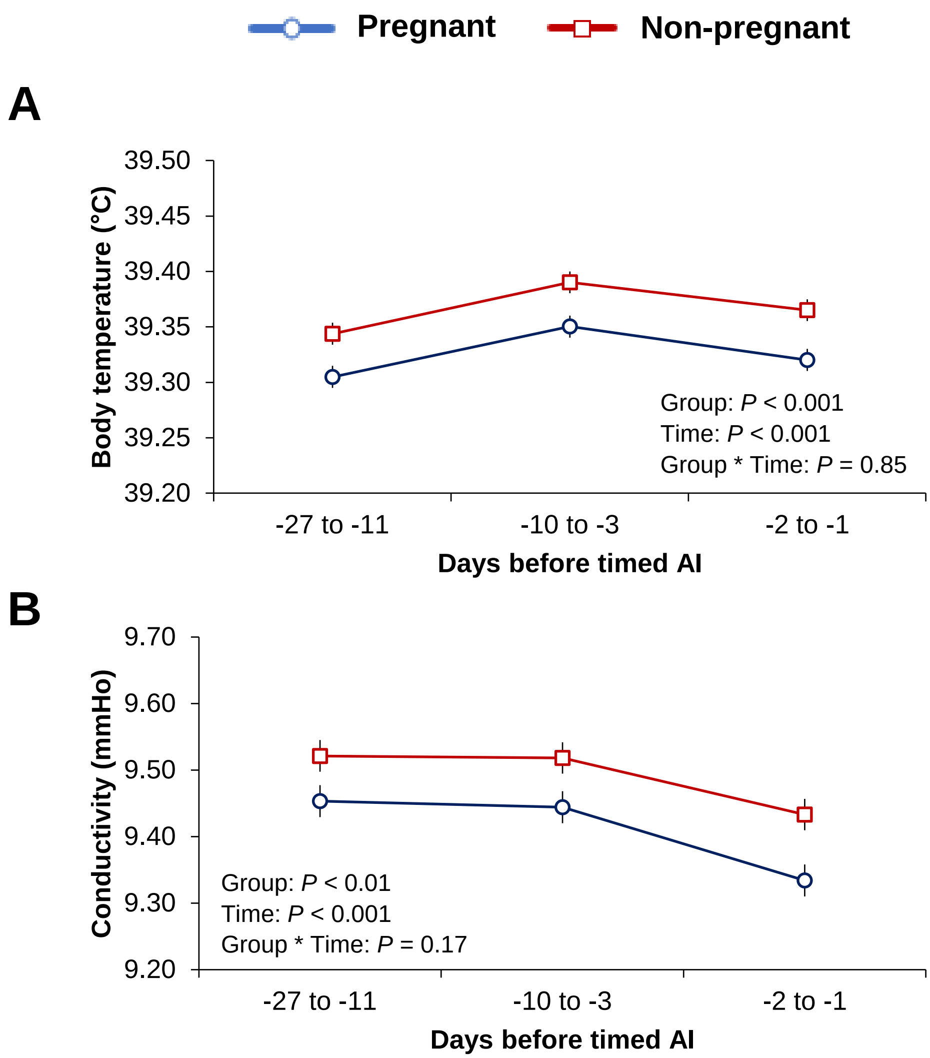

Figure 14. Body temperature (A) and milk conductivity (B) from -27 to -1 d before TAI for multiparous cows that were pregnant or not after first service. For body temperature data was available from 391 pregnant and 358 non-pregnant cows and for milk conductivity data was available from 902 pregnant and 959 non-pregnant cows. Values are presented as LSM ± SEM

Physical activity. There was a tendency for a group by time interaction (*P* = 0.06) for physical activity from -14 to 56 d after calving, whereby non-pregnant cows had greater physical activity up to calving but lesser physical activity starting the first week after calving (Figure 15A). There was also an effect of time (*P* < 0.001) but there was no effect of group (*P* = 0.51; pregnant 4.46 ± 0.04 AU and non-pregnant cows 4.45 ± 0.04 AU). Cows without health disorders (4.40 ± 0.04 AU) recorded up to 56 d after calving tended to have lesser (*P* = 0.06) physical activity than cows with health disorders (4.51 ± 0.05 AU). There was a group by time interaction (*P* < 0.001) for physical activity from -27 to -1 d before TAI whereby non-pregnant cows had greater physical activity levels (pairwise comparisons *P* <0.05 in spite of significant interaction). (Figure 16A). There was also an effect of time (*P* < 0.005) but there was no effect of group (*P* = 0.44; pregnant 4.34 ± 0.04 AU and non-pregnant cows 4.38 ± 0.04 AU). In addition, there was an effect of season of calving (*P* = 0.02), whereby cows that calved during the warm season had lesser activity (4.29 ± 0.05 AU) than cows that calved during the cold season (4.44 ± 0.04 AU). Cows with 2 lactations tended to have greater (*P* = 0.06) physical activity (4.42 ± 0.04 AU) than cows with ≥3 lactations (4.30 ± 0.05 AU).

Walking activity. Walking activity from 0 to 56 d after calving (Figure 15B) tended to be greater (*P* = 0.06) for pregnant (195 ± 1.24 steps/h) than non-pregnant cows (192 ± 1.21 steps/h), differed over time (*P* < 0.001), but there was no group by time interaction (*P* = 0.36). In addition, there was an effect of season of calving (*P* = 0.02), whereby cows that calved during the cold season had lesser walking activity (192 ± 0.93 steps/h) than cows that calved during the warm season (196 ± 1.59 steps/h). Cows with health disorders (189 ± 1.38 steps/h) recorded up to 56 d after calving had lesser (*P* < 0.001) walking activity than cows without health disorders (198 ± 1.08 steps/h). Cows with 2 lactations had greater (*P* < 0.001) walking activity (198 ± 1.24

steps/h) than cows with ≥3 lactations (190 ± 1.20 steps/h). There was a group by time interaction (*P* < 0.005) for walking activity from -27 to -1 days before TAI (Figure 16B) because from -27 to -11 d before TAI pregnant cows had lesser protein percent than non-pregnant cows. There was an effect of time (*P* < 0.001) but there was no overall group difference (*P* = 0.50) for pregnant (161 ± 1.39 steps/h) and non-pregnant cows (162 ± 1.36 steps/h). In addition, cows without health disorders (163 ± 1.21 steps/h) recorded up to 56 d after calving had more (*P* = 0.05) walking activity than cows with health disorders (160 ± 1.58 steps/h). Cows with 2 lactations had more (*P* < 0.001) walking activity (168 ± 1.40 steps/h) than cows with ≥3 lactations (155 ± 1.36 steps/h).

Resting behavior. Resting time from 0 to 56 d after calving (Figure 15C) differed by time (*P* < 0.001) but there was no difference (*P* = 0.26) between pregnant (568 ± 3.81 min/d) and non-pregnant cows (526 ± 3.72 min/d), and no group by time interaction (*P* = 0.65). In addition, there was an effect of season of calving (*P* < 0.001), whereby cows that calved during the warm season had lesser resting time (551 ± 4.89 min/d) than cows that calved during the cold season (579 ± 2.86 min/d). Cows with health disorders (576 ± 4.25 min/d) recorded up to 56 d after calving had greater (*P* < 0.001) resting time than cows without health disorders (553 ± 3.33 min/d). Cows with ≥3 lactations had greater (*P* < 0.003) resting time (572 ± 3.69 min/d) than cows with 2 lactations (558 ± 381 min/d). There was a tendency for a group by time interaction (*P* = 0.06) for resting time from -27 to -1 d before TAI (Figure 16C), an effect of time (*P* < 0.001), and an overall effect of group as resting time was greater (*P* = 0.001) for pregnant (653 ± 4.70 min/d) than non-pregnant cows (634 ± 4.60 min/d). In addition, there was an effect of season of calving (*P* = 0.02), whereby cows that calved during the warm season had greater resting time (652 ± 5.98 min/d) than cows that calved during the cold season (635 ± 3.59 min/d).

Cows with health disorders (651 ± 5.34 min/d) recorded up to 56 d after calving had greater (P = 0.01) resting time than cows without health disorders (636 ± 4.07 min/d). Cows with ≥3 lactations had greater (P < 0.003) rest time (665 ± 4.60 min/d) than cows with 2 lactations (622 ± 4.71 min/d).

The number of lying bouts per day from 0 to 56 d after calving (Figure 15D) differed over time (P < 0.001) but there was no difference (P = 0.70) for pregnant (9.56 ± 3.81 bouts/d) and non-pregnant cows (9.51 ± 0.09 bouts/d), and there was no group by time interaction (P = 0.87). Cows that calved during the warm season tended (P = 0.06) to have more lying bouts (9.66 ± 0.12 bouts/d) than cows that calved during the cold season (9.40 ± 0.07 bouts/d). Cows with health disorders (9.71 ± 0.11 bouts/d) recorded up to 56 d after calving had more (P < 0.01) lying bouts than cows without health disorders (9.36 ± 0.08 bouts/d). Cows with 2 lactations had greater (P < 0.001) lying bouts (9.81 ± 0.09 bouts/d) than cows with ≥3 lactations (9.25 ± 0.09 bouts/ d). The number of lying bouts per day from -27 to -1 d before TAI (Figure 16D) differed over time (P < 0.001) but there was no difference (P = 0.36) for pregnant (9.95 ± 0.11 bouts/d) and non-pregnant cows (9.82 ± 0.11 bouts/d), and there was no group by time interaction (P = 0.32).

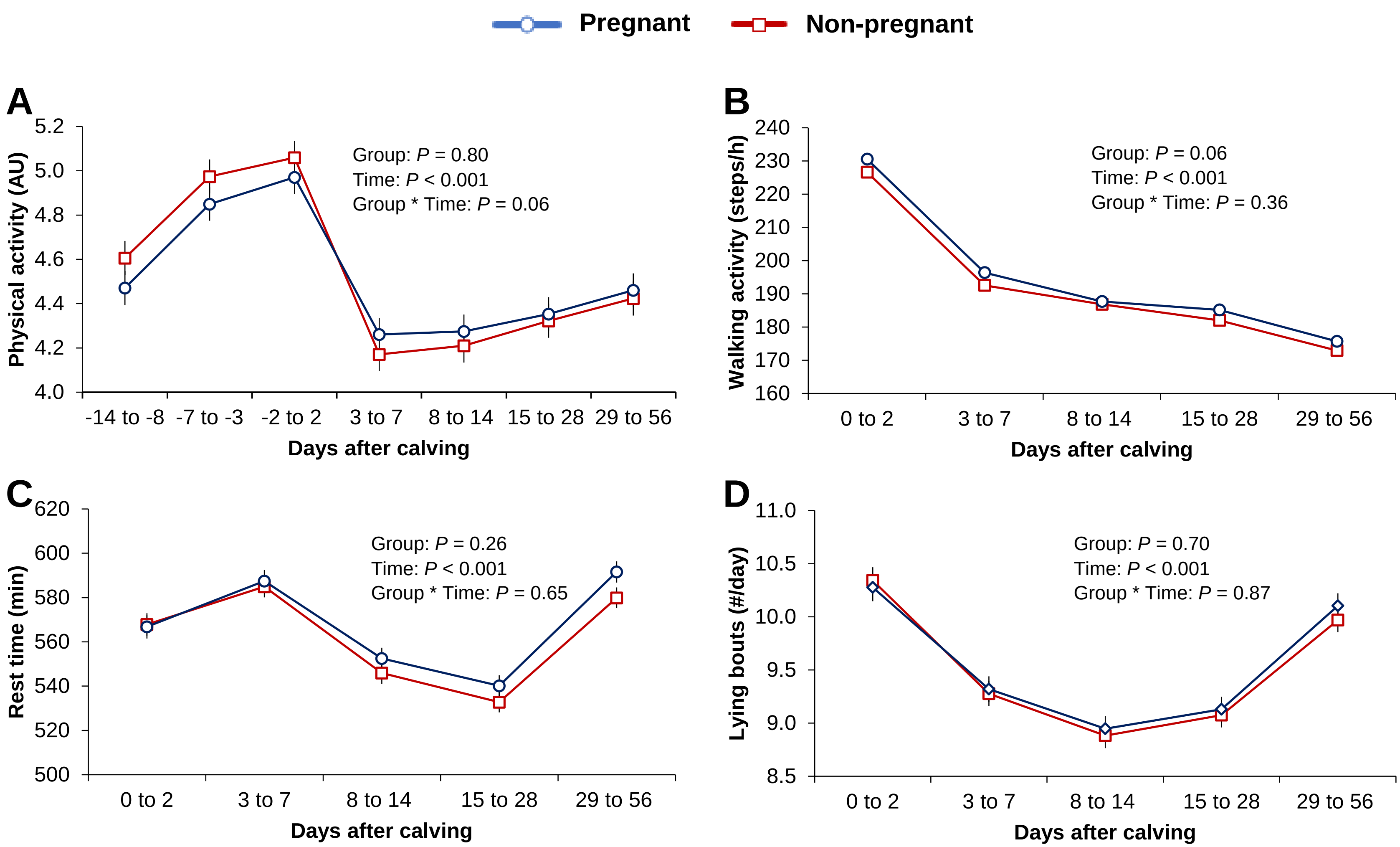

Figure 15. Physical activity (A), walking activity (B), rest time (C), and lying bouts (D) from 0 to 56 d after calving for multiparous cows that were pregnant or not after first service. For physical activity data was available from 416 pregnant and 391 non-pregnant cows, for activity, rest time and lying bouts data was available from 900 pregnant and 959 non-pregnant cows. Values are presented as LSM ± SEM

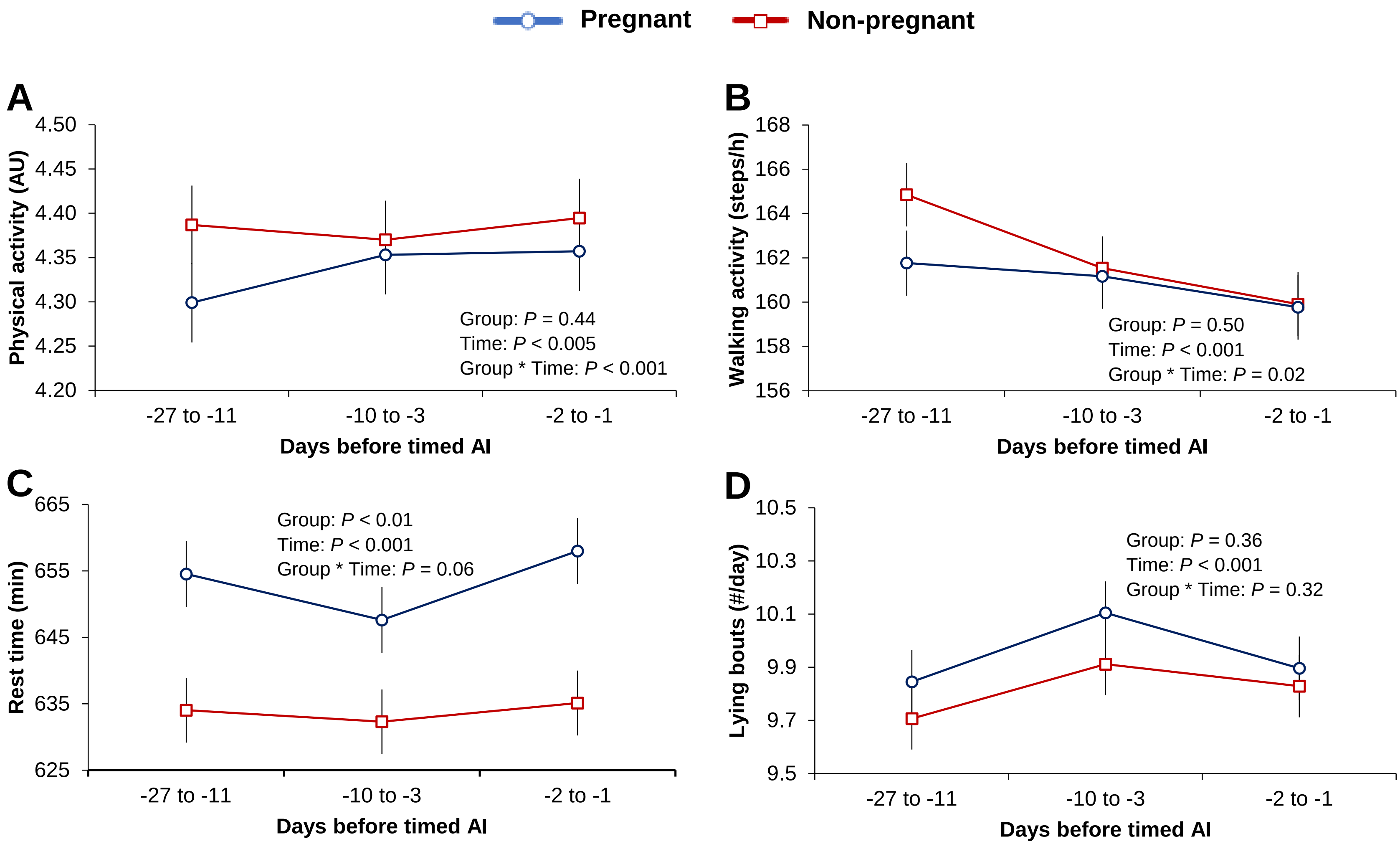

Figure 16. Physical activity (A), walking activity (B), rest time (C), and lying bouts (D) from -27 to -1 d before TAI for multiparous cows that were pregnant or not after first service. For physical activity data was available from 416 pregnant and 391 non-pregnant cows, for activity, rest time and lying bouts data was available from 900 pregnant and 959 non-pregnant cows. Values are presented as LSM ± SEM.

Body weight change after calving for primiparous and multiparous cows

Absolute BW from 0 to 56 d after calving (Figure 17A) differed with time ($P < 0.001$) as cow lost weight after calving but there was no difference ($P = 0.11$) for pregnant (558 ± 2.91 kg) and non-pregnant cows (552 ± 3.61 kg), and no group by time interaction ($P = 0.45$). In addition, cows without health disorders (562 ± 2.61 kg) recorded up to 56 d after calving had greater ($P < 0.005$) BW than cows with health disorders (549 ± 4.01 kg). Absolute BW for primiparous cows from -27 to -1 d before TAI (Figure 17B) differed by time ($P < 0.001$) as cows continued to lose weight until FS, but there was no difference ($P = 0.88$) for pregnant (567 ± 2.75 kg) and non-pregnant cows (567 ± 3.44 kg), and no group by time interaction ($P = 0.91$). In addition, cows without health disorders (572 ± 2.46 kg) recorded up to 56 d after calving had greater ($P = 0.03$) absolute body weight than cows with health disorders (562 ± 3.83 kg).

The percent BW change between 3 d after calving and the BW nadir (Figure 18) was not different ($P = 0.39$) for pregnant (-4.99 ± 0.32%) and non-pregnant (-4.58 ± 0.40%) cows. For the period between the nadir for body weight and 56 d after calving, the percent body weight change (Figure 18) was not different ($P = 0.14$) for pregnant (2.34 ± 0.21%) and non-pregnant cows (2.80 ± 0.27%). There was an effect of season of calving ($P < 0.001$), whereby cows that calved during the warm season had greater change in body weight (3.26 ± 0.29%) than cows that calved during the cold season (1.89 ± 0.19%), and cows with health disorders (3.18 ± 0.30%) recorded up to 56 d after calving had greater ($P < 0.001$) change in body weight than cows without health disorders (1.96 ± 0.19%). For the period from 3 to 56 d after calving (Figure 18) the percent change in body weight was not different ($P = 0.18$) for pregnant (-2.13 ± 0.48%) and non-pregnant (-2.88 ± 0.37%) cows.

There was no difference (*P* = 0.67) for P/AI for cows with BW loss (68.7%, n = 57), no change (63.3%, n = 100), or gain (64.1%, n = 166) between 3 d after calving and the BW nadir (Figure 20A). There was also no difference (*P* = 0.32) in P/AI for cows that had BW loss (69.6%, n = 96), no change (61.8%, n = 97), or gain (66.0%, n = 169) for the period between the body weight nadir and 56 d after calving (Figure 20A). For cows grouped based on BW change for the period between the 3 and 56 d after calving (Figure 20A), there was no difference (*P* = 0.89) in P/AI for cows that had BW loss (67.1%, n = 49), no change (65.3%, n = 93), or gain (64.7%, n = 178).

Multiparous cows. There was a group by time interaction (*P* = 0.04) for absolute body weight from 0 to 56 d after calving (Figure 17C) because pregnant cows (722 ± 3.00 kg) had lesser body weight than non-pregnant cows (729 ± 3.11 kg) at 0 to 2 d after calving but not thereafter. Body weight changed over time (*P* < 0.001) as cows lost weight until 56 DIM, but there was no difference between groups (*P* = 0.18). Average body weight for the whole period was 687 ± 2.85 and 691 ± 2.97 kg for pregnant and non-pregnant cows, respectively. Cows with ≥3 lactations had greater (*P* < 0.003) body weight (725 ± 2.70 kg) than cows with 2 lactations (653 ± 3.14 kg).

Absolute body weight from -27 to -1 d before TAI (Figure 17D) differed by time (*P* < 0.001) because cows lost BW overtime but there was no difference (*P* = 0.15) for pregnant cows (667 ± 3.37 kg) and non-pregnant cows (673 ± 3.56 kg), and no group by time interaction (*P* = 0.25). In addition, cows with ≥3 lactations had greater (*P* < 0.001) body weight (701 ± 3.18 kg) than cows with 2 lactations (639 ± 3.77 kg).

The percent body weight change between 3 d after calving and the nadir (Figure 19) was greater (*P* = 0.02) for non-pregnant (-8.51 ± 0.37%) than pregnant cows (-7.94 ± 0.34%). Cows with health disorders (-8.55 ± 0.41%) recorded up to 56 d after calving had greater (*P* = 0.02) change in percent body weight than cows without health disorders (-7.40 ± 0.31%). The percent body weight change between the nadir and 56 d after calving (Figure 19) was not different (*P* = 0.73) for pregnant (0.28 ± 0.16%) and non-pregnant cows (0.36 ± 0.18%). The change in percent body weight between 3 d after calving and 56 d after calving (Figure 19) was greater (*P* = 0.03) for non-pregnant (-8.25 ± 0.41%) than pregnant cows (-7.08 ± 0.39%). Cows with health disorders (-8.34 ± 0.46%) recorded up to 56 d after calving had greater (*P* = 0.01) change in percent body weight than cows without health disorders (-6.99 ± 0.35%).

There was no difference (*P* = 0.42) in P/AI for cows with BW loss (46.9%, n = 164), no change (51.4%, n = 149), or gain (52.9%, n = 92) body weight between 0 d after calving and 56 d after calving for body weight (Figure 20B). There was an effect of season of calving (*P* = 0.03), whereby cows that calved during the warm season had greater P/AI (58.0 ± 3.83%) than cows that calved during the cold season (48.9 ± 2.20%), and cows with 2 lactations tended to have greater (*P* = 0.07) P/AI (56.8 ± 3.13%) than cows with ≥3 lactations (50.2 ± 2.71%). For cows grouped based on body weight change between the nadir for body weight and 56 d after calving (Figure 20B), the group that lost (55.4%, n = 206) body weight tended to have greater (*P* = 0.07) P/AI than cows that had no change (47.28%, n = 174), whereas cows that gained (54.3%, n = 138) body weight had similar P/AI than cows that lost or had no change. For cows grouped based on body weight change between 3 and 56 d after calving (Figure 20B), there was no difference (*P* = 0.82) in P/AI for cows that lost (49.58%, n = 176), had no change (49.2%, n = 146), or gained (52.3%, n = 80) BW. There was an effect of season of calving (*P* = 0.02), whereby cows that calved during the cold season had greater P/AI (51.3 ± 2.25%) than cows that calved during the warm season (41.4 ± 3.82%).

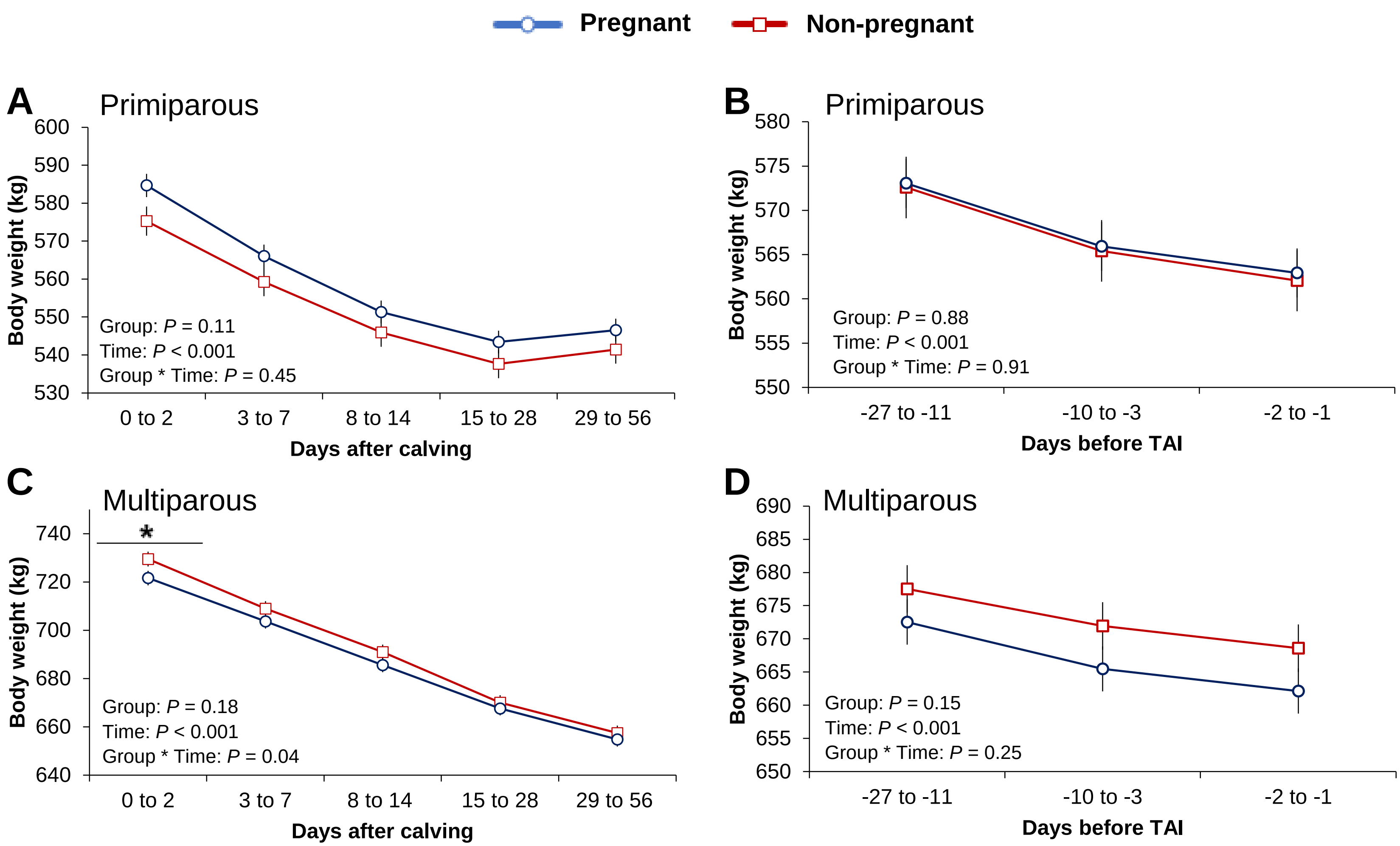

Figure 17. Body weight for pregnant and non-pregnant primiparous cows from 0 to 56 days after calving (A) and from -27 to -1 days before TAI (B). Body weight for pregnant and non-pregnant multiparous cows from 0 to 56 days after calving (C) and from -27 to -1 days before TAI (D). For primiparous cows, data was available from 395 pregnant and 217 non-pregnant cows whereas for multiparous cow data was available from 561 pregnant and 530 non-pregnant cows. Within time points, an asterisk (*) represents differences ($P \leq 0.05$) for pairwise comparisons between pregnant and non-pregnant. Values are presented as LSM ± SEM

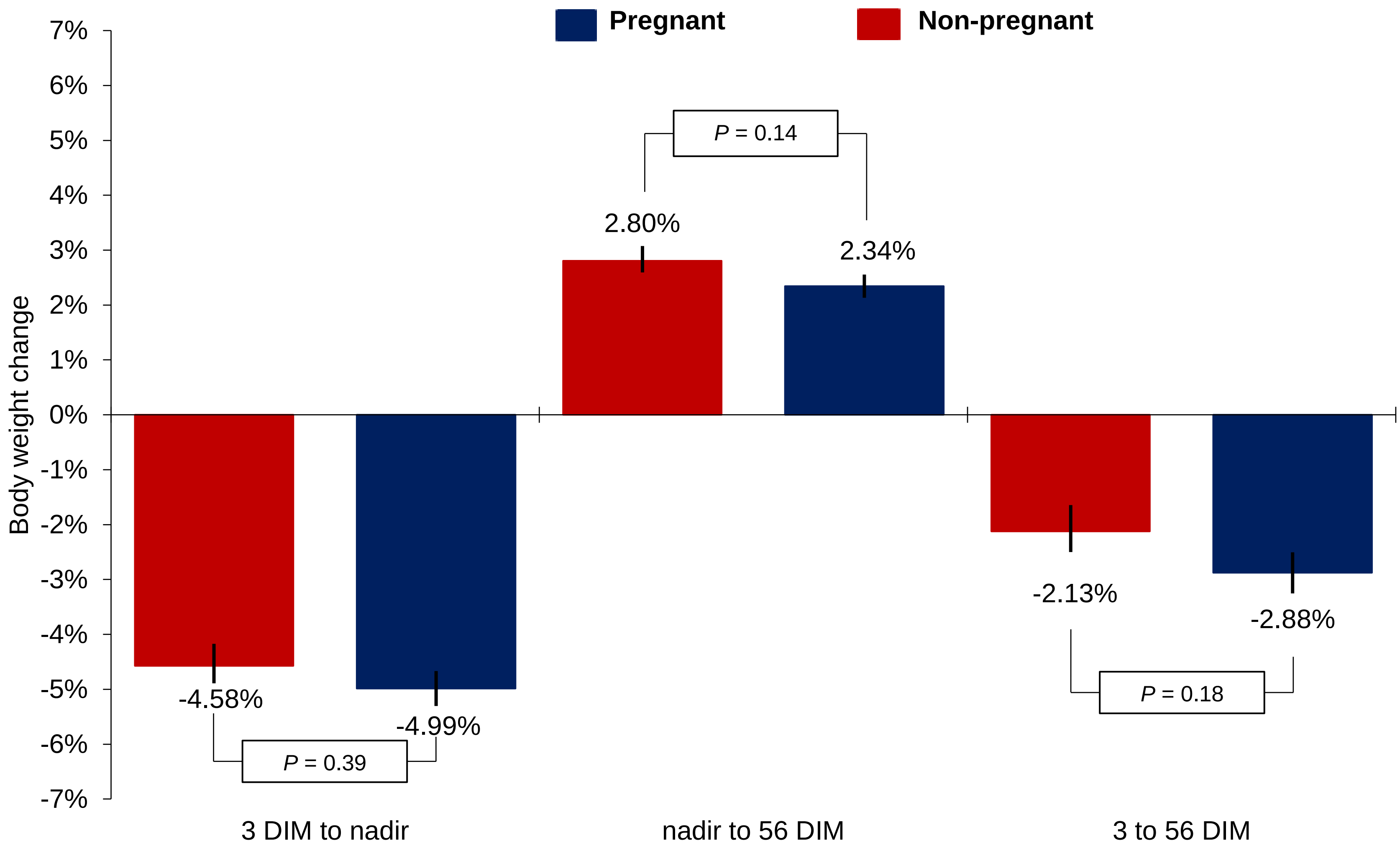

Figure 18. Percent body weight change from 3 d after calving to the nadir for body weight, nadir for body weight to 56 d after calving, and from 3 to 56 d after calving for pregnant and non-pregnant primiparous cows, data was available from 362 pregnant and 189 non-pregnant cows**.**

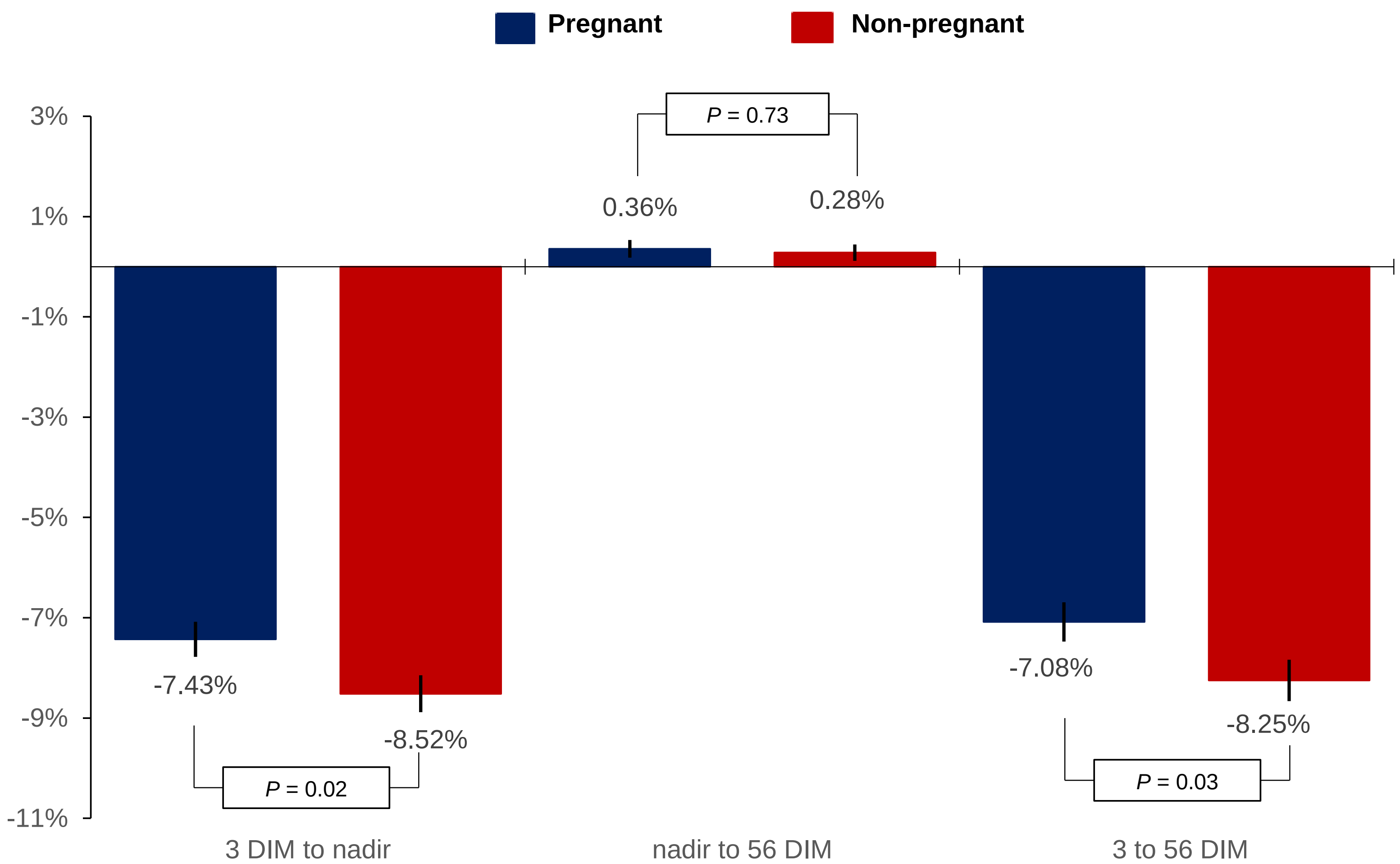

Figure 19. Percent body weight change from 3 d after calving to the nadir for body weight, nadir for body weight to 56 d after calving, and from 3 to 56 d after calving for pregnant and non-pregnant multiparous cows, data was available from 518 pregnant and 476 non-pregnant cows

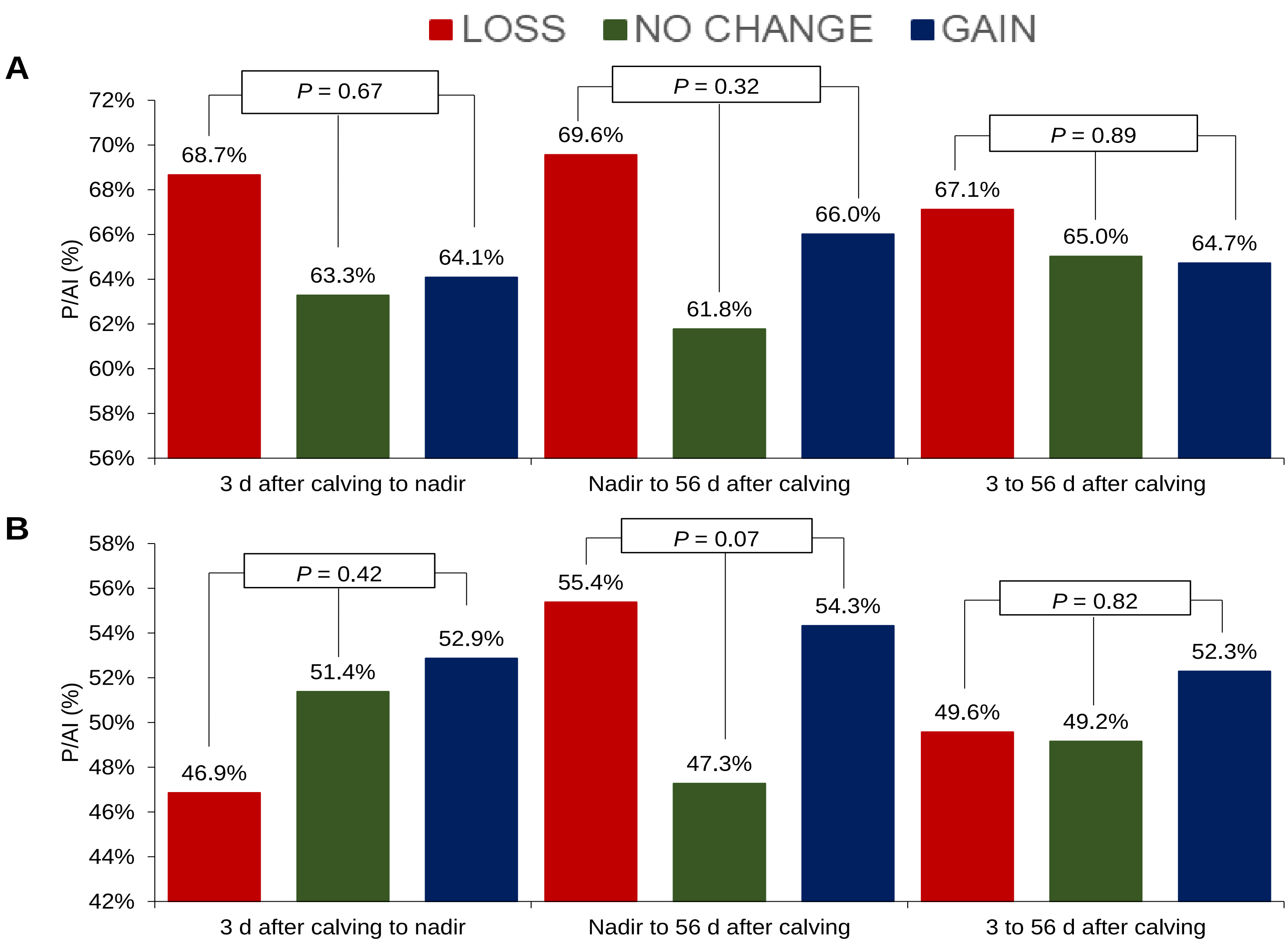

Figure 20. Association between body weight change group (i.e., loss, no change, gain) for specific time points from 0 to 56 days after calving and first service P/AI for (A) primiparous and (B) multiparous cows. For primiparous cows, data was available from 362 pregnant and 189 non-pregnant cows, for multiparous cows from 518 pregnant and 476 non-pregnant cows.

Effect of Health Disorders on Pregnancies per AI for Primiparous and Multiparous cows

Primiparous cows. There was no difference (*P* = 0.42) in P/AI (Figure 21) between cows with (60.0%, n = 132) and cows without (63.1%, n = 449) health disorders up to 56 d after calving. In addition, there was no difference (*P* = 0.44) for cows that calved during the warm (63.8 ± 3.38%) and cold season (60.9 ± 2.08%).

Multiparous cows. Cows (Figure 21) with health disorders (44.8%, n = 330) tended (*P* = 0.10) to have reduced P/AI than cows without health disorders (49.0%, n = 653). In addition, there was no difference (*P* = 0.54) for cows that calved during the warm (48.2 ± 2.29%) and cold season (46.6 ± 1.30%), and there was no difference (*P* = 0.18) for cows with 2 (48.8 ± 1.76%) or ≥3 lactations (46.1 ± 1.71%).

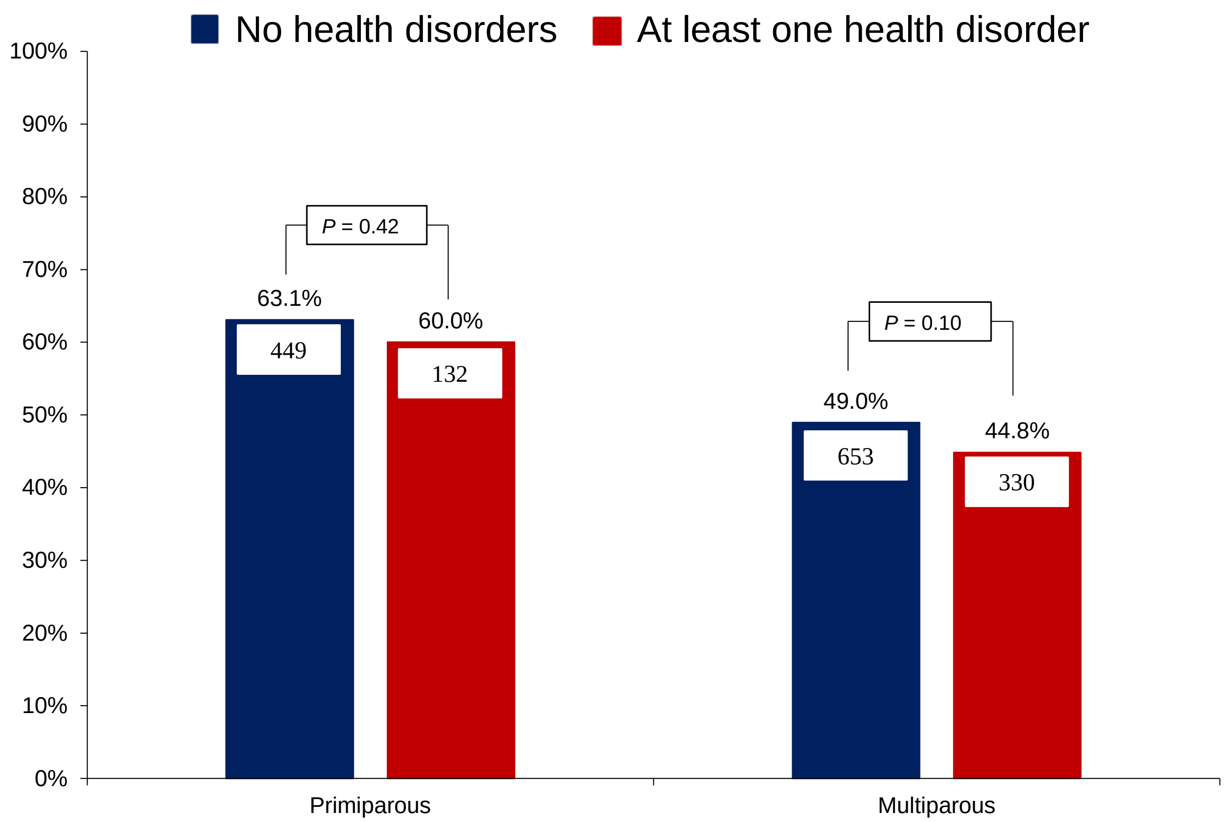

Figure 21. Association between occurrence of health disorders recorded up to 56 d after calving and first service pregnancy per AI for primiparous and multiparous cows. For primiparous cows, data was available from 712 healthy and 220 cows with at least one health disorder, for multiparous cows from 1333 healthy and 737 cows with at least one health disorder**.**

Previous Lactation Production Performance for Multiparous Cows

Previous lactation total milk yield (Figure 24) was greater (P < 0.001) for non-pregnant (12,792 ± 90.8 kg) than pregnant cows (12,335 ± 94.7 kg). In addition, cows that calved during the cold season tended to have greater (P = 0.08) milk yield (12,687 ± 69.9 kg) than cows that calved during the warm season (12,440 ± 123 kg/d). There was no difference (P = 0.47) for cows with (12,608 ± 119.9 kg/d) or without (12,519 ± 82.8 kg) health disorders up to 56 d after calving. Cows with ≥3 lactations had greater (P < 0.001) milk yield (13,410 ± 91.9 kg) than cows with 2 lactations (11,717 ± 93.9 kg).

Previous lactation 305 d milk yield (Figure 24) was greater (P = 0.001) for non-pregnant (11,950 ± 68.6 kg) than pregnant cows (11,663 ± 71.5 kg). In addition, there was an effect of season of calving (P = 0.01), whereby cows that calved during the cold season had greater 305 d milk (11,942 ± 52.8 kg) than cows that calved during the warm season (11,672 ± 92.6 kg). There was no difference (P = 0.26) for cows with (11,754 ± 79.4 kg) or without (11,859 ± 62.5 kg) health disorders. Cows with ≥3 lactations had greater (P < 0.001) milk yield (12,740 ± 69.4 kg) than cows with 2 lactations (10,873 ± 70.9 kg).

Previous lactation total fat yield (Figure 24) was greater (P < 0.001) for non-pregnant (483 ± 3.53 kg) than pregnant cows (462 ± 3.68 kg). In addition, cows with ≥3 lactations had greater (P < 0.001) fat yield (501 ± 3.57 kg) than cows with 2 lactations (444 ± 3.65 kg). There was no difference (P = 0.40) for cows that calved during the warm (470 ± 4.76 kg) and cold season (475 ± 2.72 kg), and there was no difference (P = 0.75) between cows with (472 ± 4.08 kg) or without health disorders (473 ± 3.22 kg).

Previous lactation total protein yield (Figure 24) was greater (P < 0.001) for non-pregnant (379 ± 2.58 kg) than pregnant cows (367 ± 2.69 kg). In addition, cows with ≥3 lactations had greater ($P < 0.001$) fat yield (395 ± 2.61 kg) than cows with 2 lactations (351 ± 2.66 kg). There was no difference ($P = 0.40$) for cows that calved during the warm (371 ± 3.48 kg) and cold season (375 ± 1.98 kg), and there was no difference ($P = 0.75$) between cows with (374 ± 2.98 kg) or without health disorders (372 ± 2.35 kg).

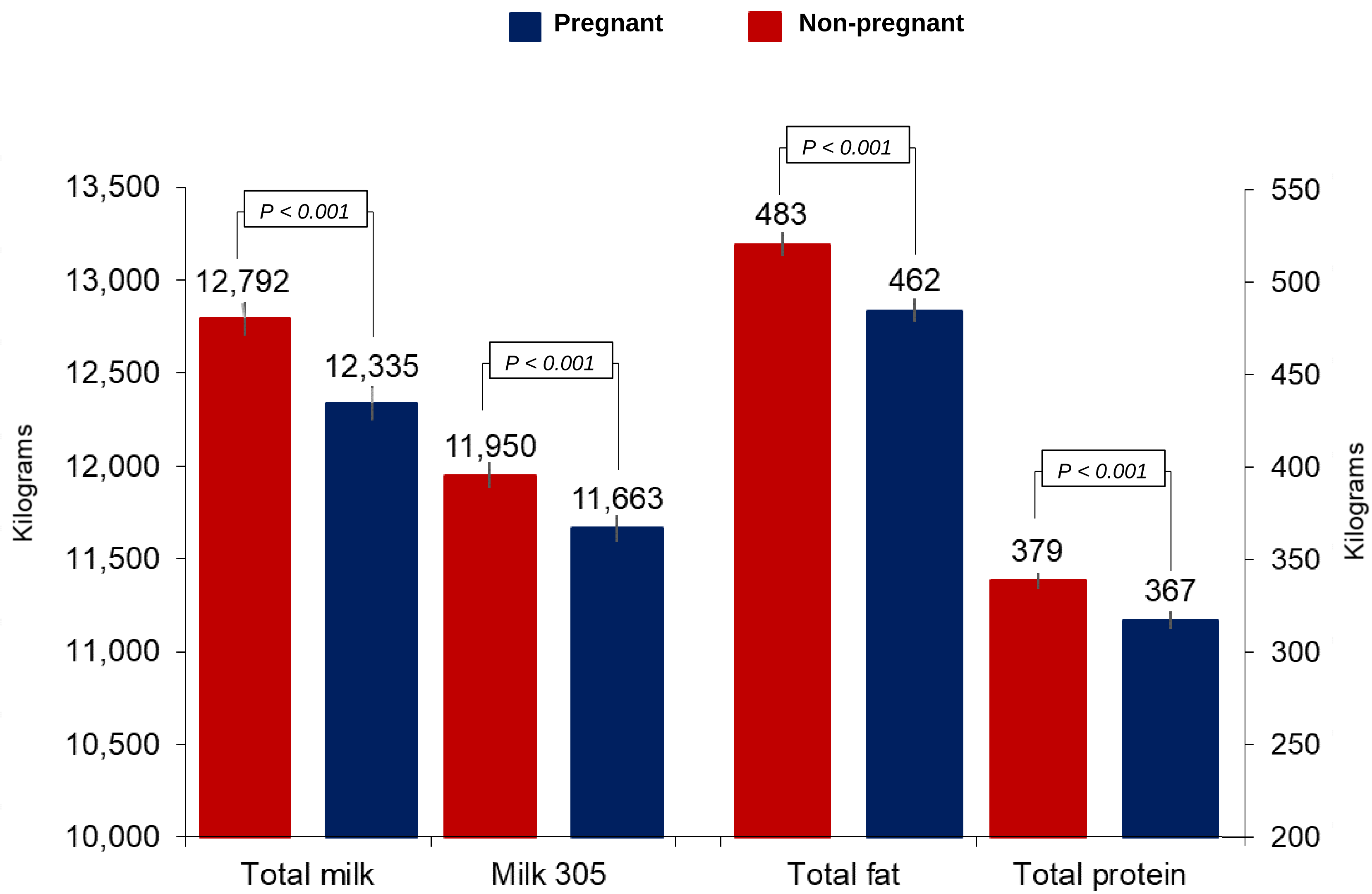

Figure 24. Previous lactation total milk, previous lactation 305 d milk, previous lactation total fat, and previous lactation total protein yield for multiparous cows that were pregnant (n = 983) or non-pregnant (n = 1,087) after first service. Values are presented as LSM ± SEM.

Previous Lactation Features and Reproductive Performance

Gestation length (Figure 25) was greater ($P < 0.001$) for non-pregnant (276.56 ± 0.18 d) than pregnant cows (275.79 ± 0.18 d). In addition, there was an effect of season of calving (P = 0.04), whereby cows that calved during the cold season had longer gestation length (276.46 ± 0.14 d) than cows that calved during the warm season (275.89 ± 0.24 d). Cows without health disorders (276.59 ± 0.16 d) recorded up to 56 d after calving had greater ($P < 0.001$) gestation length than cows with health disorders (275.75 ± 0.21 d). Cows with ≥3 lactations had greater (P < 0.001) gestation length (276.92 ± 0.18) than cows with two lactations (275.43 ± 0.18 d).

The calving interval for multiparous cows (Figure 25) was longer ($P < 0.005$) for non-pregnant (385 ± 1.39 d) than pregnant cows (380 ± 1.45 d). In addition, cows that calved during the warm season tended to have longer ($P = 0.08$) calving interval (385 ± 1.87 d) than cows that calved during the warm season (381 ± 1.07 d). Cows with health disorders (387 ± 1.60 d) recorded up to 56 d after calving had greater ($P < 0.001$) calving interval than cows without health disorders (378 ± 1.26 d). Cows with two lactations had greater ($P < 0.001$) calving interval (386 ± 1.43) than cows with ≥3 lactations (379 ± 1.40 d).

Non-pregnant cows (110 ± 1.52 d) had more ($P = 0.04$) days open in the previous lactation (Figure 25) than pregnant cows (106 ± 1.59 d). In addition, there was an effect of season of calving ($P = 0.04$), whereby cows that calved during the warm season had more days open (110 ± 2.07 d) than cows that calved during the cold season (105 ± 1.17 d). Cows with health disorders (103 ± 1.39 d) recorded up to 56 d after calving had more ($P < 0.001$) days open than cows without health disorders (113 ± 1.73 d). Cows with two lactations had greater (P = 0.001) days open (112 ± 1.57) than cows with ≥3 lactations (104 ± 1.54 d).

Multiparous cows non-pregnant after first service (69.5 ± 1.14 d) tended to have greater ($P = 0.10$) days dry (Figure 25) than pregnant cows (67.3 ± 1.08 d). In addition, there was an effect of season of calving ($P < 0.005$), whereby cows that calved during the warm season had more days dry (71.0 ± 1.54 d) than cows that calved during the cold season (65.8 ± 0.79 d). Cows with health disorders (69.9 ± 1.24 d) recorded up to 56 d after calving had more ($P = 0.03$) days dry than cows without health disorders (66.8 ± 1.00 d). Cows with ≥3 lactations had more ($P = 0.001$) days dry (71.0 ± 1.18) than cows with two lactations (65.8 ± 1.05 d).

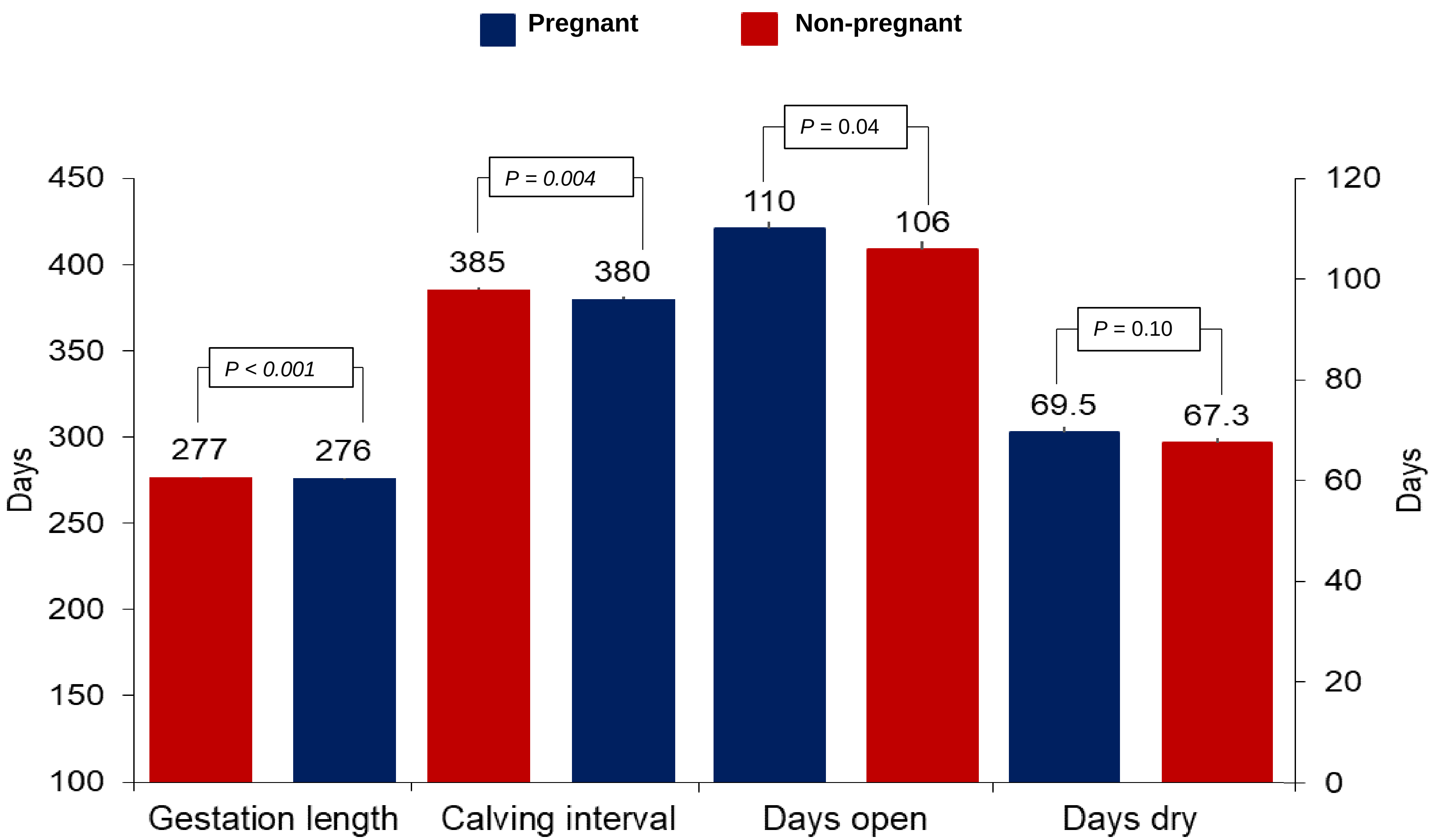

Figure 25. Gestation length, calving interval, days open, and days dry for multiparous cows that were pregnant (n = 983) or non-pregnant (n = 1,087) after first service. Values are presented as LSM ± SEM.

Association Between Temperature and Humidity Index (THI) Inside or Outside Cow Barns and Pregnancies per AI for Primiparous and Multiparous Cows

Primiparous cows. There was no difference (*P* > 0.10) in P/AI between cows exposed to THI inside of cow barns of <72 or >72 at any time point evaluated from -14 to 56 days after calving (Figure 26). Likewise, there was no difference (*P* > 0.10) in P/AI between cows exposed to THI outside of cow barns of <72 or >72 at any time point evaluated from -14 to 56 days after calving (Figure 27).

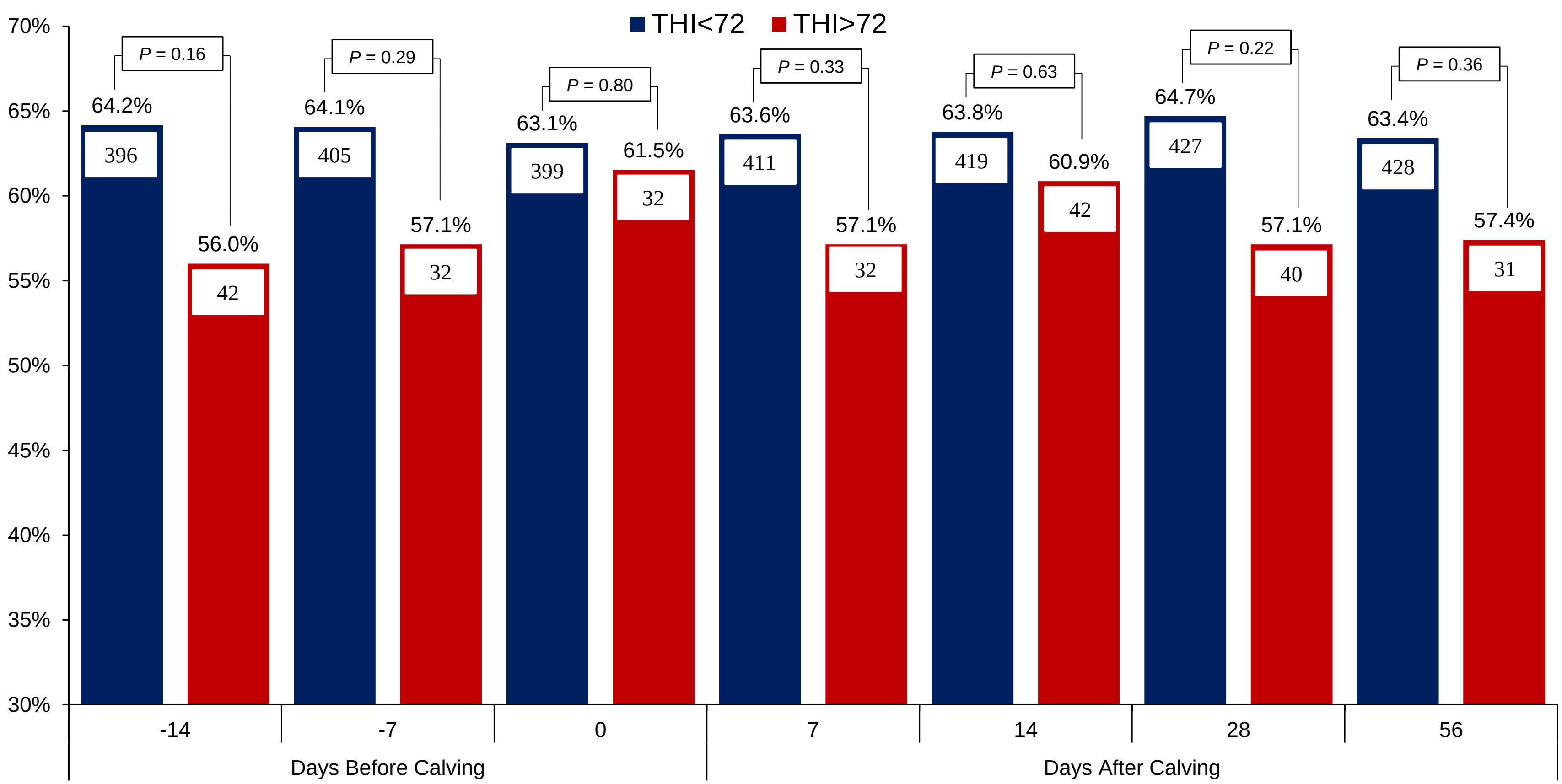

Figure 26. Association between inside of cow barns THI and pregnancies per AI for primiparous cows. Data were available from 779 cows with THI<72 and 71cows with THI>72**.**

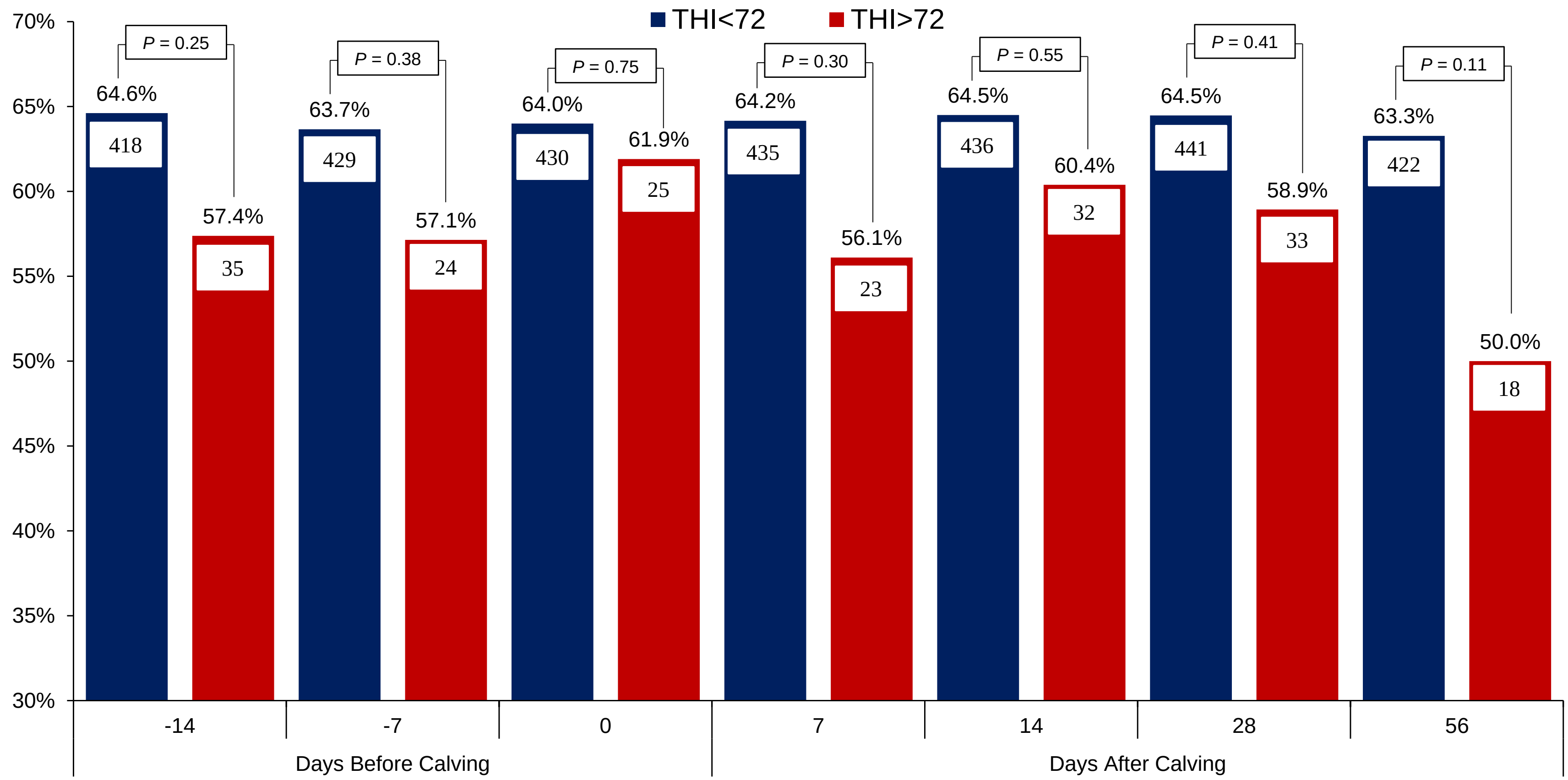

Figure 27. Association between outside of cow barns THI and pregnancies per AI for primiparous cows. Data were available from 732 cows with THI<72 and 71 cows with THI>72.

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Multiparous cows. There was no difference (*P* > 0.10) in P/AI between cows exposed to THI inside of cow barns of <72 or >72 at any time point evaluated except from 29 to 56 days after calving at which P/AI was greater (*P* = 0.01) for cows that were exposed to THI<72 than >72 (Figure 28). Likewise, there was no difference (*P* > 0.10) in P/AI between cows exposed to THI outside of cow barns of <72 or >72 at any time point evaluated except from 29 to 56 days after calving at which P/AI was greater (*P* = 0.01) for cows that were exposed to THI<72 than >72 (Figure 29).

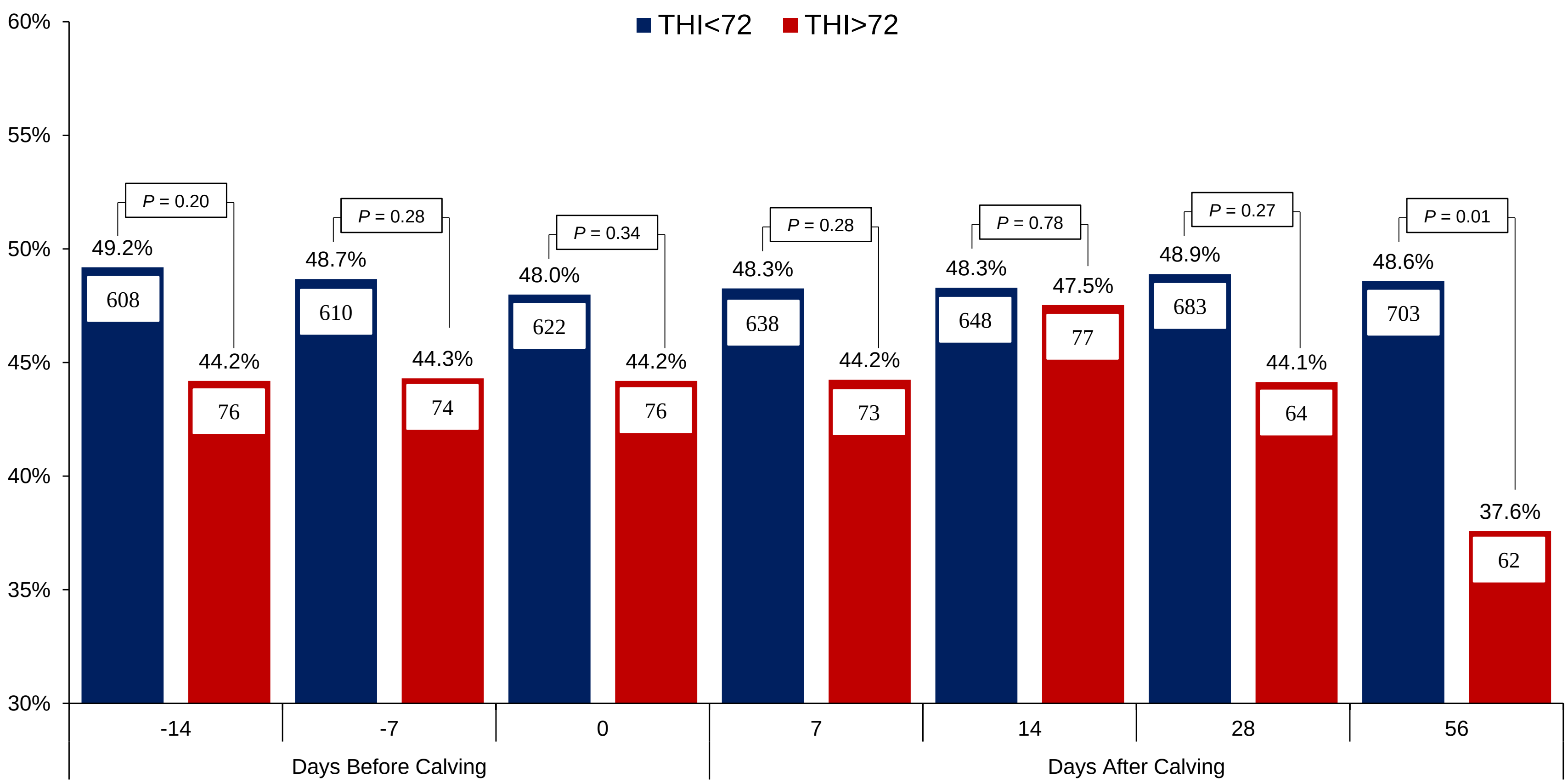

Figure 28. Association between inside of cow barns THI and pregnancies per AI for multiparous cows. Data were available from 1114 cows with THI<72 and 123 cows with THI>72.

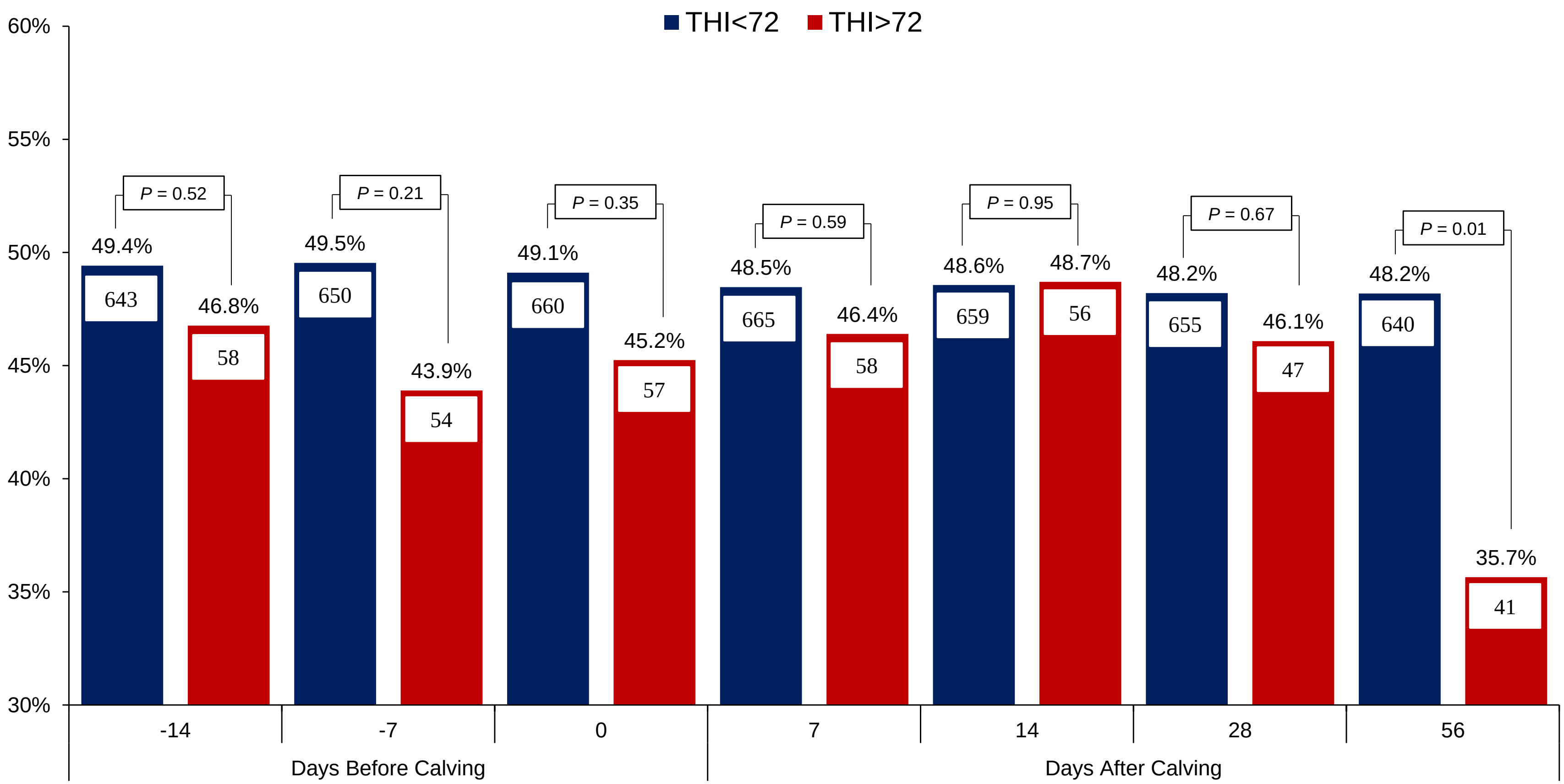

Figure 29. Association between outside of cow barns THI and pregnancies per AI for multiparous cows. Data were available from 1114 cows with THI<72 and 123 cows with THI>72.

DISCUSSION

A better understanding of associations between potential predictors of fertility and outcomes of individual AI services in lactating dairy cows might enable the implementation of targeted reproductive management (**TRM**) strategies in dairy herds (Giordano et al., 2022). As dairy cow fertility is affected by a myriad of biological, management, and environmental factors (López-Gatius, 2012), data for behavioral, physiological, and performance parameters generated by automated sensors might be useful for predicting cow fertility. Sensor parameter data might indicate the cow physiological status as well as cow responses to management and environmental conditions. To better understand these associations, we characterized the pattern of multiple behavioral, physiological, and performance parameters in cows that became pregnant or not at FS. Data were evaluated separately for primiparous and multiparous cows because of the well-known biological (Vercouteren et al., 2015), behavioral (Soriani et al., 2012), and productive (Lean et al., 1989) and reproductive performance (Bonneville-Hébert et al., 2011) differences among parities.

Although there were no differences between pregnant and non-pregnant cows for most sensor data patterns explored, or between groups of cows created based on sensor parameters of interest, several associations were observed for performance outcomes for primiparous cows. Overall, data supported an association between milk production level and pregnancy success because pregnant cows had greater milk, butterfat, protein, and lactose yield up to 56 DIM. Although not statistically significant, most outcomes presented differences of similar magnitude during synchronization of ovulation before TAI. Lack of differences in components percent (except lactose for the -27 to -1 d before TAI) and the fat-to-protein ratio indicated that greater component yield was primarily because of milk production volume. Previous studies reported conflicting results about the association between milk production and reproductive performance of lactating dairy cows with some studies reporting negative (Albarrán-Portillo et al., 2013), positive (Roxström et al., 2001), or no associations (Caraviello et al., 2006; Bello et al., 2012) with a large portion of this variation explained by farm-to-farm variation. Despite some expected within farm variation in management over time, data for the current study was from a single commercial farm in which cows were under the same general management and environment. Therefore, the greater milk and components production of pregnant primiparous cows, albeit small in magnitude, might have been linked to factors such as season of calving and health in early lactation. Indeed, calving in the cold season and not experiencing health disorders in early lactation were also positively associated with milk and components yield. Regardless of the cause, the combination of greater milk production, calving in the cold season, and not having health disorders might be good indicators of the likelihood of pregnancy in primiparous cows. This is not surprising because it is well-known that colder weather (Hansen, 2019) and better health (Fourichon et al., 2000) are associated with increased likelihood of pregnancy in lactating dairy cows. In contrast to primiparous cows, differences for milk and components yield for multiparous cows were observed only during synchronization of ovulation and the association between performance and FS outcome was the opposite than for primiparous cows. Non-pregnant cows produced more milk and milk fat during the whole synchronization period whereas protein and lactose yield were slightly greater for non-pregnant cows closer to AI. Greater yield for non-pregnant cows was observed despite lesser protein and lactose percent in milk at some time points in early lactation and during synchronization. As for primiparous cows, both season of calving and occurrence of health events were associated with milk and milk components yield. However, and unlike for primiparous cows, some of the positive effects of cold weather and better health on fertility to FS could have been overridden by the detrimental effects of the greater metabolic demands for milk production on fertility (Vercouteren et al., 2015).

The pattern of behavioral parameters monitored by sensors might be associated with pregnancy success at FS because of the direct associations between cow behavior with health and well-being. Behaviors such as rumination, eating, overall physical activity, walking activity, and resting were altered in cows with health disorders in early lactation (Liboreiro et al., 2015; Stangaferro et al., 2016; Stevenson et al., 2020) which, in turn, have been associated with decreased reproductive performance. Moreover, resting time has also been associated with cow performance and reproductive outcomes although data for this relationship has been unclear (Piñeiro et al., 2019). In our study we observed no differences between pregnant and non-pregnant primiparous cows for most behavioral parameters except for number of lying bouts per day, which was greater for non-pregnant than pregnant cows. Although non-pregnant cows had consistently more lying bouts per day from 0 to 56 DIM and the last two days before TAI, based on the small magnitude of the difference (i.e., <0.5 bouts per day) between groups, and the lack of difference in resting time and physical activity, it is difficult to attribute much of the difference in likelihood of pregnancy success to differences in this behavior. A previous study reported a negative quadratic association between lying time in early lactation (i.e., up to 14 DIM) and cyclicity by 42 DIM, but no association between lying time and the probability of pregnancy by 300 DIM for primiparous cows (Piñeiro et al., 2019). Another recent study using an ear-attached sensor that monitored resting behavior reported that cows with early ovulation (i.e., <33 DIM) had lesser resting time than cows that ovulated later (i.e., >33 DIM) after calving (Banuelos et al., 2021). Results from the latter study would suggest a negative association between resting time and some favorable reproductive outcomes, as observed for primiparous cows in our study. Overall physical activity and walking time might be associated with resting behavior because as cows are active, they cannot be resting. For primiparous cows, we observed some minor differences and interactions for physical and walking activity during synchronization of ovulation that are unlikely to help explain differences in FS outcome, or the association between resting bouts and pregnancy outcome groups. Therefore, additional research is needed to confirm our findings and determine if resting behavior and indicators of physical activity are associated with pregnancy outcome to FS in primiparous cows. Overall, pregnant and non-pregnant multiparous cows presented more differences for activity and resting behavior during synchronization of ovulation. Data indicated that non-pregnant cows had greater activity and less resting time than pregnant cows during the entire or portions of the period of synchronization of ovulation. As total resting time per day was consistently greater for pregnant than non-pregnant cows, this parameter might have predictive value of the fertility of cows.

An association between rumination and eating time with FS outcome was expected as these parameters might be indicators of overall cow health and some recent studies linked these behaviors to return to cyclicity after calving. Cows that experienced health disorders in early lactation had reduced rumination and eating time on the days immediately before and after calving (Liboreiro et al., 2015; Stevenson et al., 2020), and around clinical diagnosis of health disorders (Stangaferro et al., 2016a,b; Perez et al., 2020; Rial et al., 2021). Cows that ovulated earlier after calving had greater eating time than cows that ovulated later (Banuelos et al., 2021). The lack of difference between pregnant and non-pregnant primiparous cows for rumination and eating time indicated no association between these parameters and FS pregnancy outcome. Differences of small magnitude and in opposing directions overtime for multiparous cows also suggested lack of a strong association between rumination and eating behavior with pregnancy outcome for multiparous cows. Besides the fact that no association might exist between these parameters (as measured by the sensor used in our study) with pregnancy at FS in cows that receive TAI, there are several potential reasons for not observing differences. For example, rumination and eating behaviors typically change temporarily in cows are affected by health disorders. Thereafter, these parameters return to normal. Also, the effect of individual cows affected by health disorders at different DIM over the mean of a parameter of interest is distributed across a long period of time. Therefore, alterations to the early lactation patterns of sensor-monitored parameters such as rumination and eating time could have been masked by the normal patterns of cows not affected by health disorders, or periods of good health for cows affected by disorders. Other reasons for not observing larger differences is that rumination and eating behavior might only be associated with pregnancy success and be detectable by sensors when alterations occurs closer to the time of AI, and that the effect of cows affected by health disorders on the patterns of these behaviors was attenuated by the normal pattern from most cows not affected by health disorders. Unfortunately, rumination and eating time data were not available during the period of synchronization because of neck-attached sensor tags were removed at ~30 DIM.

Milk conductivity is a physiological parameter that was expected to differ for pregnant and non-pregnant cows because it is a marker of udder health, and both clinical and subclinical mastitis were negatively associated with P/AI at FS in dairy cows (Fuenzalida et al., 2015). If conductivity differences truly exists at certain times before AI, such differences were likely to be masked because of the same reasons than rumination and eating behavior.

Non-pregnant cows were expected to have greater body temperature than pregnant cows during late gestation and early lactation because of known long-term detrimental effects of heat stress on follicle function and oocyte fertility (Hansen, 2019). Larger and more consistent differences were expected during synchronization of ovulation because the period of final follicle and oocyte maturation before ovulation is of high risk for occurrence of physiological anomalies that lead to poor fertility (Wolfenson et al., 2019). Although small in magnitude, the greater body temperature for pregnant primiparous cows (statistical tendency observed) from -14 to 56 DIM was contrary to our expectations and disagrees with the reported negative association between elevated temperature and dairy cow reproductive performance (Lees et al., 2019; Wolfenson et al., 2019; Hansen, 2019). On the other hand, greater body temperature was consistently observed through most of early lactation and during synchronization of ovulation for non-pregnant multiparous cows. Moreover, multiparous cows exposed to THI>72 (measured within or outside cow barns) from 29 to 56 DIM had approximately a 10 percentage point reduction in P/AI as compared to cows exposed to THI<72. Thus, the current data suggested that both body temperature and THI, as measured in our study, might be reasonable indicators of FS outcome, primarily for multiparous lactating dairy cows.

Body weight was explored because of the known negative association between body tissue loss and reproductive performance in lactating dairy cows (Roche et al., 2009). Non-pregnant cows were expected to lose more BW than pregnant cows, especially during early lactation when the most dramatic changes in BW are typically observed in lactating cattle (Bello et al., 2012). Although the difference was not significant, primiparous pregnant cows were consistently heavier by about 10 kg from 0 to 56 DIM. This difference in BW, for which the biological relevance is unclear, was reduced to less than 5 kg during the synchronization of ovulation protocol. Moreover, there were also no differences in relative terms for the different periods after calving included in this study. Collectively, data suggested no association between the BW dynamic and FS outcome for primiparous cows in our dataset. Conversely, for multiparous cows, BW patterns for pregnant and non-pregnant cows were in line with expectations and suggested some potential to differentiate cows with different FS outcome. Of note, the difference in percent change in accumulated BW loss from calving to the BW nadir, and then up to 56 DIM might be used to aid in the prediction of FS outcome.

Several previous studies documented associations between calving outcomes (Pinedo et al., 2020; Pascottini et al., 2020), the occurrence of health disorders (Pascottini et al., 2020), previous performance (Shahinfar et al., 2014), and environmental conditions prior to insemination (Hansen et al., 1999; Jordan, 2003;) with the outcome of individual inseminations. Therefore, we expected to identify several similar associations between the non-sensor data and FS outcome. No association between health in early lactation and FS fertility was evident for primiparous cows whereas a 5 percentage point reduction was observed for multiparous cows with at least one health disorder recorded. Although insufficient sample size or lack of a biological association are possible reasons for the similar P/AI of primiparous cows with or without health disorders, the long voluntary waiting period and use of all-TAI after a Double-Ovsynch protocol might have contributed to the lack of difference between groups. Indeed, a long VWP with TAI at 88 DIM was associated with a greater proportion of cows without uterine health disorders, improved BCS, and a greater proportion of cyclic cows than when cows received TAI at 60 DIM (Stangaferro et al., 2018).

Although the association between milk production and reproductive performance across herds can be equivocal and affected by a myriad of herd management factors and environmental conditions (Caraviello et al., 2006; Bello et al., 2012), within herd, the association between individual cow milk yield in a previous lactation and reproductive outcomes in a subsequent lactation might be more consistent. Among several factors, the within cow association can be explained by the negative correlation between genetic potential for milk production and fertility (Carthy et al., 2016; Puangdee et al., 2017). In our study, consistent differences were observed for milk and milk components yields between pregnant and non-pregnant multiparous cows. Pregnant cows after FS produced less total milk, milk adjusted to 305 d of lactation, total fat, and total protein than non-pregnant cows. Fewer days in milk because of earlier pregnancy in the previous lactation in cows that became pregnant at FS could explain part of the difference in total milk and components yield. Nevertheless, pregnant cows also yielded 287 kg less milk adjusted to 305 d of lactation, and the 5 d shorter calving interval observed could not fully explain the 457 kg more total milk yield per lactation for non-pregnant cows. Thus, a plausible explanation for the observed differences in milk and components yield is different biological milk production potential for cows pregnant versus non-pregnant at FS. This variation in production potential might be valuable for identifying cows with different likelihood of pregnancy at FS.

Some associations between FS outcome during the lactation of interest and features and performance outcomes of the previous lactation and gestation cycle were observed. Cows pregnant at FS had shorter gestation length, shorter calving interval, longer days open, and tended to have more days dry. As most of the differences between pregnant and non-pregnant cows were small in magnitude, the potential value of these outcomes for identifying cows with different likelihood of pregnancy at FS might be limited. Nevertheless, if a biological link exists between these variables and fertility, they may add value to predictive models of FS outcome.

Although the novelty of this study was on the type, variety, and number of associations explored for multiple potential predictors of fertility monitored for prolonged periods of time and FS outcomes, there were several limitations including the approach to data analysis. Data was aggregated and compared in ways expected to be meaningful for exploring biological associations and potential practical value. For example, aggregating data from -14 to 56 d after calving was aimed at identifying variability between pregnant and non-pregnant cows for the predictors of interest at the end of the previous gestation and early lactation when cows undergo dramatic behavioral, physiological and performance changes associated with cow fertility. Data aggregated during the synchronization of ovulation protocol aimed to capture potential associations for predictors and fertility during a period of dynamic changes in ovarian function and endocrine status that directly influence the likelihood of pregnancy. For both time periods, data was averaged for specific time points chosen to capture associations with key biological and management factors that would be potentially reflected on the patterns of the parameters of interest. Collectively, the choice of period of data aggregation, data partitioning, and data summarization and analysis might have prevented identifying more consistent associations in the data. Thus, future studies should consider different ways of collecting, splitting, summarizing, and analyzing data to uncover associations of potential value for predicting FS outcome in dairy cows.

Another potential limitation of this study was that synchronization of ovulation with a fertility program such as Double-Ovsynch, and an extended VWP could have masked part of the variability in reproductive potential associated with the underlying factors captured in the sensor and non-sensor data used in this study. For example, GnRH-based fertility programs like Double-Ovsynch and an extended VWP offset part of the detrimental effects of anovulation and poor uterine health in early lactation on first service P/AI (Herlihy et al., 2012; Stangaferro et al., 2018). Other limitations of this study included use of data from a single farm, lack of data for some parameters for certain periods, and the small sample size for evaluating some outcomes such as P/AI. Therefore, larger studies with multiple farms including insemination of cows with other type of management such as insemination at detected estrus are needed.

CONCLUSIONS

In conclusion, differences in the pattern of several behavioral, physiological, and performance parameters monitored by automated sensors for cows that became pregnant or not at FS were observed. Likewise, associations with FS outcome were observed for early lactation events and environmental conditions. Collectively, these differences, which reflected underlying biological variation, and the influence of management and environmental factors on cow behavior, physiology, and performance, might be valuable for prediction of FS outcome in lactating dairy cows. Substantial variability between parities for the direction and magnitude of differences between pregnant and non-pregnant cows warrants use of parity either as a model predictor, or the development of parity-specific models when attempting to predict reproductive success of lactating dairy cows.

CHAPTER II

PREDICTING PREGNANCY IN LACTATING DAIRY COWS USING MACHINE LEARNING ALGORITHMS INCORPORATING COW FEATURES AND PATTERNS OF BEHAVIORAL, PHYSIOLOGICAL, AND PERFORMANCE PARAMETERS COLLECTED BY SENSORS

INTRODUCTION

Recent improvements in reproductive performance, availability of reproductive technologies, and market changes supports targeted reproductive management (**TRM**) whereby, dairy farmers have options to optimize the performance and profitability of cows at every artificial insemination (**AI**) service or lactation cycle (Giordano et al., 2022). Through TRM, subgroups or individual cows that share certain features or biological conditions are managed differently based on predicted probabilities of estrus, pregnancy establishment, or pregnancy loss. For example, based on predictions of reproductive outcomes and events, use of sexed versus conventional semen, use of expensive versus cheap semen, use AI versus embryo transfer, AI at detected estrus versus timed AI, and post-AI hormonal therapy to increase pregnancy success could be used. Except for real time prediction of estrus events based on some measure of physical activity (Schilkowsky et al., 2021), short- and long-term prediction of reproductive outcomes and their features in support of targeted reproductive management is not yet possible. Thus, decision-making for targeted management is not widely used, or unfortunately subjective based on parameters (e.g., monthly milk production data, predicted transmitted abilities) that do not accurately predict reproductive outcomes.

Previous research and the results presented in Chapter I of this thesis have demonstrated multiple associations of different strengths between fertility of lactating cows to individual AI services and cow features, indicators of energy balance and metabolic status, behavioral, physiological and performance parameters, environmental conditions, and herd management factors (Soriani et al., 2012; Stangaferro et al., 2016; Antanaitis et al., 2018). Some of these associations have been sufficiently consistent and explain enough variation for the outcomes of interests to the extent that single point or a few measurements in time have been helpful at predicting reproductive outcomes (Hempstalk et al., 2015). However, use of more frequent measurements of cow biological, herd, and environmental parameters that change dynamically over time (as presented in Chapter I of this thesis) may substantially increase our ability to predict reproductive outcomes with modern data analytic tools.

Machine learning algorithms (**MLA**) are a class of computer data-analytic techniques that automate prediction based on past observations. These algorithms are well suited for compressing massive data from many sources into one or a few user-friendly tools for predicting specific outcomes (Caraviello et al., 2006; Rutten et al., 2016). In dairy farming, developing MLA with data from wearable and non-wearable sensor data, cow performance records, facilities, and the environment is clearly a rich opportunity to improve management of cow health, reproduction, feeding, and milking. As for estrus, there is substantial evidence of associations of lactating dairy cow features, metabolic parameters, management factors, and environmental conditions with the probability of pregnancy after AI. Automated detection of such associations with MLA could make prediction of pregnancy possible. Indeed, previous research has shown that it is possible to predict the outcome of AI services using MLA techniques (Hempstalk et al., 2015). However, most studies (if not all) used static datasets (retrospective, non-real-time modeling) and did not use a suite of behavioral, physiological and performance sensor parameters collected at high frequency and with high time granularity. The expectation is that major gains in predictive value can be achieved by using a combination of high granularity behavioral, physiological, and performance cow data collected by automated sensors with historical and real time data of herd performance and environmental conditions.

Therefore, the primary objective of the study presented in this chapter was to evaluate the performance of supervised MLA for predicting pregnancy outcome after first service (**FS**) in lactating dairy cows using a combination cow behavioral, physiological, and performance parameters in combination with data for herd performance and farm environmental conditions. Specifically, we used the data presented in the study presented in Chapter I of this thesis to predict the outcome of the first AI service after calving in lactating dairy cows at a commercial dairy farm.

EXPERIMENTAL PROCEDURES

Data for behavioral, physiological, and performance parameters collected by the automated sensor systems for cows used in the study described in Chapter I were used for this study. Sensor data collected from -14 to 56 d after calving were summarized as either accumulated or average daily values regardless of the frequency of data collection [milk yield (accumulated), milk components percent (average), milk components yield (accumulated), fat-to-protein ratio (average), total rumination and eating activity (count of AU), body temperature (average), milk conductivity (average), physical activity (count of AU), walking activity (count of steps), rest time (accumulated), lying bouts (count of events), and BW (average)].

Thereafter, datasets were created for training and evaluation of MLA for prediction of first service (**FS**) outcome for primiparous and multiparous cows and for both parity groups combined. A multi period (**MultiP**) dataset was generated using daily data summarized as the average of the following seven time periods in relationship to calving: -14 to -8, -7 to -3, -2 to 2, 3 to 7, 8 to 14, 15 to 28, 29 to 56 d. This dataset contained 139 predictors in total. A single period (**SingleP**) dataset was created by calculating the average of all data available from 3 to 56 DIM. This dataset contained 90 predictors in total. Finally, a synchronization period (**SynchP**) dataset was generated using data collected during synchronization of ovulation. For this dataset, data were summarized in three time periods during synchronization of ovulation: -27 to -11, -10 to -3, -2 to -1 d before TAI. This dataset contained 199 predictors in total.

All data for cow features, calving event features, health events, previous lactation production and reproductive performance, and environmental conditions as collected and summarized in Chapter I were also used in this study. These data were added to the MultiP, SingleP, and SynchP datasets to include sensor and non-sensor predictors in the same datasets.

Finally, imputation with the mean for each variable (Zhang, 2016) was used to compensate for missing data for individual cows and certain periods of time for some sensor parameters. Algorithms were built and tested with datasets with or without imputation.

Using the MultiP, SingleP, and SynchP datasets, four types of MLA were built and evaluated for primiparous (**PP**) and multiparous (**MP**) and for both PP and MP combined. The MLA methods used were Decision trees (**DT**), Support Vector Machine (**SVM**), Logistic Regression (**LR**), and Extreme Gradient Boosting (**XGBoost**). For model building and testing, the MultipP, SingleP, and SynchP datasets were randomized and split into two independent datasets used for training (80% of data) and testing (20% of data). All algorithms were built and evaluated using packages in RStudio Team (2020) using the RStudio: Integrated Development for R (RStudio, PBC, Boston, MA). The sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy for each model was estimated.

Description and Parametrization of Machine Learning Algorithms

Decision trees. This supervised algorithm method uses a graphical representation of different choices and correlations between predictor variables and the dependent variable of interest. This type of classification algorithm (method = ‘class’) generates a model to calculate the probability of a cow being pregnant or not after FS (1 or 0). No restrictions were applied for maximum depth of the tree. RStudio packages used were the Recursive Partitioning and Regression Trees (rpart) to run the model, the Plot 'rpart' Models: An Enhanced Version of 'plot.rpart' (rpart.plot) to plot and print the visual trees of the model, and 'dplyr' Back End for Databases (dblyr) as a general utility functions tool to manage the database.

Support Vector Machine (SVM). This supervised algorithm uses non-linear decision boundaries and creates an extra dimension to analyze data on a tree dimensional mapping. To this end, the algorithm uses a procedure to calculate all possible dimensions known as the kernel trick which creates non-linear boundaries between classes. Basically, kernels transform the data to classify it in a three-dimensional plane. For analysis, we used the following parameters: SVM-Type: eps-regression (no restriction applies with value predetermined by the algorithm); SVM-Kernel: radial (no restriction applies with value predetermined by the algorithm); Cost: 1 (no restriction applies with value predetermined by the algorithm); Gamma: value determined by the algorithm specific for each model; and Epsilon: value determined by the algorithm specific for each model. Because of the three-dimensional method used by the algorithm, there is no direct probabilistic interpretation for the non-linear class separation. One of the packages used was the Misc Functions of the Department of Statistics, Probability Theory Group (Formerly: E1071) TU Wien. This package manages imports: graphics, grDevices, class, stats, methods, utils; and suggests: cluster, mlbench, nnet, randomForest, rpart, SparseM, xtable, Matrix, MASS, and slam; (e1071), Tools: Moving Window Statistics, GIF, Base64, ROC AUC, etc. We also used the (caTools) package which contains several basic utility functions including: moving (rolling, running) window statistic functions, read/write for GIF and ENVI binary files, fast calculation of AUC, LogitBoost classifier, base64 encoder/decoder, round-off-error-free sum and cumsum, etc.. The 'dplyr' Back End for Databases (dblyr) was used as a general utility functions tool to manage the database.

Logistic Regression. This supervised algorithm uses the logistic or sigmoid function to establish the relationship between the independent variables and the output. This algorithm can manage multiple independent variables and calculates the probability of an event to happen or not (0 or 1). For this analysis the following parameters were used: family = binomial and link = logit. The was a 'dplyr' Back End for Databases (dblyr) as a general utility functions tool to manage the database.

Extreme Gradient Boosting (XGBoost). This algorithm is an efficient implementation of the gradient boosting framework from Chen & Guestrin (2016). This package is its R interface and includes efficient linear model solver and tree learning algorithms. The package automatically runs parallel computation on a single machine which could be more than 10 times faster than existing gradient boosting packages. The package supports various objective functions, including regression, classification, and ranking. For this analysis we used the following parameters: maximum depth of the tree (max_depth) = 4, learning rate (eta) = 0.25, number of threads used during training (nthread) = 2, number of trees or boosting iterations (nrounds) = 30. To set a binary classifier model (objective) = binary: logistic and to control the loss reduction required to create a new leaf-node (gamma) = 0.5.

RESULTS

Performance metrics (Se = sensitivity, Sp = specificity, PPV = positive predictive value, NPV = positive predictive value, and overall accuracy) for all MLA for all analyses conducted for primiparous, multiparous, and primiparous and multiparous cows combined are presented in Tables 1, 2, and 3, respectively. Results for the top five performing algorithms for each parity group and for both parity groups combined are highlighted in the text.

Primiparous Cows

Support Vector Machine using the SingleP dataset with imputation had Se of 96.8%, Sp of 96.7%, PPV of 98.4%, NPV of 93.7%, and accuracy of 96.8%. Parameters used to generate these results were: SVM-Type: eps-regression, SVM-Kernel: radial, cost: 1, gamma: 0.02083333, epsilon: 0.1, Number of Support Vectors: 690 (Table 1).

Support Vector Machine using the SynchP dataset with imputation had Se of 96.7%, Sp of 90.8%, PPV of 95.1%, NPV of 93.7%, and accuracy of 94.6%. Parameters: SVM-Type: eps-regression, SVM-Kernel: radial, cost: 1, gamma: 0.005714286, epsilon: 0.1, Number of Support Vectors: 746, Number of Fisher Scoring iterations: 4 (Table 1).

Logistic Regression using the SingleP dataset with imputation had Se of 97.3%, Sp of 81.1%, PPV of 88.6%, NPV of 95.2%, and accuracy of 90.9%. (Table 1).

XGBoost using the SynchP dataset with missing data, had Se of 83.9%, Sp of 69.4%, PPV of 84.6%, NPV of 68.3%, and accuracy 79.0%. (Table 1).

XGBoost using the SynchP dataset with imputation had Se of 88.5%, Sp of 62.2%, PPV of 74.8%, NPV of 80.9%, and accuracy of 76.9%. (Table 1).

Multiparous Cows

Support Vector Machine using the MultiP dataset with imputation had Se of 45.4%, Sp of 70.3%, PPV of 60.0%, NPV of 56.8%, and accuracy of 58.0%. Parameters used were: SVM-Type: eps-regression, SVM-Kernel: radial, cost: 1, gamma: 0.01694915, epsilon: 0.1, number of Support Vectors: 1656 (Table 2).

XGBoost using the SingleP dataset with imputation had Se of 49.8%, Sp of 63.6%, PPV of 57.3% and NPV of 57.7%, and accuracy of 57.7% (Table 2).

XGBoost using the MultiP dataset with imputation had Se of 49.8%, Sp of 65.6%, PPV of 58.6%, NPV of 54.1%, and accuracy of 56.8% (Table 2).

Decision Tree using the SynchP dataset with missing data had Se of 51.2%, Sp of 61.7%, PPV of 56.8%, NPV of 56.3%, and accuracy of 56.5% (Table 2).

XGBoost using the SingleP dataset with imputation had Se of 50.7%, Sp of 61.2%, PPV of 56.2%, NPV of 55.9%, and accuracy of 56% (Table 2).

Table 1. Type of algorithm, type of dataset, use of imputation, and performance metrics observed for prediction of first service pregnancy outcome of primiparous cows using different types of machine learning algorithms.

Parity 1												
Machine Learning Algorithm	Dataset	Imputation	TN	TP	FN	FP	sensitivity	specificity	PPV	NPV	Accuracy
Decision Tree	SingleP	Yes	10	94	29	53	76.4%	15.9%	63.9%	25.6%	55.9%
Decision Tree	MultiP	Yes	20	82	41	43	66.7%	31.7%	65.6%	32.8%	54.8%
Decision Tree	SynchP	Yes	19	92	31	44	74.8%	30.2%	67.6%	38.0%	59.7%
Decision Tree	MultiP	No	12	78	45	51	63.4%	19.0%	60.5%	21.1%	48.4%
Decision Tree	SynchP	No	6	107	16	57	87.0%	9.5%	65.2%	27.3%	60.8%
Decision Tree	SingleP	No	10	104	19	53	84.6%	15.9%	66.2%	34.5%	61.3%
Logistic Regression	SynchP	Yes	39	88	24	35	78.6%	52.7%	71.5%	61.9%	68.3%
Logistic Regression	MultiP	Yes	48	93	15	30	86.1%	61.5%	75.6%	76.2%	75.8%
Logistic Regression	SingleP	Yes	60	109	3	14	97.3%	81.1%	88.6%	95.2%	90.9%
SVM	MultiP	Yes	2	120	3	61	97.6%	3.2%	66.3%	40.0%	65.6%
SVM	SynchP	Yes	59	117	4	6	96.7%	90.8%	95.1%	93.7%	94.6%
SVM	SingleP	Yes	59	121	4	2	96.8%	96.7%	98.4%	93.7%	96.8%
XGBoost	SingleP	Yes	11	97	26	52	78.9%	17.5%	65.1%	29.7%	58.1%
XGBoost	MultiP	Yes	19	89	34	44	72.4%	30.2%	66.9%	35.8%	58.1%
XGBoost	SynchP	Yes	51	92	12	31	88.5%	62.2%	74.8%	81.0%	76.9%
XGBoost	MultiP	No	18	94	29	45	76.4%	28.6%	67.6%	38.3%	60.2%
XGBoost	SingleP	No	17	99	24	46	80.5%	27.0%	68.3%	41.5%	62.4%
XGBoost	SynchP	No	43	104	20	19	83.9%	69.4%	84.6%	68.3%	79.0%

Metrics used to evaluate MLA: TN (True Negatives), TP (True Positives), FN (False Negatives), FP (False Positives), PPV (Positive Predictive Value) NPV (Negative Predictive Value) in the testing dataset.

Table 2. Type of algorithm, type of dataset, use of imputation, and performance metrics observed for prediction of first service pregnancy outcome of multiparous cows using different types of machine learning algorithms.

Parity 2												
Machine Learning Algorithm	Dataset	Imputation	TN	TP	FN	FP	sensitivity	specificity	PPV	NPV	Accuracy
Decision Tree	SingleP	Yes	142	85	120	67	41.5%	67.9%	55.9%	54.2%	54.8%
Decision Tree	MultiP	Yes	124	97	108	85	47.3%	59.3%	53.3%	53.4%	53.4%
Decision Tree	SynchP	Yes	141	88	117	68	42.9%	67.5%	56.4%	54.7%	55.3%
Decision Tree	MultiP	No	165	64	141	44	31.2%	78.9%	59.3%	53.9%	55.3%
Decision Tree	SynchP	No	129	105	100	80	51.2%	61.7%	56.8%	56.3%	56.5%
Decision Tree	SingleP	No	153	69	136	56	33.7%	73.2%	55.2%	52.9%	53.6%
Logistic Regression	SynchP	Yes	71	96	138	109	41.0%	39.4%	46.8%	34.0%	40.3%
Logistic Regression	MultiP	Yes	75	93	134	112	41.0%	40.1%	45.4%	35.9%	40.6%
Logistic Regression	SingleP	Yes	73	101	136	104	42.6%	41.2%	49.3%	34.9%	42.0%
SVM	MultiP	Yes	147	93	112	62	45.4%	70.3%	60.0%	56.8%	58.0%
SVM	SynchP	Yes	59	91	150	114	37.8%	34.1%	44.4%	28.2%	36.2%
SVM	SingleP	Yes	64	86	145	119	37.2%	35.0%	42.0%	30.6%	36.2%
XGBoost	SingleP	Yes	137	102	103	72	49.8%	65.6%	58.6%	57.1%	57.7%
XGBoost	MultiP	Yes	133	102	103	76	49.8%	63.6%	57.3%	56.4%	56.8%
XGBoost	SynchP	Yes	68	88	141	117	38.4%	36.8%	42.9%	32.5%	37.7%
XGBoost	MultiP	No	134	97	108	75	47.3%	64.1%	56.4%	55.4%	55.8%
XGBoost	SingleP	No	128	104	101	81	50.7%	61.2%	56.2%	55.9%	56.0%
XGBoost	SynchP	No	78	89	131	116	40.5%	40.2%	43.4%	37.3%	40.3%

Metrics used to evaluate MLA: TN (True Negatives), TP (True Positives), FN (False Negatives), FP (False Positives), PPV (Positive Predictive Value) NPV (Negative Predictive Value) in the testing dataset.

Primiparous and Multiparous Cows Combined

Support Vector Machine using the SynchP dataset with imputation had Se of 61.6%, Sp of 62.2%, PPV of 68.2%, NPV of 55.1%, and accuracy of 61.8%. Parameters: SVM-Type: eps-regression, SVM-Kernel: radial, cost: 1, gamma: 0.005050505, epsilon: 0.1, Number of Support Vectors: 2402 (Table 3).

Support Vector Machine using the SingleP dataset with imputation had Se of 59.7%, Sp of 60.5%, PPV of 68.2%, NPV of 51.4%, and accuracy of 60.0%. Parameters were: SVM-Type: eps-regression, SVM-Kernel: radial, cost: 1, gamma: 0.01694915, epsilon: 0.1, Number of Support Vectors: 2275 (Table 3).

XGBoost using the SingleP dataset with imputation had Se of 63.3%, Sp of 55.8%, PPV of 60.2%, NPV of 59.1%, and accuracy 59.7% (Table 3).

XGBoost using the SingleP dataset with missing data had Se of 66.9%, Sp of 48.6%, PPV of 57.9%, NPV of 58.2%, and accuracy 58% (Table 3).

Logistic Regression using the SingleP dataset with imputation had Se of 58.1%, Sp of 57.5%, PPV of 64%, NPV of 51.4%, and accuracy of 57.8% (Table 3).

Table 3. Type of algorithm, type of dataset, use of imputation, and performance metrics observed for prediction of first service pregnancy outcome of primiparous and multiparous cows combined using different types of machine learning algorithms.

Parity 1 and Parity 2+												
Machine Learning Algorithm	Dataset	Imputation	TN	TP	FN	FP	sensitivity	specificity	PPV	NPV	Accuracy
Decision Tree	SingleP	Yes	153	177	131	139	57.5%	52.4%	56.0%	53.9%	55.0%
Decision Tree	MultiP	Yes	144	198	110	148	64.3%	49.3%	57.2%	56.7%	57.0%
Decision Tree	SynchP	Yes	97	227	81	195	73.7%	33.2%	53.8%	54.5%	54.0%
Decision Tree	MultiP	No	111	220	88	181	71.4%	38.0%	54.9%	55.8%	55.2%
Decision Tree	SynchP	No	138	192	116	154	62.3%	47.3%	55.5%	54.3%	55.0%
Decision Tree	SingleP	No	95	247	61	197	80.2%	32.5%	55.6%	60.9%	57.0%
Logistic Regresion	SynchP	Yes	75	91	141	95	39.2%	44.1%	48.9%	34.7%	41.3%
Logistic Regresion	MultiP	Yes	141	186	151	122	55.2%	53.6%	60.4%	48.3%	54.5%
Logistic Regresion	SingleP	Yes	150	197	142	111	58.1%	57.5%	64.0%	51.4%	57.8%
SVM	MultiP	Yes	134	213	95	158	69.2%	45.9%	57.4%	58.5%	57.8%
SVM	SynchP	Yes	161	210	131	98	61.6%	62.2%	68.2%	55.1%	61.8%
SVM	SingleP	Yes	150	210	142	98	59.7%	60.5%	68.2%	51.4%	60.0%
XGBoost	SingleP	Yes	149	196	112	143	63.6%	51.0%	57.8%	57.1%	57.5%
XGBoost	MultiP	Yes	163	195	113	129	63.3%	55.8%	60.2%	59.1%	59.7%
XGBoost	SynchP	Yes	134	181	158	127	53.4%	51.3%	58.8%	45.9%	52.5%
XGBoost	MultiP	No	153	188	120	139	61.0%	52.4%	57.5%	56.0%	56.8%
XGBoost	SingleP	No	142	206	102	150	66.9%	48.6%	57.9%	58.2%	58.0%
XGBoost	SynchP	No	122	182	170	126	51.7%	49.2%	59.1%	41.8%	50.7%

Metrics used to evaluate MLA: TN (True Negatives), TP (True Positives), FN (False Negatives), FP (False Positives), PPV (Positive Predictive Value) NPV (Negative Predictive Value) in the testing dataset.

DISCUSSION

In the present study, we evaluated the performance of four different supervised MLA for predicting the outcome of the FS after calving in lactating Holstein cows. We compared the predictive ability of several different algorithms using a dataset in which data from 14 d before to 56 d after calving was summarized for multiple periods, or a dataset that summarized data in a single value from 3 to 56 d after calving. Both datasets aimed to capture potential cow biological variation and the influence of management and environment on cow biology during early lactation that could be associated with FS outcome. A third dataset that relied primarily on data collected during synchronization of ovulation before first service TAI was used to explore the potential predictive value of algorithms using data that more closely reflects cow biology and the influence of management and environment but closer to insemination. These different approaches to summarize data were used because there are no standard procedures, guidelines, or known best practices to build and test MLA for prediction of pregnancy outcome in lactating dairy cattle.

As for any test used for predicting outcomes of interest for dairy reproductive management or any other area of dairy herd management, the interpretation of performance metrics depends upon the implications of the decisions made based on the test outcome. Moreover, depending on the type of decision, the implications of greater sensitivity, specificity or the positive and negative predictive values would be different. In this regard, because the type and possible combinations of management strategies that can be implemented as part of TRM programs is vast, the interpretation of the results of the current study depend upon the context in which models would be used in practice. For example, in some cases it may be more valuable to use predictive models that yield better sensitivity whereas in other cases greater specificity would be more valuable. Regardless of the potential use of model predictions, the results of this study demonstrated that large variation can be expected in MLA performance even when the same data is used for model development and testing. Our results also suggested that the same MLA methods can present dramatic variation in performance when data for predictors are summarized differently as was the case with the MultiP, SingleP, and SynchP datasets. Another interesting observation of this study was the effect of data imputation. This method of compensating for missing data had mixed effects on MLA performance. Although in general algorithms performed better with imputation, substantial improvements in MLA performance were not always observed or were of small magnitude.

To the best of our knowledge, this is one of the first studies aimed at training MLA for predicting FS outcome in lactating dairy cows using behavioral, physiological, and performance data collected with high time granularity by automated wearable and non-wearable sensors. Therefore, direct comparisons with previous studies is difficult. Most of the previous studies used datasets with limited behavioral and physiological data or included performance data from monthly tests or whole lactations (Caraviello et al., 2006; Rutten et al., 2016). Most studies also developed algorithms for primiparous and multiparous cows combined (Hempstalk et al., 2015; Ghiasi et al., 2016) and for all AI services rather than FS only (Caraviello et al., 2006). Despite these limitations, there were some similarities between some of these previous studies and our study. For example, large variation was observed among different types of MLA using the same datasets (Shahinfar et al., 2014; Hempstalk et al., 2015; Wełeszczuk et al., 2022) and data imputation did not dramatically change algorithm performance (Zhang et al., 2010; Zhang, 2016).

Overall, we observed that MLA trained with a combination of automated sensor cow behavioral, physiological and performance data, as well as herd performance and environmental data from a single commercial farm presented a wide range of performance for predicting pregnancy success of FS after calving for primiparous and multiparous cows. The best performing algorithms were primarily those developed for primiparous cows. Among those, Support Vector Machine (**SVM**) and Logistic Regression (**LR**) had the best better performance among all MLA developed and tested. Of note, a SVM algorithm yielded performance metrics in the mid-to-high 90% range with an interesting balance of high Se and Sp (both above 95%). If repeatable under commercial farm conditions when data is fed in real time to this type of MLA, these results are encouraging because the model might be suitable for use in practice. Nevertheless, because we used a relatively small dataset from a single commercial farm and the lack of validation with an independent dataset, additional research with larger, independent datasets, and ideally from more than one commercial farm is needed before this type of models can be deployed for use on farms.

Two other algorithms with the highest performance for predicting FS outcome in primiparous cows were developed with LR and SVM algorithms with the SingleP or SynchP dataset, respectively. These algorithms had similar Se and NPV but substantially lower Sp and PPV than the best performing algorithm for primiparous cows. Thus, SVM, and potentially LR might be the most suitable for predicting FS outcome for primiparous cows with similar type of data than that used in this study. More interestingly, was the fact that three algorithms with best performance used the SingleP and SynchP datasets. These observations would suggest that summarizing data in a single value that accumulates or averages all values from the early postpartum period or during synchronization of ovulation for first service, might yield better results for predicting FS outcome than when using data summarized in multiple periods for late gestation and early lactation, as done for the MultiP dataset. In agreement, the two other algorithms in the top five performing algorithms used the SynchP dataset. The latter, which were developed with XGBoost, had substantially lower Sp and NPV as compared with the best performing SVM and LR algorithms. Thus, their potential value would be limited compared to other algorithms developed by other methods.

Developing different MLA models for primiparous and multiparous cows was explored because of the potential effects of known and expected differences in biology, performance, and reproductive outcomes for these two groups of cows. In agreement with these expectations, there were dramatic differences in MLA performance not only when data for primiparous and multiparous cows was used separately, but also when data for the different parity groups were combined. Compared with results for primiparous cows, the performance of algorithms for predicting FS outcome for multiparous cows was poor and likely of low value for practical application. None of the top five performing algorithms had performance metrics above 70%, with most values in the range of ~45 to 60%. These contrasting results for primiparous and multiparous cows are intriguing because the same type of data, which was collected and summarized using the same methods, and was offered to the same MLA methods generated highly disparate outcomes. Therefore, it is plausible to speculate that a main reason for the differences in MLA performance between parity groups was the underlying patterns, relationships, and dependencies in the input data used for predictions for each parity group. Although not entirely surprising as MLA do not use the same relationships and patterns in the data to generate predictions than traditional statistical methods, it was interesting that algorithms for primiparous cows performed better than for multiparous cows considering the larger number, greater variety, and greater magnitude of differences observed in the analysis of patterns and associations between input data and FS outcome presented in Chapter I. Certainly, there is a myriad of other factors that could explain the observed differences in MLA performance. Nevertheless, those are more difficult to identify and elucidate because of the complex manners in which the input data with its underlying, patterns, relationships, and dependencies are used by the functions of different MLA to predict the outcome of interest.

CONCLUSIONS

In conclusion, supervised MLA trained with a combination of cow behavioral, physiological, and performance parameters collected by automated wearable and non-wearable sensors, and data for herd performance and farm environmental conditions from a single commercial farm presented large variation in performance. As parity group was a major source of variation in MLA predictive ability, different models might have to be developed for predicting FS outcome for primiparous and multiparous cows. Our results also demonstrated that the same MLA methods can present dramatic variation in performance when input data for predictors are summarized differently.

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CHAPTER III

OVERALL CONCLUSIONS AND FUTURE RESEARCH

1. OVERALL CONCLUSIONS

The overarching objectives of the research presented in this thesis were to characterize associations between cow, herd, and environmental data with the outcome of individual inseminations, and develop and evaluate MLA to predict the outcome of the first service (**FS**) after calving in lactating dairy cows. A better understanding of associations between potential predictors of fertility and outcomes of individual AI services in lactating dairy cows might enable the implementation of targeted reproductive management (**TRM**) strategies in dairy herds.

The primary objective of the study presented in Chapter I of this thesis was to compare the pattern of behavioral, physiological, and performance parameters collected by automated sensors for cows that became pregnant or not at FS. A secondary objective was to evaluate the association between pregnancy outcome at FS and previous gestation and early lactation performance and events, as well as environmental conditions before insemination. Therefore, we conducted an observational retrospective cohort study using data from a commercial dairy farm. In this study, we observed that for primiparous cows there were no differences between pregnant and non-pregnant cows for most sensor data patterns explored, or between groups of cows created based on sensor parameters of interest. Nevertheless, some associations were observed for performance outcomes because pregnant cows had greater milk, butterfat, protein, and lactose yield up to 56 DIM. Contrary to our expectations, we observed no differences between pregnant and non-pregnant primiparous cows for most behavioral parameters except for number of lying bouts per day, which was greater for non-pregnant than pregnant cows.

For multiparous cows, we observed consistent differences for milk and milk components yields between pregnant and non-pregnant mostly during the previous lactation cycle. Pregnant cows after FS produced less total milk, milk adjusted to 305 d of lactation, total fat, and total protein than non-pregnant cows. Fewer days in milk in the previous lactation because of earlier pregnancy in cows that became pregnant at FS seemed to explain part of the difference in total milk and components yield. Some associations were also observed between FS outcome during the lactation of interest and features and performance outcomes of the previous lactation and gestation cycle. Cows pregnant at FS had shorter gestation length, shorter calving interval, longer days open, and tended to have more days dry. However, because most of these differences were small in magnitude, the potential value of these outcomes for identifying cows with different likelihood of pregnancy at FS might be limited.

Unlike for primiparous cows, a few more associations were observed for behavioral and physiological parameters in multiparous cows. Most notable was the greater body temperature observed through most of early lactation and during synchronization of ovulation for non-pregnant multiparous cows. Moreover, multiparous cows exposed to THI>72 (inside or outside barns) in early lactation had a substantial reduction in P/AI as compared with cows exposed to THI<72. Thus, the current data suggested that both body temperature and THI, as measured in our study, might be reasonable indicators of FS outcome for multiparous lactating dairy cows. Moreover, BW patterns for multiparous pregnant and non-pregnant cows were in line with expectations and suggested some potential to differentiate cows with different FS outcome. Of note, the difference in percent change in accumulated BW loss from calving to the BW nadir, and then up to 56 DIM might be used to aid in the prediction of FS outcome.

The overaching objective of the study presented in Chapter II of this thesis was to evaluate the performance of supervised MLA for predicting pregnancy outcome after FS in lactating dairy cows using a combination behavioral, physiological, and performance parameters in combination with data for herd performance and farm environmental conditions. We used the data presented in Chapter I of this thesis to predict the outcome of the first AI service after calving. We compared the predictive ability of several different MLA using dataset that captured biological, herd performance and environmental conditions variation at different stages of the lactation cycle and prior to first insemination in dairy cows. In this exploratory research, we used different approaches to summarize data because of the lack standard procedures, guidelines, or known best practices to process input data and build and test MLA for prediction of pregnancy outcome in lactating dairy cattle. Based on the performance of the multiple MLA developed and tested, we learned that a wide range of performance for predicting pregnancy success of FS after calving can be expected. Interestingly, we observed large variation in performance between primiparous and multiparous cows. Some models for primiparous cows had Se, Sp, PPV, and NPV in excess of 90 to 95% whereas none of the models for multiparous cows had values for performance metrics above 70%. Also, some types of MLA had greater overall performance than others despite the use of the same data for training of all algorithms. For this study, we concluded that supervised MLA trained with a combination of cow behavioral, physiological, and performance parameters collected by automated wearable and non-wearable sensors, and data for herd performance and farm environmental conditions are likely to present large variation in performance. Moreover, because parity group was a major source of variation in MLA predictive ability, different models might have to be developed for predicting FS outcome for primiparous and multiparous cows.

In summary, a main contribution of the research presented in this thesis was to better understand associations between multiple cow, herd, and environmental parameters with the outcome of the FS postpartum in lactating dairy cows. Elucidation of these relationships provides new opportunities to explore novels methods to group cows for targeted reproductive management based on their probability to conceive after AI. Another contribution was to improve our understanding of the process of developing and testing MLA for prediction of pregnancy outcome at FS. Overall, we learned that many challenges and bottlenecks remain in the process of MLA development for reproductive outcome predictions.

2. FUTURE RESEARCH

The studies presented in this thesis had several limitations. Among the most notable were the use of data from a single dairy farm, the methods used to aggregate and summarize data for analysis of association with FS pregnancy outcome and development of MLA, lack of data for all periods evaluated for some parameters, and no accounting for data interactions for the evaluation of associations between input data and FS pregnancy outcome. The use of data from a single farm reduced the confounding effects of the between-herds variability observed when data from multiple herds are included. However, using a single farms limited the interpretation of results and the scope of inference to the herd in which the study was conducted. Thus, future studies should be designed to include data from multiple commercial dairy farms that generate the same type of data for the cow, herd, and environmental parameters of interest. Future studies including data for patterns of behavioral, physiological, and performance parameters collected by automated sensors over periods of days, weeks, and months should explore different methods and strategies to aggregate and summarize data for exploring associations with pregnancy outcome, and for developing MLA to predict pregnancy outcome. Rather than using averages or accumulated values for periods of time before AI, data for the same periods could be used to categorize cows into groups with levels that reflect cow biological, physiological, or performance outcomes. For example, cows could be grouped based on the observation of peaks, nadirs, increase and decrease rates overt time, or the duration of periods of no change for specific parameters collected by sensors. Moreover, metrics of data variation such as the coefficient of variation, standard deviation, number of standard deviations above or below the mean for a group could be used to group cows or as the direct input data for analysis. Issues related to lack of data for all cows and all periods could be addressed by ensuring that all cows for which outcomes will be evaluated or predictions generated have functional wearable sensors attached and their data is properly collected from non-wearable sensors. Designing prospective studies could help mitigate this issue provided that access to all sensor systems is possible. A potential solution to explore interactions between multiple parameters and the outcome of interest is to run multivariable models for exploring two- and three-way interactions in the data. A caveat of running such models is the complexity of some of the models if all variables are to be offered at the same time and the difficulties of interpreting data from some interactions.

Future research should also explore associations between the parameters of interest and pregnancy outcome for all AI services rather than FS only. Although there might be enough interest on the development and implementation of targeted reproductive management strategies for first service, there might be value on developing the same type of management strategies for second and greater AI services.

Lastly, models developed with MLA to predict pregnancy outcome should be evaluated in prospective studies under real world conditions at commercial dairy farms with data from cows that were not used for training the models used for prediction. Evaluating the performance of these models with independent datasets in commercial farms will help determine their true potential value dairy herd reproductive management.

SUPPLEMENTARY DATA, TABLES, AND FIGURES

Decision Tress Outputs

Variables used in tree construction for SingleP parity 1 dataset with imputation (figure 1A): changebwnadir_dim56, difbw3_5, difdim3_dim56, eatlast_3, fatyield8, lactyield8, protein8, pywcr, restbout8, restperbout8, resttime8, thi_inside, thi_outside, warmcold, yield8.

Variables used in tree construction for MultiP parity 1 dataset with imputation (figure 1B): conductivity2, conductivity3, fatyield2, lactose1, lactose5, restbout2, restbout4, restperbout5, thi_inside, yield4, yield5.

Variables used in tree construction for SynchP parity 1 dataset with imputation (figure 1C): activity, activity2, afc, animalactivity2, conductivity3, dcc, difnadir_dim56, eatlast_2, eatlast1, fatyield2, fatyieldsynch3, fpratio4, fpratiosynch3, lactosesynch3, milkingtime4, protyield4, protyieldsynch3, restbout2, restbout3, restperbout2, restperbout3, restperboutsynch1, restperboutsynch3, rumlast3, yield2, yield3.

Variables used in tree construction for SingleP parity 1 dataset with missing data (figure 1D): conductivity8, difbw2_5, difnadir_dim56, eatlast_3, fatyield8, fpratio8, lactose8, lactyield8, restbout8, restperbout8, resttime8, rumlast_3, rumlast8, thi_inside, yield8.

Variables used in tree construction for MultiP parity 1 dataset with missing data (figure 2A): eatlast_1, fat4, fatyield2, fatyield4, fpratio1, lactose1, lactose5, milkingtime5, protein5, restbout4, restperbout1, rumlast3, yield5

Variables used in tree construction for SynchP parity 1 dataset with missing data (figure 2B): activity4, animalactivity2, animalactivitysynch1, bolustempsynch1, conductivity4, fatyield2, lactose1, lactosesynch3, protyield4, restperbout2, restperbout3, restperbout4, restperboutsynch2.

Variables used in tree construction for SingleP parity 2 dataset with imputation (figure 3A): afc, changebwnadir_dim56, ddry, difbw345, pltotf, thi_inside, thi_outside.

Variables used in tree construction for MultiP parity 2 dataset with imputation (figure 3B): changebwnadir_dim56, ddry, eatlast3, lactose1, restbout5, restperbout3, resttime5, thi_inside, thi_outside.

Variables used in tree construction for SynchP parity 2 dataset with imputation (figure 3C): afc, lactyieldsynch3, pltotf, protyield3, protyieldsynch1, pywcr, restperbout1, weight1.

Variables used in tree construction for SingleP parity 2 dataset with missing data (figure 3D): animalactivity_3, cr4wkavg, pltotf, protein8, pywcr.

Variables used in tree construction for MultiP parity 2 dataset with missing data (figure 4A): pltotf, protein5, pywcr, resttime1.

Variables used in tree construction for SynchP parity 2 dataset with missing data (figure 4B): animalactivity_2, cr4wkavg, eatlast2, fpratio3, fpratiosynch2, lactose1, lactosesynch3, pltotf, pywcr, restperbout1, yield4.

Variables used in tree construction for SingleP parity 1 and 2+ dataset with imputation (figure 5A): ddry, lact, pltotf, pywcr.

Variables used in tree construction for MultiP parity 1 and 2+ dataset with imputation (figure 5B): activity4, ddry, fatyield2, lactyield5, pltotf, pltotp, pywcr, rumlast_1.

Variables used in tree construction for SynchP parity 1 and 2+ dataset with imputation (figure 5C): conductivitysynch3, eatlast_2, milkingtime2, pltotf, pywcr, restperboutsynch3, weightsynch3.

Variables used in tree construction for SingleP parity 1 and 2+ dataset with missing data (figure 5D): lact, pltotf, protein8, pywcr.

Variables used in tree construction for MultiP parity 1 and 2+ dataset with missing data (figure 6A): lact, pltotf, protein5, pywcr, resttime1.

Variables used in tree construction for SynchP parity 1 and 2+ dataset with missing data (figure 6B): cr4wkavg, fpratio3, fpratiosynch2, lact, lactose1, pltotf, pywcr.

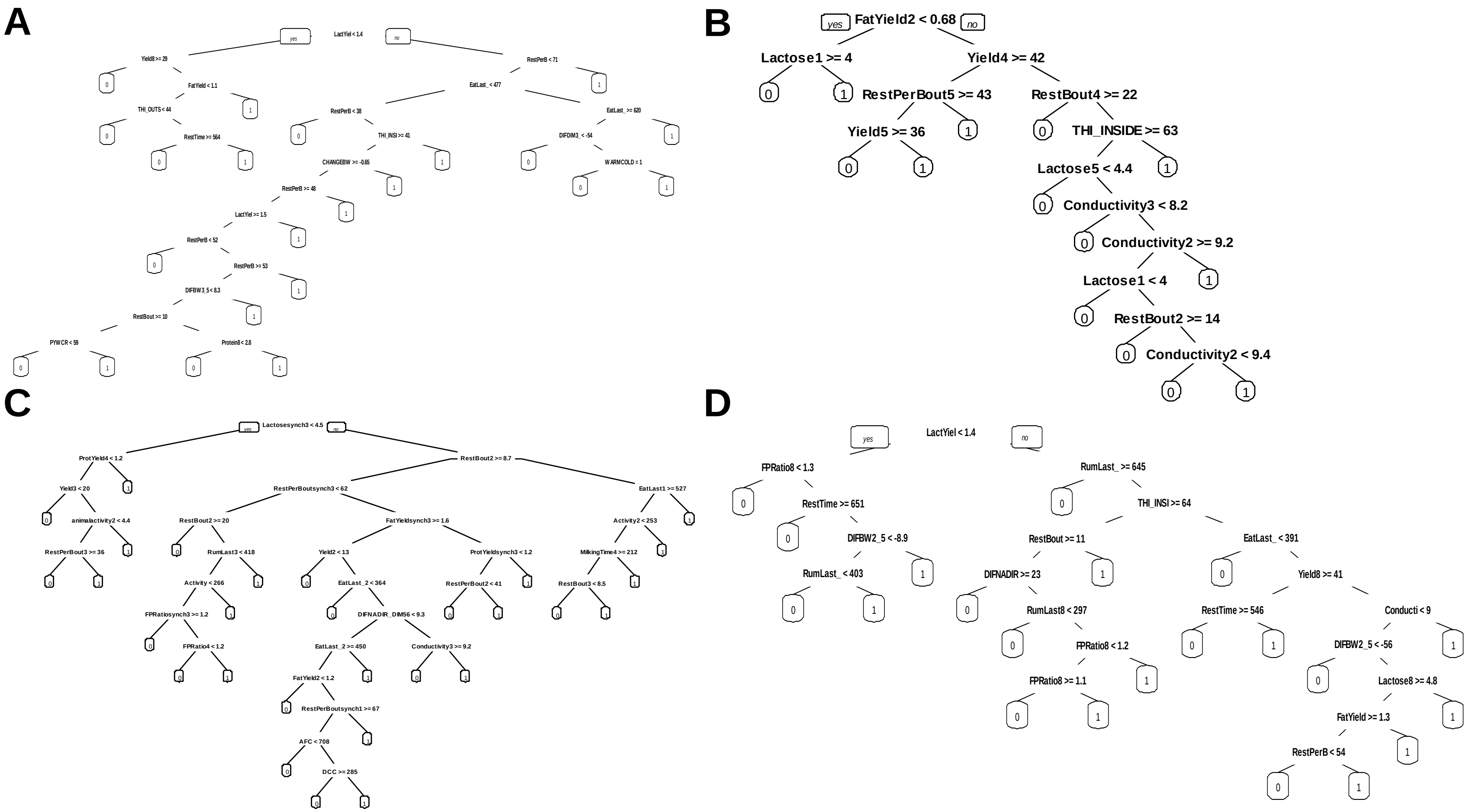

Figure 1. Variables used in tree construction for: SingleP parity 1 dataset with imputation (A), MultiP parity 1 dataset with imputation (B), SynchP parity 1 dataset with imputation (C), and SingleP parity 1 with missing data (D), data from 609 primiparous lactating dairy cows.

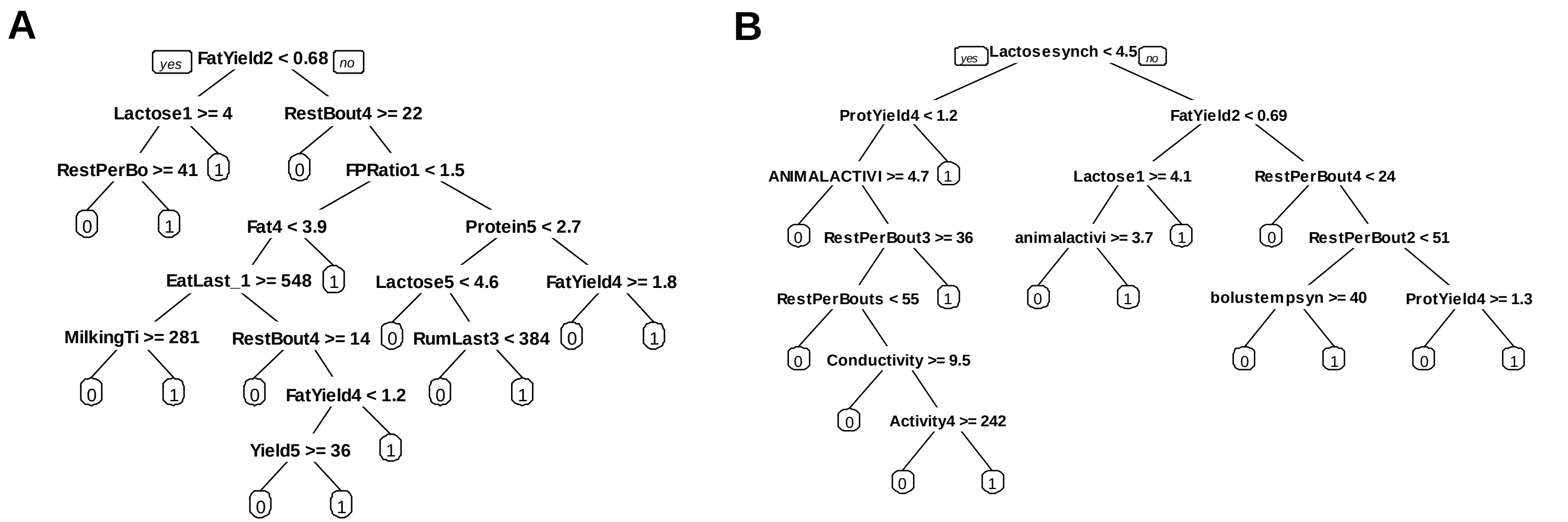

Figure 2. Variables used in tree construction for: MultiP parity 1 with missing data (A), SynchP parity 1 dataset with missing data (B), data from 609 primiparous lactating dairy cows.

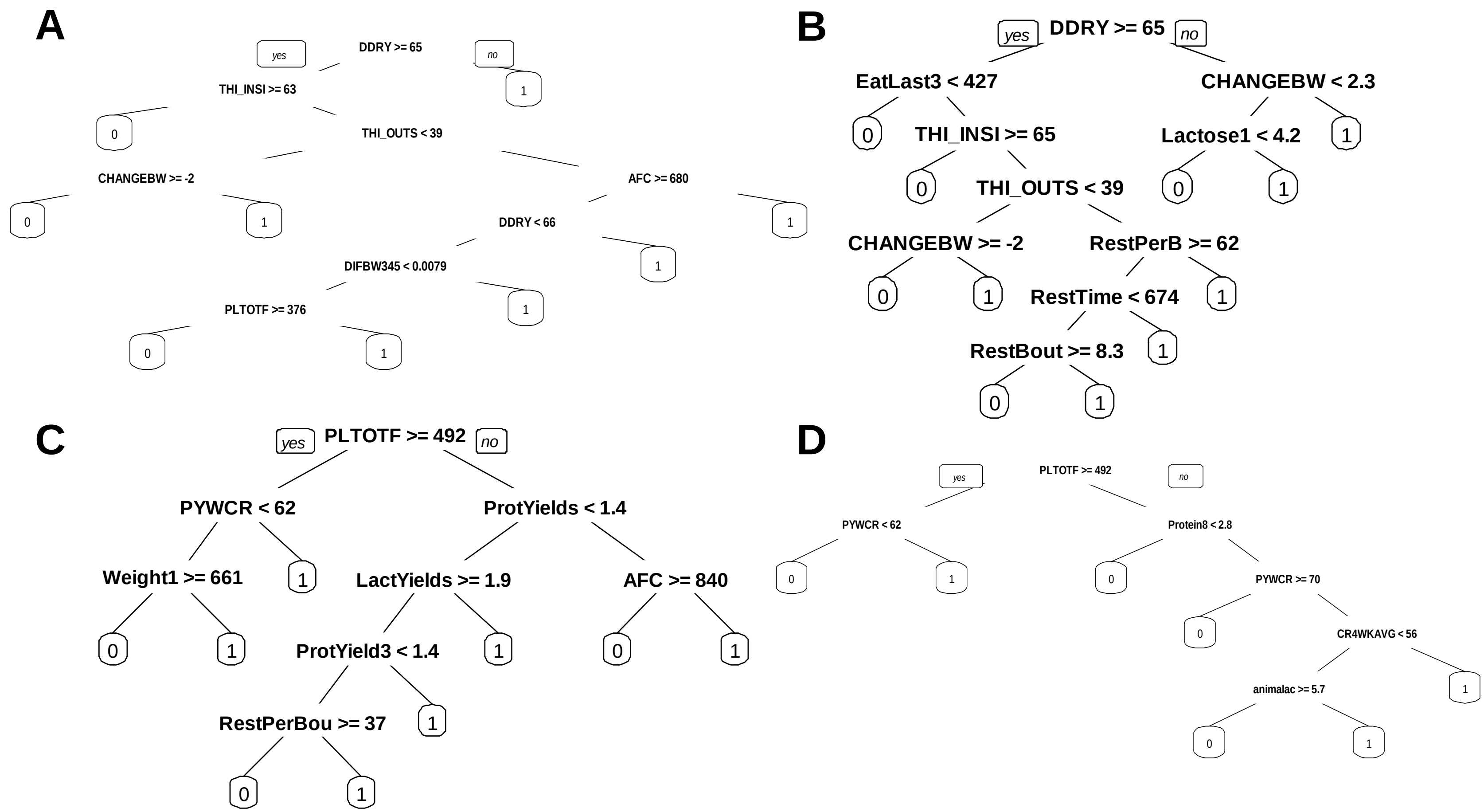

Figure 3. Variables used in tree construction for: SingleP parity 2 dataset with imputation (A), MultiP parity 2 dataset with imputation (B), SynchP parity 2 dataset with imputation (C), and SingleP parity 2 with missing data (D), data from 1010 multiparous lactating dairy cows.

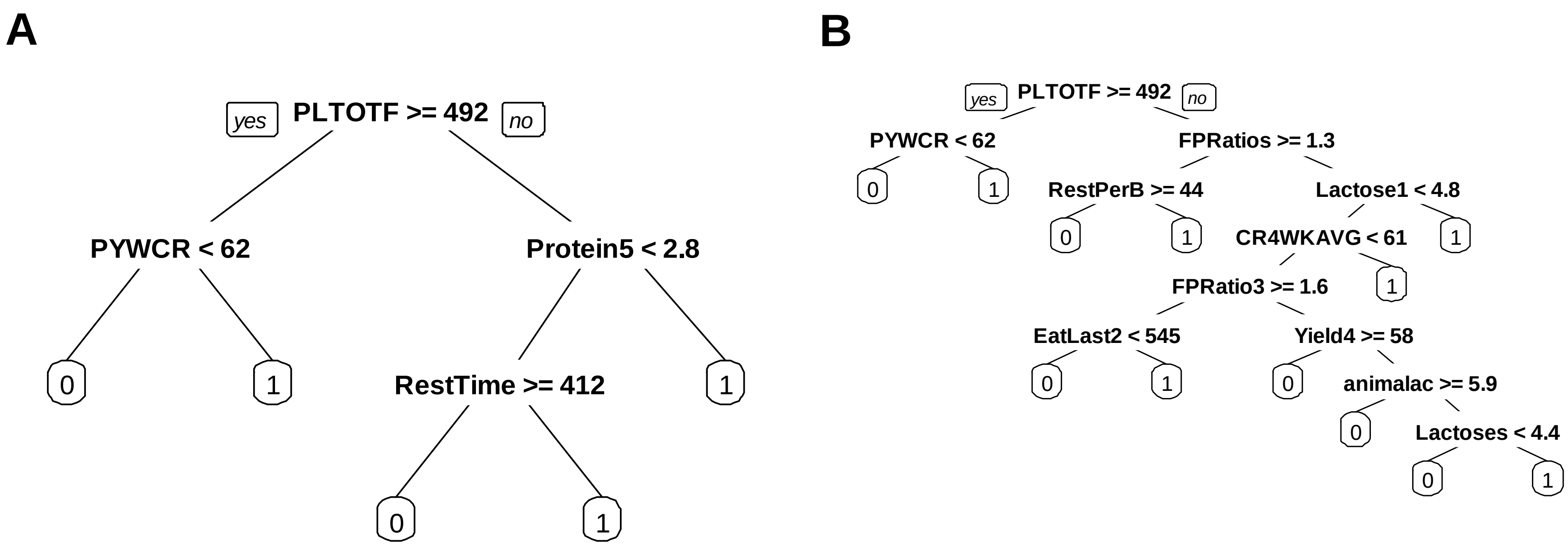

Figure 4. Variables used in tree construction for: MultiP parity 2 with missing data (A), SynchP parity 2 dataset with missing data (B), data from 1010 primiparous lactating dairy cows.

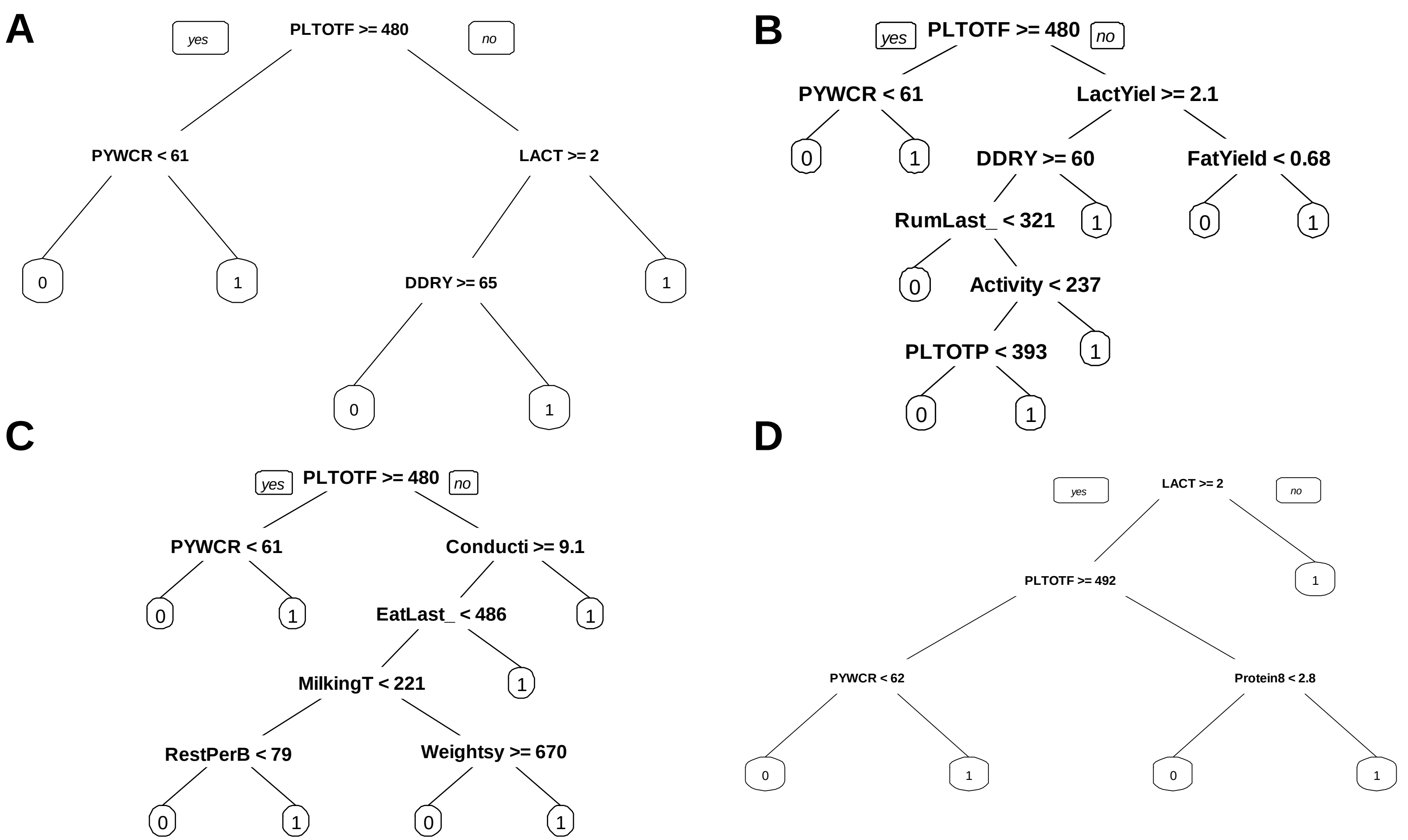

Figure 5. Variables used in tree construction for: SingleP parity 1 and 2+ dataset with imputation (A), MultiP parity 1 and 2+ dataset with imputation (B), SynchP parity 1 and 2+ dataset with imputation (C), and overlap parity 1 and 2+ with missing data (D), data from 1,619 lactating dairy cows (n = 609 primiparous; n = 1,010 multiparous).

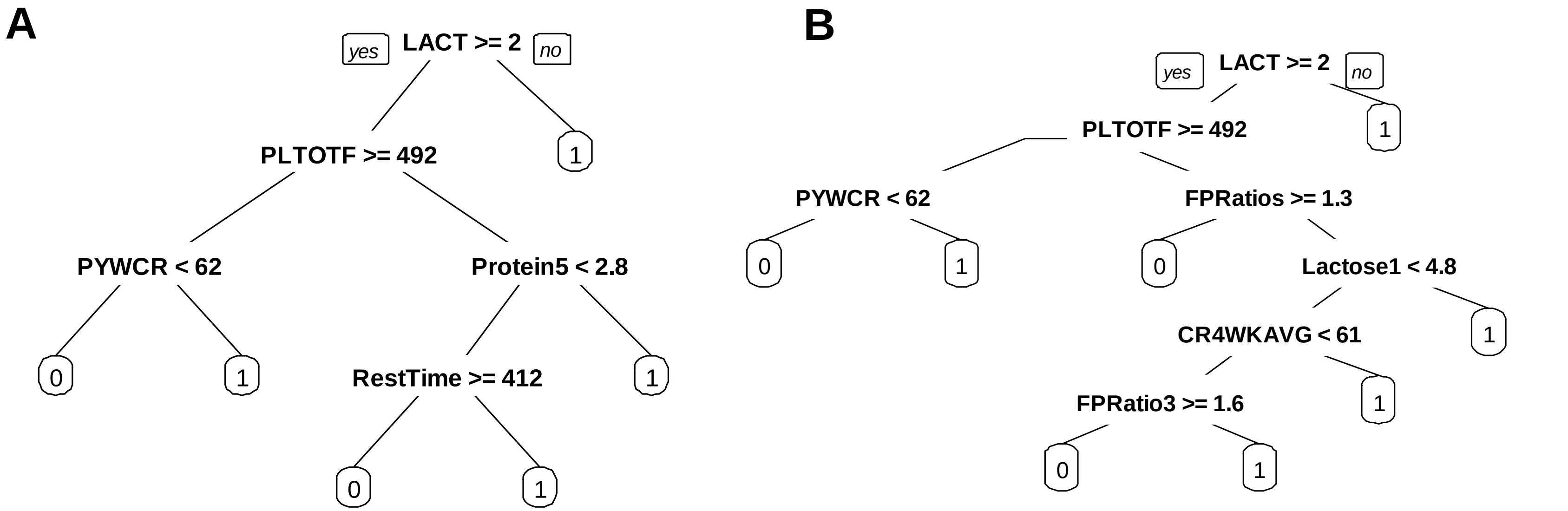

Figure 6. Variables used in tree construction for: MultiP parity 1 and 2+ dataset with missing data (A), SynchP parity 1 and 2+ dataset with missing data (B), data from 1,619 lactating dairy cows (n = 609 primiparous; n = 1,010 multiparous).

Logistic Regression Outputs

Table 4. Coefficients for Logistic Regression for primiparous cows with SingleP dataset using imputation for the missing data.

SingleP dataset with imputation for Parity 1					
Coefficients:	Estimate Std.	Error	z value	Pr(>|z|)	
protyield8	1.36E+01	5.64E+00	2.416	0.0157	*
ce	2.35E+00	1.19E+00	1.978	0.0479	*
(Intercept)	-8.99E+01	5.00E+01	-1.796	0.0725	.

Signif. codes: 0.01 ‘*’ 0.05 ‘.’

Table 5. Coefficients for Logistic Regression for primiparous cows with MultiP dataset using imputation for the missing data.

MultiP dataset with imputation for Parity 1					
Coefficients:	Estimate Std.	Error	z value	Pr(>|z|)	
restperbout4	-7.22E-02	2.68E-02	-2.697	0.007	**
animalactivity_2	5.79E-01	2.24E-01	2.583	0.00979	**
activity3	-9.54E-03	4.60E-03	-2.075	0.03799	*
ce	2.55E+00	1.26E+00	2.027	0.04264	*
animalactivity_1	-6.54E-01	3.36E-01	-1.946	0.05165	.
restperbout5	5.30E-02	2.82E-02	1.878	0.06044	.
(intercept)	-1.10E+02	6.01E+01	-1.829	0.06744	.
fat1	3.73E+00	2.07E+00	1.802	0.07147	.
animalactivity2	7.27E-01	4.07E-01	1.787	0.0739	.
animalactivity3	-9.15E-01	5.17E-01	-1.771	0.07656	.
fpratio1	-1.07E+01	6.41E+00	-1.666	0.0958	.
fat2	-4.96E+00	3.00E+00	-1.65	0.09897	.

Signif. codes: 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’

Table 6. Coefficients for Logistic Regression for primiparous cows with SynchP dataset using imputation for the missing data.

SynchP dataset with imputation for Parity 1					
Coefficients:	Estimate Std.	Error	z value	Pr(>|z|)	
animalactivitysynch1	-4.93E+00	1.35E+00	-3.644	0.000269	***
conductivitysynch3	-1.89E+00	5.56E-01	-3.402	0.00067	***
conductivitysynch1	2.28E+00	7.13E-01	3.204	0.001356	**
animalactivity_2	8.60E-01	2.72E-01	3.158	0.001589	**
(intercept)	-2.47E+02	8.49E+01	-2.915	0.003562	**
animalactivity2	1.38E+00	5.01E-01	2.75	0.00596	**
activitysynch1	-2.10E-02	7.90E-03	-2.662	0.007772	**
animalactivity_1	-9.76E-01	3.81E-01	-2.565	0.010321	*
lactyieldsynch2	-4.66E+00	2.05E+00	-2.273	0.023031	*
animalactivitysynch2	3.62E+00	1.64E+00	2.211	0.027015	*
bolustempsynch1	-6.01E+00	2.78E+00	-2.161	0.030729	*
metdig_30_n	-3.28E+00	1.53E+00	-2.141	0.032272	*
lng2_5	-9.34E-01	4.44E-01	-2.101	0.0356	*
bolustempsynch2	7.91E+00	3.79E+00	2.089	0.036748	*
difdim3_dim56	3.28E-02	1.60E-02	2.056	0.039806	*
thi_inside	-4.61E-02	2.26E-02	-2.044	0.040928	*
restbout2	-2.12E-01	1.05E-01	-2.016	0.043832	*
thi_outside	3.50E-02	1.78E-02	1.961	0.049857	*
restperbout4	-5.40E-02	2.78E-02	-1.943	0.052013	.
ce	2.83E+00	1.47E+00	1.93	0.053572	.
restboutsynch2	4.58E-01	2.40E-01	1.911	0.055972	.
restperboutsynch2	7.48E-02	3.98E-02	1.877	0.060539	.
resttime2	4.98E-03	2.65E-03	1.875	0.06078	.
protyield4	-2.68E+00	1.47E+00	-1.825	0.067944	.
activitysynch2	1.60E-02	8.83E-03	1.814	0.069687	.
protyieldsynch1	2.53E+00	1.42E+00	1.778	0.075361	.
protein3	-9.74E+00	5.54E+00	-1.756	0.079026	.
lactose3	2.17E+00	1.30E+00	1.677	0.093478	.
yieldsynch2	1.78E-01	1.06E-01	1.675	0.094021	.

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’

Table 7. Coefficients for Logistic Regression for multiparous cows with SingleP dataset using imputation for the missing data.

SingleP dataset with imputation for Parity 2+					
Coefficients:	Estimate Std.	Error	z value	Pr(>|z|)	
resttime8	7.08E-03	1.92E-03	3.686	0.000228	***
thi_inside	-3.19E-02	9.50E-03	-3.361	0.000777	***
thi_outside	2.30E-02	7.05E-03	3.266	0.00109	**
pltotf	-3.42E-03	1.13E-03	-3.03	0.002443	**
restbout8	-3.20E-01	1.09E-01	-2.924	0.003456	**
restperbout8	-4.91E-02	1.75E-02	-2.803	0.005065	**
pltotm	-2.88E-04	1.25E-04	-2.31	0.020888	*
cr4wkavg	1.93E-02	8.77E-03	2.194	0.028212	*
plm305	2.22E-04	1.03E-04	2.149	0.031621	*
changebwdim3_dim56	7.23E-01	3.46E-01	2.09	0.036659	*
changebwdim3_nadir	-7.36E-01	3.81E-01	-1.931	0.053514	.
pltotp	5.83E-03	3.26E-03	1.786	0.074103	.
difdim3_dim56	-7.60E-02	4.54E-02	-1.672	0.094502	.

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’

Table 8. Coefficients for Logistic Regression for multiparous cows with MultiP dataset using imputation for the missing data.

MultiP dataset with imputation for Parity 2+					
Coefficients:	Estimate Std.	Error	z value	Pr(>|z|)	
changebwnadir_dim56	-0.1682211	0.0553111	-3.041	0.00236	**
thi_outside	0.0233387	0.0079796	2.925	0.00345	**
thi_inside	-0.0322547	0.0110808	-2.911	0.0036	**
pltotf	-0.0034583	0.0012284	-2.815	0.00487	**
changebwdim3_dim56	0.1664522	0.0620613	2.682	0.00732	**
changebwdim3_nadir	-0.1774954	0.0679487	-2.612	0.009	**
pltotm	-0.0003438	0.000135	-2.547	0.01086	*
weight2	-0.0097272	0.0038794	-2.507	0.01216	*
pltotp	0.0088579	0.00357	2.481	0.01309	*
eatlast3	-0.004009	0.00162	-2.475	0.01333	*
(intercept)	62.0878426	28.322579	2.192	0.02837	*
fat2	3.9701407	1.8148922	2.188	0.0287	*
milkingtime2	0.0065341	0.0029921	2.184	0.02898	*
bolustemp3	-1.0012771	0.4904263	-2.042	0.04119	*
fat1	-2.409918	1.2054816	-1.999	0.04559	*
plm305	0.0002142	0.0001086	1.972	0.04858	*
bolustemp_1	1.0751974	0.5566556	1.932	0.05342	.
cr4wkavg	0.0184164	0.0096755	1.903	0.05699	.
activity3	-0.0056545	0.0030126	-1.877	0.06052	.
fatyield2	-3.6680709	1.9592183	-1.872	0.06118	.
lact	-0.1468522	0.080239	-1.83	0.06722	.
conductivity3	0.350557	0.1979996	1.77	0.07664	.

Signif. codes: 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’

Table 9. Coefficients for Logistic Regression for multiparous cows with SynchP dataset using imputation for the missing data

SynchP dataset with imputation for Parity 2+					
Coefficients:	Estimate Std.	Error	z value	Pr(>|z|)	
thi_outside	2.67E-02	8.66E-03	3.082	0.00206	**
(intercept)	1.21E+02	4.24E+01	2.841	0.0045	**
fat1	-5.66E-01	2.08E-01	-2.726	0.00642	**
thi_inside	-3.25E-02	1.21E-02	-2.701	0.00692	**
activitysynch1	-1.29E-02	4.93E-03	-2.617	0.00886	**
animalactivitysynch1	-1.73E+00	6.92E-01	-2.506	0.01223	*
pltotf	-3.14E-03	1.34E-03	-2.351	0.01871	*
animalactivitysynch2	1.95E+00	8.74E-01	2.231	0.02566	*
lame_30_n	-8.94E-01	4.01E-01	-2.229	0.02582	*
fpratiosynch1	4.08E+00	1.83E+00	2.223	0.02622	*
bolustempsynch1	-3.15E+00	1.42E+00	-2.211	0.02704	*
fatsynch3	1.95E+00	8.89E-01	2.19	0.02855	*
lactose4	-1.52E+00	7.30E-01	-2.08	0.03752	*
pl_metdig_30_n	-1.22E+00	5.88E-01	-2.077	0.03777	*
lngnadir_dim56	6.89E-01	3.33E-01	2.068	0.03866	*
milkingtime2	6.47E-03	3.17E-03	2.04	0.04132	*
lact	-1.70E-01	8.42E-02	-2.019	0.0435	*
activitysynch3	8.81E-03	4.39E-03	2.004	0.04506	*
fpratio4	-3.02E+00	1.52E+00	-1.994	0.04618	*
pl_mast_30_n	-1.97E+00	1.02E+00	-1.929	0.05369	.
pltotm	-2.64E-04	1.41E-04	-1.871	0.06128	.
weight4	6.37E-03	3.47E-03	1.839	0.06592	.
yieldsynch2	-1.48E-01	8.04E-02	-1.838	0.06611	.
fatsynch1	-1.87E+00	1.03E+00	-1.816	0.06937	.
conductivity3	3.72E-01	2.07E-01	1.796	0.07253	.
bolustemp_1	1.04E+00	5.82E-01	1.788	0.07382	.
activity3	-5.70E-03	3.22E-03	-1.771	0.07655	.
weight2	-6.32E-03	3.63E-03	-1.743	0.08139	.
pltotp	6.64E-03	3.82E-03	1.742	0.08157	.
cr4wkavg	1.79E-02	1.05E-02	1.706	0.08808	.
pl_lame_30_n	-1.38E+00	8.20E-01	-1.688	0.09143	.
protein4	-1.93E+00	1.14E+00	-1.682	0.09249	.
lactose1	6.06E-01	3.60E-01	1.682	0.09261	.
changebwdim3_nadir	2.65E-01	1.58E-01	1.68	0.09295	.
bolustempsynch2	2.57E+00	1.53E+00	1.676	0.09366	.
conductivitysynch3	-4.90E-01	2.93E-01	-1.672	0.09457	.

metdig_30_n	-6.31E-01	3.79E-01	-1.666	0.09579	.
bolustempsynch3	-1.93E+00	1.17E+00	-1.647	0.09959	.

Signif. codes: 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’

Table 10. Coefficients for Logistic Regression for primiparous and multiparous cows with SingleP dataset using imputation for the missing data.

SingleP dataset with imputation for Parity 1 and Parity 2+					
Coefficients:	Estimate Std.	Error	z value	Pr(>|z|)	
thi_outside	1.65E-02	5.64E-03	2.918	0.00352	**
pltotf	-3.24E-03	1.12E-03	-2.879	0.00399	**
parity	-4.86E-01	1.83E-01	-2.654	0.00795	**
thi_inside	-1.96E-02	7.58E-03	-2.583	0.00978	**
pltotp	7.83E-03	3.20E-03	2.447	0.01439	*
pldcc	-3.27E-02	1.55E-02	-2.112	0.03473	*
resttime8	2.61E-03	1.27E-03	2.056	0.03981	*
pltotm	-2.19E-04	1.16E-04	-1.889	0.0589	.
twns	-8.60E-01	5.08E-01	-1.695	0.09011	.
restbout8	-1.08E-01	6.43E-02	-1.672	0.09449	.

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

Table 11. Coefficients for Logistic Regression for primiparous and multiparous cows with MultiP dataset using imputation for the missing data.

MultiP dataset with imputation for Parity 1 and Parity 2+					
Coefficients:	Estimate Std.	Error	z value	Pr(>|z|)	
changebwnadir_dim56	-1.37E-01	4.10E-02	-3.332	0.000863	***
pltotf	-3.45E-03	1.20E-03	-2.885	0.00392	**
thi_outside	1.68E-02	6.04E-03	2.788	0.005296	**
pltotp	8.85E-03	3.40E-03	2.602	0.009267	**
changebwdim3_dim56	1.15E-01	4.56E-02	2.519	0.011772	*
activity3	-5.98E-03	2.39E-03	-2.5	0.012427	*
protyield3	9.07E+00	3.87E+00	2.34	0.019266	*
conductivity3	3.88E-01	1.69E-01	2.301	0.021407	*
thi_inside	-1.93E-02	8.55E-03	-2.26	0.023852	*
eatlast3	-2.72E-03	1.22E-03	-2.229	0.025781	*
changebwdim3_nadir	-1.05E-01	4.74E-02	-2.22	0.026436	*
yield4	-8.29E-02	3.97E-02	-2.088	0.036817	*
pldcc	-3.27E-02	1.62E-02	-2.016	0.043792	*
changebw4_5	-8.63E-02	4.32E-02	-1.996	0.045917	*
activity5	4.41E-03	2.28E-03	1.937	0.052768	.
bolustemp3	-7.57E-01	3.98E-01	-1.901	0.057292	.
lactyield1	3.74E+00	1.99E+00	1.879	0.060177	.
restperbout1	-1.27E-02	6.78E-03	-1.867	0.061886	.
yield1	-1.16E-01	6.32E-02	-1.838	0.066069	.
twns	-9.62E-01	5.25E-01	-1.834	0.066695	.
bolustemp_1	8.14E-01	4.46E-01	1.825	0.068007	.
protyield5	-5.77E+00	3.22E+00	-1.791	0.073343	.
lact	-1.14E-01	6.55E-02	-1.737	0.082355	.
resttime5	2.01E-03	1.19E-03	1.687	0.091684	.
protein4	-5.31E+00	3.16E+00	-1.679	0.093246	.
ce	8.86E-01	5.31E-01	1.67	0.095013	.
eatlast4	1.76E-03	1.06E-03	1.665	0.095931	.
eatlast2	1.62E-03	9.74E-04	1.662	0.096465	.

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’

Table 12. Coefficients for Logistic Regression for primiparous and multiparous cows with SynchP dataset using imputation for the missing data.

SynchP dataset with imputation for Parity 1 and Parity 2+					
Coefficients:	Estimate Std.	Error	z value	Pr(>|z|)	
(intercept)	1.53E+02	4.23E+01	3.62	0.000295	***
fat1	-6.16E-01	1.98E-01	-3.103	0.001915	**
lngnadir_dim56	9.89E-01	3.38E-01	2.928	0.003416	**
bolustempsynch1	-4.07E+00	1.42E+00	-2.856	0.004296	**
thi_outside	2.25E-02	8.57E-03	2.621	0.008775	**
lame_30_n	-1.02E+00	3.93E-01	-2.594	0.009483	**
animalactivitysynch1	-1.66E+00	6.44E-01	-2.571	0.010131	*
utd_30_n	-8.39E-01	3.31E-01	-2.536	0.011222	*
pl_metdig_30_n	-1.36E+00	5.45E-01	-2.498	0.012505	*
fpratiosynch1	4.05E+00	1.73E+00	2.34	0.019292	*
tbrd	-6.90E-01	2.99E-01	-2.309	0.020934	*
twns	-1.47E+00	6.60E-01	-2.225	0.026102	*
yield4	-6.25E-02	2.82E-02	-2.217	0.026633	*
pl_mast_30_n	-2.45E+00	1.11E+00	-2.209	0.027176	*
activity4	6.95E-03	3.17E-03	2.194	0.028207	*
metdig_30_n	-7.72E-01	3.55E-01	-2.173	0.029801	*
animalactivitysynch2	1.73E+00	8.08E-01	2.143	0.032145	*
pltotp	8.10E-03	3.80E-03	2.131	0.033073	*
thi_inside	-2.51E-02	1.18E-02	-2.12	0.03398	*
protyield1	2.48E+00	1.17E+00	2.116	0.034374	*
fpratio1	1.36E+00	6.47E-01	2.097	0.036033	*
fatsynch1	-2.60E+00	1.27E+00	-2.05	0.040327	*
dz30_n	1.10E+00	5.40E-01	2.033	0.042033	*
pl_lame_30_n	-1.63E+00	8.05E-01	-2.023	0.043093	*
pltotf	-2.66E-03	1.33E-03	-2.001	0.045446	*
pl_dz30_n	1.18E+00	5.92E-01	1.986	0.047058	*
changebwdim3_nadir	2.07E-01	1.06E-01	1.958	0.050182	.
activitysynch1	-9.54E-03	4.89E-03	-1.95	0.051117	.
pl_other_30_n	1.92E+00	9.96E-01	1.926	0.05406	.
activity3	-6.18E-03	3.27E-03	-1.89	0.0588	.
mast_30_n	-8.32E-01	4.44E-01	-1.873	0.061083	.
lactyield1	-1.27E+00	6.85E-01	-1.849	0.064389	.
lactose1	6.25E-01	3.44E-01	1.817	0.069221	.
resttime4	2.85E-03	1.58E-03	1.799	0.072	.
cr4wkavg	1.85E-02	1.03E-02	1.791	0.073234	.
pl_utd_30_n	-9.72E-01	5.47E-01	-1.779	0.075201	.

lactosesynch3	2.15E+00	1.21E+00	1.77	0.076649	.
resttimesynch1	4.83E-03	2.73E-03	1.77	0.076755	.
yieldsynch2	-1.31E-01	7.43E-02	-1.759	0.078647	.

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’