Investigation of dry period length and transition period intervention strategies to increase ruminant productivity in the subsequent lactation

by

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B.S., California Polytechnic State University - San Luis Obispo, 2014
M.S., Kansas State University, 2017

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
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Abstract

Successful transition from gestation to lactation sets the stage for lifetime productivity in ruminants. Several factors affecting successful transition are due to characteristics of the dry period. Optimal dry period length for dairy cattle has long been debated, but no study has ever evaluated performance associations with dry period length while differentiating between reasons for the deviation from target. We used 32,182 lactations from 16 farms in a retrospective observational study to determine if biological versus management reasons for a short or long dry period have the same associations with subsequent lactation productivity. Dry period length (**DPL**) and gestation length (**GL**) were each categorized as short or long and combined to generate 7 study groups. Cows with both a short DPL and GL had the worst early and whole lactation milk and component yields. Although not as severe, similar decreases for cows with an average DPL but short GL indicated short GL is a greater contributor to poor performance than DPL itself. Long GL, independent of DPL, did not impact productivity. Cows subjected to a long DPL based on management decisions experienced issues related to excessive lipid mobilization that did not affect milk production but manifested in greater hazard of leaving the herd. Intervention strategies have targeted the depressed feed intake and postpartum inflammation that characterizes the transition period. *Saccharomyces cerevisiae* fermentation product was fed from -29 ± 5 to 42 d relative to calving to evaluate the effects on feed intake, milk production, and metabolism. Supplementation increased meals per d with less time between meals, increased milk fat concentration, altered cholesterol metabolism, and increased incidence of subclinical ketosis, but early lactation milk yield and metabolism (plasma free fatty acids, β-hydroxybutyrate, glucose, and insulin) were generally unaffected. Postpartum treatment with the non-steroidal anti-inflammatory drug, meloxicam, has previously been evaluated in dairy cattle,

but this intervention strategy has not been applied to sheep. After lambing, 36 Hampshire and Hampshire × Suffolk ewes were sequentially assigned within type of birth to control or meloxicam treatment on d 1 and 4 of lactation. Postpartum meloxicam treatment of ewes decreased plasma concentrations of haptoglobin (marker of inflammation) and several oxylipids, with the greatest impact in ewes with biomarkers reflecting a greater inflammatory state before treatment. Overall, the transition from gestation to lactation can be impacted by differences in individual biology and management, with some aspects of the transition improved through use of feed additives and drug interventions.

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Approved by:

Major Professor
Dr. Barry J. Bradford

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Table of Contents

List of Figures

List of Tables

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Dedication

To my parents, John and Connie Olagaray. They instilled in me one heck of a work ethic and a fire to follow my passions.

Chapter 1 - **Literature Review - Utilization of herd data to determine associations and directionality of factors related to disease at different levels of the data hierarchy**

Developments in dairy technologies and growing sophistication of data recording systems have created dairy data hubs containing information at the herd, cow, and even mammary gland levels. These data hubs contain information regarding nutrition, reproduction, health, management, genetics, and housing, amongst other factors, and will continue to grow as more precision technologies are implemented on farms. These data are routinely collected for the main purpose of internal farm management and decision making (Bello et al., 2018), but represent a great opportunity for observational research.

Utilization of such herd data presents the advantages of large samples sizes and realistic representation of commercial dairies, thus providing a broad scope of inference (Bello et al., 2018). Observational studies can be employed to estimate disease incidence rates and risk factors of disease, to develop predictive models, and to develop hypotheses regarding causal relationships between variables in complex biological systems (Rosa and Valente, 2013; Bello et al., 2018).

Dairy data is often clustered, which must be considered during experimental design and analysis. A cluster refers to a group of observations that share common features (Stryhn and Christensen, 2014). Within dairy data, spatial clustering occurs, as cows within a pen or dairy are more likely to be similar than others. Additionally, repeated measures from the same individual are considered clustered. With each of these types of clustering, animals closer together in time

or space are more likely to have highly correlated phenotypic data than those far apart (Dohoo, 2008).

Due to the inherent clustering within dairy data, associations between risk factors or explanatory variables and outcomes of interest can be assessed at different hierarchical levels (i.e. region, herd, cow, quarter). Not only is it necessary to properly account for the hierarchical structure of data in the statistical model (Stryhn and Christensen, 2014), but such structure presents opportunity - beyond increased statistical power with greater sample sizes – for greater external validity through the incorporation of multiple herds. The hierarchical structure allows for assessment of risk factors at each hierarchical level. Additionally, the proportion of total variance at each level can be used to determine which level intervention strategies may be most effective (Dohoo, 2008). Most often, hierarchical data is analyzed in linear mixed models in which all but the observational level are included as random effects (Stryhn and Christensen, 2014). Although multiple traits can be investigated in mixed models at multiple levels of the data architecture, only associations between variables can be determined. The findings are limited in that they cannot assess the directionality of correlations.

Recently, structural equation models (**SEM**) have been adapted to a mixed model framework (Gianola et al., 2004), allowing for their application in observational data. They essentially combine graph theory and probability theory (Pearl, 2009) to assess interrelations between explanatory variables while also determining directionality and potential causality between variables in the biological system being investigated (Wu et al., 2010; Rosa et al., 2011; Bello et al., 2012). These models have been increasingly applied in the animal genetics and genomics field, and are slowly becoming implemented in veterinary epidemiology.

The objective of this review is twofold. First, I will discuss the use of multilevel mixed effects linear or logistic regression models to determine associated factors related to automated milking systems, lameness, and mastitis. Secondly, I will review the progression of SEM models in dairy research and potential future applications of the modeling system.

Directed acyclic graphs

Directed acyclic graphs (**DAG**) are causal diagrams that contain explanatory variables and the outcomes of interest in a manner that illustrates plausible casual structure (Dohoo et al., 2009). As extensively described by Bello et al. (2018), nodes or variables of interest are connected or not by directed edges (i.e. arrows). An arrow from one variable to another represents a direct effect of the variable at the arrow tail on the variable at the arrow head. The arrows also denote temporality as the variable at the arrow tail must occur before the variable at the arrow head. Just as important, especially in terms of studying causality, lack of an arrow between two variables represents the absence of any causal direct effect, meeting the independence constraints between variables in a multivariable joint distribution (Pearl, 2010). Creation of a DAG is imperative to both multivariable mixed models and SEM. For mixed models, DAG can be used to identify potential confounder and intervening variables that should or should not be included in the model. In the case of SEM, a DAG illustrates the causal relationship that exists - or is believed to exist - between variables (Bello et al., 2018). In either case, DAG are constructed based on previous literature, expert knowledge, and known temporal relationships between variables (Pearl, 2009; Cha et al., 2017).

Multivariable mixed models

Automated milking systems. Implementation of automated milking systems (**AMS**) has grown throughout the world during the last 20 years. With this new system of milking has come questions regarding overall herd management for optimal productivity. What is the ideal approach to cow flow on the dairy? How many robots per pen is best? What kind of manure removal system should I use? Although recommendations had been available previously, it wasn't until the analysis of Tremblay and colleagues (2016) that facilities and management practices for optimal production in AMS herds were scientifically explored. Data from 635 North American dairy farms with AMS were analyzed to determine herd-level factors associated with daily milk production per cow and per AMS unit. Numerous variables and their interactions were tested, which warrants cautious interpretation, as the model was likely over-parameterized. Although many factors were deemed significant, two results were especially significant to the industry when considering the transition to milking via AMS: 1) free flow traffic was associated with 1.11 kg/d greater milk yield per cow compared to guided flow, and 2) milk yield may not reach its peak until 4 years following installation in retrofitted barns, whereas newly built AMS farms do not experience a change in production over time relative to installation.

Additional studies assessing herd-level housing and management practices in Canadian AMS herds have reported a negative association between milking frequency and number of cows per AMS unit (Deming et al., 2013; King et al., 2016). Each 10 additional cows/AMS beyond the mean (49.4 cows) reduced milking frequency by 0.22 times per cow daily (King et al., 2016). It has been suggested that feed bunk requirements for cows milked in an AMS may be less that the standard 0.61 m/cow due to less synchronization of feeding activity (Wagner-Storch and Palmer,

2003); however, little research supports this. Despite such logic, Deming et al. (2013) reported greater space at the feed bunk was positively associated with milk yield in AMS systems.

The aforementioned studies provide some data related to herd-level factors associated with greater productivity in AMS herds. Although the study by Tremblay and others (2016) provides critical information for the industry, over-parameterization of the model creates concern about the accuracy of the coefficients generated. Re-evaluation of those data and additional studies that perhaps more appropriately analyze the numerous potential factors associated with maximizing production are necessary.

Lameness. Lameness is a major health and welfare concern in the dairy industry. Prevalence of lameness in North America is between 21-55% in conventional freestall herds (von Keyserlingk et al., 2012; Solano et al., 2015). To compound this issue, many producers underestimate the prevalence of lameness in their herd (Bennett et al., 2014). Although there are several cow-level risk factors for this condition, there are several herd management and facility design factors that also play a role. Due to the multifaceted nature of the condition (Holzhauer et al., 2006), lameness research has been one of the largest subject areas within dairy science to employ hierarchical analysis.

Body condition score has been associated with lameness, as thinner cows are at greater risk of lameness regardless of barn type (tie-stall, freestall, or AMS; Westin et al., 2016; Jewell et al., 2019) or stage of lactation (lactating or dry; Daros et al., 2019; Jewell et al., 2019). Underconditioned cows also had lower odds of curing lameness during the dry period, making it unsurprising they were also at greater risk of chronic lameness (Daros et al., 2019). Underconditioned cows have decreased thickness of the sole cushion which decreases hoof support (Bicalho and Oikonomou, 2013) and increases risk of noninfectious lesions (Machado et

al., 2011), often resulting in lameness. Hoof durability is also an important consideration, as softer claws are at greater risk of developing severe hoof lesions (Borderas et al., 2004). There is some evidence to suggest that exposure to urine and fecal contamination causes the hooves to swell and soften (Gregory et al., 2006). Wet bedding for cows housed in tie-stalls increased odds of lameness by 2.5 times compared to those with dry bedding (Jewell et al., 2019). The same study also evaluated freestall herds; however, there was no metric that might similarly depict hoof exposure moisture. Frequency of manure removal in freestall herds was measured by Chapinal et al. (2013) and odds of severe lameness decreased by 26% for each additional time manure was removed from the pen each day.

Cows with previous lameness bouts are more likely to become lame again (Green et al., 2014) which coincides with greater odds of lameness in older cows (King et al., 2017; Jewell et al., 2019). Odds of lameness increased with increasing DIM, which could be the result digital cushion thickness gradually decreasing (Bicalho and Oikonomou, 2013) or recurrence of lameness in cows previously lame during the dry period and early lactation (Green et al., 2004; Daros et al., 2019). Additional cow-level factors associated with increased lameness were presence of hock lesions (Westin et al., 2016) and leg cleanliness (Jewell et al., 2019), both of which may be somewhat related to management factors.

Herd-level factors associated with lameness varied by housing system and region. Jewell and colleagues (2019) evaluated herd-level factors associated with lameness in Canadian dairy herds, but conducted separate analyses for tie-stall and freestall herds due to factors of interest varying between barn types. Unique to tie-stall herds, odds of lameness increased when bedding material was wet compared to dry. For freestall herds, when time in the holding pen awaiting milking exceeded 3 h/d, odds of lameness increased by 2.11 times. Common to both housing

systems, odds of lameness decreased when cows were housed on a bedded pack vs. freestall or tie-stall barn during the dry period. A similar analytical approach was used by Chapinal et al. (2013), as their evaluation of lameness was stratified into two U.S. regions. Since there are large management and facility differences between the Northeastern U.S. and California dairies, rather than including region as a random effect, separate models were built for the two regions. In the Northeast, odds of clinical lameness decreased with access to pasture, but odds of both clinical and severe lameness increased with increasing herd size. This relationship was actually opposite for California herds, as odds of clinical lameness decreased with increased herd size. Odds of lameness also decreased with rubber in the alley to the parlor, but increased by 10% for each additional 10% increase for percent of stalls with fecal contamination. Severe lameness in California also increased with greater presence of fecal contamination and decreased with greater frequency of pen manure removal. Similarly, greater frequency of alley scraping decreased risk of clinical lameness in Canadian AMS herds (King et al., 2016).

Westin et al. (2016) carried out one of the few studies to detect associations between both pen and stall factors and lameness. Narrow feed alleys (the alley between the feed bunk and stalls; < 430 cm) and obstructed lunge spaces increased odds of lameness. Sand-bedded stalls were determined to have the lowest odds of lameness compared to other bedding material, including mattresses (Westin et al., 2016; Jewell et al., 2019). Additional measures regarding general management, pen and stall characteristics, bedding, water trough and bunk space, milking specifications, and lameness management were tested, but few associations were determined. It is important to remember that failure to find an association does not necessarily mean an association does not exist, but its lack of detection could be due to a lack of variation in the predictor across farms in the dataset (Chapinal et al., 2013).

Many herd-level factors associated with lameness can be modified to prevent lameness. Changes in housing and management may help decrease the prevalence of lameness on dairy farms, but key risk factors vary across housing systems and regions. Hierarchical analysis of factors both at the cow- and herd-levels provide recommendations for reducing lameness on commercial farms.

Mastitis. Hierarchical analysis of herd data as it relates to mastitis and somatic cell count has been used quite extensively in Europe to determine at what level (quarter, cow, herd) preventative measures should be applied to control mastitis prevalence. Bulk tank somatic cell count was positively associated with intramammary infection (**IMI**) in early lactation heifers (De Vliegher et al., 2004; Piepers et al., 2011) and whole lactation incidence rate of clinical mastitis in cows (Tomazi et al., 2018). Reducing overall exposure to infection should reduce occurrence of new infections (De Vliegher et al., 2004; Dufour et al., 2012). Most practices associated with *Staphylococcus aureus* IMI were related to milking procedure (Dufour et al., 2012). Both pre-milking teat disinfectant and wearing gloves decreased *S. aureus* IMI incidence and prevalence. Wearing gloves also increased IMI elimination. Overcrowding during early lactation (< 60 DIM) and use of a '3 strikes and you're out' clinical mastitis culling policy decreased odds of *S. aureus* IMI elimination (Dufour et al., 2012). Poor hygienic practices such as cleaning the calving area less than once per month, scrapping the gathering yard less than 2 ×/d, poor heifer hygiene, and ineffective fly control increased risk of IMI (Peeler et al., 2000; Piepers et al., 2011).

Although these studies illustrate the importance of management factors in the prevention and control of IMI, variance components presented in De Vliegher et al. (2004) and Piepers et al. (2011) revealed most variation in IMI occurs at the heifer and quarter levels as opposed to the herd level. For IMI with contagious and environmental pathogens, 39 and 32% of variance

occurred at the heifer level and 58 and 68% of the variation at the quarter-level (Piepers et al., 2011). Even more extreme, De Vliegher et al. (2004) reported 97.3% of total variance was explained at the heifer level compared to 2.6% at the herd level. Similarly, only about 10% of variation in milk leakage was explained at the herd level (Klaas et al., 2005). The majority of variance for factors contributing to IMI (i.e. milk leakage) and IMI itself is explained at the cow or heifer level; therefore, practices and assessments at the cow level will be most advantageous to reduce IMI occurrence. However, that is not to say that udder health improvement is beyond the control of the farmer (De Vliegher et al., 2004).

Although these studies provide valuable information, they were conducted on small European or Canadian dairies, so little can be extrapolated to modern commercial dairies in the United States. A similar study in the United States was not identified in our literature search, and is therefore necessary to determine relevant associations between IMI and regional, herd, cow, and quarter-level factors.

Critique of hierarchical studies

In the studies discussed herein, analyses of hierarchical herd data successfully determined factors associated with either productivity in AMS herds or disease. Although it is beneficial to describe such relationships, these statistically-significant associations were not structured to determine directionality or causality. Jewell et al. (2019) was the only study to present a DAG to help illustrate both herd- and cow-level effects investigated. While others mentioned making attempts to identify confounders, it was noted that residual confounders may still bias the observed results to some extent. In some cases, factors with significant associations may have been the result of a reverse order directionality. For example, Dufour et al. (2012) reported

greater odds of prevalent *S. aureus* IMI for herds where milking system vacuum pressure was checked at least daily. Rather than the act of checking vacuum pressure causing greater IMI prevalence, it is more probable that dairymen struggling with high IMI prevalence increased surveillance on their milking system in response (Dufour et al., 2012). Issues such as associations with reverse order directionality could be alleviated by proper development of DAG that are either used to select explanatory variables included in the model or used in the context of SEM.

Studying large datasets with many variables also comes with the increased risk of finding associations by chance (Dohoo et al., 1996). This was likely the case with the analysis of Tremblay et al. (2016) in which 18 main predictors and more than 20 interactions remained in the final multivariable model. Although multicollinearity was assessed and confounding effects evaluated, use of a univariable screen to determine variables to include in their multivariable model likely would have reduced risk of overparameterizing their model. The model also could have benefited from stratification by country. Milk production goals differ between the United States and Canada due to the quota system in Canada. Although included as a fixed effect, stratifying the analysis by country would have decreased risk of overparameterized models while also increasing interpretive ability. Similar to the suggested approach, Chapinal et al. (2013) investigated factors associated with herd-level lameness separately for herds in the Northeastern United States and those in California, and Jewell et al. (2019) analyzed lameness factors separately for tie-stall and freestall herds.

One of the main advantages of hierarchical data analysis is the evaluation of variance components and their use to identify the hierarchical level with the most potential for intervention (Dohoo, 2008). For example, the iconic Reunion Island paper utilized a dataset with

four-level hierarchical structure consisting of region, herds within region, cows within herd, and lactation within cow to determine which level explained the most variance in calving to first service interval (Dohoo et al., 2001; Dohoo, 2008). Variance components calculated for each level and from region to lactation were as follows: 0.1, 1.6, 2.0, and 13.2%. The greatest amount of variation in calving to first service interval was explained at the lactation level. Therefore, to influence calving to first service interval, interventions or implementation of new strategies would be most fruitful for factors that vary by lactation (i.e., diseases of the reproductive tract or return to positive energy balance after calving; Dohoo, 2008). Despite the value of variance components, they often go unreported (Dohoo, 2008). In accordance with such observation, only 3 of the studies discussed herein reported variance components. Each were European studies investigating factors associated with IMI (De Vliegher et al., 2004; Piepers et al., 2011) or milk leakage (Klaas et al., 2005). The greatest implications would be for the associated factors at the level explaining the greatest variance; therefore, future studies should report variance components by hierarchical level.

Structural equation modeling

A simplified example. Implementation of SEM is a relatively new statistical technique utilized in animal science. To facilitate a better understanding of the approach in an animal production setting, the relatively simple mixed-model-based SEM to investigate interrelations of bovine respiratory disease (**BRD**) and performance (Cha et al., 2017) will be discussed. Outcomes of interest were arrival weight (**AW**), BRD-related treatment costs (**Trt$**), and average daily gain (**ADG**). A DAG was constructed based on expert knowledge and temporal order of the outcomes (Figure 1). Authors hypothesized a direct effect of AW on ADG, a direct

effect of AW on Trt$, and an indirect effect of AW on ADG through Trt$ (**SEM1**). Three additional models were created that represented alternate relationships between the 3 variables. All models specified the random effect of contemporary group, or lots if cattle were managed in clusters, thereby generating multilevel correlation patterns. Models were compared by Akaike Information Criteria (**AIC**) and Bayesian Information Criteria (**BIC**) and the model with the lowest values was considered to have the best model fit. Two standard linear mixed models were employed, one analogous to the best fitting model represented in Figure 1 (Linear1), and the other similar in all aspects except it excluded Trt$ (Linear2).

Lowest AIC and BIC values indicated best model fit for SEM1, shown in Figure 1. Structural coefficients were estimated, from which the indirect effect of AW on ADG mediated through Trt$ can be calculated as the product of the corresponding direct effects. In this example, the direct effect of AW on ADG estimated ADG increased by 0.002 ± 0.0001 kg/head daily for every 1 kg/head increase in AW. The direct effect of AW on Trt$ was -0.0783 ± 0.0061 dollars per head for every 1 kg/head increase in AW and the direct effect of Trt$ on ADG was -0.0038 kg/head daily for every dollar spent per head on treatment. Therefore, the indirect effect of AW on ADG was a daily increase of 0.0003 kg/head (-0.0783 × -0.0038) per every 1 kg/head increase in AW. The total effect of AW on ADG was a 0.0023 ± 0.0001 kg increase per head daily for every 1 kg increase in AW per head.

Comparison of SEM and linear models indicate that when Trt$ was not included in the model (Linear1) the coefficient denoting the effect of AW on ADG is the same as the total effect of AW on ADG determined by SEM (0.0023 ± 0.0001 kg/head daily). In Linear2, when Trt$ was included in the model, the regression coefficient was similar to only the direct effect generated by SEM. Thus, linear regression models including both AW and Trt$ not only represent the

direct effects of the variable on the outcome, but also fail to address the relationship between AW and Trt$. As demonstrated by this example, SEM was able to partition the overall effect of AW on ADG into direct and indirect effects. Such deconstruction is not possible with the linear mixed model (Cha et al., 2017). The ability to simultaneously explore both direct and indirect effects of a variable, and thus gain insight into the network of interrelated outcomes, is a main advantage of SEM (Cha et al., 2017).

SEM examples in dairy cattle. Does greater milk yield lead to greater risk of mammary infection or does infection lead to lower milk yield? The relationship between milk yield and somatic cell score (**SCS**) may involve both recursive and simultaneous effects between traits (de los Campos et al., 2006). This relationship and determination of the directionality of such relationship cannot be addressed using linear mixed models, but can using SEM approaches described by Gianola et al. (2004). Consequently, this has been the main question explored using SEM within dairy production science.

This dilemma was first addressed by de los Campos et al. (2006) though 4 SEM designed to study the relationships between SCS and milk yield in first lactation Norwegian Red cows using a sire model. The models evaluated no recursive effect (Model 1), the recursive effect of SCS on milk yield (Model 2), the recursive effect of milk yield on SCS (Model 3) and the simultaneous effects between milk yield and SCS (Model 4). Model 2 had the lowest BIC, thus indicating the negative association between milk yield and somatic cell score is more likely due to infection (measured indirectly by somatic cell score) decreasing production rather than vice versa. A key limitation of the ISREL (linear structural relationships) software used was the inability to include random effects. The data had to be pre-corrected for the random effects

obtained from univariate mixed linear models where sire, cow, and herd were random. Nonetheless, this study provided inroads for SEM use in the field of dairy science.

Similarly, Wu et al. (2007) investigated the relationship between milk yield and SCS, but extended the recursive and simultaneous models to account for potential population heterogeneity. Additionally, stratum-specific estimates were generated for degree of milk production (high or low) and stage of lactation (first 60 DIM or 60-120 DIM). Their results again indicated large negative direct effect of SCS on milk yield and little reciprocal effects of milk yield on SCS. Based on their stratum-specific estimates, they determined greater effects of SCS on milk yield during the first 60 d of lactation and for cows of greater production level. This study had a similar limitation to de los Campos et al. (2006) in that adjustments for herd effects were conducted using best linear unbiased predictors generated from univariate mixed models.

Wu et al. (2008) took SEM modeling a step further and proposed a Gaussian-threshold model within the general framework of SEM to investigate recursive and simultaneous models of relationships between binary and continuous variables. Authors used a Bayesian analysis via Markov Chain Monte Carlo implementation to deduce parameters of interest. They applied such methods to investigate the carryover effects of clinical mastitis on milk yield across three 60-d periods for the first 180 DIM. Since then, recursive Gaussian-threshold models have been employed to continue the study of the interrelationships between mastitis and milk yield (Rehbein et al., 2013) as well as other health disorders and milk yield (Dhakal et al., 2015).

The latest applications of SEM in the mastitis field have examined relationships between herd-level factors and mastitis (Detilleux et al., 2012), estimated direct and indirect level of tolerance to mastitis (Detilleux et al., 2013), and estimated the direct and indirect milk losses due to clinical mastitis in dairy cattle (Detilleux et al., 2015). Herd-level factors associated with

mastitis have been explored previously (De Vliegher et al., 2004) and are discussed above; however, those characteristics are interrelated and it becomes hard to distinguish between spurious and factual associations and between direct and indirect effects. A complex DAG was created to illustrate the direct and indirect effects of herd-level factors on the latent state of udder health. One of the most meaningful results as it applies to the advantages of SEM was the indirect positive effect of post-milking teat disinfection on mastitis, mediated through treatment of subclinical ketosis. Use of post-milking teat disinfectant had a positive direct effect (+0.10 SCS) on treatment of subclinical mastitis and treatment of subclinical mastitis had a direct positive effect (+0.07 SCS) on herd-level mastitis. Thus, through its direct effect on subclinical mastitis treatment, post-milking teat disinfectant had a positive indirect effect (+0.01 SCS) on mastitis. The direct (-0.12 SCS) and total effects (-0.11 SCS) were intuitively negative. The study again highlights the advantage of SEM to differentiate between direct and indirect effects as well as indicating directionality of the associations.

The use of SEM in animal science is relatively new, but provides great opportunity for analyzing observational data in complex biological systems. They also provide an avenue to infer causal associations. Reviews by Rosa and Valente (2013) and Bello and colleagues (2018) both discuss analytical techniques in which causation can be inferred in observational data. The ability to do so is complex due to potential confounding effects and certain assumptions must be met (Rosa and Valente, 2013). Those assumptions include the following: 1) causal sufficiency, 2) invokes the Markov condition, and 3) meets assumption of faithfulness (Bello et al., 2018). Another crucial component to making casual assumptions is substantive knowledge of the scientific core that is used to create DAGs. As stated by Rosa and Valente (2013), the advancements and developments in statistical techniques, coupled with the increasing volume

and variety of operational data generated on dairy farms, leave much to be learned from observational data.

SUMMARY

Huge amounts of data are generated on dairies daily, and that amount is only expected to increase with the implementation of more precision technologies. These operational data represent great opportunity for observational studies. Multilevel models can reveal associations amongst explanatory variables at different levels of the data structure and variance components can be used to determine at which level an intervention could be most effective. Structural equation models can also be used to evaluate associations in dynamic biological systems with advantages including separation of direct and indirect effects as well as determining directionality of associations. Operational data provides ample opportunity for observational studies that can not only determine associations within biological systems but also potentially allow for causal inference.

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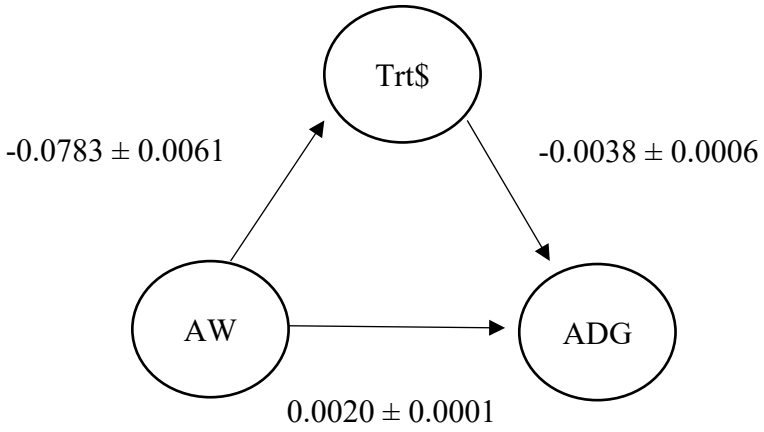
FIGURES

Figure 1.1. Directed acyclic graph illustrated the hypothesized causal path between arrival weight (AW), treatment costs associated with Bovine Respiratory disease (Trt$), and average daily gain (ADG).

Arrows indicate a direct effect of the outcome at the end of the arrow tail to the outcome at the head of the arrow. The indirect, Trt$-mediated, effects of AW on ADG were 0.0003 ± 0.0001. The total effect of AW on ADG was 0.0023 ± 0.0001 kg/head daily. Adapted from (Cha et al., 2017).



Chapter 2 - **Do biological and management causes of a short or long dry period induce the same effect on dairy cattle productivity?**

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ABSTRACT

The dry period is a well-established factor for determining lactation success. A retrospective observational study utilized 32,182 lactations from 16 farms to determine if management versus biological reasons for a short or long period have the same associations with subsequent lactation performance. Herd inclusion criteria were Holstein cows, herd size ≥ 900 cows, breeding by artificial insemination, and (minimally) bimonthly milk testing. Dry period (**DP**) length and gestation length (**GL**) were each categorized as short (> 1 SD below mean) or long (> 1 SD above mean) and combined to generate the following 7 study groups: short DP, short GL (**S_DS_G**, n = 2,123); short DP, average GL (**S_DA_G**, n = 1,418); average DP, short GL (**A_DS_G**, n = 1,759); average DP, average GL (**A_DA_G**, n =19,265); average DP, long GL (**A_DL_G**, n =3,325); long DP, average GL (**L_DA_G**, n = 2,573); and long DP, long GL (**L_DL_G**, n =1,719). Responses evaluated included milk and component yields at first test and over the whole lactation, days to first service, first service conception risk, days open, and herd retention through 60 and 365 days in milk (DIM). Continuous data were analyzed by mixed models and time to event data by Cox proportional hazard models, both accounting for clustering at the herd level. First test and whole lactation milk and component yields were lowest for S_DS_G. Within cows that experienced calving difficulty, rate of receiving first service was 13 and 20% less for S_DS_G and A_DS_G compared to A_DA_G. Hazard of leaving the herd by 60 DIM was 34% greater for A_DS_G than A_DA_G. Similar effects between S_DS_G and A_DS_G but not S_DA_G indicated short GL was a greater contributor to poor performance than DP length itself. Overall production was similar between A_DA_G and S_DA_G; however, somatic cell linear score at first test was greater for S_DA_G and milk yield at first test was lesser for S_DA_G cows with greater milk at last test before dry-off. While short DP might work for some herds or cows, cows with high milk yield at dry-off should not be

subjected to a short dry period. Long DP or GL did not impact early lactation or whole lactation milk yield. Cows with a long DP due to management factors (L_DA_G) likely experienced issues related to excessive lipid mobilization as milk fat concentration and fat:protein ratio at first test were greater and hazard of leaving the herd was 30 and 24% greater compared to A_DA_G by 60 and 365 DIM, respectively. In conclusion, deviations in DP length caused by biology (short GL) were associated with greater impacts than management causes of short DP, whereas management reasons for long DP were associated with more negative outcomes than long GL.

INTRODUCTION

Optimal dry period length (**DPL**) has been long debated (Annen et al., 2004a). As far back as 1936 and especially from the 1970s-1990s, retrospective observational studies conducted to identify optimal DPL (Arnold and Becker, 1936; Schaeffer and Henderson, 1972; Keown and Everett, 1986) generally found negative associations between short dry periods and subsequent milk production. However, nonrandom assignment of cows (Bachman and Schairer, 2003) and failure to account for factors other than DPL in statistical models (Wiggans et al., 2002) created speculation regarding validity of these results. As Bachman and Schairer (2003) discussed, there is inherent bias in these retrospective study groups because they represent unplanned short or long dry periods. Many of the unplanned short dry periods in these studies were likely the result of various reasons that caused cows to calve early (i.e. carrying twins, heat stress, etc.); factors that are known to decrease cow productivity independent of DPL. Similarly, a large portion of cows with an unplanned long DPL were likely dried off early because of their inability to maintain a minimum level of milk production in late lactation, and this low productivity is likely carried over to the next lactation (e.g., inferior genetics). To account for at least some of the aforementioned bias, Funk and others (1987) and Makuza and McDaniel (1996) demonstrated the necessity of including previous lactation days open and milk yield when evaluating the effect of DPL on subsequent milk yield; however, even after such adjustments, both provided evidence supporting an optimal DPL between 60-69 d.

Production potential of cows has changed considerably over the last 20 years. Such high levels of milk production has resulted in relatively high milk yield at 220 d of gestation (~24-30 kg/d; Annen et al., 2004a), leading to concerns about mammary gland health and animal welfare. Shortening the dry period has been proposed as a mechanism to reduce milk yield at the time of

dry-off (Annen et al., 2004a), promoting reexamination of dry period length for cows in the 21st century.

More recent retrospective analyses aligned with previous studies, reporting negative associations between short or long dry periods and subsequent lactation performance (Pinedo et al., 2011; Atashi et al., 2013). Although these studies accounted for other explanatory variables to reduce the biases associated with nonrandom assignment of cows to DPL categories, neither were conducted in the United States, nor did they differentiate among causes for the deviations in DPL. It is known that short or long gestation lengths (**GL**) are associated with greater incidence of dystocia, stillbirth, retained placenta, and metritis, and lower milk production (Vieira-Neto et al., 2017). As such, it is possible the adverse effects associated with short or long DPL reported by Pinedo et al. (2011) and Atashi et al. (2013) were actually related to biological factors causing early or late parturition rather than impacts of DPL per se.

To our knowledge, no study has ever evaluated the lactation outcomes associated with DPL while accounting for the reasons underlying the deviation from the target DPL. Incorporation of GL as a reason for DPL deviation may provide insight into which DPL associations are linked to underlying/pre-existing biological processes versus those mainly resulting from management decisions (i.e. early dry-off, incorrect recording, etc.). The objective of this study was to determine if biological and management reasons for a short or long dry period are associated with different effects on subsequent lactation productivity. Our hypothesis was that subsequent lactation productivity would be most hindered by short or long DPL caused by deviations in GL rather than management factors.

MATERIALS AND METHODS

Dairy herds and animals

A convenience sample of 16 dairies from Western and Midwestern regions of the United States were used in a retrospective cohort design. Participating herds were identified through nutrition consultants and included based on their willingness to share herd records and meet the following herd inclusion criteria: Holstein cows, herd size ≥ 900 cows, use of artificial insemination or the ability to exclude bull breedings, and at least bimonthly Dairy Herd Improvement Association testing. Descriptive statistics of dairies are presented in Table A.1. Medians for herd size, herd turnover rate, and previous lactation 305-d mature equivalent (**ME**) milk yield were 2,769 cows (range: 928-10,047), 44.3% (range: 29.1 – 53.4%) and 12,816 kg (range: 11,655-18,615 kg), respectively.

Data collection

Previous lactation and subsequent lactation data for cows calving in 2016 were extracted from archived herd records (DairyComp 305, Valley Ag Software, Tulare, CA). Data cleaning was performed on individual dairy datasets in SAS (v. 9.4, Cary, NC) to remove observations that did not satisfy cow level inclusion criteria. The complete dataset totaled 60,773 observations, 28,591 of which were removed for the following reasons: first lactation cows (38.8%), cows not specifically defined as Holstein (2.7%), duplicate data or information for the second lactation for cows that calved twice in 2016 (2.5%), cows missing gestation length data (< 0.01%), cows without a dry period or that were missing dry period length data (0.26%), and cows with dry period length > 280 d (0.003%). As gestations < 260 are generally considered to

end in abortions (Gädicke and Monti, 2013), a post hoc removal of observations with GL < 260 was performed (116 observations, 0.003%).

Treatment category determination

Treatment categorization was conducted on an individual farm basis to account for differences in management and genetics that are reflected in the varied distributions of DPL and GL across farms (Figure A.1). The UNIVARIATE procedure of SAS (v. 9.4, Cary, NC) was run twice for both DPL and GL; the first to remove outliers for both (> 3 standard deviations [**SD**] from the mean) and the second to determine short (> 1 SD below the mean), average (within ± 1 SD of the mean), and long (> 1 SD above the mean) categories within each herd. Mean DPL and GL used to categorize cows in each farm are listed in Table 2.1 with their respective SD. Mean DPL for all farms were within industry norms. A 3 × 3 factorial design for DPL and GL categories was used to generate the following 9 study groups: 1) short DP, short GL (**S_DS_G**, n = 2,123); 2) short DP, average GL (**S_DA_G**, n = 1,418); 3) short DP, long GL (**S_DL_G**, n = 50); 4) average DP, short GL (**A_DS_G**, n = 1,759); 5) average DP, average GL (**A_DA_G**, n =19,265); 6) average DP, long GL (**A_DL_G**, n =3,325); 7) long DP, short GL (**L_DS_G**, n = 310**)**; 8) long DP, average GL (**L_DA_G**, n = 2,573); and long DP, long GL (**L_DL_G**, n =1,719). Categories S_DL_G and L_DS_G were not analyzed due to the small number of observations in each and lack of biological plausibility. A total of 32,182 lactating cows from the 16 dairies were used for analysis.

Outcome variables

First test day parameters evaluated were milk yield (**MILK1**), milk fat and protein concentrations and yields, somatic cell count. Somatic cell count is reported as somatic cell

linear score (**LSC1**) calculated as $\log_2$(somatic cell count/100)+3 (Shook, 1993). Observations were restricted to those with a first test between 5-35 DIM. As there were no observations with a first test on d 5 or 6 of lactation, DIM at first test actually ranged from 7 to 35 DIM. Similarly, whole lactation parameters investigated included milk yield, milk fat and protein concentrations and yield, and average linear somatic cell count (**LSC**). For insight into whole lactation milk production, 305-d ME milk yield, predicted at 3rd test of lactation was used (**ME305**). The 3rd test was chosen because it included a large proportion of observations (avoiding survivor bias) while also having the precision of data from multiple tests (see Table A.2). Excluding first test data, milk component concentrations and LSC were averaged across lactation. Whole lactation milk fat and protein yields were calculated as the average component values multiplied by ME305.

Reproduction was evaluated by the following outcome variables: 1) DIM at first service, 2) 1st service conception risk, and 3) days open, defined as the number of days between calving and the breeding that resulted in pregnancy. Herd retention was analyzed as hazard of leaving the herd either through death or live culling was assessed within the first 60 d after calving and through 365 DIM.

Explanatory variables

The explanatory variables can be grouped to consider 3 periods: 1) previous lactation, 2) dry-off, and 3) calving and subsequent lactation. Variables from the previous lactation included previous days carried calf, 305-d ME milk yield (**PRVME**), days open (**PDOPN**), and both milk yield and LSC at last test before dry-off (**LMILK** and **LLSC**, respectively). The last test before dry-off must have occurred within 40 d before dry-off to be considered. Dry period information

included DPL and days in the close-up pen. Calving and subsequent lactation information included parity group (categorized as 2 or 3+), fresh month, calf description (female, male, twin), calf dead on arrival (**DOA**), calving difficulty (**CFDIF**; yes or no), mastitis at first test defined as LSC > 4.5 (yes or no), and predicted transmitting ability for milk yield (**PTAM**), fat yield, and protein yield. Milk yield at first test was tested as an explanatory variable for first test milk fat and protein models. Quadratic terms were created for all continuous variables and tested in all models for which the respective linear term was included.

Statistical analysis

To determine significant predictors of study group assignment, multinomial logistic regression was conducted for each dairy using the LOGISTIC procedure of SAS (v. 9.4, SAS Institute, Cary, NC) with the glogit link function. Fixed effects evaluated were parity group, fresh month, calf description, DOA, previous lactation GL, PRVME, and LMILK.

Associations between study group and outcomes of interest were all assessed in STATA/IC 12.1 (StataCorp LLC, College Station, TX). Continuous parameters of interest were assessed by multivariable mixed effects regression models, and first service conception risk by a mixed logistic regression mode. Time to event data (days to first service, days open, survival) were analyzed by Cox proportional hazard models and visualized with Kaplan Meier survival graphs. The hierarchical structure of the data consisted of cows nested within herds. To account for clustering at the herd level, herd was included as a random intercept in mixed models and as a shared frailty term for Cox models.

Multivariable models were constructed for each outcome of interest using variables significant at the 20% level ($P < 0.20$) in the univariable screen. The main predictor of interest,

study group, was forced into all models. All 2-way interactions between study group and other predictors were tested. Manual backwards elimination was used to remove all nonsignificant variables ($P > 0.05$) unless they were part of a significant interaction term or their quadratic term was significant. Models were assessed for collinearity using Spearman's correlation coefficient; however, there were no correlated variables (> |0.80|) for any of our final models.

Diagnostics of final multivariable linear and logistic mixed effects models involved examination of residuals both at the dairy and cow level. Predicted values (best linear unbiased predictors; BLUP) were generated for the random effect (herd), and standardized residuals were calculated for lower-level residuals (cow). Both levels of residuals were visually examined to assess model fit and identify potential outliers. Potential outliers were individually assessed for biological plausibility. Those with biologically implausible values were removed from the analysis. Results are presented as the coefficients for all significant variables with means also generated for categorical variables. Pairwise comparisons among study groups were adjusted for multiple comparisons by the method of Bonferroni. Intra-class correlation coefficients (**ICC**) were calculated to determine the proportion of total variance in the population that could be attributed to variation between groups.

Time to event data (days to first service, days open, herd retention) were analyzed by Cox proportional hazard models and visualized with Kaplan Meier survival graphs. The proportional hazards assumption was evaluated both graphically and via statistical assessment using Schoenfeld residuals. To fully understand the main effect of study group and obtain hazard ratios that would represent the Kaplan Meier graph, a univariable Cox model containing only study group was run for survival models. Kaplan-Meier survival curves were generated for each study group and tested for homogeneity by the Wilcoxon test. To further determine which study groups

differed, the univariable Cox model was run several times, each with a different study group as the referent.

RESULTS

Data analyzed in this study represented 32,182 cows from 16 dairy herds across the Midwestern and Western United States. Distributions of DPL and GL for each study group are shown in Figure 2.1. Medians and interquartile ranges for DPL (S_DS_G=44, 40-48 d; S_DA_G = 46, 43-50 d; A_DS_G=53, 48-56 d; A_DA_G=56, 51-60 d; A_DL_G=60, 57-64 d; L_DA_G=77, 70-85 d; L_DL_G=70, 67-77 d) and GL (S_DS_G=268, 266-270 d; S_DA_G = 273, 272-275 d; A_DS_G=270, 268-271 d; A_DA_G=277, 275-279 d; A_DL_G=284, 283-285 d; L_DA_G=277, 275-280 d; L_DL_G=284, 283-287 d) reflect appropriate DPL and GL categorization.

As study group classification was determined on an individual herd basis, associated factors were also investigated at the individual herd basis. Factors that predicted study group were generally similar across herds; however, the direction of prediction for the study group was not consistent across dairies (Table A.3). Predictive factors (PRVME, PDOPN, previous lactation GL, LMILK, DOA, calf description, and parity group; $P < 0.05$) are expressed as the proportion of herd models for which the variable was retained in Figure 2.2. Generally, cows in their 3+ lactation had greater odds of being in L_DA_G, L_DL_G, and A_DL_G, cows bearing male calves had greater odds of being in a long GL group, twin-bearing cows had greater odds of being in either short GL group, cows with a DOA calf had greater odds of being in either of the short GL groups and A_DL_G, greater milk yield at last test was associated with greater odds of being in either short DPL group or A_DL_G, and greater previous lactation GL was associated with greater odds of the long GL groups. Predicted transmitting ability for milk production was tested but not

retained for any herd. There was only one dairy in which the aforementioned variables did not predict study group (dairy E).

First test lactation parameters

Full final regression models for all milk lactation outcomes are available in Tables A.4-10. The main effects of study group, interactions with study group, and study group means for lactation outcomes at first test are presented in Tables 2.2 and 2.3, respectively.

The association between MILK1 and study group was dependent on PRVME, LMILK, and PDOPN ($P < 0.001$; Figures 2.3A-C). There was a positive relationship between MILK1 and PRVME, but the magnitude was less for cows with a short DPL or GL, and least for cows with both a short DPL and GL. The study group × LMILK and study group × PDOPN interactions for MILK1 were similar. While MILK1 either increased or was similar across the LMILK and PDOPN ranges for other study groups, MILK1 progressively decreased for S_DA_G cows as LMILK or PDOPN increased. Although the main effect of study group was not significant ($P = 0.16$), S_DS_G had the least mean MILK1, followed by A_DS_G and S_DA_G; these means are consistent with the results shown in the interaction graphs (Figure 2.3). Milk at first test was greater for 3+ lactation cows than 2nd-lactation cows (43.4 vs. 40.7 ± 0.96 kg), less for cows bearing twins (39.5 vs. 42.2 ± 1.01 kg) or a dead calf (40.0 v. 42.3 ± 0.97 kg), decreased with mastitis at first test (41.1 vs. 42.7 ± 0.97), and varied by month of parturition (all $P < 0.001$).

Milk fat concentration decreased with increasing milk yield at first test. The slope reflecting this relationship was similar across study groups, except for a steeper slope for L_DA_G (Figure 2.4A). The study group × PDOPN interaction indicated relatively constant milk fat concentration across PDOPN (A_DA_G and A_DS_G) or increases as PDOPN increased (S_DS_G, S_DA_G, A_DL_G, L_DA_G, L_DL_G; Figure 2.4B). Again the interaction was most drastic for L_DA_G. First test

milk fat concentration was greater for 3+ lactation cows (4.01 vs. 4.09 ± 0.10%), lowest for cows calving in summer months, less for twin-bearing cows (3.90 vs. 4.07 ± 0.10%), greater for cows with mastitis at first test (4.03 vs. 4.15 ± 0.10%), increased with increasing PTA fat yield, but decreased with PTAM and PRVME (all $P < 0.001$).

Milk fat yield at first test was greatest for L_DL_G and A_DL_G, intermediate for S_DA_G, A_DA_G, L_DA_G, decreased for A_DS_G, and least for S_DS_G ($P < 0.001$). Milk fat yield at first test was greater for 3+ lactation cows (1.66 vs. 1.81 ± 0.10 kg/d), less for cows calving twins (1.75 vs. 1.59 ± 0.06 kg/d), cows with a calf DOA (1.74 vs. 1.65 ± 0.06 kg/d), and cows with mastitis at first test (1.75 vs. 1.71 ± 0.06 kg/d; all $P < 0.001$). Milk fat yield increased with LMILK, PRVME, and PDOPN ($P < 0.001$); however, the negative coefficient for the quadratic term of PDOPN ($P < 0.001$) indicates a threshold for the positive relationship between PDOPN and first test milk fat yield.

Differences in milk protein concentration at first test by study group were generally inversely related to differences in MILK1 ($P < 0.001$), with greatest concentrations for S_DS_G, least for L_DA_G and L_DL_G, and intermediate for S_DA_G, A_DS_G, A_DA_G, and A_DL_G. Other factors associated with milk protein concentration at first test also reflected dilution by milk yield and included decreases for cows in their 3rd lactation or greater (3.09 vs. 3.11 ± 0.02%), increased milk protein concentration for cows with calves DOA (3.10 vs. 3.14 ± 0.03%) and with mastitis at first test (3.08 vs. 3.17 ± 0.02%), and differences by fresh month ($P \leq 0.002$). Milk protein concentration decreased with increasing MILK1 and PRVME, but increased with increases in PTA protein percent and PTA protein yield ($P < 0.001$). Previous lactation days open and LMILK were positively related to milk protein concentration at first test, but quadratic terms indicate a threshold for which the relationship remains positive ($P \leq 0.01$).

Milk protein yield at first test was least for S_DS_G and A_DS_G, intermediate for S_DA_G and L_DL_G, and greatest for A_DA_G, A_DL_G, and L_DA_G ($P < 0.001$). There was an interaction with PTA protein yield in which milk protein yield increased as PTA protein yield increased; however, the slope was greatest for S_DS_G ($P = 0.03$; Figure 2.5A). Protein yield was either similar across PTAM range (A_DS_G and L_DL_G), or decreased with increasing PTAM (S_DS_G, A_DA_G, A_DL_G, and L_DA_G), especially for S_DS_G ($P < 0.001$; Figure 2.5B). Milk protein yield was equivalent across treatments at PRVME of 15,000 kg, but S_DA_G and A_DL_G had greater protein yield among cows with greater PRVME, yet S_DA_G had the least protein yield among cows with lesser PRVME (Figure 2.5C). Interactions with LMILK and PDOPN largely reflected observed interactions for MILK1. Increases in LMILK were associated with increased milk protein yield for all study groups except for S_DA_G which decreased ($P < 0.001$; Figure 2.5D). Similarly, while there was little difference across PDOPN for most study groups, increased PDOPN was associated with decreased milk protein yield at first test for S_DA_G ($P = 0.02$; Figure 2.5E).

Overall, LSC1 was positively related to LLSC at last test before dry-off, but LSC1 was greatest for S_DA_G cows across the range of LLSC ($P = 0.004$; Figure 2.6). LSC1 was greater in 3+ lactation cows (2.69 vs. 2.06 ± 0.17) and cows that experienced calving difficulty (2.46 vs. 2.38 ± 0.17), lesser in cows with a calf DOA (2.12 vs. 2.41 ± 0.18), and varied by fresh month. LSC1 was least for October calvings, greatest for January calvings, and intermediate the remainder of the year ($P \leq 0.05$). Milk yield at first test was inversely associated with LSC1 while PDOPN were positively associated with LSC1.

Associations between study group and fat:protein ratio at first test (**FP1**) were investigated to provide some insight into early lactation metabolic status. Study groups did not differ in FP1 overall ($P = 0.54$), but rather interacted with MILK1, PRVME, and PDOPN ($P \leq$

0.01; Figures 2.7A-C). The L_DA_G study group had greater FP1 when MILK1 was less than 40 kg, was similar across the PRVME range, but increased greatly with increased PDOPN.

Whole lactation parameters

Full final regression models for whole-lactation parameters are shown in Tables A.11-16 with study group means summarized in Table 2.3. Overall ME305 was less for S_DS_G cows compared to all other study groups ($P < 0.001$). The association between milk production and study group did however interact with fresh month, calf description, PRVME, and PDOPN ($P \leq 0.02$). No clear pattern in the study group × fresh month interaction was detected. Study group differences for cows birthing female calves mirrored overall differences, with 305ME lower for S_DS_G compared to all other study groups. Within cows birthing male calves, 305ME was again least for S_DS_G, but greatest for A_DL_G, and intermediate for all other study groups. There was no difference in milk production among study groups for twin-bearing cows. Unsurprisingly, 305ME was positively associated with PRVME, but slopes were greater for cows with any combination of average and long DPL and GL compared to cows with short GL (Figure 2.8A). As PDOPN increased, milk production decreased; however, cows with long GL (A_DL_G and L_DL_G) were least impacted by increasing PDOPN (Figure 2.8B). Predicted 305-d ME milk was lesser for 3+ compared to 2nd lactation cows (11,833 vs. 12,045 ± 250 kg), cows with a calf DOA (12,160 vs 12,502 ± 256 kg), and cows with mastitis at first test (12,197 vs. 12,604 ± 250 kg; all $P < 0.001$). Milk increased with PTAM and LMILK ($P < 0.001$).

Average fat concentration varied by study group and interacted with both parity group and previous lactation 305-d ME milk production ($P \leq 0.02$). There were no study group differences amongst 2nd lactation cows, but for cows in their 3rd lactation or greater, average milk

fat concentration was greatest for A_DA_G and A_DL_G cows, least for L_DA_G, and intermediate for the others. Average fat concentration decreased with increased PRVME, with decreases greatest for S_DS_G and A_DL_G (Figure 2.9). Additional factors associated with increases in average fat concentration were PTA fat yield and LMILK, whereas PTAM and PRVME were associated decreases ($P \leq 0.03$), and differed by fresh month.

Whole lactation fat yield was least for S_DS_G, intermediate for A_DS_G, and greater for other study groups ($P = 0.03$). Although whole lactation fat yield increased with PRVME, the degree of increase was less for S_DS_G and A_DS_G ($P = 0.02$; Figure 2.10A). Whole lactation fat yield decreased with greater LLSC, with the decrease greatest for A_DS_G (Figure 2.10B). Decreased fat yield was associated with parity 3+ (456.7 vs. 445.7 ± 14.3 kg), calf DOA (441.9 vs. 451.2 ± 14.6 kg), mastitis at first test (446.2 vs. 452.1 ± 14.4 kg), PTAM, and PDOPN, and increased fat yield was associated with LMILK and DIM when cows left the herd ($P \leq 0.03$).

Average protein concentration was greatest for cows with a short dry period, intermediate with an average dry period, and lowest for cows with a long dry period ($P < 0.001$). Additional associations (all $P \leq 0.01$) such as decreased protein concentration for 3+ lactation cows (3.10 vs. 3.12 ± 0.03%) and cows bearing live singletons (3.11 vs. 3.13 ± 0.03%) could be attributed to dilution as milk yield effects were opposite. Milk protein concentration was positively associated with PTA protein and yield parameters and LMILK, but negatively associated with PTA milk yield, PRVME, and PDOPN ($P \leq 0.01$).

Whole lactation protein yield was greatest for A_DA_G and A_DL_G, least for S_DS_G and L_DA_G, and intermediate for L_DL_G, S_DA_G, A_DS_G ($P < 0.001$). Protein yield was less for cows in lactation 3+ (371.8 vs. 383.2 ± 7.8 kg) and for cows with mastitis at first test (375.8 vs. 377.8 ± 7.8 kg), and differed by fresh month (all $P \leq 0.02$). Additional factors associated with lactation protein

yield included positive relationships with PTA protein concentration and yield, PRVME, LMILK and DIM when cows left the herd ($P < 0.01$). Previous lactation days open was the only factor with a negative association ($P < 0.001$).

Associations between study group and average LSC throughout lactation was dependent on fresh month, mastitis at first test, and LLSC ($P < 0.01$). The interaction with fresh month showed no clear pattern. Average LSC increased with increasing LLSC for all study groups except A_DS_G (Figure 2.11). The positive relationship was greatest for A_DL_G. There was no difference in average LSC between study groups in 2nd lactation cows, but in 3+ lactation cows was greatest for A_DL_G cows, least for A_DA_G, and intermediate for other study groups. Overall average LSC was greater for cows with mastitis at first test (3.26 vs. 2.15 ± 0.16) and 3+ lactation cows (2.61 vs. 2.10 ± 0.16; $P < 0.001$). Average LSC decreased with PRVME and LMILK, while it increased with LLSC ($P \leq 0.01$).

Reproductive parameters

Difference for days to first service between study groups were dependent on calving difficulty ($P = 0.01$; Table 2.5). While there was no difference between study groups for cows without calving difficulty, hazard of insemination (reduced calving to first service interval) was greatest for A_DA_G and S_DA_G, least for A_DS_G, and intermediate for others. Overall, median days to first service was 67. Conception risk at first service was not associated with study group ($P = 0.06$; Table A.16). First service conception risk was greater for cows in their 2nd lactation, that had carried female calves born alive, and varied by fresh month ($P < 0.001$). Factors positively associated with first service conception risk were MILK1 and DIM at first service, while LMILK and PDOPN were negatively associated ($P \leq 0.001$).

Days open (up to 365 DIM) was associated with study group; hazard of pregnancy was less for A_DL_G compared with A_DA_G, but days open did not differ for any other study group compared to A_DA_G (Table 2.4). Overall cows with a calf DOA had poorer fertility ($P < 0.001$); and although there was no fertility difference between study groups for cows with calves born alive, within cows that had a calf DOA, fertility was greater for A_DA_G compared to all other study groups ($P = 0.02$). Fertility was less for cows in their 3+ lactation and cows that had twins, was positively associated with MILK1, and negatively associated with PRVME, PDOPN, and fat:protein concentration at first test.

Herd retention

Hazard of removal from the herd by either death or culling within in the first 60 DIM differed by study group, was greater for 3+ parity cows and those with mastitis at first test, increased with PDOPN and decreased with increased MILK1 ($P \leq 0.04$; Table 2.5). Results of the multinomial model indicated hazard of removal was 34 and 30% greater for A_DS_G and L_DA_G compared to A_DA_G ($P < 0.05$), but no significant difference was detected for S_DS_G despite being the group with the greatest hazard of removal in the Kaplan Meier curve (Figure 2.12A). Since MILK1 was least for S_DS_G, it is probable that the incongruence between the Kaplan Meier curve and the Cox model is due to a large proportion of the S_DS_G effect being explained by the MILK1 term in the Cox model.

When analyzed through 365 DIM, herd removal differed by study group, was greater for 3^{rd}+ lactation cows and those with mastitis at first test, was positively related to PDOPN, but negative related to MILK1 and LMILK ($P < 0.001$). Similar to the 60-d model, differences for study groups did not align between the multivariate analysis and the Kaplan Meier graph (Figure 2.12B). The multivariate model indicated 24% greater hazard of removal for L_DA_G compared to

A_DA_G while hazard was similar for all other study groups compared to A_DA_G. The 33% greater hazard of removal generated for S_DS_G in the univariate Cox models is likely explained by other variables included in the multivariate model.

DISCUSSION

Previous retrospective studies that investigated the associations between DPL and subsequent lactation productivity failed to consider potential reasons for the deviation in DPL (Makuza and Mcdanlelt, 1996; Pinedo et al., 2011; Santschi and Lefebvre, 2014). In those studies, cows with a shortened dry period were likely cows that calved early for various reasons (carrying twins, abortion), many of which are independently associated with poor performance (Bachman and Schairer, 2003; Overton, 2005). Further stratification of DPL by GL allowed us to begin to tease apart the effects of DPL coupled with involuntary GL deviations from those more directly associated with DPL.

Although target DPL was 60 d for all but 1 herd (dairy I = 55 d), there was considerable variation in DPL distribution across farms (Figure A.1A). For example, the DPL ranges for the herds with the tightest and widest DPL distribution were 34-83 d compared to 16-135 d. The ICC obtained from a univariate model for DPL indicated 14.4% of the variability in DPL occurred at the herd level. Similarly, GL distributions varied by herd (Figure A.1B), although only 2.6% of the variation was explained by herd. Our objective was to segregate DPL effects as caused by biological versus management reasons. Thus, DPL and GL categorization at the farm level was deemed most appropriate to meet our objective rather than categorizing across the distribution of the entire dataset or using predefined thresholds determined by literature.

Long dry period associations

Coupled with a long or average dry period, there was little evidence that extended GL hampered subsequent lactation productivity. The interaction of study group with PRVME for MILK1 suggested the most positive relationship for L_DL_G, suggesting that cows with greater lactation potential actually benefited from the longer DP and GL. Long GL was again associated with most positive relationship between PRVME and 305ME milk in the following lactation, but for long gestation cows with average DPL. Little effect of long GL with an average or long DP is consistent with the lack of effect of long GL on milk yield through 300 DIM reported by Vieira-Neto et al. (2017). The only relationship between long gestation and milk components was less milk protein yield for L_DL_G compared to A_DA_G and A_DL_G.

Negative associations with long DP were exhibited in L_DA_G cows and closely aligned with the adverse consequences of excessive body condition at calving. Extended dry periods increase the odds of BCS gain (Chebel et al., 2018) which in turn can lead to enhanced lipolysis and metabolic stress at calving (Weber et al., 2015). Mammary uptake of these mobilized fatty acids subsequently results in greater milk fat concentration and a greater milk fat to protein ratio (De Vries and Veerkamp, 2000). Both milk fat concentration and FP1 were greater for low-producing L_DA_G cows and decreased with greater milk yield more than any other group. Thus, despite being challenged with excessive lipid mobilization due to likely excessive body condition, L_DA_G cows with greater initial levels of production were able to transition more smoothly. The low production coupled with greater milk fat and milk FP1 indicates a subgroup of L_DA_G struggled. Additionally, both milk fat and FP1 had strong positive relationships with PDOPN, which provides more support for the focus on over-conditioning, as it is a greater risk for cows conceiving later in lactation (De Vries et al., 2010).

Despite potential challenges with lipid metabolism in early lactation, neither first test milk yield nor 305-d ME milk yield were different for L_DA_G cows compared to A_DA_G. The likely metabolic issues did, however, manifest in a 30 and 24% greater risk of death or culling by 60 and 365 DIM, respectively, compared to A_DA_G. Previous studies have associated greater early lactation fat:protein ratio with greater odds of postpartum disease (retained placenta, displaced abomasum, and metritis) and increased risk of culling, especially within the first 30 d of lactation (Toni et al., 2011). Heuer et al. (1999) reported that even though milk production was greater, cows with FP ratio > 1.5 had greater risk of disease and poorer reproductive performance (decreased first service conception risk, increased calving to conception interval, and increased services per conception). Despite indications of metabolic stress and the greater odds of removal from the herd, reproductive parameters were not different for L_DA_G cows. The greater early lactation cull rate likely removed cows that would've had poor reproductive performance, had they remained in the herd longer and been subjected to reproductive protocols.

Short dry period associations

In general, subsequent lactation performance was the worst for cows with both a short DPL and GL. Both early lactation and whole lactation milk and component yields were least for S_DS_G compared to other groups. Comparisons of S_DS_G with S_DA_G and A_DS_G suggests GL is likely a larger contributor to poor performance than short DPL. The A_DS_G study group was also associated with lesser MILK1, and milk fat and protein yield at first test were intermediate between S_DS_G and A_DA_G. Unlike S_DS_G, hampered performance did not completely extend throughout lactation among A_DS_G cows, as 305ME was similar to other study groups; however, differences in milk fat yield did continue, with whole lactation yield intermediate to S_DS_G and

A_DA_G. Kaplan Meier survival graphs illustrated greater removal from the herd within the first 60 d of lactation for both S_DS_G and A_DS_G relative to both A_DA_G and S_DA_G. After adjusting for parity group, mastitis at first test, milk at first test, LMILK, and PDOPN, hazard of removal was 34% greater for A_DS_G, but only 7% greater for S_DS_G (both relative to A_DA_G). As MILK1 was positively associated with survival and MILK1 was less for S_DS_G, a large proportion of variance that would've been explained by the S_DS_G study group was likely captured by the MILK1 variable. Days to first service was also greater for both S_DS_G and A_DS_G cows that had calving difficulty. The main difference between S_DA_G and A_DA_G was greater LSC1 for S_DA_G. Thus, our dataset suggests that although there are some negative effects of a short dry period, the parallels between S_DS_G and A_DS_G point to short GL as a greater contributor to poor performance, which can be further compounded by a short DP.

The performance parallels between S_DS_G and A_DS_G seem to support our hypothesis that dry periods at least in part caused by shorter GL result in poorer performance. As noted previously, the poor performance associations with short DPL in past retrospective studies were likely because of factors that stimulated early calving and thus resulted in the involuntary short DP (Bachman and Schairer, 2003; Overton, 2005). Known factors associated with reduced GL, carrying twins, female vs. male calves, heifers vs. cows (Norman et al., 2009), and dry period heat stress (Tao et al., 2012), are also associated with lower production and increased perinatal morbidity (Tao et al., 2012; Damaso et al., 2018). Even after controlling for GL, parity, season of calving, and sex of calves, Vieira-Neto et al. (2017) reported short gestation length (mean = 266, range=256-269) was associated with greater incidence of stillbirth, retained placenta, metritis, rate of removal from the herd (38% greater through 300 DIM), decreased milk yield, and a smaller proportion of cows receiving at least one artificial insemination. Therefore, short GL

seems to be the primary culprit for poor performance that had previously been attributed to short dry periods.

The lack of differences for lactation parameters, survival, and reproduction between S_DA_G and A_DA_G cows aligns with suggestions that a shorter dry period may be better for the modern high-producing dairy cow (Kuhn et al., 2005). Several controlled studies comparing short DPL (30-35 d) to traditional DPL (55-60 d) showed either no difference in subsequent milk yield (Bachman, 2002; Gulay et al., 2003; Rastani et al., 2005), or milk yield decreases that were restricted to 2nd lactation cows (Annen et al., 2004b; Watters et al., 2009; Pezeshki et al., 2010; Santschi et al., 2011).

At first glance, main effects for S_DA_G in our study support the idea that short dry periods may be appropriate for today's high yielding dairy cows; however, our observed interactions support the caution of Santschi and Lefebvre (2014) that the practice may not be suitable for all cows or herds. Milk yield at last test before dry-off was negatively associated with MILK1 for S_DA_G cows, and LSC1 was greater for S_DA_G across all levels of LLSC. Although milk yield has increased by 30-40% in the last 4 decades (Thornton, 2019), sudden dry-off is still the most common management practice on commercial dairy farms (Bertulat et al., 2015). Milk yield at last test before dry-off (restricted to within 40 d of dry-off) ranged from 0.9 to 78.5 kg (median = 28.1, IQR= 21.8-34.5 kg). Greater milk yield at dry-off is associated with greater prevalence of milk leakage and slower formation of the keratin plug, which leaves the teat canal open for bacterial entry, increasing susceptibility to intramammary infections (Rovai et al., 2007). The negative interaction between length of previous lactation (PDOPN) and MILK1 suggests that S_DA_G cows would benefit from earlier dry-off. While it might be common practice to extend lactation for high producing cow at the expense of DPL, our evidence suggests this practice

actually hinders performance. Thus, our data supports Dingwell and others (2002) in that cows with high levels of production at dry-off should not be subjected to a short dry period.

Cows with the greatest genetic potential seemed to be most sensitive to short GL and DPL. The positive relationship between PRVME and MILK1 was weakest for S_DS_G cows, and intermediate for S_DA_G and A_DS_G compared to cows with combinations of average or long DPL and GL. The study group interaction for first test protein yield and PRVME indicated lower milk protein yield for the groups with worst early lactation survival (S_DS_G, A_DS_G, and L_DA_G) as PRVME increased. Predicted 305-d ME milk yield and whole lactation fat yield increased with increasing PRVME, but to the lowest degree for S_DS_G and A_DS_G. Thus, cows with the greatest milking potential, based on PRVME, were most negatively impacted by short gestation.

Our data do support previous studies that suggest shortened dry periods (approximately 40 d) may be an option for today's high-producing dairy cows; however, cow production potential, calving interval, and milk yield at dry-off should all be considered when making such a decision. When making decisions related to DPL, it should be realized that natural variation in actual versus expected calving date and the normal distribution of GL present within every farm will inevitably result in cows having shorter or longer DPL than managed for (Overton, 2005).

Has gestation length shortened?

A 280-d gestation has been the hallmark for Holstein dairy cows and is used in most herd management software, unless manually adjusted, to determine expected calving date. Such is supported by Norman and others (2009) with their dataset of 11 million parturitions from 1999-2006 that generated GL means of 277.8 and 279.4 d for Holstein heifers and cows, respectively. More recently, Vieira-Neto et al. (2017) reported a GL mean and SD of 276 ± 6 d from their

dataset of primiparous and multiparous cows from 2 California herds. In our data, GL mean and SD of 277 ± 5 d was slightly greater, as it only included multiparous cows, but supports their findings and together suggests that GL for Holstein cows may be decreasing. It is important to note that our dataset and that of Vieira-Neto et al. (2017) has been cleaned and excludes extreme GLs (shorter or longer than 3 SD from the mean) that may otherwise inflate or deflate a raw herd average. In further support, the 5 million Holstein bulls (born since 1995) used to develop the PTA for GL reported a base GL of 277 d and SD of 1.4 d (Wright and VanRaden, 2017).

Study limitations

Farms included in this study represent a convenience sample of dairy farms in the Western and Midwestern United States. Thus, there is some selection bias present as the farms do not represent a random sample of farms from our target population (large commercial dairy farms in the United States). Inclusion of more herds, especially herds from the Eastern U.S. would have increased our external validity while also allowing for investigation of regional and herd level effects.

To account for seasonality, month of calving was included in all our models. We considered further categorization by season but felt it may be misleading for our dataset given the differences in climates across herds. Although differences in weather characteristics are also present when comparing months across region, we felt its inclusion would best account for seasonality while also decreasing risk for misinterpretation. If our dataset allowed, a variable categorized by temperature and humidity index or the ability to account for region would have strengthened our interpretative ability of seasonality in our models.

Many explanatory variables were included in the models to account for potential biases across study groups; however, we have identified additional variables that should be accounted for in the future. Stocking density of dry pens could largely influence both management decisions regarding timing of dry-off and biological stress on the animal that could contribute to early calving. A metric related to stocking density would be helpful to account for such variation across study groups as well as to account for its direct effect on cow performance in the subsequent lactation. The bias of milk production potential across groups is somewhat accounted for by the inclusion of PRVME in models; however, reproductive protocols (i.e. use of sexed semen) also vary by production potential but were not accounted for in our study. A variable for sexed vs. conventional semen could be implemented when evaluating reproductive efficiency across groups, so that the decreased reproductive efficiency associated with sexed semen is not unintentionally attributed to the study group. Inclusion of these variables would further strengthen future models.

CONCLUSION

Retrospective observational studies investigating effects of DPL need to account for causes underlying the deviations in DPL. In stratifying DPL by GL, we determined short GL to be the main factor associated with poor performance in the subsequent lactation. Short dry period did appear to compound the effects of short GL, as lactation performance and herd retention rate were least for S_DS_G cows compared to any other study group. Overall productivity was similar between S_DA_G and A_DA_G, which supports utilization of shorter dry periods (~40 d); however, based on interactions with PRVME and LMILK, short dry period should not be applied to cows with high production potential or high milk at dry-off. Long gestation length, independent of

DPL, had little effect on subsequent lactation performance. Greater milk fat concentration and FP1 in cows subjected to a long dry period due to management decisions (L_DA_G) indicated issues with early lactation lipid metabolism that ultimately resulted in decreased herd retention. In conclusion, deviations in DPL caused by biology have greater impacts as it relates to short DPL, while management reasons for DPL deviation have the greatest effect when causing long dry periods.

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TABLES AND FIGURES

Table 2.1. Mean and standard deviations for dry period and gestation length used to calculate study group by each dairy.

		Dry period length		Gestation length	
Dairy	**n**	**mean**	**sd**	**mean**	**sd**
A	1550	59.2	12.7	276.8	5.1
B	713	54.2	7.5	274.3	5.2
C	1763	59.5	13.7	277.2	5.3
D	1422	50.2	11.9	275.9	4.9
E	590	61.8	7.1	276.1	5.0
F	1313	48.0	11.7	276.2	5.4
H	780	52.4	8.1	276.2	5.4
I	1602	56.3	12.3	276.8	5.3
J	766	58.9	15.7	278.0	5.6
K	6345	58.1	7.4	276.9	5.1
M	2854	56.0	10.6	276.5	5.9
N	1566	63.3	5.6	277.3	4.9
O	3848	60.2	15.1	277.1	5.5
P	2875	63.1	10.7	276.5	5.5
Q	2263	56.4	11.6	276.7	4.8
R	2408	56.0	5.8	276.7	5.1
Overall	32,182	57.6	11.1	276.9	5.1

Table 2.2. Mean values for lactation performance parameters at first test (between 7-35 DIM) for cows by study group.

	Study group[1]										
First test parameters	**S_DS_G**	**S_DA_G**	**A_DS_G**	**A_DA_G**	**A_DL_G**	**L_DA_G**	**L_DL_G**	**SEM[2]**	***P*-value[3]**	**Interactions[4]**	**ICC[5]**
Milk yield, kg	38.72	41.58	39.78	42.37	42.91	42.63	43.11	1.00	0.16	prvme, lmilk, pdopn	0.141
Milk fat concentration, %	4.04	4.10	4.08	4.05	4.07	4.08	4.04	0.10	0.002	milk1, pdopn	0.210
Milk fat yield, kg	1.61^{c}	1.72^{ab}	1.69^{b}	1.75^{a}	1.77^{a}	1.74^{ab}	1.75^{ab}	0.06	< 0.001	-	0.171
Milk protein concentration, %	3.17^{a}	3.14^{ab}	3.12^{bc}	3.10^{c}	3.09^{c}	3.05^{d}	3.03^{d}	0.02	< 0.001	-	0.069
Milk protein yield, kg	1.27	1.31	1.29	1.33	1.34	1.34	1.31	0.03	0.65	Ptapy, ptam, prvme, lmilk, pdopn	0.088
LSC[6]	2.37	2.59	2.39	2.38	2.47	2.41	2.41	0.18	0.08	llsc	0.098
Fat:protein concentration	1.29	1.32	1.32	1.32	1.33	1.34	1.34	0.03	0.54	milk1, prvme, pdopn	0.175

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

[2]SEM = standard error of the mean

[3]*P*-value for the main effect of study group

[4]Interactions with study group: llsc = linear somatic cell count at last test before dry-off; lmilk = milk yield at last test before dry-off; milk1 = milk yield at first test; pdopn =previous lactation days open; prvme= previous lactation 305-d mature equivalent milk; ptam = predicted transmitting ability for milk yield; ptapy = predicted transmitting ability for protein yield

[5]ICC=inter-cluster correlation coefficient represents the amount of variation attributed to dairy (cluster variable).

[6]LSC= linear somatic cell score

Table 2.3. Mean values for whole lactation production parameters by study group.

	Study group[1]										
Whole lactation parameters	**S_DS_G**	**S_DA_G**	**A_DS_G**	**A_DA_G**	**A_DL_G**	**L_DA_G**	**L_DL_G**	**SE**	***P*-value[2]**	**Interactions[3]**	**ICC[4]**
305-ME milk yield[5], kg	12024[b]	12526[a]	12408[a]	12537[a]	12505[a]	12296[a]	12542[a]	255.1	< 0.001	freshmo, csex, prvme, pdopn	0.225
Milk fat concentration[6], %	3.68	3.69	3.66	3.67	3.70	3.65	3.67	0.07	0.005	paritygr, prvme	0.249
Milk fat yield[7], kg	436.2[b]	451.0[a]	446.2[ab]	452.5[a]	455.4[a]	448.0[a]	453.8[a]	14.5	0.03	prvme, llsc	0.379
Milk protein concentration[6], %	3.16	3.13	3.11	3.11	3.10	3.08	3.07	0.03	< 0.001	-	0.241
Milk protein yield[7], kg	371.9[c]	378.2[ab]	374.5[abc]	378.6[a]	379.7[a]	371.9[c]	372.6[bc]	7.9	< 0.001	-	0.318
LSC[8]	2.43	2.29	2.41	2.36	2.39	2.43	2.33	0.17	0.17	freshmo, mast1, llsc	0.158

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

[2]SEM = standard error of the mean

[3]Interactions with study group: csex = calf description (female, male, twin); freshmo = fresh month; llsc = linear somatic cell count at last test before dry-off; mast1 = mastitis at first test; paritygr = parity group; pdopn =previous lactation days open; prvme= previous lactation 305-d mature equivalent milk

[4]ICC=inter-cluster correlation coefficient represents the amount of variation attributed to dairy (cluster variable).

[5]305-d mature equivalent milk yield estimated at the third test.

[6]Average of variable from tests throughout lactation, excluding first test measures

[7]Calculated as average concentration from all tests throughout lactation except first test x 305-d mature equivalent milk yield estimated at 3rd test.

[8]LSC= linear somatic cell score

Table 2.4. Cox proportional hazards for days to first service and days open (through 365 DIM).

	Days to first service					Days open				
Variable[1]	**Hazard ratio**	**SE**	**95% CI**	**Median**	***P*-value**	**Hazard ratio**	**SE**	**95% CI**	**Median**	***P*-value**
Study group[2]					0.15					0.04
S_DS_G	1.08	0.04	1.00, 1.15	66	0.04	0.991	0.038	0.92, 1.07	132	0.81
S_DA_G	1.02	0.04	0.95, 1.10	68	0.54	1.020	0.041	0.94, 1.10	126	0.62
A_DS_G	1.06	0.04	0.98, 1.13	66	0.12	1.032	0.040	0.96, 1.11	133	0.41
A_DA_G	Ref	Ref	Ref	67	Ref	Ref	Ref	Ref	121	Ref
A_DL_G	0.98	0.03	0.94, 1.04	67	0.55	0.913	0.026	0.84, 0.97	132	0.001
L_DA_G	0.96	0.03	0.91, 1.02	66	0.22	1.005	0.034	0.94, 1.07	134	0.87
L_DL_G	1.02	0.04	0.95, 1.10	67	0.54	0.956	0.037	0.89, 1.03	135	0.25
Parity group					< 0.001					< 0.001
2	Ref	Ref	Ref	69		Ref	Ref	Ref	116	
3+	0.95	0.01	0.92, 0.97	69		0.885	0.015	0.86, 0.92	127	
Calf description					< 0.001					
Female	Ref	Ref	Ref	69	Ref	Ref	Ref	Ref	117	Ref
Male	0.97	0.01	0.94, 1.00	69	0.04	0.984	0.017	0.95, 1.02	122	0.36
Twin	0.88	0.03	0.83, 0.95	70	< 0.001	0.703	0.030	0.65, 0.77	152	< 0001
Calf issue[3]					0.07					
No	Ref	Ref		70		Ref	Ref	Ref	120	Ref
Yes	1.04	0.03	1.00, 1.10	66		0.623	0.052	0.53, 0.73	156	0.623
Study group[2] × calf issue					0.01					0.02
S_DS_G	0.87	0.06	0.76, 1.00		0.04	1.119	0.220	0.76, 1.64		0.57
S_DA_G	1.10	0.10	0.93, 1.31		0.26	1.357	0.321	0.85, 2.16		0.20
A_DS_G	0.80	0.06	0.69, 0.92		0.002	1.222	0.335	0.71, 2.09		0.46
A_DA_G	Ref	Ref	Ref		-	Ref	Ref	Ref		Ref
A_DL_G	0.94	0.05	0.85, 1.04		0.22	1.814	0.308	1.30, 2.53		< 0.001
L_DA_G	0.98	0.06	0.87, 1.10		0.71	1.507	0.380	0.92, 2.47		0.10
L_DL_G	1.03	0.08	0.89, 1.20		0.66	1.396	0.330	0.88, 2.22		0.16
Fresh month					< 0.001					< 0.001
Jan	Ref	Ref	Ref	69	Ref	Ref	Ref	Ref	120	Ref

Feb	0.97	0.03	0.91, 1.03	69	0.33	0.953	0.040	0.88, 1.03	117	0.25
Mar	1.04	004	0.97, 1.12	68	0.25	1.038	0.045	0.95, 1.13	120	0.38
Apr	0.99	0.04	0.92, 1.06	69	0.79	0.999	0.046	0.91, 1.09	135	0.98
May	0.96	0.03	0.90, 1.03	69	0.24	0.961	0.042	0.88, 1.05	133	0.37
June	1.05	0.04	0.98, 1.12	68	0.20	0.969	0.041	0.89, 1.05	132	0.46
July	1.08	0.04	1.01, 1.15	68	0.03	1.091	0.047	1.00, 1.19	118	0.04
Aug	1.10	0.04	1.04, 1.18	68	0.001	1.135	0.044	1.05, 1.23	120	0.001
Sept	0.95	0.03	0.90, 1.01	69	0.12	1.129	0.042	1.05, 1.22	118	0.001
Oct	0.89	0.03	0.84, 0.94	69	< 0.001	1.121	0.041	1.04, 1.20	116	0.002
Nov	0.85	0.03	0.81, 0.91	69	< 0.001	1.074	0.040	1.00, 1.16	118	0.06
Dec	0.80	0.03	0.75, 0.85	70	< 0.001	1.065	0.043	0.98, 1.15	121	0.12
Mastitis at first test					< 0.001					
No	Ref	Ref	Ref	68						
Yes	0.91	0.02	0.88, 0.95	70						
Milk at first test, kg						1.004	0.001	1.00, 1.01		< 0.001
Prvme, kg						1.000	0.000	1.00, 1.00		< 0.001
Pdopn, per 100 d	99.94	0.01	99.92, 99.96		< 0.001	99.76	0.014	99.73, 99.79		< 0.001
FP1						0.94	0.03	0.89, 0.997		0.04
Theta[4]	0.46	0.17			< 0.001	0.02	0.03			< 0.001

[1]Prvme = previous lactation 305-d mature equivalent milk production; pdopn = previous lactation days open, FP1 = fat:protein concentration at first test.

[2]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G = average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

[3]Calf issue for days to first service was calving difficulty whereas the calf issue for the days open model was calf dead on arrival.

[4]Estimate used to measure the degree of within group correlation. Significant likelihood ratio test of frailty variance indicates correct inclusion of herd as a random effect.

Table 2.5. Proportional hazards of being sold or dying within the first 60 DIM or through 365 DIM

	60 DIM				365 DIM			
Variable[2]	Hazard ratio	SE	95% CI[1]	*P*-value	Hazard ratio	SE	95% CI[1]	*P*-value
Study group[3]				0.04				< 0.001
S_DS_G	1.07	0.13	0.84, 1.36	0.57	0.97	0.05	0.87, 1.07	0.50
S_DA_G	0.83	0.14	0.60, 1.15	0.26	0.92	0.05	0.82, 1.03	0.15
A_DS_G	1.34	0.17	1.04, 1.71	0.02	0.99	0.06	0.89, 1.10	0.83
A_DA_G	Ref	Ref	Ref	-	Ref	Ref	Ref	-
A_DL_G	1.13	0.12	0.92, 1.39	0.26	1.07	0.04	0.99, 1.15	0.10
L_DA_G	1.30	0.14	1.06, 1.60	0.03	1.24	0.05	1.14, 1.35	< 0.001
L_DL_G	1.03	0.16	0.77, 1.39	0.84	1.01	0.06	0.90, 1.13	0.93
Parity group				< 0.001				< 0.001
2	Ref	Ref	Ref		Ref	Ref	Ref	
3+	2.29	0.17	1.99, 2.64		1.73	0.05	1.64, 1.82	
Mastitis at first test				< 0.001				< 0.001
No	Ref	Ref	Ref		Ref	Ref	Ref	
Yes	2.22	0.17	1.91, 2.56		1.38	0.04	1.30, 1.46	
MILK1, kg	0.94	0.002	0.93, 0.94	< 0.001	0.98	0.001	0.97, 0.98	< 0.001
LMILK, kg	-	-	-	-	0.99	0.001	0.99, 0.996	< 0.001
Pdopn, per 100 d	10.06	0.01	10.03, 10.09	< 0.001	10.03	0.005	10.02, 10.04	< 0.001
Pdopn sq, per 100 d	99.999	0.0003	99.9984, 99.9997	0.005	9.9995	1.14E-04	99.9993, 99.9998	< 0.001
Theta[4]	1.31	0.41		< 0.001	1.34	0.42		< 0.001

[1]95% confidence interval

[2]Milk1 = milk yield at first test; lmilk = milk yield at last test before dry-off; pdopn = previous lactation days open.

[3]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G = average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

[4]Estimate used to measure the degree of within group correlation. Significant likelihood ratio test of frailty variance indicates correct inclusion of herd as a random effect.

Figure 2.1. Box and whiskers plots of A) dry period length (DP) and B) gestation length (GL) for each study group.

The horizontal red line indicates the dataset mean (DP = 57.6, GL = 276.9). For the box plots, line within the box indicates the median; top and bottom of the box indicate 75th and 25th percentiles, respectively; whiskers above and below the box indicates the upper adjacent value (quartile 3+1.5[interquartile range]) and lower adjacent value (quartile 1 – 1.5([interquartile range)]), respectively; and dots outside the whiskers represent outliers. Study groups were categorized as S_DS_G = short DP, short GL; S_DA_G = short DP, average GL; A_DS_G = average DP, short GL; A_DA_G = average DP, average GL; A_DL_G = average DP, long GL; L_DA_G = long DP, average GL; L_DL_G = long DP, long GL.

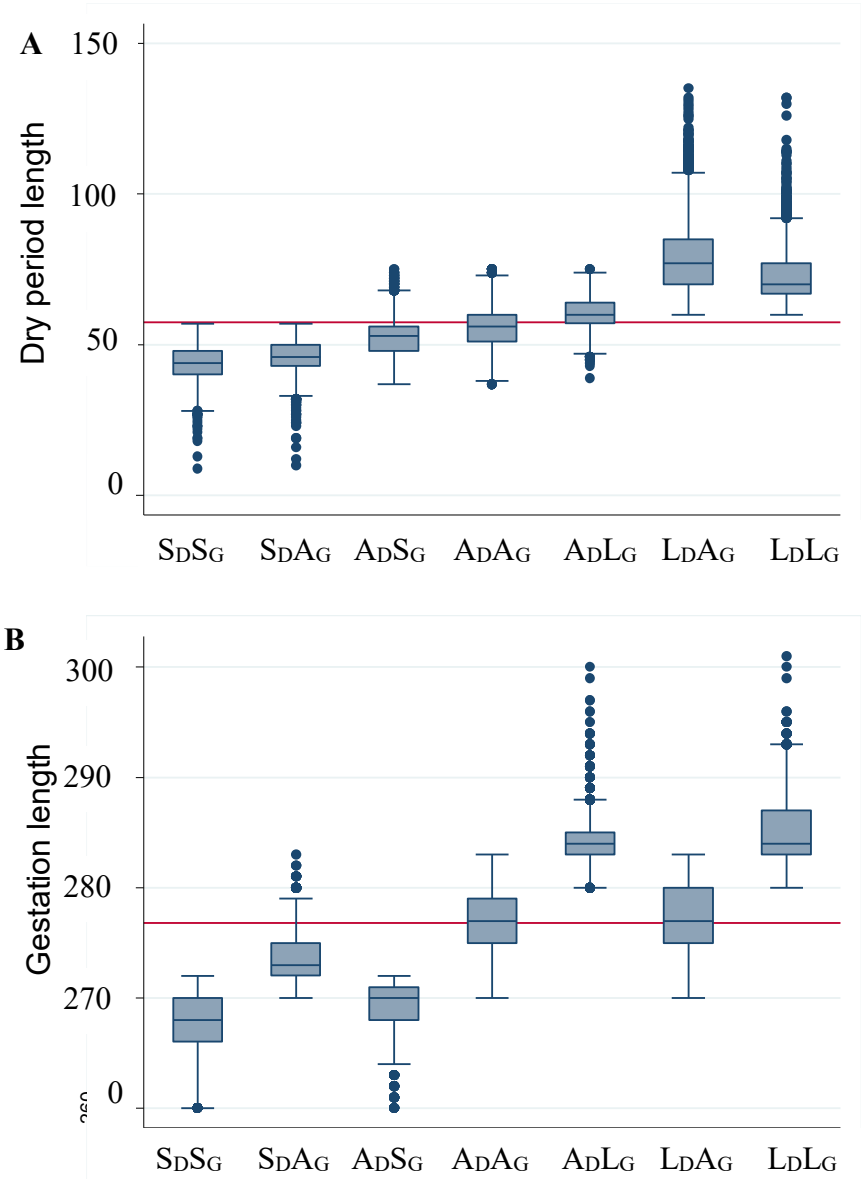


Figure 2.2. Multinomial logistic regression was performed for each of the 16 dairies to determine significant predictors of study group.

Predictor variables (PRVME = previous lactation 305-d mature equivalent milk yield, PDOPN = previous lactation days open, GL = gestation length, LMILK = milk yield at last test before dry-off) are expressed as a proportion of the herds for which they significantly predicted study group. Study groups were categorized as follows: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length).

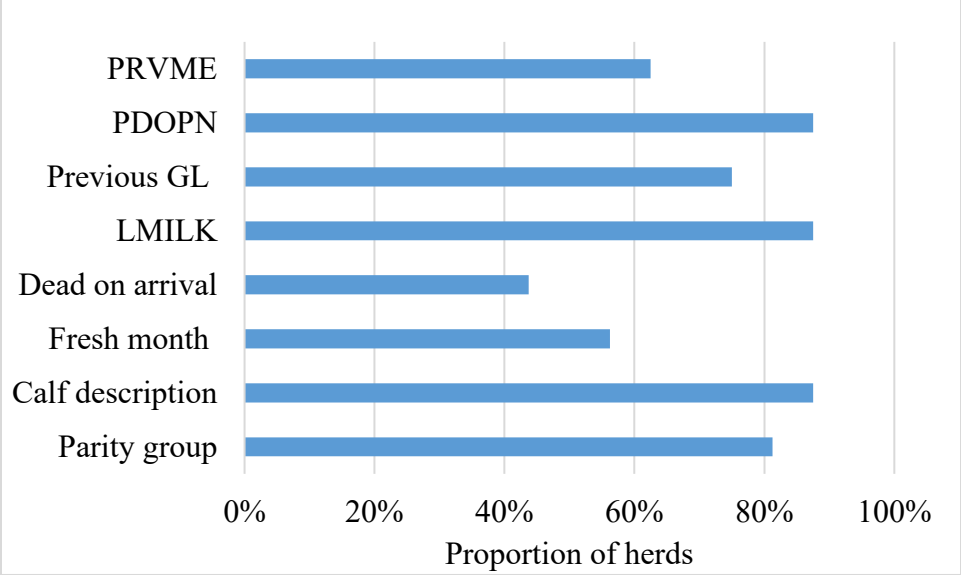
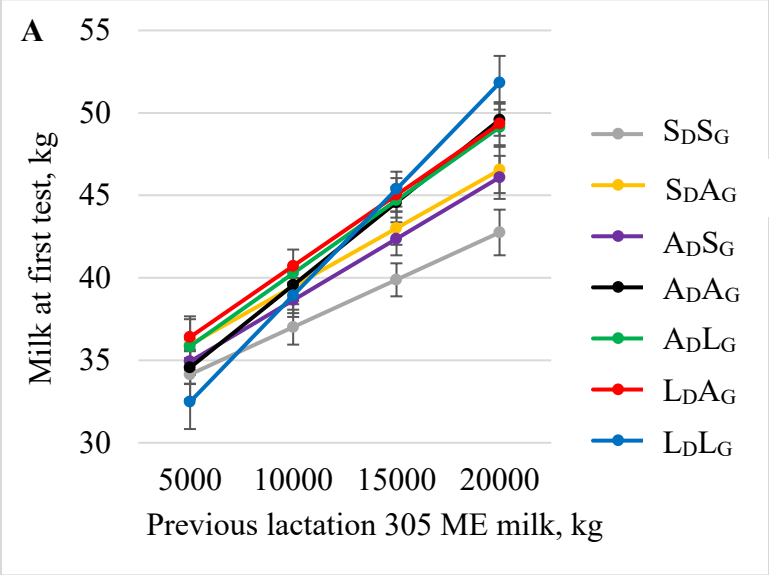
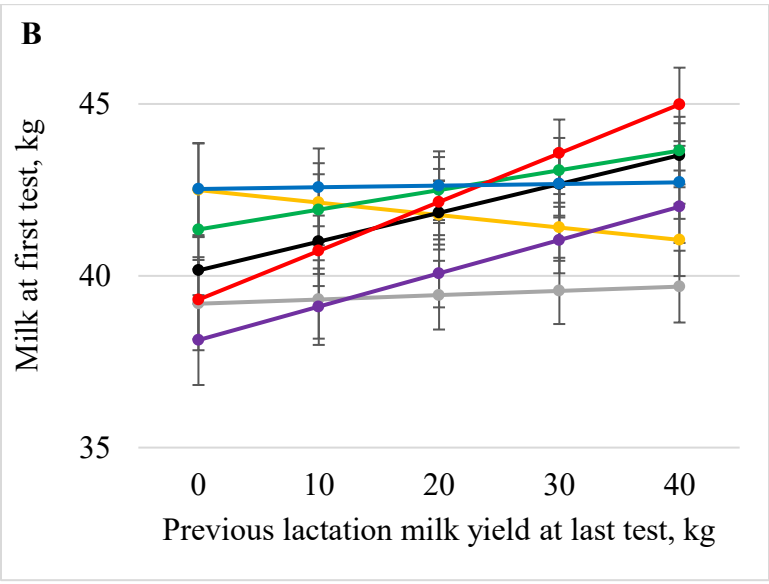


Figure 2.3. The association between milk yield at first test and study group was dependent on A) previous lactation 305-ME milk yield, B) milk yield at last test before dry-off, and C) previous lactation days open (all $P < 0.001$).

Study group was categorized as follows: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length.





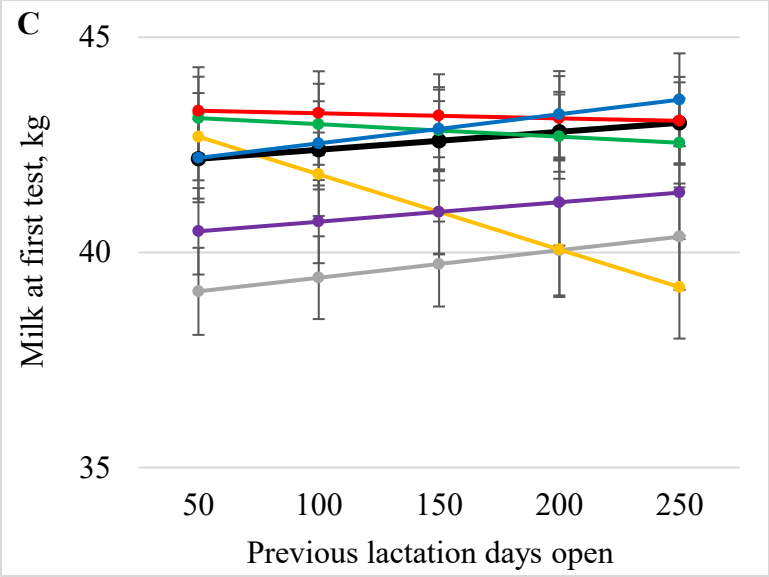

C
45
40
35
Milk at first test, kg
50
100
150
200
250
Previous lactation days open

Figure 2.4. Association between study group and milk fat concentration at first test depended on A) milk yield at first test and B) previous lactation days open.

Study group was categorized as follows: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length)

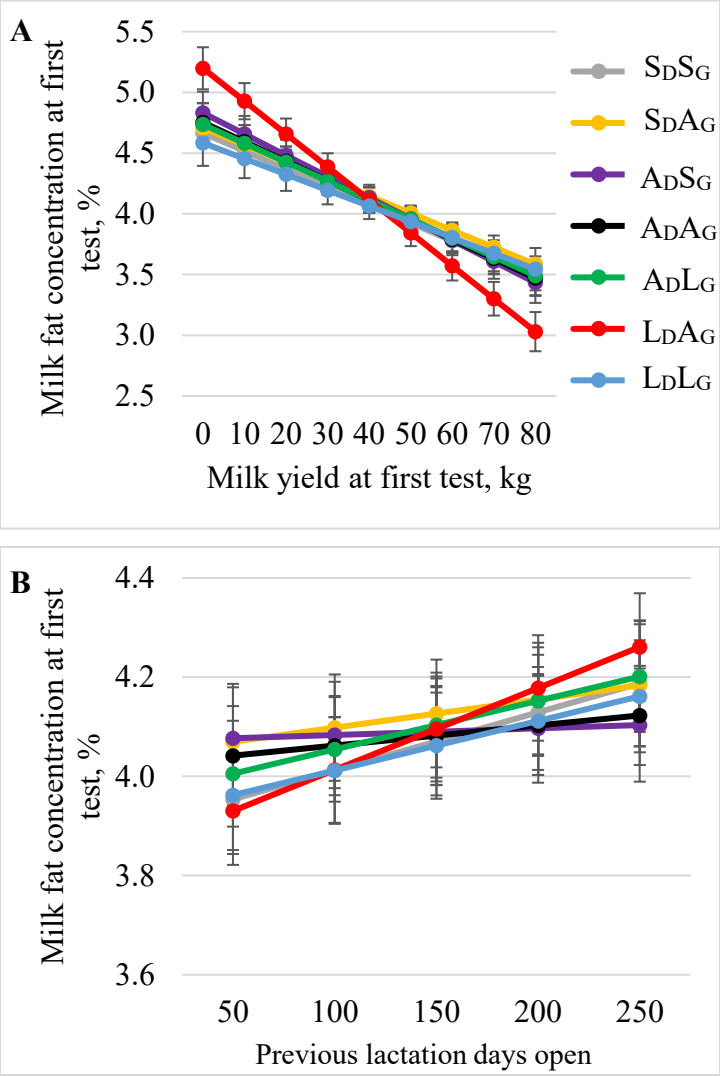
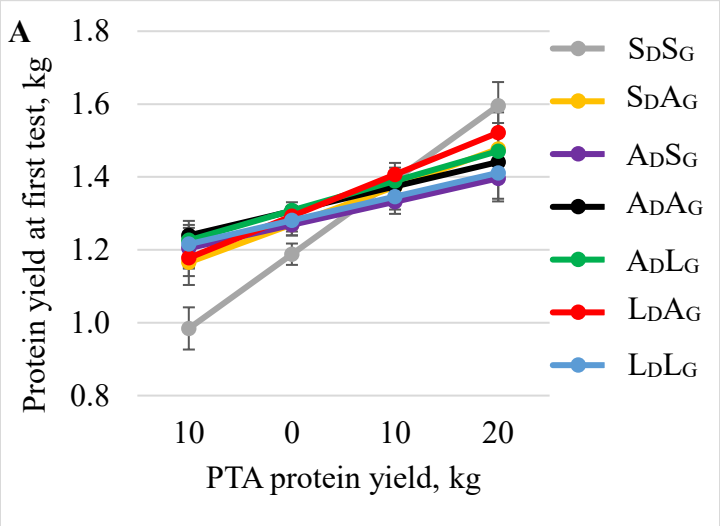
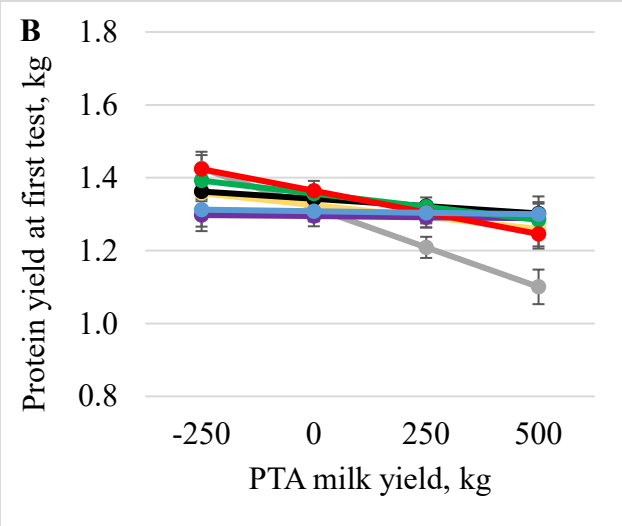
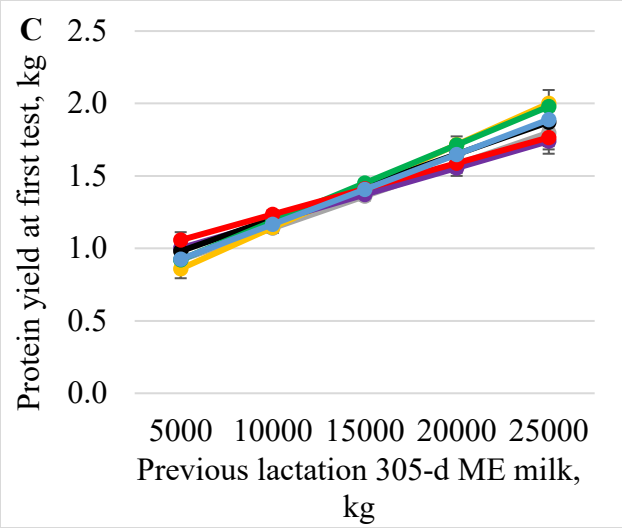


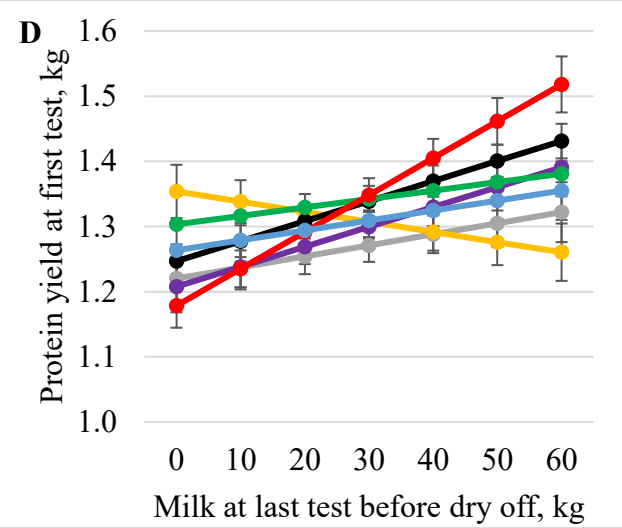
Figure 2.5. Associations between study group and milk protein yield at first test A) PTA protein yield (*P* =0.03), B) PTA milk yield (*P* = 0.02), C) previous lactation 305-d ME milk yield (*P* < 0.001), D) milk at last test before dry-off (*P* < 0.001), E) previous lactation days open (*P* = 0.02).

Study group was categorized as follows: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length.






C
Protein yield at first test, kg
2.5
2.0
1.5
1.0
0.5
0.0
5000
10000
15000
20000
25000
Previous lactation 305-d ME milk, kg


D
Protein yield at first test, kg
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0
10
20
30
40
50
60
Milk at last test before dry off, kg

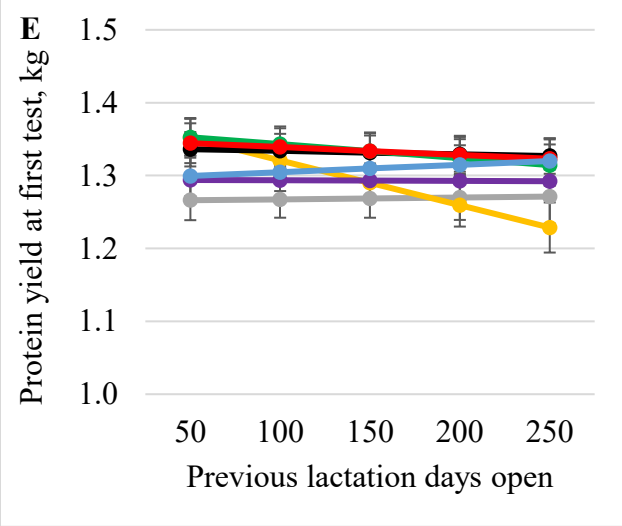

E
Protein yield at first test, kg
1.5
1.4
1.3
1.2
1.1
1.0
50
100
150
200
250
Previous lactation days open

Figure 2.6. Association between linear somatic cell score (LSC) at first test and study group depended on LSC at last test before dry-off.

Study group was categorized as follows: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length)

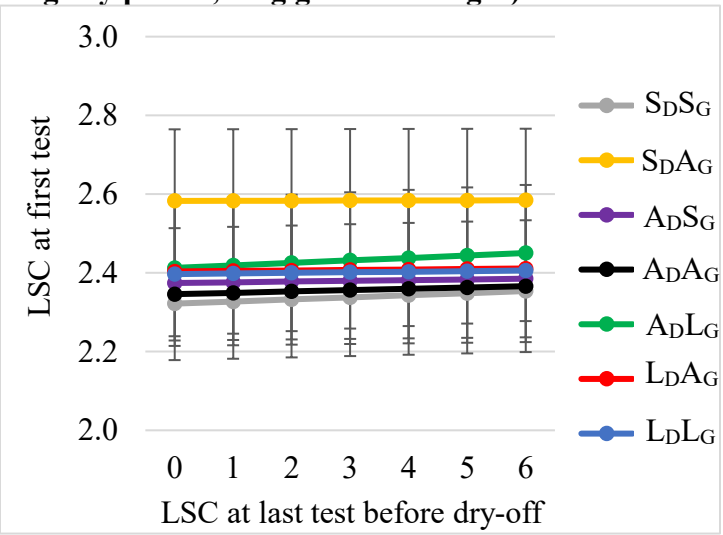
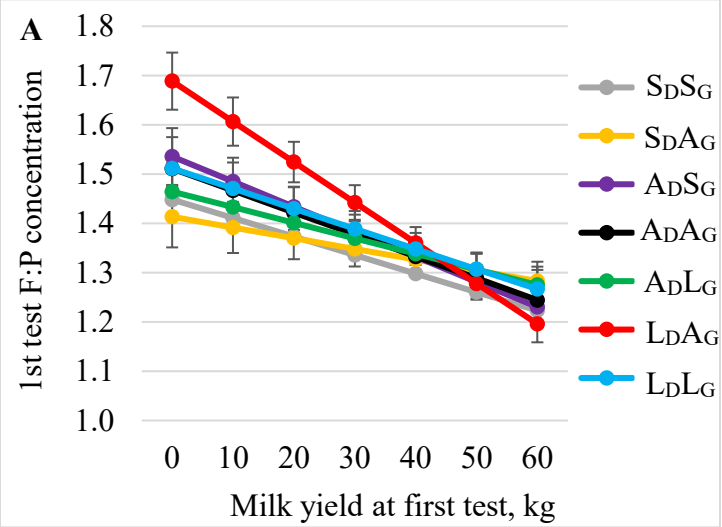
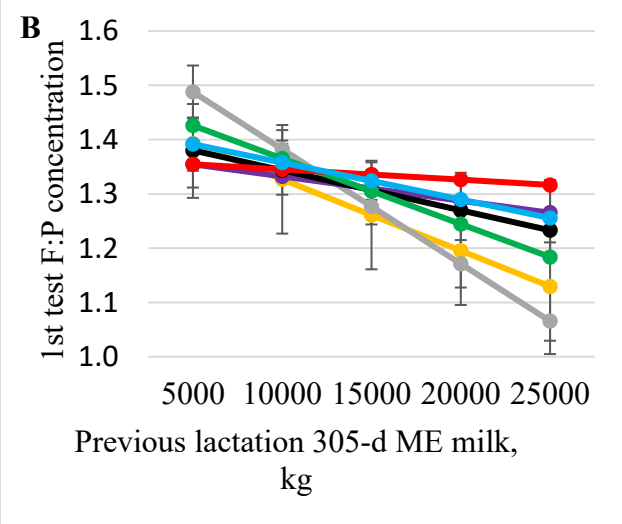


Figure 2.7. Association between study group and fat:protein concentration at first test of lactation. A) milk yield at first test, B) previous lactation days open, C) previous lactation 305-d mature equivalent milk yield.

Study group was categorized as follows: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length.





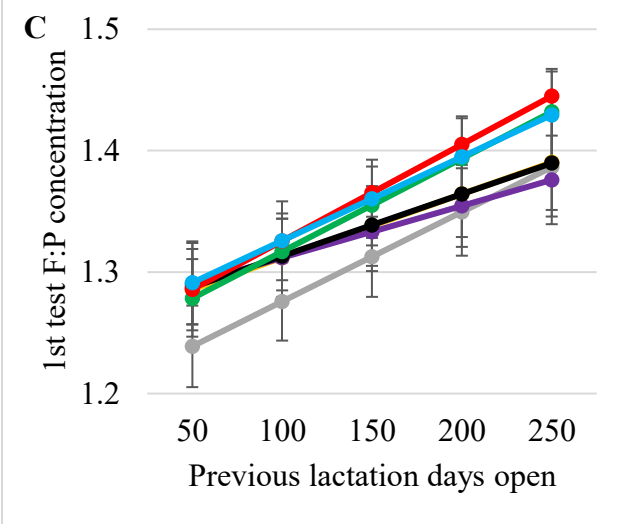


Figure 2.8. Association between study group and 305-d mature equivalent milk yield predicted at third test of lactation interacted with A) previous lactation 305-d mature equivalent milk production and B) previous lactation days open.

Study group was categorized as follows: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length.

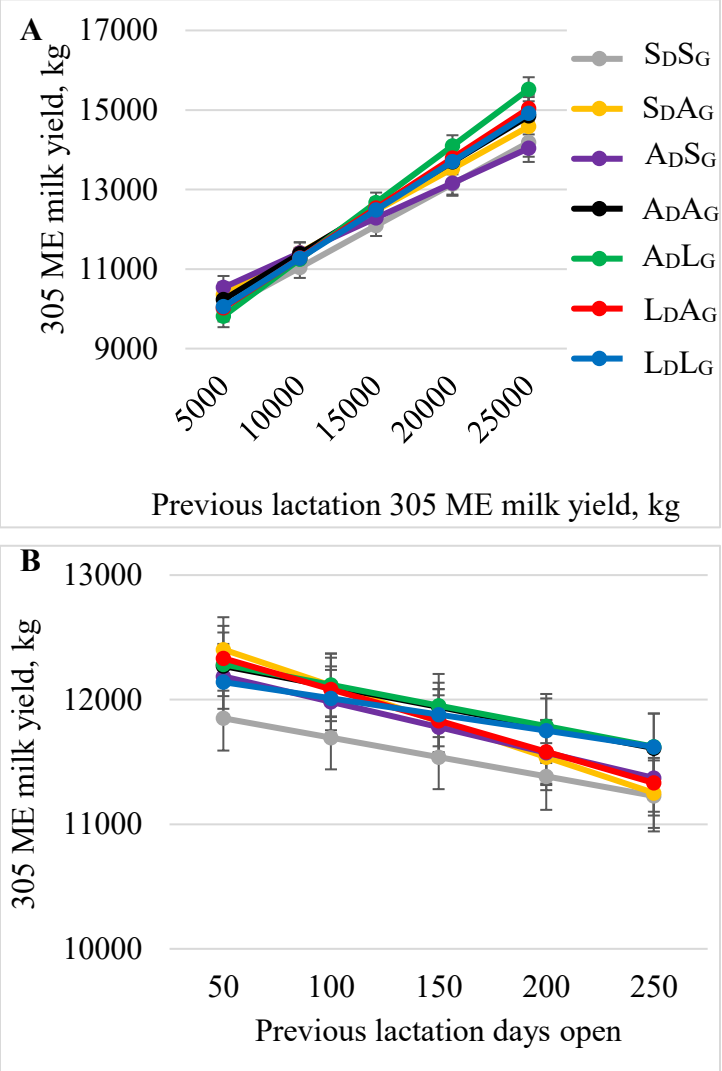


Figure 2.9. Association between study group and average milk fat concentration throughout lactation.

Study group categorized as follows: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length.

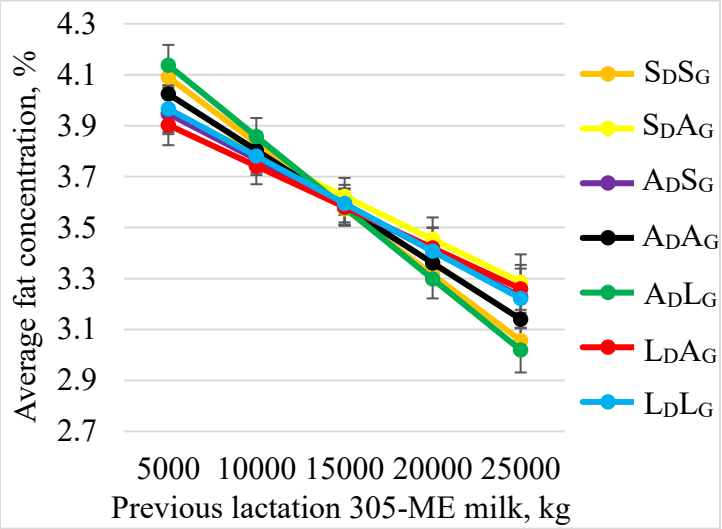
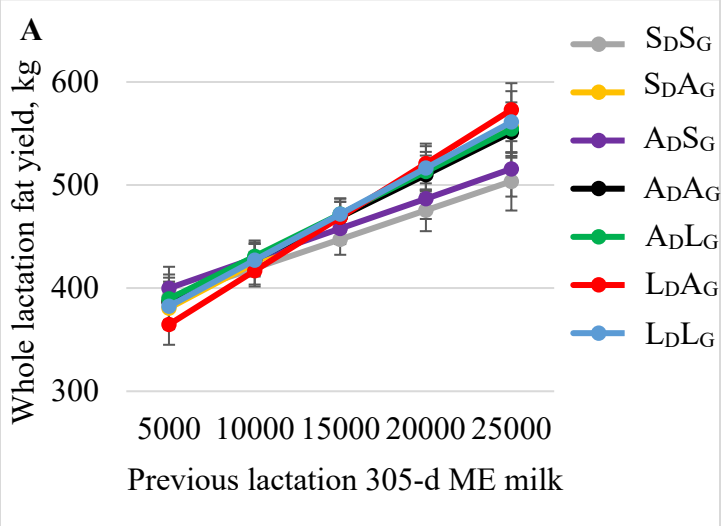


Figure 2.10. Association between study group and whole lactation milk fat yield interacted with A) previous lactation 305-d mature equivalent milk production and B) LSC at last test before dry-off (LLSC).

Study group categorized as follows: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length.



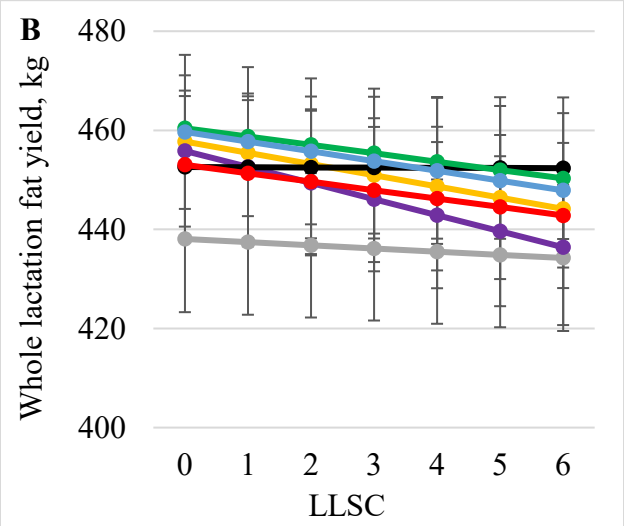


Figure 2.11. Association between study group and average linear somatic cell score (LSC) depended on linear somatic cell score at last test before dry-off (LLSC).

Study groups were categorized as follows: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length.

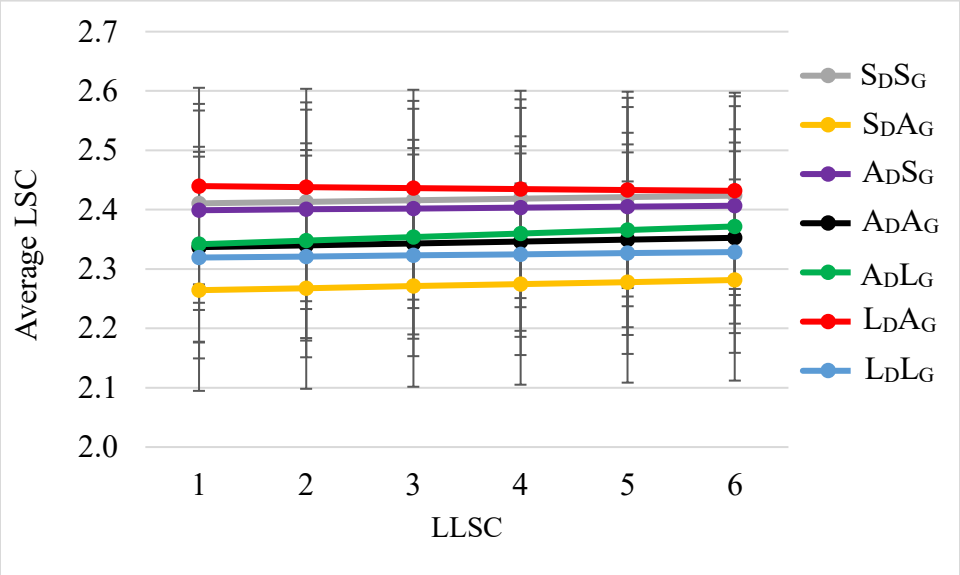
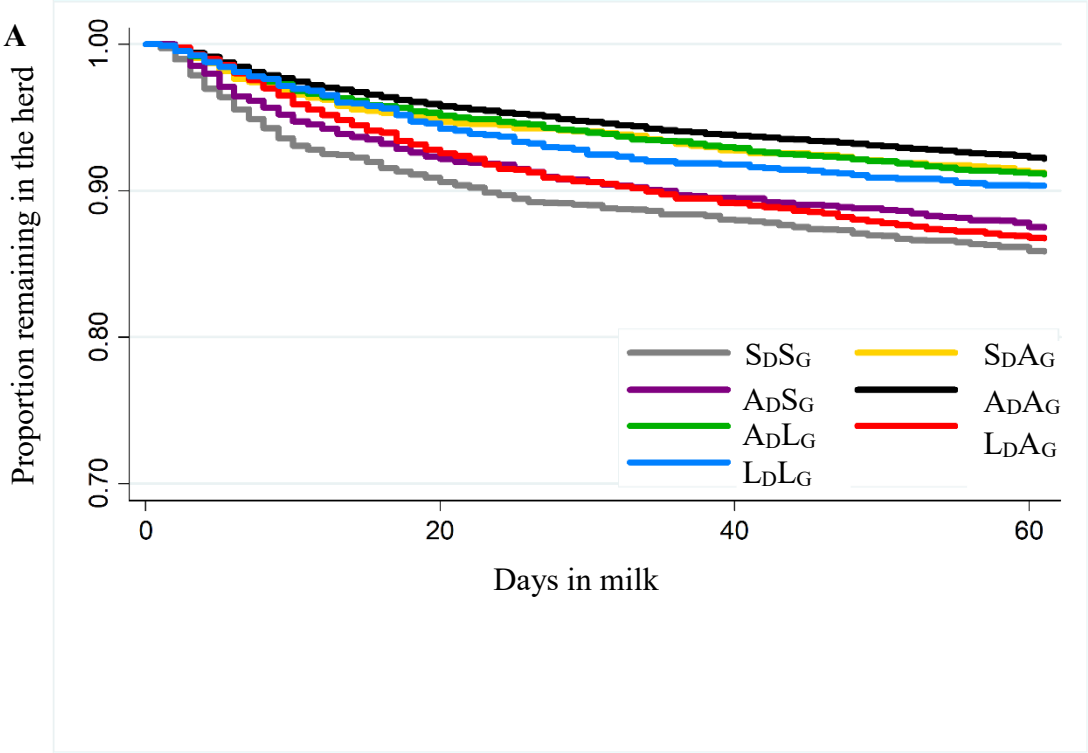
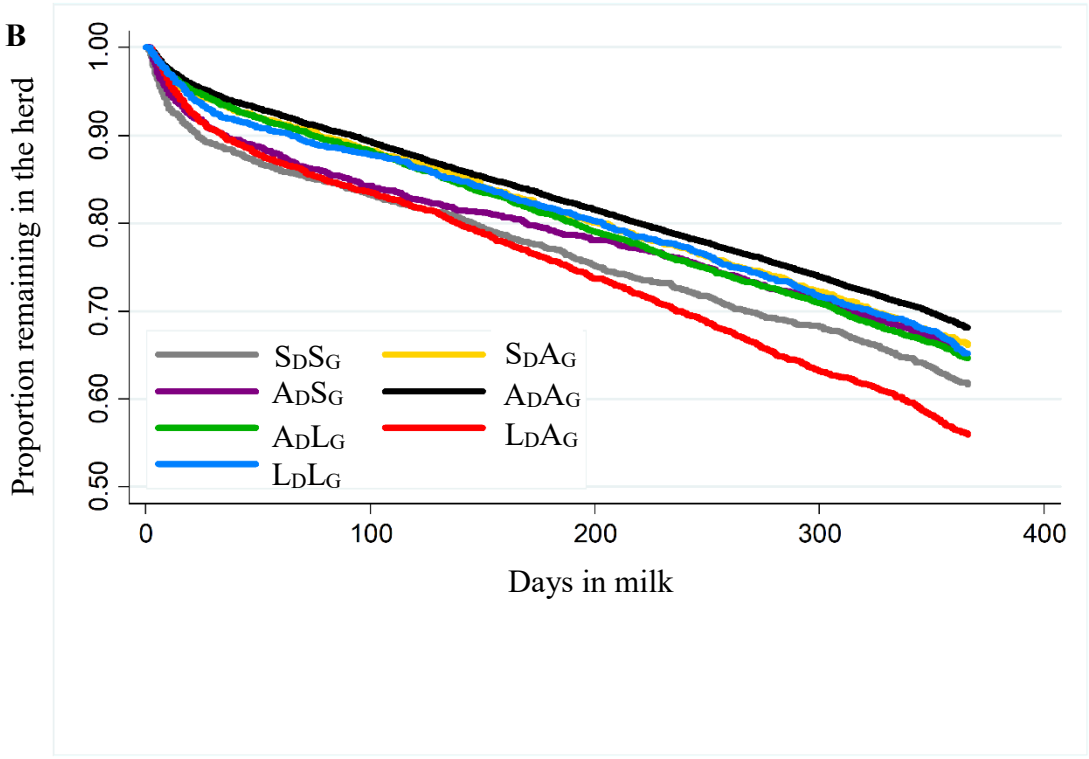


Figure 2.12. Kaplan Meier survival curves for time to leave the herd by either death or culling to A) 60 DIM or B) 365 DIM.

Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length.




B
Proportion remaining in the herd
1.00
0.90
0.80
0.70
0.60
0.50
0
100
200
300
400
Days in milk
SDSG
ADSG
ADLG
LDLG
SDAG
ADAG
LDAG

Chapter 3 - **Impact of *Saccharomyces cerevisiae* fermentation product on feed intake parameters, lactation performance, and metabolism of transition dairy cattle**

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ABSTRACT

The transition period in dairy cattle is characterized by many stressors including an abrupt diet change, but yeast product supplementation can alter the rumen environment to increase dairy cattle productivity. *Saccharomyces cerevisiae* fermentation product (**SCFP**) was fed from -29 ± 5 to 42 d relative to calving (**RTC**) to evaluate the effects on feed intake, milk production, and metabolism. Treatments were control (**CON**, *n* = 30) or SCFP (*n* = 34) incorporated into a total mixed ration. Cows were individually fed 3×/d prepartum and 2×/d postpartum. Blood samples were collected once during each of the following time points RTC: d -28 to -24 (wk -4), d -14 to -10 (wk -2), d 3 to 7 (wk 1), d 12 to 16 (wk 2), and d 31 to 35 (wk 5). Liver biopsies were taken once between d -19 to d -12 (wk -3) and at 14 d in milk. Cows were milked 2×/d with samples taken 2d/wk for composition analysis. Dry matter intake did not differ by treatment, but SCFP increased meals/d and decreased time between meals. Body weight (measured at enrollment, d 0, and d 42 RTC) and body condition score (scored weekly) were not affected by treatment. Milk, energy-corrected milk, and fat-corrected milk yields did not differ by treatment. Milk fat concentration was greater in SCFP with significant differences in wk 4 and 5. Milk lactose concentration tended to be greater for CON and milk urea nitrogen tended to be lesser for CON, but there were no treatment effects on milk protein concentration or somatic cell count. Assuming equal digestibility, energy balance deficit was greater for SCFP than CON (-6.15 vs. -4.34 ± 0.74 Mcal/d) with significant differences in wk 4 and 5. Plasma concentrations of free fatty acids, β-hydroxybutyrate, glucose, and insulin did not differ with treatment, but cholesterol was greater for SCFP. Liver triglyceride increased while liver cholesterol decreased with time. Liver TG did not differ by treatment, but liver cholesterol tended to be lesser in SCFP. Relative mRNA abundance of cholesterol related genes (*SREBF2, HMGCS1, HMGCR, MTTP, SPOB100,*

APOA1), *FGF21*, and *CPT1a* did not differ by treatment, but *PCK1* tended to be greater for SCFP. The ketogenic transcript *HMGCS2* was greater for SCFP which aligns with SCFP increasing incidence of subclinical ketosis; however, *BDH* did not differ between treatments. In conclusion, SCFP supplementation increased meals/d with less time between meals, increased milk fat concentration, altered cholesterol metabolism, and increased incidence of subclinical ketosis, but early lactation milk yield and metabolism were generally unaffected.

INTRODUCTION

Feeding yeast culture products to dairy cattle can alter the rumen environment to increase populations of microbes associated with fiber digestion (Mullins et al., 2013), increase lactic acid utilization, and increase ruminal pH (Piva et al., 1993). Milk production responses have been variable with reports of increased milk production in some studies (Zaworski et al., 2014; Acharya et al., 2017; Dias et al., 2018a), but not others (Dann et al., 2000; Schingoethe et al., 2004; Yuan et al., 2015a). Possible explanations for varying production responses to *Saccharomyces cerevisiae* fermentation product (**SCFP**) include differences in dietary NDF concentration (Robinson and Erasmus, 2009), forage to concentrate ratio (Piva et al., 1993), DMI and level of milk production (Allen and Ying, 2012), and stage of lactation (Poppy et al., 2012) across studies.

Saccharomyces cerevisiae fermentation products (**SCFP**) have been particularly advantageous during periods of stress, reducing fluctuations in rumen pH during subacute ruminal acidosis (Li et al., 2016) and increasing DMI during the transition period (3 wk before and after calving; Poppy et al., 2012). The meta-analysis by Poppy et al. (2012) determined early lactation SCFP supplementation increased DMI by 0.62 kg/d and energy-corrected milk (**ECM**) by 1.65 kg/d. Mechanisms behind these responses to SCFP supplementation during the transition period are not fully known, but could be attributed to altered rumen microbial population and thus changes in ruminal VFA production (Poppy et al., 2012), altered feeding behavior (DeVries and Chevaux, 2014; Yuan et al., 2015a) and/or improved immune function (Zaworski et al., 2014).

The SCFP evaluated in this study is a new product that contains added antioxidants and polyphenols. Based on previous SCFP studies and the anti-inflammatory and anti-oxidant effects

of polyphenols (Middleton et al., 2000), we hypothesized that feeding this SCFP product during the transition period would alter feeding behavior. The primary objective of this study was to determine the effect of this new SCFP product (NutriTek, Diamond V, Cedar Rapids, IA) on feeding behavior during the transition period in dairy cows; the secondary objective was to evaluate the effects on milk production and composition, energy balance, and metabolism.

MATERIALS AND METHODS

Experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee (protocol #3759.2).

Cows and treatments

Sixty-four prepartum Holstein cows (50 multiparous, 14 primiparous) were used in a randomized complete block design. Cows were blocked by parity, expected calving date, and previous 305ME yield, then randomly assigned to treatment within block. Treatments were either control (n = 30) or SCFP (16.0 ± 0.7 g/d prepartum and 18.9 ± 0.5 g/d postpartum; n = 34; NutriTek, Diamond V, Cedar Rapids, IA) incorporated into a TMR. The TMR was formulated for an expected average DMI of 11.1 kg/d prepartum and 20 kg/d postpartum. Differences between expected and observed intakes resulted in slight deviations from the manufacturer's recommended SCFP dose of 19 g/d. Across the entire period, SCFP consumption averaged 17.4 ± 0.6 g/d. We evaluated the power of the study to detect difference in immunoglobulin response to ovalbumin vaccination, our key measure of adaptive immune function (results not shown here). We used variance data from our past work (Yuan et al., 2015b) and found that 60 cows (30/treatment) would provide 90% power to detect a 0.06 difference in optical density for anti-

ovalbumin IgG. This magnitude matches the difference in means observed in our previous study (Yuan et al., 2015b), and at 15% of the mean optical density, reflects sufficient sensitivity to detect a meaningful difference in antibody production. Treatments were fed from -29 ± 5 to 42 days relative to calving (**RTC**). Time points referencing time RTC, refer to d before expected calving date for prepartum data and d relative to actual calving date for postpartum data. Average BCS (3.61 ± 0.06) and BW (689 ± 13 kg) were not different between treatment groups at enrollment ($P \geq 0.41$).

Diets were formulated to meet NRC (2001) requirements for a 650-kg Holstein cow; the lactation diet was formulated to support 45 kg/day of milk production at 20 kg/day DMI. Feed ingredient samples were collected once weekly, composited by 4 months (12 month experiment), and analyzed by wet chemistry methods for dry matter, neutral detergent fiber, starch, crude protein, ether extract, and ash content (Dairy One, Ithaca, NY). Dry matter content was determined by oven drying for 3 h at 105°C. Crude protein was determined (Etheridge et al., 1998) by oxidation and detection of N_2 (Leco Analyzer, Leco Corp., St. Joseph, MI). Concentrations of ash-free NDF and ADF (Van Soest et al., 1991) were determined using an Ankom Fiber Analyzer (ANKOM Technology, Macedon, NY) and filter bags with a pore size of 25 µm. Sodium sulfite was added to the detergent solution for the ash-free NDF analysis. Crude fat was determined by ether extraction (method 2003.05; AOAC, 2012). Ash concentration was determined using AOAC method 942.05 (AOAC, 2012). Chemical analyses of individual feed ingredients were used for determination of TMR nutrient composition (Table 3.1).

To evaluate adaptive immunity, cows were injected on d 7 and 21 RTC with an innocuous protein, ovalbumin (0.5 mg OVA; Sigma-Aldrich, St. Louis, MO) along with 0.25 mg

Quil-A adjuvant dissolved in 1.0 mL saline (# vac-quil; Invivogen, San Diego, CA). Results for this protocol are not presented here.

Data and sample collection

Prepartum cows were fed treatment diets 3x/d (0900, 1300, and 1700 h) using an electronically gated feeding system (Roughage Intake System; Insentec B.V., Marknesse, the Netherlands). All cows on a given dietary treatment were allowed access to 4 feed bins assigned to that treatment, and no more than 6 animals shared those 4 bins at any given time. After calving, cows were moved to a tie-stall facility where they were fed individually twice daily (0500 and 1700 h). Both feeding systems electronically recorded individual feed consumption and meal patterns. As-fed feed intake was recorded daily and adjusted by TMR DM for determination of meal and daily DMI. Analysis of individual feeding behavior data was performed according to Yuan et al. (2015a). Specifications for feeding behavior parameters such as a minimum inter-meal interval (**IMI**) of 12 min and minimum meal weight of 0.4 kg were based on Mullins et al. (2012). Meals considered biologically infeasible (eating rate > 1.8 kg/min) were removed prior to analysis of meal weight, length, and count. In addition to electronically recorded DMI determination, feed refusals and water intake were measured daily postpartum.

Cows were milked 2x/d (0400 and 1600 h) and with milk weights recorded at each milking. Milk samples were collected at each milking 2 d/wk and analyzed for concentrations of fat, true protein, lactose (B-2000 Infrared Analyzer; Bentley Instruments, Chaska, MN), MUN (MUN spectrophotometer, Bentley Instruments), and somatic cells (SCC 500, Bentley Instruments) by MQT labs (Kansas City, MO). Somatic cell linear score (**SCLS**) was calculated

as described by Schukken et al. (2003): SCLS = $\log_2$[somatic cell count (1000 cells/mL)/100] + 3. Energy-corrected milk was calculated as (0.327 × milk yield) + (12.95 × fat yield) + (7.65 × protein yield), and fat-corrected milk (**FCM**) was calculated according to the NRC (2001) as (0.432 × milk yield) + (16.216 × fat yield).

Body condition score was recorded weekly by 3 trained investigators. Body weight was measured at enrollment (d -29 ± 5 RTC), after calving, and at 42 DIM. Prepartum energy balance was calculated according to the NRC (2001) as net energy (**NE**) intake – (NE maintenance + NE pregnancy) where NE intake is DMI × NE_L of prepartum ration, NE maintenance (Mcal/d) is $BW^{0.75}$ × 0.08, and NE for pregnancy (Mcal/d) is {(2 × 0.00159 × days pregnant – 0.0352) × [(mature weight × 0.06275)/45]}/ 0.14. Postpartum energy balance was calculated as NE intake – (NE maintenance + NE milk). NE intake and NE maintenance were calculated as described above, but used NE_L of postpartum diets and BW was adjusted with a constant weekly rate of BW loss between d 0 and 42 DIM. NE in milk was calculated as 0.75 × ECM.

A total of 5 jugular blood samples (60 mL) were taken from each cow throughout the experiment. The days (RTC) of sampling were as follows: d -28 to -24 (wk -4); d -14 to -10 (wk -2); d 3 to 7 (wk 1); d 12 to 16 (wk 2); d 31 to 35 (wk 5). From the 60 mL sample, 50 mL was used for neutrophil isolation and oxidative burst assay (results not shown here), with the remaining 10 mL allocated to two tubes containing either K_3EDTA or sodium fluoride. Vacutainers were immediately placed on ice. Plasma was separated by centrifugation (1,500 × *g* for 15 min) and stored in microcentrifuge tubes at -20˚C until analyses. Plasma samples were analyzed for free fatty acids (**FFA**; NEFA-HR; Wako Chemicals USA Inc., Richmond, VA), BHB (kit #H7587-58; Pointe Scientific Inc., Canton, MI), and glucose (kit #439-90901; Wako Chemicals USA Inc.) by enzymatic assays. Insulin was measured by a bovine-specific sandwich

ELISA (#10-1201-01; Mercodia AB, Uppsala, Sweden). Total plasma cholesterol was measured using a fluorometric assay kit (item no. #10007640; Cayman Chemical, Ann Arbor, MI).

Liver biopsies were collected by percutaneous biopsy according to Mullins et al. (2012) once between d -19 and d -12 RTC (allowing prepartum biopsies to occur only one d/wk) and at 14 DIM for analysis of transcriptional and metabolic responses to treatment. Liver tissue was immediately frozen in liquid nitrogen. Samples were stored in 1.5 mL microcentrifuge tubes either alone (for triglyceride (**TG**) and cholesterol analysis) or with 1 mL TriZol (ref. no. 15596018; ThermoFisher Scientific, Waltham, MA) for mRNA analysis; both were stored at -80°C until analysis. Liver TG content was measured as described by Yuan et al. (2013) and cholesterol concentrations were measured using an enzymatic assay (#ab65390; Abcam, Cambridge, MA). Concentrations are expressed both relative to wet weight and relative to total protein content. Liver total protein concentration was evaluated by the Bradford method using a colorimetric kit (kit #23236; Thermo Scientific Pierce, Rockford, IL).

Hepatic transcripts evaluated include fibroblast-growth factor-21 (***FGF21***), carnitine palmitoyltransferase 1A (***CPT1A***), phosphoenolpyruvate carboxykinase (***PCK1***), sterol regulatory element binding factor 2 (***SREBF2***), microsomal triglyceride transfer protein (***MTTP***), apolipoprotein B 100 (***APOB100***), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (***HMGCR***), 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (***HMGCS1***), apolipoprotein A 1 (***APOA1***), 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2 (***HMGCS2***), beta-hydroxybutyrate dehydrogenase (***BDH***), and internal control genes *β*-actin (***ACTB)*** and 40S ribosomal protein S15 (***RPS15***). RNA was isolated from liver tissue using the Direct-zol RNA mini prep kit (cat. no. r2072; Zymo Research, Irvine, CA). Quantitative real-time PCR was performed as previously described (Yuan et al., 2013). Briefly, 2 µg of total RNA per sample

was used as a template for the reverse transcriptase reaction using random primers (High-Capacity cDNA RT Kit; Bio-Rad Laboratories, Hercules, CA). Quality of RNA (integrity number = 7.62 ± 0.14 for a random subset of 12 samples) was verified with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Quantitative real-time PCR was run in duplicate using 5% of the cDNA product on 96-well plates with 200 nM of gene-specific primers (Table 3.2) and SYBR Green master mix (cat. no. 172_5120; BioRad Laboratories, Hercules, CA). All target genes in samples were normalized against the control genes *β-actin* (***ACTB***) and *RPS15;* Ct values of these targets were not affected by treatment ($P \geq 0.40$). Messenger RNA abundance was calculated as $(2 \times \text{PCR efficiency})^{-\Delta Ct}$ (Pfaffl, 2001) with results scaled such that the wk -3 control mean equaled 1 for each transcript.

Cow health was evaluated daily by visual inspection, rectal temperature measurement, and assessment of urine acetoacetic acid concentration (KetoCare, TRUEplus, Trividia Health, Fort Lauderdale, FL). Cows were monitored for disorders including subclinical ketosis, milk fever, displaced abomasum, retained placenta, metritis, and mastitis. Subclinical ketosis was defined as urine acetoacetic acid concentration > 40 mg/dL, and other diseases were diagnosed according to the definitions of Kelton et al. (1998). Cows diagnosed with subclinical ketosis were treated for 3 d with 300 mL propylene glycol (Oral Keto Energel, Aspen Veterinary Resources, Ltd., Greely, CO).

Cow exclusion criteria and attrition

A total of 90 cows were enrolled in the study, but data were used from just 64 cows, 59 of which completed the entire study. Five cows were removed from the study due to periparturient health issues that developed > 4 DIM ($n = 2$) or because of administration of an

initial adjuvant for OVA injection that caused a dramatic febrile response (n = 3); for these cows, all prepartum data were used in analyses. The remaining cows that did not complete the study were removed due to >15% consumption of the incorrect diet prepartum, generally by overpowering the pneumatic gates (n = 13), inability to adjust to the feeding system (n = 1), calving with < 14 d on treatment diets (n = 3), twin calving (n = 4), calving paralysis (n = 1), chronic periparturient illness developed < 4 DIM (n = 3), and death < 4 DIM (n = 1).

Statistical analysis

Results were analyzed using the Mixed Procedure of SAS (SAS Institute, Cary, NC). Models included fixed effects of treatment, time, parity, and 2- and 3-way interactions with treatment, and the random effects of block and cow. Interactions with parity were tested and removed from the model when $P > 0.20$. Repeated measures within cow were modeled with autoregressive or heterogeneous autoregressive covariance structures when data points were equally spaced, selected based on the least Bayesian Information Criterion value. Unequally spaced data points (e.g., plasma data) were modeled with spatial power covariance structures. Prepartum data for DMI and feeding behavior were analyzed separately from postpartum data, due to the different housing systems employed. DMI and milk data were summarized by week for statistical analysis. Outliers were excluded when the Studentized residual exceeded an absolute value of 4, which represents less than 0.01% of a normal population. Hepatic transcript abundance for all genes except *SREBF2* were log transformed prior to analysis to achieve a normal residual distribution, with results presented after back-transformation. Significance was declared when $P < 0.05$, and tendencies at $0.05 \leq P < 0.10$. When treatment interactions were $P < 0.05$, the slice option of SAS was used to test treatment effects at each measurement time or within parity.

RESULTS AND DISCUSSION

Dry matter intake and feeding behavior

Unsurprisingly, primiparous cows consumed less DM than multiparous cows both prepartum (10.3 vs. 13.0 ± 0.6 kg/d; $P < 0.01$) and postpartum (18.4 vs. 22.9 ± 0.62 kg/d; $P < 0.001$). Parity effects on prepartum feeding behavior included greater meal weight for multiparous cows (1.48 vs. 1.12 ± 1.3 kg; $P < 0.01$) and a longer IMI for multiparous cows (0.82 vs. 0.74 ± 0.02 h; $P = 0.04$). Postpartum meal length was shorter (22.7 vs. 27.0 ± 1.4 min; $P < 0.05$) and IMI longer for primiparous than multiparous cows (1.85 vs. 1.57 ± 0.76 h; $P = 0.07$).

As shown in Table 3.3 and Figure 3.1A, DMI increased with the progression of lactation ($P < 0.001$); however, DMI did not differ with SCFP supplementation ($P > 0.69$). In agreement, the only other two published studies evaluating this product (to our knowledge) did not observe an effect on DMI in transition cows (Shi et al., 2019) or mid-lactation cows (Acharya et al., 2017).

Despite no overall effects on DMI, SCFP supplementation did modulate feeding behavior both pre- and postpartum in the present study. All feeding behavior parameters except IMI ($P = 0.28$) were influenced by day RTC ($P < 0.01$; Table 3.3). SCFP tended to increase prepartum meal count ($P = 0.06$; Figure 3.1A) and decreased the time between meals ($P = 0.03$; Figure 3.1C), specifically during the 10 d preceding calving. A treatment x day RTC interaction for meal weight ($P = 0.03$) indicated that control cows consumed larger meals from d -7 to -4 relative to calving. These data suggest SCFP cows consumed lighter meals more often, with less time between meals, leading up to calving. Interestingly, a prepartum treatment x parity interaction for meal count and IMI ($P \leq 0.03$) suggested this altered feeding behavior with SCFP

mainly applied to primiparous cows. Meal count was greater (9.7 vs. 8.5 ± 0.4 d^{-1}) and IMI lesser (0.65 vs. 0.82 ± 0.04 h) for primiparous SCFP vs. control cows, but both were similar for multiparous SCFP and control cows (8.8 vs. 8.9 ± 0.2 d^{-1} and 0.82 vs. 0.81 ± 0.02 h). Postpartum, SCFP cows continued to consume more meals ($P = 0.04$) with a tendency for less time between meals ($P = 0.07$). Meal size and length did not differ by treatment ($P \geq 0.20$).

Such modulation of feeding behavior has also been documented in previous transition cow studies supplementing either yeast culture (Yuan et al., 2015a) or active dry yeast (Bach et al., 2007; DeVries and Chevaux, 2014). The more frequent meals may contribute to improved rumen function. In yeast-supplemented late lactation cows with increased meal frequency, rumen pH was also greater (Bach et al., 2007). DeVries and Chevaux (2014) debated that while yeast supplementation could modulate feeding behavior and such alterations can stabilize rumen pH and fermentation, it is also possible that feeding behavior is a secondary effect in these circumstances. In such case, yeast supplementation may result in more consistent VFA production, improved fiber digestibility, and therefore a more rapid return to eating. Through either mechanism, stabilization of rumen pH by SCFP (Shen et al., 2018) would support its supplementation during the transition period when cows are at high risk for experiencing low rumen pH (Penner et al., 2014).

Body weight, body condition, milk production, and energy balance

Cows experienced the typical decrease in BCS and BW during the transition to lactation ($P < 0.001$); however, there was no effect of treatment or treatment x time for either ($P > 0.50$). On average, from the time of enrollment through 42 DIM cows lost 0.7 BCS units (3.6 to 2.9) and from calving through 42 DIM BW decreased by 46 kg (646 to 600 kg). As shown in Table

3.4 and Figure 2A, milk production expressed as milk, energy-corrected milk, or fat-corrected milk yield was unaffected by treatment ($P \geq 0.32$). Several studies have failed to observe effects of SCFP on milk yield (Dann et al., 2000; Yuan et al., 2015a), while others have reported positive effects (Ramsing et al., 2009; Zaworski et al., 2014; Acharya et al., 2017; Dias et al., 2018a). The variation in production responses has been attributed to differences in DIM (Poppy et al., 2012), dietary NDF concentration (Robinson and Erasmus, 2009), and product evaluated (Yuan et al., 2015a). Studies also differ in power to detect treatment effects on milk yield. Milk fat concentration increased ($P = 0.01$) and milk fat yield tended to increase ($P = 0.10$; Figure 3.2B) for SCFP cows, with significant differences in wk 4 and 5 (Figure 3.2B). We observed no differences for milk protein yield and content, lactose yield, or milk somatic cell linear score ($P > 0.15$). Milk lactose concentration tended to be greater for control ($P = 0.06$) and MUN concentration tended to be greater for SCFP ($P = 0.06$). Although the net economic impacts depend on market conditions, one partial budget analysis that accounted for milk income and costs of feed, SCFP, and ketosis treatment suggested responses to SCFP supplementation in this study generated a positive financial return (Olagaray et al., 2019).

Greater milk fat content in early lactation can indicate greater fat mobilization; however, lack of effects on BCS and timing of the milk fat response (wk 4 and 5) make that unlikely. Cows are at greater risk for ruminal acidosis after parturition (Penner et al., 2007). Supplementation with SCFP has been shown to attenuate the associated drop in milk fat content (Li et al., 2016); but again, timing of our observed response does not appear to align with this mechanism. Recent analysis of a feeding behavior database revealed milk fat yield was associated with meal frequency (+0.02 kg/meal; Johnston and DeVries, 2018). As discussed previously, cows with greater meal frequency in response to SCFP supplementation also

exhibited increased rumen pH (Bach et al., 2007). Even without changes in feeding behavior, SCFP increased rumen pH and reduced ruminal lactate concentrations (Dias et al., 2018b). *Saccharomyces cerevisiae* fermentation product is believed to contain growth factors that stimulate ruminal microbial growth, especially for lactate utilizers (Callaway and Martin, 1997; Chaucheyras-Durand et al., 2008). Therefore, through its effects on microbial populations, SCFP can increase rumen pH. Greater rumen pH prevents shifts in the rumen biohydrogenation pathways (Bauman and Griinari, 2001), decreasing risk of milk fat depression. Fiber-digesting bacteria that largely produce acetate are also more productive and prolific at greater rumen pH. Wiedmeier et al. (1987) observed an increase in cellulolytic bacterial populations with SCFP, which could lead to increase acetate supply. Acetate is one of the main lipogenic precursors for de novo fatty acid synthesis, and increasing its supply via enhanced cellulolytic bacteria productivity could be partially responsible for our observed increased milk fat content. The recent experiment by Urrutia et al. (2017) supports this hypothesis as increased ruminal acetate supply via ruminal infusion increased milk fat concentration through increased C16 and de novo fatty acid yields. Although rumen function influences milk fat, the time effect makes explanations for this effect puzzling.

Energy balance calculated assuming equal digestibility differed by parity ($P < 0.01$), wk ($P < 0.001$) and treatment ($P = 0.03$). A parity x wk interaction ($P < 0.001$) indicated tendencies for greater energy balance during wk -4 and -3 RTC but significantly lesser energy balance during wk 1-5 RTC for multiparous compared to primiparous cows. Overall, energy balance was less in multiparous compared to primiparous cows (-7.02 vs. -3.47 ± 0.83 Mcal/d, $P < 0.01$). Energy balance was less for SCFP cows (-6.15 vs. -4.34 ± 0.74 Mcal/d), but despite this, body condition loss did not differ between treatments. This incongruence between calculated energy

balance and BCS change could indicate greater diet digestibility and/or metabolizability with SCFP. It is known that SCFP can affect diet digestibility (Allen and Ying, 2012; Dias et al., 2018a), providing a plausible mechanism by which NE harvest could be improved.

Metabolic signaling

Temporal patterns for plasma FFA, BHB, insulin, and glucose reflected the typical metabolic and endocrine changes during the transition period ($P < 0.001$). The metabolic profile was not altered by SCFP supplementation ($P > 0.35$; Figure 3.3). Several cows were treated for subclinical ketosis around the time of blood sampling, and therefore their BHB results could be biased by the administration of glucogenic precursors. Thus, cows treated within 1 d of sampling were excluded from the BHB analysis presented in Figure 3B. The analysis was also performed with all cows included. Similar to previous results, there was no effect of treatment ($P = 0.57$) or treatment x wk interaction ($P = 0.12$). Minimal alterations to metabolism have been reported by other studies investigating yeast culture supplementation in transition cows (Ramsing et al., 2009; Zaworski et al., 2014; Yuan et al., 2015a). Most notable was that yeast supplementation increased plasma BHB with a quadratic dose effect in Yuan et al. (2015a). Potential explanations provided by authors included increased ruminal butyrate production or increased hepatic ketogenesis. The latter was consistent with a tendency for increased plasma FFA concentration and decreased (quadratic dose effect) plasma glucose.

Hepatic metabolism

Liver TG increased from prepartum to postpartum, but there was no effect of SCFP ($P > 0.40$; Figure 3.4A). Liver TG was greater in multiparous than primiparous cows (1.14 vs. 1.01 ±

0.01 % of wet weight; $P = 0.01$). Typically, lipid mobilization is greater in multiparous cows; thus, it is logical that we observed greater liver TG in multiparous cows.

Our findings of increased *FGF21* relative mRNA abundance from prepartum to postpartum ($P < 0.001$), the time when energy balance was negative, corresponds with upregulation during fasting (Fisher and Maratos-Flier, 2016). Interestingly, relative abundance of *FGF21* was greater for primiparous than multiparous cows (1.00 vs. 0.34 ± 0.59; $P = 0.03$). Relative mRNA abundance of *PCK1* tended to be greater for SCFP ($P = 0.08$). Transcript abundance for the mitochondrial fatty acid transport enzyme *CPT1a* did not differ by treatment ($P = 0.19$) or wk ($P = 0.54$), but was greater in multiparous cows (3.10 vs. 1.00 ± 1.39; $P = 0.04$). To our knowledge this is the first study to investigate the effects of SCFP on these particular transcripts involved in lipid and glucose metabolism. Further investigation is required to explain the tendency for increased *PCK1* abundance that occurred in SCFP cows.

Cholesterol metabolism

Interestingly, liver total cholesterol concentration decreased from wk -3 to 2 RTC ($P < 0.001$) and wk 2 liver TG and cholesterol concentrations were negatively correlated ($R^2 = 0.18$; $P < 0.01$). These results are contrary to the pattern reported previously in which hepatic total cholesterol content increased from wk -3 to wk 1 with subsequent decreases (Schlegel et al., 2012). Similarly, Bobe et al. (2003) reported peak hepatic free cholesterol at 12 DIM, at which point it decreased to prepartum levels by wk 5. The different forms of liver cholesterol analyzed between our study and Bobe et al. (2003) could account for liver cholesterol concentrations increasing after parturition in Bobe et al. (2001) but decreasing in ours; however, Schlegel et al. (2012) also analyzed total cholesterol and reported time effects similar to Bobe et al. (2003).

Liver cholesterol concentration tended to be lesser in SCFP than control ($P < 0.10$; Figure 3.4B). To our knowledge, this is the first study to investigate the effects of SCFP on hepatic cholesterol content. Decreased hepatic cholesterol content could be the result of increased very low density lipoprotein formation and lipid export; however, SCFP did not decrease liver TG content. Additional potential explanations for decreased cholesterol content include less cholesterol synthesis, less uptake into the liver, and increased transformation to bile acids (Schlegel et al., 2012).

SCFP effects on cholesterol metabolism also included increased plasma cholesterol concentration throughout the study period ($P = 0.02$). Other studies evaluating effects of yeast products on plasma cholesterol reported no treatment differences (Piva et al., 1993; Yalcin et al., 2011); however, mid-lactation cows were used. Cholesterol metabolism is affected by nutrient and energy deficiency and therefore differs with stage of lactation (Gross et al., 2015). The change in plasma cholesterol, decreasing as parturition approached, with a nadir at wk 1 and a subsequent increase through wk 5 ($P < 0.001$), was similar to previously reported patterns (Bernabucci et al., 2004; Schlegel et al., 2012; Kessler et al., 2014). Decreased plasma cholesterol concentrations within the first wk of lactation could be the result of less liver synthesis of cholesterol, decreased very low density lipoprotein secretion from the liver, increased cholesterol uptake by the mammary gland (Kessler et al., 2014) and other tissues (Schlegel et al., 2012), and less reverse cholesterol transport (Kessler et al., 2014).

It has been suggested that plasma cholesterol concentration is mainly influenced by hepatic cholesterol synthesis (van Dorland et al., 2009). We investigated several genes transcripts involved in cholesterol metabolism including those involved in biosynthesis (*SREBP2, HMGCR, HMGCS1*), transport (*APOA1, APOB100*), and construction of lipoproteins

(*MTTP*). The only transcript investigated that differed with time was *APOA1*, which increased from wk -3 to wk 2 ($P < 0.001$). Nascent high density lipoprotein particles are formed by lipidation of *APOA1* in the liver (Vaughan and Oram, 2006). Therefore, greater mRNA abundance postpartum when liver cholesterol content was lesser could suggest increased production of reverse cholesterol transport machinery/components. Previous studies have reported increased mRNA abundance of genes involved in cholesterol synthesis at the onset of lactation (Schlegel et al., 2012; Weber et al., 2013; Kessler et al., 2014), making it surprising that we did not observe any effects of time. It is possible our d 14 sample was too late to capture the expected postpartum increase in these transcripts. Relative mRNA abundance was not altered by SCFP supplementation for any of the genes ($P > 0.10$; Table 3.5).

Further investigation is required to understand the associations between plasma and hepatic levels of cholesterol with dairy cow health and productivity. Over-interpretation is a risk when examining changes in gene expression to provide explanation for systemic changes. Schlegel et al. (2012) observed no association between expression of hepatic enzymes of cholesterol synthesis and plasma cholesterol concentration; expression of *HMGCR* and *HMGCS1* was greatest at wk 1 when plasma cholesterol concentration was least (Schlegel et al., 2012).

Health outcomes

Incidence of common periparturient diseases occurring throughout the study period are provided in Table 6. No metabolic diseases recorded, except for subclinical ketosis (**SCK**), differed by treatment ($P > 0.10$). Incidence of SCK was greater in cows supplemented with SCFP compared to control cows (38% vs. 12%, $P = 0.02$, Fisher's exact test) and days of glucogenic treatment were greater (1.7 vs. 0.4 ± 0.3 d; $P = 0.01$). To understand the observed

increase in SCK incidence, despite little evidence of an overall treatment effect on ketone concentrations in plasma or decreased energy balance during the window of time when ketosis was observed, additional analyses were conducted. The majority of ketosis diagnosis occurred between 10 and 20 DIM (n = 8, SCFP = 6, control = 2). Because of the timing, wk 2 data from all cows were used to try to understand potential mechanisms underlying this effect. Appropriate diagnosis of SCK by urine acetoacetic acid concentrations is supported by greater wk 2 plasma BHB concentrations in cows diagnosed with SCK compared with those that were not (2068.3 ± 135.0 vs. 792.5 ± 62.3 μM; $P < 0.001$). Analysis of wk 2 BHB concentrations independently demonstrated a parity x treatment interaction ($P = 0.02$; Figure 3.5). Treatment did not impact wk 2 plasma BHB in primiparous cows, but SCFP increased BHB concentrations in multiparous cows.

To further investigate the SCK effect, we analyzed hepatic mRNA abundance for two enzymes in the ketogenic pathway; *HMGCS2*, which facilitates the rate-limiting formation of HMG-CoA, and *BDH*, which reduces acetoacetate to produce BHB (Hegardt, 1999). Aligning with incidence of SCK, *HMGCS2* was greater for SCFP cows compared to control ($P = 0.03$). Despite this alignment of treatment effects, *HMGCS2* did not differ by parity or time RTC ($P > 0.35$). Relative *BDH* abundance did not differ by treatment, time, or parity ($P > 0.30$). Previous studies have showed increased relative *BDH* abundance in response to nutrition-induced ketosis (Loor et al., 2007), however, abundance of *HMGCS2* and *BDH* have not always coincided with increased plasma BHB concentrations (van Dorland et al., 2009, 2014; Graber et al., 2010). In a study that examined cows with different metabolic loads (defined by plasma BHB, FFA, and glucose concentrations), *BDH2* and *HMGCS2* did not differ between the two groups even though plasma BHB did (van Dorland et al., 2014). It is possible that small changes at the mRNA level,

although not statistically significant for *BDH*, were sufficient to generate phenotypic changes (Graber et al., 2010). It is also possible that BHB synthesis is more heavily regulated by posttranslational mechanisms (Quant et al., 1990).

It is possible the greater incidence of ketosis with SCFP contributed to our observed differences in cholesterol metabolism. Both *SREBF2* and *HMGCS1* (cholesterol biosynthesis) were downregulated in cows during nutrition-induced ketosis (Loor et al., 2007). Because cholesterol synthesis and ketogenesis share a common pathway utilizing the enzyme *HMGCS,* it is unsurprising that the metabolism of the two may be related. Although SCFP did not alter *SREBF2* and *HMGCS1* mRNA abundance in this study, it is interesting that the tendency for less liver cholesterol and increased plasma cholesterol coincided with greater SCK.

Occurrence of ketosis is typically concerning as it is associated with depressed feed intake and decreased performance (Duffield et al., 2009; Ospina et al., 2010); however, recent BHB infusion data suggests elevated BHB in early lactation may not necessarily be problematic. Infusion of BHB for 48 hours at rates that elevated plasma BHB to levels of SCK (1.5 to 2.0 mmol/L) decreased plasma glucose concentrations, but had no effect on DMI, milk yield, or ECM (Zarrin et al., 2013). Despite epidemiological evidence linking decreased cow performance with SCK (Ospina et al., 2010), more recent studies have reported increased milk yield and fat concentration (Rathbun et al., 2017) and greater first test day milk (Vanholder et al., 2015) in cows diagnosed with SCK during the first 2 wk of lactation. These observational studies point to greater early lactation milk yield for SCK cows, although associations with peak milk yield are more variable. This disconnect between epidemiological studies and responses to infused BHB may be that most cases of SCK in transition cows are secondary to other events (e.g. mastitis or retained placenta) that may account for the negative effects on health and productivity (Ospina et

al., 2010; Zarrin et al., 2013). Thus, the elevated BHB observed in cows diagnosed with SCK in the current study might not be detrimental to cow productivity.

Increased SCK could also be the result of shifts in rumen fermentation that resulted in greater plasma BHB. Greater supply of ruminal acetate increased plasma BHB (Urrutia and Harvatine, 2017), largely due to microbial conversion of acetate to butyrate and metabolism of butyrate to BHB in the rumen epithelium (Sutton et al., 2003). As previously discussed, SCFP can modulate rumen fermentation toward increased acetate production. Ruminally infused butyrate also increased plasma BHB concentration that was accompanied by a decrease in plasma glucose (Herrick et al., 2018). Although not measured in our study, it is possible that SCFP caused shifts in ruminal fermentation that partially contributed to increased systemic concentrations of BHB, and thus increased incidence of SCK. Since only the greatest doses of ruminal acetate (15 mol/d; Urrutia and Harvatine, 2017) and butyrate (2g/kg BW; Herrick et al., 2018) infusion increased plasma BHB concentrations to levels near the 1.2 mM cut-point for SCK (1.175 and 1.45 mM, respectively), it is likely any shifts in ruminal fermentation by SCFP would have only partially contributed to the increased ketone concentrations used to diagnose SCK. Likewise, if altered ruminal fermentation is a key underlying mechanism for responses to SCFP, it is difficult to explain why the timing of SCK and milk fat yield responses differed. Several possible explanations for increased incidence of subclinical ketosis with SCFP have been presented, but the exact mechanisms remain unknown.

CONCLUSIONS

SCFP supplementation during the transition period altered prepartum and postpartum feeding behavior, with increased meals per day and decreased time between those meals. Although no

effects were detected for DMI, milk yield, milk protein, or SCLS, milk fat content was increased by approximately 13% in cows receiving SCFP, with differences beginning after the time period that lipid mobilization is greatest during the transition period. Body weight, BCS, and energy metabolites were unaffected by treatment. Liver metabolic signals were mostly unaffected by treatment, but there was a tendency for SCFP to increase *PCK1* mRNA abundance. Supplementation with SCFP led to shifts in cholesterol metabolism, tending to decrease hepatic cholesterol and increasing plasma cholesterol. Treatment also increased incidence of SCK, and increased the rate-limiting ketogenic enzyme *HMGCS2,* but did not affect *BDH*, encoding the final enzyme in the ketogenesis pathway. Overall, SCFP supplementation during the transition period increased meals/d with less time between meals, increased milk fat concentration, altered cholesterol metabolism, and increased incidence of SCK, but early lactation milk yield and metabolism were otherwise unaffected.

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TABLES AND FIGURES

Table 3.1: Ingredient and nutritional composition of the prepartum and postpartum diets.

	Prepartum		Postpartum	
	Control	**SCFP[1]**	**Control**	**SCFP**
Ingredient, % of DM				
Alfalfa hay[2]	-		9.57	
Alfalfa hay[3]	-		9.57	
Grass hay	38.61		1.66	
Corn silage	21.15		24.06	
Wet corn gluten feed[4]	18.09		23.56	
Cotton seed	-		3.98	
Ground corn	7.73	7.69	17.16	17.11
Micronutrient premix[5-8]	14.42	14.48	10.42	10.48
Nutrient, % of DM (unless otherwise specified)				
DM, % as-fed	63.3		59.7	
CP	12.9		17.0	
ADF	25.0		17.8	
Ash-free NDF	43.1		31.3	
NFC	30.1		37.6	
Starch	15.3		22.6	
Crude fat	5.1		6.3	
NE_L[9], Mcal/kg	1.42		1.66	

[1]*Saccharomyces* cerevisiae fermentation product
[2]Lower quality alfalfa with 22.1% CP
[3]Higher quality alfalfa with 23.9% CP
[4]Sweet Bran (Cargill Inc., Blair, NE)
[5]Prepartum control micronutrient premix consisted of 37.5% SoyChlor (Anionic feed supplement (West Central Cooperative, Ralston, IA), 34.3% soybean meal, 7.51% calcium propionate, 6.44% calcium sulfate, 5.36% Ca salts of long-chain fatty acids (Megalac R, Arm & Hammer Animal Nutrition, Princeton, NJ), 2.68% Reashure (28.8% choline chloride, Balchem Corp., New Hampton, NY), 2.14% vitamin E (20 kIU/g), 1.29% stock salt, 1.03% Niashure (65% niacin, Balchem Corp.), 0.54% magnesium oxide, 0.33% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.21% vitamin A premix (30 kIU/g), 0.19% selenium,), 0.15% Zinpro 120 (Zinpro Corp.), 0.10% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.09% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), 0.06% vitamin D premix (30 kIU/g), 0.02% ethylenediamine dihydroiodide premix (3.65% I).

[6]Prepartum SCFP micronutrient premix consisted of 37.2% SoyChlor (Anionic feed supplement (West Central Cooperative, Ralston, IA), 34.0% soybean meal, 7.44% calcium propionate, 6.37% calcium sulfate, 5.31% Ca salts of long-chain fatty acids (Megalac R, Arm & Hammer Animal Nutrition, Princeton, NJ), 2.66% Reashure (28.8% choline chloride, Balchem Corp., New Hampton, NY), 2.12% vitamin E (20 kIU/g), 1.27% stock salt, 1.02% Niashure (65% niacin, Balchem Corp.), 0.94% SCFP (NutriTek, Diamond V, Cedar Rapids, IA), 0.53% magnesium oxide, 0.33% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.21% vitamin A premix (30 kIU/g), 0.19% selenium,), 0.15% Zinpro 120 (Zinpro Corp.), 0.10% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.09% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), 0.06% vitamin D premix (30 kIU/g), 0.02% ethylenediamine dihydroiodide premix (3.65% I).

[7]Postpartum control micronutrient premix consisted of 59.9% expeller soybean meal (SoyBest, Grain States Soya, West Point, NE), 12.0% limestone, 10.5% sodium bicarbonate, 7.48% Ca salts of long-chain fatty acids (Megalac R, Arm & Hammer Animal Nutrition, Princeton, NJ), 2.40% magnesium oxide, 1.50% stock salt, 1.50% trace mineral salt, 1.50% potassium chloride, 1.50% vitamin E (20 kIU/g), 0.94% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), 0.25% selenium premix (0.06%), 0.23% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.15% vitamin A premix (30 kIU/g), 0.12% Zinpro 120 (Zinpro Corp.), 0.06% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.04% vitamin D premix (30 kIU/g), 0.01% ethylenediamine dihydroiodide premix (3.65% I).

[8]Postpartum SCFP micronutrient premix consisted of 59.4% expeller soybean meal (SoyBest, Grain States Soya, West Point, NE), 11.9% limestone, 10.4% sodium bicarbonate, 7.42% Ca salts of long-chain fatty acids (Megalac R, Arm & Hammer Animal Nutrition, Princeton, NJ), 2.37% magnesium oxide, 1.48% stock salt, 1.48% trace mineral salt, 1.48% potassium chloride, 1.48% vitamin E (20 kIU/g), 0.93% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), 0.87% SCFP (NutriTek, Diamond V, Cedar Rapids, IA), 0.25% selenium premix (0.06%), 0.23% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.15% vitamin A premix (30 kIU/g), 0.12% Zinpro 120 (Zinpro Corp.), 0.06% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.04% vitamin D premix (30 kIU/g), 0.01% ethylenediamine dihydroiodide premix (3.65% I).

[9]NE_L = 0.703 × ME (Mcal/kg) – 0.19 + [(0.097 × ME, Mcal/kg, + 0.19)/97] × [ether extract, %, − 3].

Table 3.2: Gene primers for quantitative reverse-transcriptase PCR of liver samples.

Trancript[1]	Primer	Primer Sequence (5' to 3')	Accession Number[2]	Efficiency, %
FGF21	Forward	GCCAGGCGTCATTCAGATCT	AC_000175.1	92
	Reverse	GAAAGCTGCAGGCTTTGGG		
CPT1a	Forward	CTTCCCATTCCGCACTTTC	DV820520	100
	Reverse	CCATGTCCTTGTAATGAGCCA		
PCK1	Forward	CGAGAGCAAAGAGATACGGTGC	NM_174737.2	103
	Reverse	TGACATACATGGTGCGACCCT		
SREBF2	Forward	GATGCACAAGTCTGGCGTTC	NM_001205600.1	70
	Reverse	GTCGATGCCCTTCAGGAGTT		
HMGCS1	Forward	ACAGTGAGGTGGGTAACTTTGA	NM_001206578.1	90
	Reverse	GCTGCTTTCTTGCCTAAACTGT		
HMGCR	Forward	GCTGCTGGTCGACCTTTCTA	NM_001105613.1	70
	Reverse	TCCCACGAGCAATGTTCTCC		
MTTP	Forward	TGGGTGTCACTTCGAAAGCC	NM_001101834.1	70
	Reverse	GCTCCAGTTTCTGCCTCGAT		
APOB100	Forward	CTGGAGAGTGGAACGGATGC	XM_015473552.1	95
	Reverse	GCACGTGGTCTGTCTGATGT		
APOA1	Forward	GGAGAGCCTCAAGGTCAGCATC	NM_174242.3	73
	Reverse	ATCTCACTGGGCGTTCAGCTT		
HMGCS2	Forward	GGCGTCCCGTTTAAAGATATG	XM_010803104.3	64
	Reverse	AGTTGAAAGAGGGCAGACGTT		
BDH	Forward	AGGGTCTTCGAGAAGGAAACG	NM_001034600.2	138
	Reverse	GGTTCCCAAAACAAACTGGCG		
ACTB	Forward	ACGACATGGAGAAGATCTGG	NM_173979.3	83
	Reverse	ATCTGGGTCATCTTCTCACG		
RPS15	Forward	GGCGGAAGTGGAACAGAAGA	NM_001024541.2	97
	Reverse	GTAGCTGGTCGAGGTCTACG		

[1]*FGF21* = Hepatic fibroblast growth factor-21; *CPT1a* = carnitine palmitoyltransferase 1A; *PCK1* = phosphoenolpyruvate carboxykinase; *SREBF2* = sterol regulatory element binding factor 2; *HMGCS1* = 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1, *HMGCR* = 3-hydroxy-3-methylglutaryl-coenzyme A reductase*; MTTP* = microsomal triglyceride transfer protein, *APOB100* = apolipoprotein B 100, *APOA1* = apolipoprotein A 1, *HMGCS2* = 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2; *BDH* = beta-hydroxybutyrate dehydrogenase; *ACTB* = *β*-actin; *RPS15* = 40S ribosomal protein S15

[2]From NCBI Nucleotide Database (https://www.ncbi.nlm.nih.gov/nucleotide/)

Table 3.3: Feed intake, water intake, and feeding behavior parameters for control cows and cows supplemented with a *Saccharomyces cerevisiae* fermentation product (SCFP) from -29 ± 5 days relative to calving through 42 DIM.

				P-values				
	Control	SCFP	SEM[1]	Trt	Time[2]	Trt x Time	Parity	Trt x Parity
Prepartum measure								
DMI, kg/d	11.52	11.72	0.50	0.70	< 0.001	0.76	< 0.01	> 0.20
Meal count, d^{-1}	8.66	9.27	0.22	0.06	< 0.001	0.44	0.52	0.03
Meal weight, kg	1.31	1.29	0.06	0.75	< 0.001	0.03	< 0.01	> 0.20
Meal length, min	28.28	29.49	0.94	0.28	< 0.001	0.03	0.91	> 0.20
Inter-meal interval, h	2.26	2.09	0.05	0.03	0.28	0.10	0.04	0.01
Postpartum measure								
Water intake, L/d	104.3	109.7	3.7	0.16	< 0.001	0.60	< 0.001	> 0.20
DMI, kg/d	20.58	20.69	0.52	0.84	< 0.001	0.75	< 0.001	> 0.20
Meal count, d^{-1}	11.32	12.60	0.45	0.04	< 0.001	0.66	0.45	> 0.20
Meal weight, kg	2.00	1.91	0.12	0.59	< 0.001	0.34	0.28	> 0.20
Meal length, min	23.81	25.90	1.30	0.20	< 0.001	0.81	< 0.05	> 0.20
Inter-meal interval, h	1.81	1.62	0.09	0.07	< 0.001	0.55	0.07	> 0.20

[1]Pooled standard error of the mean
[2]Time is by wk for DMI and by day relative to calving for feeding behavior parameters

Table 3.4: Lactation performance and energy balance for control cows and cows supplemented with a *Saccharomyces cerevisiae* fermentation product (SCFP) from 29 ± 5 days prepartum to 42 DIM.

	Control	SCFP	SEM[1]	*P*-values[2]		
				Trt	Wk	Trt x wk
Milk, kg/d	41.35	40.23	1.26	0.43	< 0.001	0.24
Milk fat, %	3.96	4.32	0.11	0.01	< 0.001	< 0.05
Milk fat, kg/d	1.64	1.77	0.08	0.10	< 0.001	0.09
Milk protein, %	3.03	3.12	0.04	0.16	< 0.001	< 0.01
Milk protein, kg/d	1.24	1.21	0.04	0.48	< 0.001	0.61
Milk lactose, %	4.93	4.87	0.02	0.06	< 0.001	0.70
Milk lactose, kg/d	2.04	1.97	0.06	0.29	< 0.001	0.41
Milk urea nitrogen, mg/dL	11.51	12.42	0.38	0.06	< 0.001	0.21
Milk somatic cell linear score[3]	2.32	1.94	0.28	0.29	< 0.001	0.55
Energy-corrected milk, kg/d	43.89	45.21	1.66	0.41	< 0.001	0.09
Fat-corrected milk, kg/d	43.90	45.93	1.90	0.32	< 0.001	0.20
Body weight change[4], kg	-41.2	-44.5	7.9	0.74	-	-
Body condition score change[5]	-0.61	-0.73	0.07	0.22	-	-
Energy balance, Mcal/d	-4.34	-6.15	0.74	0.03	< 0.001	0.20

[1]Pooled standard error of the mean
[2]Parity was significant for all parameters except milk protein concentration ($P = 0.10$) and milk urea nitrogen ($P = 0.86$), and none had a parity × trt interaction (all $P > 0.14$).
[3]SCLS = $\log_2$(somatic cell count/100) + 3
[4]Body weight change from calving through 42 DIM
[5]Body condition score change from enrollment (d -29± 5) to 42 DIM

2

Table 3.5: Liver lipid concentrations and relative mRNA abundance of transcripts involved in lipid metabolism and ketogenesis for control cows and cows supplemented with a *Saccharomyces cerevisiae* fermentation product (SCFP) from 29 ± 5 days prepartum to 42 DIM.

Variable	Treatment	Wk -3	Wk 2	SEM[1]	*P*-values				
					Trt	Wk	Trt x wk	Parity	Trt x Parity[2]
Lipid Concentrations									
TG, % wet weight	Control	0.50	1.33	0.91	0.59	< 0.001	0.63	0.01	NS
	SCFP	0.42	1.33	0.88					
TG, mg/g protein	Control	110	320	30.5	0.41	< 0.001	0.67	0.04	NS
	SCFP	96	283	32.0					
Cholesterol, % wet weight	Control	0.16	0.09	0.01	0.09	< 0.001	0.39	0.50	NS
	SCFP	0.13	0.09	0.01					
Cholesterol, mg/g protein	Control	38.4	15.8	2.69	< 0.10	< 0.001	0.31	0.88	NS
	SCFP	32.4	14.8	2.67					
Transcripts[3]									
FGF21	Control	1.00	3.18	2.18	0.11	< 0.001	0.41	0.03	0.12
	SCFP	1.24	5.88	3.80					
CPT1a	Control	1.00	0.84	0.94	0.37	0.72	0.27	0.04	NS
	SCFP	0.95	1.34	1.18					
PCK1	Control	1.00	1.04	1.05	0.08	< 0.05	0.75	0.85	NS
	SCFP	1.05	1.11	1.12					
SREBF2	Control	1.00	1.08	0.94	0.91	0.38	0.85	0.21	NS
	SCFP	0.99	1.11	0.94					
HMGCR	Control	1.00	1.02	0.98	0.21	0.75	0.81	0.55	0.13
	SCFP	1.38	1.60	1.45					
HMGCS1	Control	1.00	0.82	0.87	0.46	0.86	0.54	0.29	NS
	SCFP	0.70	0.78	0.69					
MTTP	Control	1.00	1.10	1.00	0.14	0.61	0.75	0.84	NS
	SCFP	1.33	1.36	1.27					
APOB100	Control	1.00	1.36	1.15	0.23	0.90	0.20	0.55	0.12

	SCFP	2.73	1.89	2.17					
APOA1	Control	1.00	3.61	2.16	0.59	< 0.001	0.77	0.76	NS
	SCFP	1.05	4.31	2.43					
HMGCS2[6]	Control	1.00	1.43	1.04	0.04	0.37	0.28	0.45	NS
	SCFP	2.07	2.00	1.68					
BDH	Control	1.00	1.35	1.04	0.90	0.84	0.42	0.31	NS
	SCFP	1.64	1.00	1.13					

[1]Pooled standard error of the mean.

[2]NS= Not Significant; Interaction not included in final model because $P > 0.20$.

[3]*FGF21* = Hepatic fibroblast growth factor-21; *CPT1a* = carnitine palmitoyltransferase 1A; *PCK1* = phosphoenolpyruvate carboxykinase; *SREBF2* = sterol regulatory element binding factor 2; *HMGCS1* = 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1, *HMGCR* = 3-hydroxy-3-methylglutaryl-coenzyme A reductase*; MTTP* = microsomal triglyceride transfer protein, *APOB100* = apolipoprotein B 100, *APOA1* = apolipoprotein A 1, *HMGCS2* = 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2; *BDH* = beta-hydroxybutyrate dehydrogenase

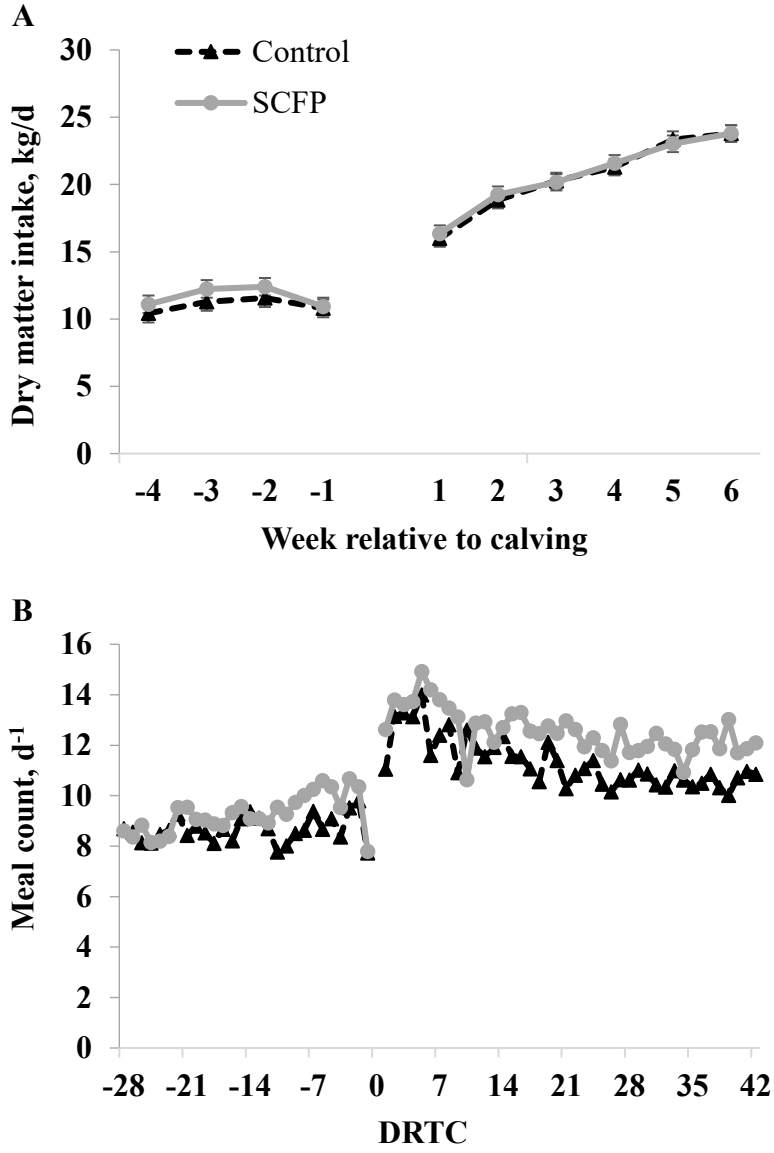
Table 3.6: Disease incidence through 42 DIM for control cows and cows supplemented with a *Saccharomyces cerevisiae* fermentation product (SCFP) from 29 ± 5 days prepartum to 42 DIM.

	Control	SCFP
At-risk[1]	30	34
Fever	9	5
Displaced abomasum	0	2
Retained placenta	2	0
Ketosis	4	12*
Mastitis	2	1
Other[2]	4	1

[1]Includes all cows that surpassed the exclusion criteria at calving. Cows excluded from analysis due to periparturient issues were included.
[2]Other includes 1 case of peritonitis resulting in death (control), 3 foot injuries (2 control, 1 SCFP), and 1 diarrhea/digestive upset at calving (control).
*Fisher's exact test: $P = 0.02$. No other conditions were significantly affected by treatment.

Figure 3.1: DMI (A), meal count (B), and inter-meal interval (IMI; C) for control cows and cows supplemented with *Saccharomyces cerevisiae* fermentation product (SCFP) from -29 ± 5 days relative to calving through 42 DIM.

An effect of time was present both prepartum and postpartum for all measures ($P < 0.001$). A). DMI did not differ by treatment ($P \geq 0.75$). B) SCFP cows tended to consume more meals per day prepartum ($P = 0.06$) and increased meals per day postpartum ($P = 0.03$). Prepartum standard error of the means = 0.22, postpartum standard error of the means = 0.45. C). SCFP decreased time between meals prepartum ($P = 0.03$) and tended to decrease inter-meal interval postpartum ($P = 0.07$). Prepartum standard error of the means = 0.05, postpartum standard error of the means = 0.09.



C

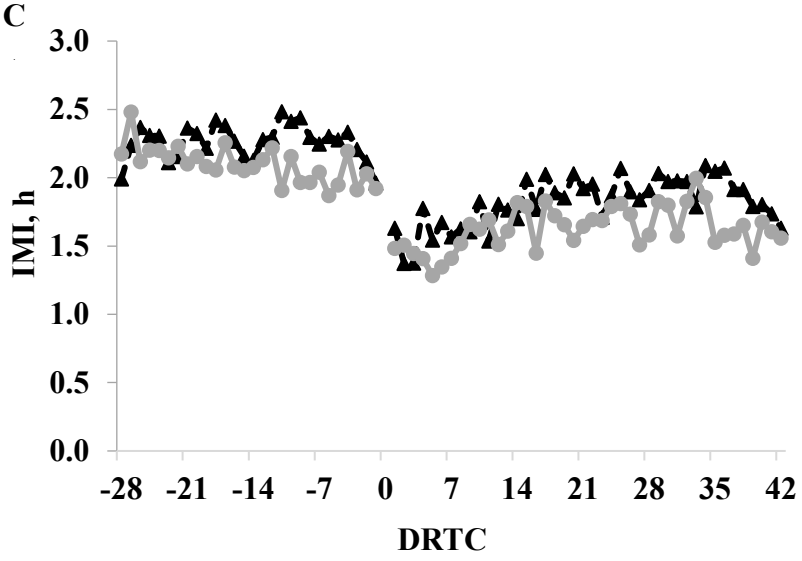


Figure 3.2: Milk and milk fat yield for control cows and cows supplemented with *Saccharomyces cerevisiae* fermentation product (SCFP) from d -29 ± 5 relative to calving through 42 DIM.

Milk yield was not different ($P = 0.43$). There was an effect of wk ($P < 0.001$), but no treatment x wk interaction ($P = 0.24$). Weekly milk fat yield was not different for cows supplemented with SCFP compared to control cows ($P = 0.10$). Milk fat yield differed by wk ($P < 0.001$), and there was a tendency for a treatment x wk interaction ($P = 0.09$). Treatment differences are indicated by *($P < 0.05$) and †($0.05 \leq P < 0.10$). Values are LS means; error bars represent standard errors.

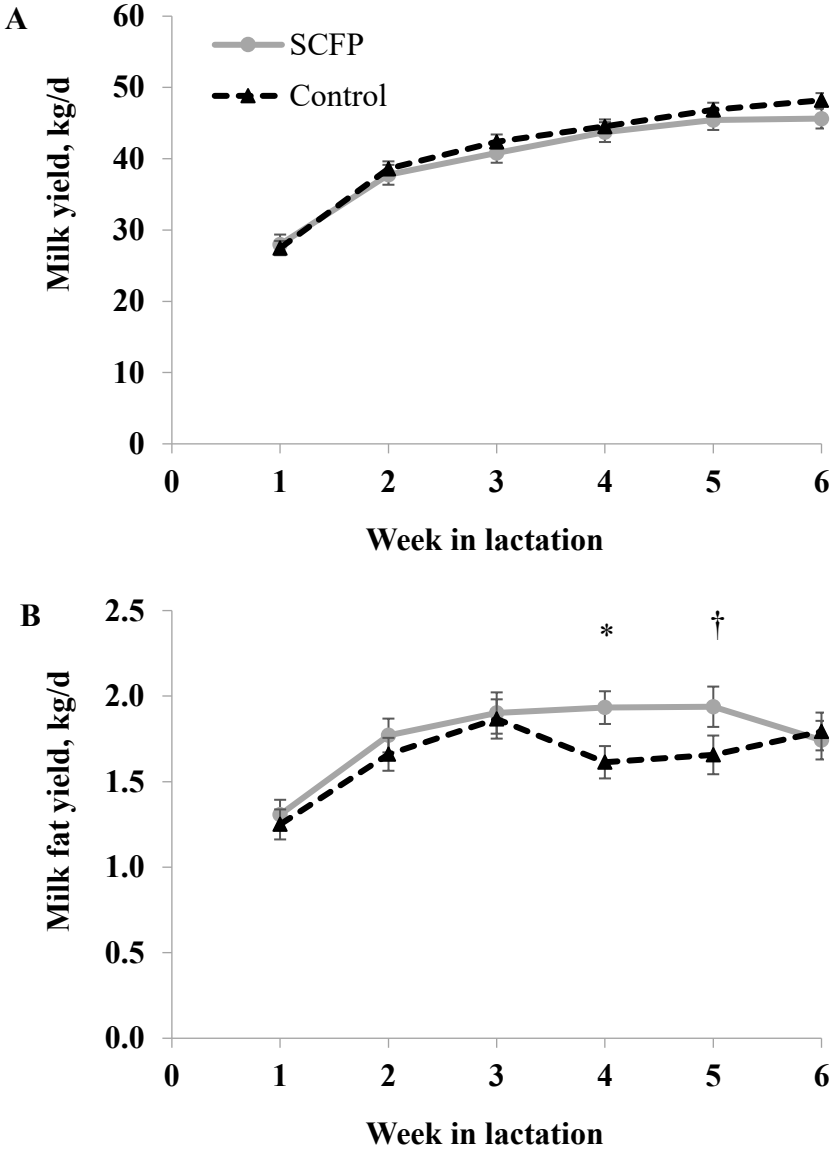


Figure 3.3: Plasma concentrations of free fatty acids (A), BHB (B), glucose (C), and insulin (D) in cows supplemented with *Saccharomyces cerevisiae* fermentation product (SCFP) from d - 29 ± 5 relative to calving through 42 DIM.

Values are LS means; error bars represent standard errors.

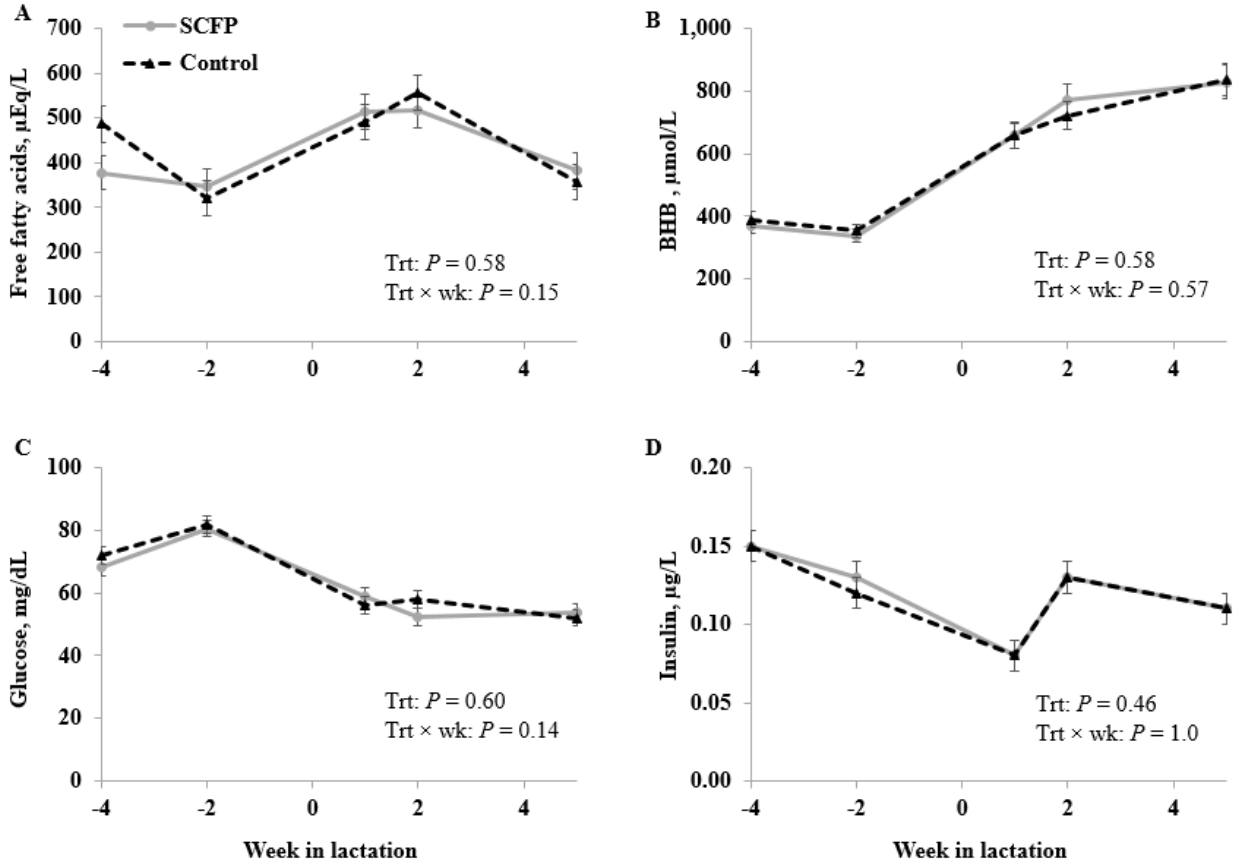
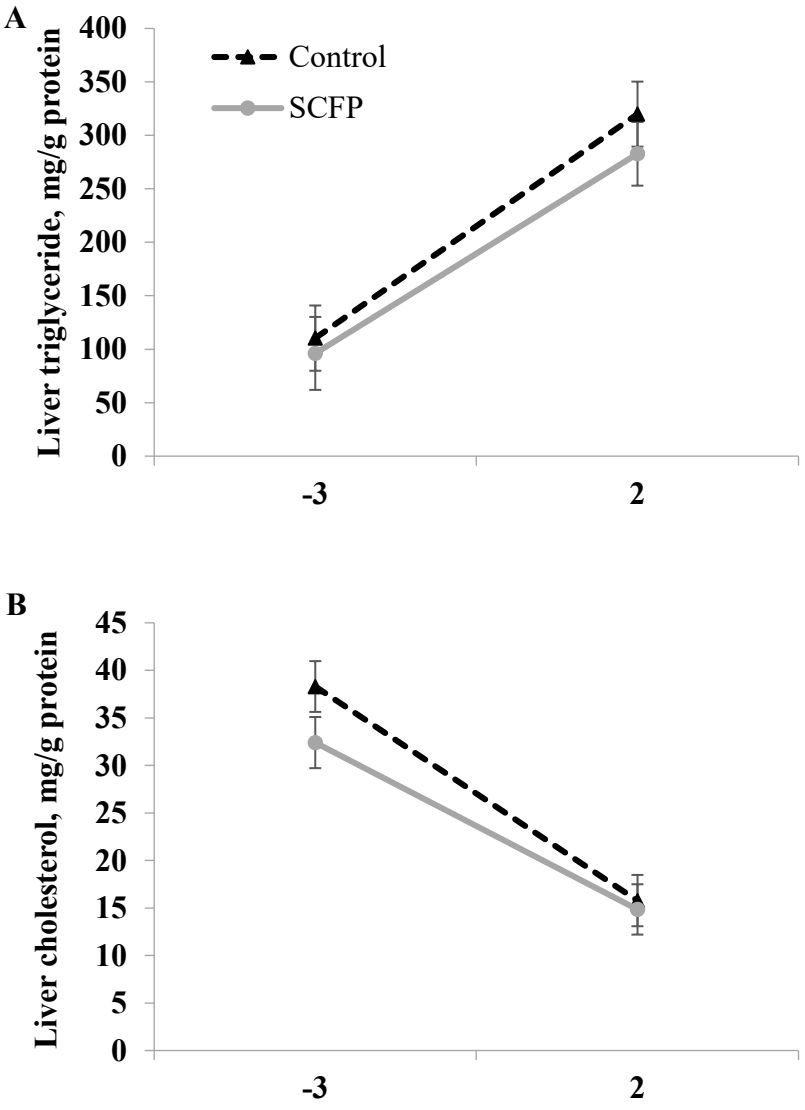


Figure 3.4: Liver triglyceride (A), liver cholesterol (B), and plasma cholesterol (C) content in cows supplemented with *Saccharomyces cerevisiae* fermentation product (SCFP) from d - 29 ± 5 relative to calving through 42 DIM.

A) Liver triglyceride concentration did not differ by treatment ($P > 0.41$). There was an effect of wk ($P < 0.001$), but no treatment x wk interaction ($P = 0.67$). B) Liver cholesterol content tended to be less in cows compared to control cows ($P < 0.10$). There was an effect of wk ($P < 0.001$), but no effect of treatment x wk ($P > 0.30$). C) Plasma cholesterol was greater for SCFP ($P = 0.02$), differed by wk ($P < 0.001$), but there was no treatment x wk interaction ($P = 0.20$). Values are LS means; error bars represent standard errors.



C

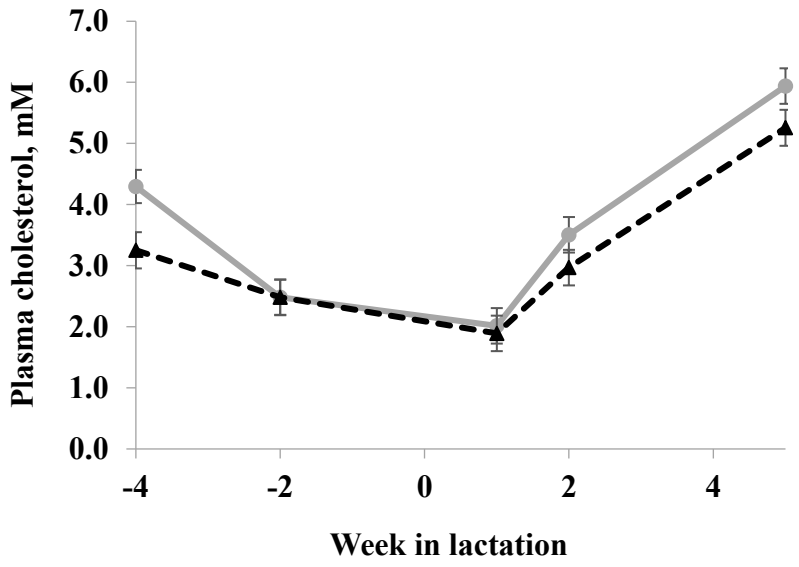
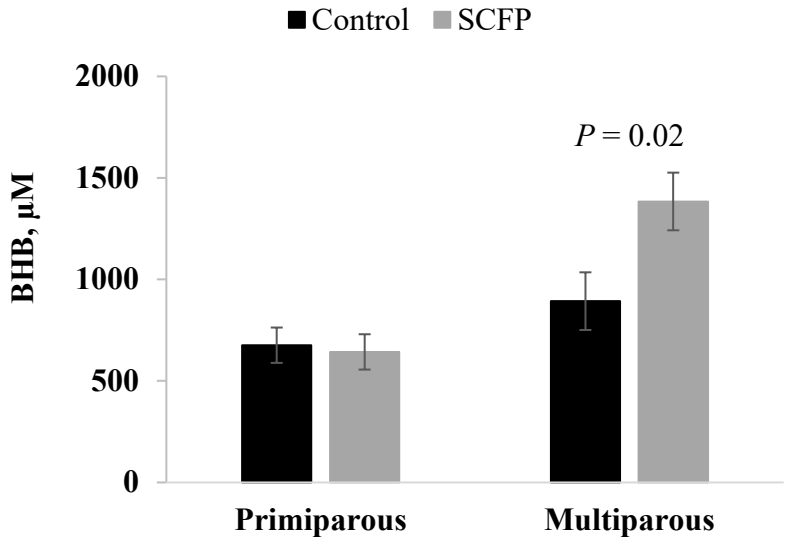

7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Plasma cholesterol, mM
-4
-2
0
2
4
Week in lactation

Figure 3.5: Plasma BHB concentrations for control cows and cows *Saccharomyces cerevisiae* fermentation product (SCFP) from d - 29 ± 5 relative to calving through 42 DIM.

Analyzed independently, wk 2 BHB concentrations demonstrated a significant parity x treatment interaction. Although supplementation SCFP did not impact BHB in primiparous cows, SCFP increased BHB in multiparous cows. Values are LS means; error bars represent standard errors.



Chapter 4 - **Postpartum meloxicam administration reduced ewe inflammatory status and altered plasma fatty acid and oxylipid concentrations but did not influence lamb growth**

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ABSTRACT

Postpartum inflammation is a natural and necessary response; however, a dysfunctional inflammatory response can be detrimental to animal productivity. The objective of this study was to determine the effect of a non-steroidal anti-inflammatory drug (meloxicam) on ewe inflammatory response, plasma fatty acid (**FA**) and oxylipid concentrations, and lamb growth. After lambing, 36 Hampshire and Hampshire × Suffolk ewes were sequentially assigned within type of birth to control (n = 17) or meloxicam orally administered on d 1 and 4 of lactation (**MEL**; 90 mg, n = 19). Milk and blood was sampled on d 1 (prior to treatment) and d 4. Milk was analyzed for glucose-6-phosphate (**G6P**) and glucose and blood for haptoglobin (**Hp**), FA, and oxylipids. Lamb weights at 30, 60, 90, and 120 d were standardized and evaluated as the amount of lamb produced per ewe. Results were analyzed using mixed models with d 1 values as covariates for plasma and milk parameters. Milk G6P was not affected by MEL. Plasma Hp concentrations tended to be less for MEL ewes; control ewes with greater d 1 Hp concentrations were also elevated on d 4, but this was not the case for MEL-treated ewes. Among FA, MEL increased arachidonic acid concentration by more than 4-fold in ewes rearing singles. MEL decreased concentrations of 9,10-dihydroxyoctadecenoic acid, prostaglandin $F_{2\alpha}$, 8-iso-prostaglandin E_2, and 8,9-dihydroxyeicosatetraenoic acid. Nine oxylipids had interactions of treatment with d 1 Hp, all of which revealed positive associations between d 1 Hp and d 4 oxylipid concentrations for CON, but neutral or negative relationships for MEL. MEL decreased 13-hydroxyoctadecadienoic acid (**HODE**):13-oxooctadecadienoic acid ratio, tended to increase 9-HODE:9-oxooctadecadienoic acid ratio (both dependent on d 1 values), and tended to decrease 13-HODE:9-HODE ratio (depending on d 1 Hp), indicating progressive metabolism of linoleic acid-derived oxylipids occurred by enzymatic oxidation after MEL treatment. Meloxicam

reduced oxylipids generated across oxygenation pathways, potentially due to an improved redox state. Overall, postpartum MEL treatment of ewes decreased plasma concentrations of Hp and several oxylipids, with the greatest impact in ewes with biomarkers reflecting a greater inflammatory state before treatment.

INTRODUCTION

Inflammation is a natural and necessary response after parturition; however an uncontrolled inflammatory response can be detrimental to animal productivity. Postpartum inflammation has been well established in dairy cattle (Bradford et al., 2015) with greater degrees of inflammation associated with decreased milk production (Bertoni et al., 2008), increased innate immune response (Nightingale et al., 2015), and decrease hazard of conception (Huzzey et al., 2015). Use of nonsteroidal anti-inflammatory drugs (**NSAID**) to attenuate early lactation inflammation has been successful to increase both early lactation (Shock et al., 2018; Swartz et al., 2018) and whole-lactation milk production (Carpenter et al., 2016).

Increased plasma concentrations of the positive acute phase proteins α1-acid glycoprotein, haptoglobin, and ceruloplasmin, suggests sheep also experience postpartum inflammation (Sheldon et al., 2003). To our knowledge, associations of postpartum inflammatory biomarkers with health and productivity of ewes and lambs have not been evaluated, nor have there been any studies that investigated anti-inflammatory intervention strategies. Meloxicam is non-steroidal anti-inflammatory drug approved for use in Australia, New Zealand, and Canada, but the scope of research has been limited to its analgesic application during events such as lameness (Colditz et al., 2019), castration, tail docking (Small et al., 2014), and mulesing (Paull et al., 2008). If postpartum meloxicam administration induces responses in ewes similar to those reported on with dairy cattle (Carpenter et al., 2016; Swartz et al., 2018), there is potential to improve ewe health and milk production with subsequent increases to lamb growth.

Meloxicam is an NSAID that decreases inflammation by inhibiting cyclooxygenase-2 (**COX-2**), the enzyme responsible for converting fatty acids (**FA**) to oxylipids that include prostaglandins, thromboxanes, and leukotrienes (Steinmeyer, 2000). The inhibitory action of

meloxicam on COX-2 is known, but much remains to be elucidated in regards to how meloxicam changes physiology. The few plasma parameters that have been measured (Hp and serum amyloid A) in response to postpartum meloxicam treatment in cows were unaltered (Mainau et al., 2014; Carpenter et al., 2016; Newby et al., 2017). Despite the direct effect of meloxicam on one of the enzymatic pathways responsible for oxylipid synthesis, to our knowledge investigation of how meloxicam might create shifts within the oxylipid network has been limited to knee synovial fluid in horses (de Grauw et al., 2011) and humans (Valdes et al., 2018).

Our primary objectives were to determine if postpartum meloxicam administration to ewes would reduce systemic inflammation, and if so, potential mechanisms through shifts in plasma FA and oxylipid profiles. Our secondary objective was to determine the effect of meloxicam on offspring growth. We hypothesized postpartum meloxicam administration to ewes would reduce systemic inflammation via reductions in COX-2 derived oxylipids, which in term would increase ewe milk production, leading to greater lamb growth. Moreover, we hypothesized these effects to be more pronounced in ewes rearing twins compared to a single lamb.

MATERIALS AND METHODS

Experimental procedures were approved by the Iowa State University Institutional Animal Care and Use Committee (protocol #5-17-8532-O).

Animals and treatments

Thirty-six ewes lambing during the 2018 winter lambing season at the Iowa State University Sheep Teaching Farm used in a randomized design. At lambing ewes were

sequentially assigned within type of birth to control (**CON**; n = 17) or treatment with meloxicam (**MEL**; n = 19). A dose of 90 mg of meloxicam, approximately 1 mg/kg BW (six 15 mg tables in a #13 veterinary capsule; Torpac Inc., Fairfield, NJ) was administered orally within 24 h of lambing (d 1) and again on d 4 of lactation. Administration time points were chosen for the first to be most proximal time to lambing without hindering placenta expulsion (Newby et al., 2017), and the second to follow 3 d later, based on the 72 h clearance rate in sheep (Shukla et al., 2007; Stock et al., 2013).

Ewes and lambs were housed in a drylot barn from the time of birth until weaning. Ewes typically lambed in communal lambing pens and were then moved into individual 4’ x 5’ postpartum acclimation pens for no more than 48 h. If a lamb was not thriving, the ewe and lamb(s) may have stayed in the individual postpartum pen for up to 96 h. The number of lambs born and reared were recorded. Fewer lambs were reared than born because of lamb death and removal of lamb(s) from ewes with insufficient milk. Descriptive statistics of ewes and lambs pre-treatment are presented in Table 4.1.

To avoid the potential stress associated with frequent sorting and weighing, which could impact lamb growth performance, lambs were weighed and weaned in groups. Lambs were weighed at approximately 30 d of age (32 ± 2 d), weaning (61 ± 6 d of age), and approximately 30 and 60 d post-weaning (90 ± 5 and 120 ± 6 d of age, respectively). To reflect potential treatment effects on the amount of weight gained per ewe, birth weights were removed and lamb weights standardized. For example, 30-d lamb weight gain was calculated by first subtracting lamb birth weight, and then dividing the resulting value by the lamb’s age (in d) to acquire an average daily gain (**ADG**). The lamb’s ADG was then multiplied by 30 to achieve a 30-d weight

gained. Since effect of ewe meloxicam treatment on lamb growth was evaluated as lamb weight gained per ewe, for twin-rearing ewes it represents the combined weight gain of each twin lamb.

Sampling and analysis

Blood samples were collected from each ewe within 24 h of lambing (immediately prior to MEL treatment) and again 3 d later. Samples were collected into 2 evacuated tubes (Thermo Fisher Scientific Inc.,Waltham, MA), one containing heparin and the other K_3EDTA, inverted several times, and placed on ice. Samples were centrifuged at 3,000 x *g* for 15 min, plasma transferred to 1.5 mL microcentrifuge tubes, and stored at -80°C until analyses. Prior to storing, plasma from EDTA tubes were snap frozen in liquid nitrogen for FA and oxylipid analyses. Milk samples were also collected from ewes on d 1 (to avoid colostrum) and 4 of lactation. Milk samples were centrifuged at 1,380 x *g* for 20 min at 4°C. The fat layer was removed, and skim milk was stored at -20°C until analysis of glucose and glucose-6-phosphate (**G6P**).

Milk glucose and G6P concentrations were measured by a fluorometric assay as previously described (Silanikove et al., 2014; Zachut et al., 2016). In short, G6P was determined through enzymatic oxidation by G6P dehydrogenase using NADP+ and the total (both glucose and G6P) was determined by enzymatic oxidation by both G6P dehydrogenase and hexokinase. Results are presented as both G6P concentration and G6P as a percent of total glucose available for phosphorylation.

Haptoglobin (**Hp**) was measured according to the method of (Cooke and Arthington, 2013), a colorimetric assay that measures Hp-hemoglobin complexing via differences in peroxidase activity. Haptoglobin concentrations from the colorimetric assay were validated using a commercial ELISA kit (cat#HAPT-11; Life Diagnostics Inc., West Chester, PA). Trolox

equivalent antioxidant capacity (**TEAC**) was measured using a commercial antioxidant assay kit (#709001; Cayman Chemical; Ann Arbor, MI).

Plasma FA were analyzed with LC-MS and oxylipids by LC-MS/MS according to (Putman et al., 2019). Briefly, 1 mL plasma was mixed with an antioxidant reducing agent mixture (50% methanol, 25% ethanol, and 25% water), butylhydroxytoluene (0.9 mM), EDTA (0.54 mM), triphenyphosphine (3.2 mM), and indomethacin (5.6 mM) to prevent exvivo lipid peroxidation and oxidation of preformed oxylipids (Mavangira et al., 2015). The following internal standards were added to each sample: 5(S)-hydroxyeicosatetraenoic acid-d_8 (0.25 µM), 15(S)-hydroxyeicosatetraenoic acid-d_8 (0.25 µM), 8,9-epoxyeicosatrienoic acid-d_{11} (0.5 µM), prostaglandin E_2-d_9 (0.5 µM), 8,9-dihydroxyeicosatrienoic acid-d_{11} (0.25 µM), arachidonic acid-d_8 (50 µM), 2-arachidonoyl glycerol-d_8 (2 µM), and arachidonoyl ethanolamide-d_8 (0.25 µM) in 15 µL. A 7-point standard curve was generated with a mix of standards and internal standards for quantification.

Solid phase extraction was used for both FA and oxylipids (Putman et al., 2019). Samples were reconstituted in a 2:1 methanol:HPLC-grade water mixture and passed through Acrodisc GHP 13 mm GHP 0.2m syringe filters (Waters, Milford, MA) to remove any particulates. Samples were transferred to glass chromatography vials with glass inserts.

Fatty acids were quantified using a reverse phase LC on a Waters Acquity UPLC with a Supleco (State College, PA) Ascentis Express C18 10 cm × 2.1 mm, 2.7 µm column with a flow rate of 0.35 mL/min at 50˚C coupled to a quadrupole mass spectrometer. Mobile phases included A = acetonitrile, B = methanol, and D = 0.1% formic acid. The gradient mobile phase was programmed as follows (A/B/D ratio): time 0 to 0.2 min (45/22/33), to (80/19/1) at 4.0 min and maintained to 5.0 min, to (45/22/33) at 6 min and held until 8 min. Fatty acids were identified

and quantified by matching mass-1 and retention time with corresponding internal standard and calibrated using a linear 7-point standard curve ($R^2 > 0.99$).

Oxylipids were quantified using a Waters Acquity UPLC connected to a Waters Xevo-TQ-S tandem quadrupole mass spectrometer using multiple reaction monitoring. The Ascentis Express C18 HPLC column (Sigma Aldrich) was set at 50˚C and the autosampler at 10˚C. Flow rate was 0.3 mL/min. Eluents included 0.1% formic acid in water (A) and acetonitrile (B). The 15 min run time was programmed with a linear gradient as follows (A:B ratio): time 0 to 0.5 min (99:1), to (60:40) at 2.0 min, to (20:80) at 8.0 min, to (1:99) until 13.0 min, then returned to (99:1) at 13.01 min, and held until 15.0 min. Oxylipids were detected using electrospray ionization in negative-ion mode. Cone voltage and collision voltages were optimized for each analyte using Waters QuanOptimize software (Mavangira et al., 2015), and data analysis was carried out with Waters TargetLynx software.

Statistical analysis

Ewe plasma data (d 4) were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute, Cary, NC) with the fixed effects of treatment, d 1 covariate values, number of lambs born, and the two-way interactions of these variables, the cubic term for d 1 covariate values and its interaction with treatment, the d 1 covariate for Hp, and the Hp covariate x treatment interaction. Ewe was included as a random effect. Treatment and covariates for outcomes of interest were retained in all models. Unless part of a significant interaction, all other variables were removed from the model when $P > 0.20$. Residual plots were assessed visually for normality. Any parameters violating that assumption were log-transformed prior to analysis with reported data back-transformed.

Weight of lamb raised per ewe at approximately 30, 60, 90, and 120 d of lamb age was analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute, Cary, NC) with the fixed effects of treatment, number of lambs reared, lamb sex, and their two-way interactions, and with the random effect of ewe. Variables were removed from the model when $P > 0.20$.

All models utilized variance components as the covariance structure, and removed observations when Studentized residuals were ≤ -3 or ≥ 3. Significance was declared at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$.

RESULTS

Ewe inflammatory, oxidative balance, and energy balance biomarkers

Plasma Hp was lesser for MEL ewes, indicating reduced inflammatory status of ewes ($P < 0.05$); however, magnitude of the treatment response was dependent on d 1 Hp concentrations ($P = 0.04$; Figure 4.1). As expected, there was a positive relationship between d 1 and d 4 Hp concentrations in control ewes, but meloxicam treatment eliminated this association. Ewes treated with MEL had similar d 4 Hp concentrations regardless of pre-treatment (d 1) concentration. Plasma TEAC, a measure of antioxidant capacity, did not differ with MEL ($P = 0.82$). An indirect indicator of energy balance, milk G6P was unaltered by MEL whether expressed as a concentration or as a percent of milk glucose ($P \geq 0.12$; Table 4.2).

Ewe plasma fatty acid concentrations

Quantified plasma FA included polyunsaturated FAs (**PUFA** [linoleic acid (**LA**), arachidonic acid (**ArA**), eicosapentaenoic acid, dihomolinolenic acid, adrenic acid, and docosahexaenoic acid], the monounsaturated FA oleic acid, and saturated FA (lauric, palmitic,

and stearic acids). Plasma FA are reported as concentrations in Table 4.3 and as a percent of total FAs in Table 4.4. Among FA, ArA was the only FA altered by MEL, with concentrations increased by more than 4-fold in ewes rearing singles ($P < 0.01$ main effect and interaction; Figure 2).

Expressed as a percent of total FA, treatment only altered proportions of palmitic and oleic acids. Overall proportion of oleic acid was greater for control, but had opposite changes with time, decreasing for control but increasing in MEL ewes after treatment ($P = 0.01$). Similarly, proportion of palmitic acid was greater for control ewes, but as degree of initial inflammation increased, the proportion of palmitic acid tended to decrease in control but increase in MEL ewes ($P = 0.05$). Proportions of lauric acid and ArA were greater in ewes rearing a single lamb vs. twins (8.75 vs. 4.41 ± 0.93% and 3.25 vs. 2.08 ± 0.35%, respectively).

Ewe oxylipid profile

Plasma samples were analyzed for 57 oxylipids, 36 of which were detected, and 31 statistically analyzed. Although detectable, concentrations of PGD_2, 11,12- epoxy-eicosatrienoic acid [**EET**], 14,15-EET, 6-keto-prostaglandin (**PG**) $F_{1\alpha}$, and 10,17- dihydroxydocosahexaenoic acid were very low and lacked sufficient variation to detect treatment differences. Effects of MEL on oxylipids are summarized by biosynthesis pathway in Tables 4.5-9. Overall shifts to the oxylipid network can be visualized in Figure 4.3.

The interaction between treatment and initial Hp concentration was significant or tended to be significant for many oxylipids (12- hydroxyeicosatrienoic acid [**HHTrE**], 11-hydroxyeicosatetraenoic acid [**HETE**], 9-hydroxyoctadecadienoic acid [**HODE**], 9-oxooctadecadienoic acid [**oxoODE**], 15-HETE, 9,10-epoxyoctadecenoic acid [**EpOME**], 20-

HETE, 14,15-dihydroxyeicosatetraenoic acid [**DHET**], and 9-HETE; $P < 0.05$; Figure 4.4; 8,9-DHET and 19,20- dihydroxydocosapentaenoic acid [**DiHDPA**]; $P < 0.10$). Generally, the observed interaction indicated a positive association between initial inflammatory status and oxylipid in control ewes, but a negative relationship for MEL ewes.

The detected COX-derived oxylipids were all metabolites of ArA (Table 4.5). The only main effect of MEL on COX-derived oxylipids was decreased $PGF_{2\alpha}$.

The lipoxygenase (**LOX**)-derived oxylipid concentrations are shown in Table 4.6. Treatment effects on the LA metabolites 13-HODE and 9-HODE depended or tended to depend on d 1 covariate values ($P < 0.01$ and = 0.07). Greater 13-HODE concentrations were positively related to d 4 concentrations for control, but negatively associated for MEL. Concentrations of 9-HODE were greater for MEL, and again there was a positive relationship between d 1 and 4 values in control ewes; however, post-treatment concentrations in MEL were, independent of d 1 covariate values (cov × trt: $P < 0.01$). The ALA metabolite 13-hydroxyocctadecatrienoic acid (**HOTrE**) also tended to have a covariate × treatment interaction, with greater d 1 values related to decreased concentrations on d 4 for control, but little difference for MEL ($P = 0.08$). Ewes rearing twins had greater concentrations of 9-HODE (39.9 vs. 57.4 ± 4.8 μM; $P = 0.03$), 9-oxoODE (10.7 vs. 16.2 ± 1.3 μM), and 5-HETE (-0.12 vs. 0.51 ± 0.23 μM) compared to singles. Concentration of 17- hydroxyl-docosahexaenoic acid (**HDoHE**) tended to have a treatment × lamb interaction with MEL attenuating the drop in HDoHE otherwise seen in twin bearing ewes (MEL: 1.03 vs. 0.96 ± 0.29 μM, CON: 1.92 vs. 0.66 ± 0.33; $P = 0.07$).

Effect of MEL on cytochrome P450 derived oxylipids are show in Table 4.7. MEL tended to decrease 17,18- dihyroxy-eicosatetraenoic acid (**DiHETE**; $P = 0.07$). Concentrations of 9,10-DiHOME decreased with MEL, with the largest effect in ewes with greater

concentrations initially ($P = 0.03$). MEL also decreased the ArA metabolite 8,9-DHET, and the cov^2 × treatment interaction showed MEL prevented the decrease in d 4 concentrations for ewes with greater initial 8,9-DHET ($P = 0.05$). Ewes raising twins had greater concentrations of 9,10-dihydroxyoctadecenoic acid (**DiHOME**; 18.0 vs. 21.7 ± 1.1 μM), 8,9-DHET (0.66 vs 0.81± 0.18 μM), 17,18-DiHETE (36.5 vs. 47.8 ± 2.5 μM), and 19,20-DiHDPA (1.83 vs. 2.96 ± 0.24 μM; all $P \leq 0.04$), and a tendency for greater concentrations of 14,15-DHET and 14,15-DiHETE (2.34 vs. 2.85 ± 0.16 μM and 4.78 vs. 5.92 ± 0.40 μM, respectively; $P = 0.07$) compared to ewes rearing a single lamb.

MEL effects on oxylipids formed through non-enzymatic (**NE**) oxidation were mostly dependent on number of offspring reared and initial inflammatory status (Table 4.8). Concentrations of 8-iso-PGE_2 were similar amongst control ewes raising singles, control ewes raising twins, and MEL ewes raising twins (0.51, 0.97, and 0.48 ± 1.5 μM, respectively), but lesser for MEL ewes raising singles (0.06 ± 1.4 μM). Control ewes with twins had the least 8-iso-PGA_2 concentration (0.28 ± 0.07 μM), control and MEL ewes with singles were intermediate (0.52 and 0.41 ± 0.09 μM) and MEL ewes with twins were the greatest (0.49 ± 0.07 μM). Treatment effect on 9-HETE was based on covariate values, in which 9-HETE decreased from initial values for control, but remained greater at d 4 for those treated with MEL ($P = 0.04$; Figure 4H).

The effect of MEL on the relative contribution of enzymatic (LOX) or NE oxidation of LA was assessed by the ratio of 13-HODE:9-HODE. Both can be generated through LOX, but 9-HODE is largely derived from NE oxidation (Mavangira et al., 2015). There was a tendency for a Hp × treatment interaction in which the ratio increased in response to MEL for ewes with greater initial inflammation in contrast to the decrease seen in control ($P = 0.08$; Figure 4.5A).

Thus, during an inflammatory state, MEL tended to increase the relative proportion of LA oxidized enzymatically versus non-enzymatically.

Differences in the progressive metabolism of oxylipids within certain pathways was assessed through ratios of hydroxyl oxylipids to their ketone derivatives (HODE to oxoODE) and epoxides to vicinal diols (EpOME to DiHOME). Ratios of 9-HODE:9-oxoODE and 13-HODE:13-oxoODE were both greater than, which signifies greater abundance of the hydroxyl oxylipids relative to their ketone derivatives. Initial 9-HODE:9-oxoODE tended to be positively related to values on d 4 for MEL but negatively related for control ($P = 0.06$; Figure 4.5B). This ratio also tended to be related to initial inflammatory status with greater initial Hp related to slight increases for MEL and decreases for control ($P \leq 0.10$). In contrast, the 13-HODE:13oxoODE ratio was less with MEL, with initial values positively related to d 4 values in control but negatively related in MEL ($P = 0.04$; Figure 4.5C). Progressive metabolism of 9,10-EpOME tended to be lesser for MEL ($P = 0.06$); however, unlike the HODE:oxoODE ratios, there was a greater proportion of the downstream metabolite (ratio < 1).

Lamb growth

Lamb weight produced per ewe did not differ by treatment at 30, 60, 90, or 120 d of life (Table 4.10). Unsurprisingly, type of rearing affected lamb weight produced, with ewes rearing twins producing more lamb weight than singles for all time points ($P < 0.001$).

DISCUSSION

Postpartum inflammation is a natural and necessary response to parturition; however, a dysfunctional inflammatory response has been attributed to health disorders and decreased

animal productivity(Bradford et al., 2015; Mavangira and Sordillo, 2018). Several studies in dairy cattle have investigated NSAID administration around parturition to attenuate inflammation, but to our knowledge this is the first study to apply this intervention strategy to sheep. Our 2-dose regimen of MEL on d 1 and 4 after lambing decreased plasma haptoglobin concentrations, and the reduction most pronounced for ewes with greater biomarkers of inflammation prior to treatment.

Although meloxicam is a selective inhibitor of COX-2 (Dennis and Norris, 2015), typically treatment strategies that only target one enzymatic oxygenation pathway also have unpredicted effects on the entire oxylipid network (Sordillo, 2018). For a thorough understanding of MEL effects on plasma oxylipid concentrations, we examined differences at each of the following levels that control oxylipid biosynthesis: 1) substrate (PUFA) availability, 2) products of multiple oxygenation pathways, and 3) the degree to which intermediate metabolites were catabolized to their end products (Willenberg et al., 2015).

The only difference we observed for PUFA concentrations was a 4-fold increase in ArA for MEL ewes bearing singletons. Although PUFA can be oxidized by each pathway, some pathways have a substrate preference (Sordillo, 2018). In the case of COX-2, ArA is preferentially oxidized. Given MEL's inhibition of COX-2, it is seemingly logical for the substrate to accumulate; however, ArA oxidation is expected to shift to other pathways in this scenario (Dennis and Norris, 2015). Additionally, it is unclear why MEL treatment decreased, rather than increased, ArA concentration in ewes with greater initial Hp concentrations.

Direct downstream effects of COX inhibition were observed through the overall reduction in $PGF_{2\alpha}$ and decreased HHTrE in MEL ewes with greater pretreatment inflammation. As all COX-derived oxylipids detected in this study were metabolites of ArA, the observed

effect for HHTrE could partially be attributed to reduced substrate availability. Effects on $PGF_{2\alpha}$ but not other prostanoids (PGD_2, PGE_2, PGI_2) generated from the same substrate (PGH) could be due to the timing of our sample relative to the stage of inflammation (Tilley et al., 2001). For example, PGE_2 is elevated during the early stages of inflammation compared to PGD_2, which is more prevalent during the final stages of the response (Gilroy et al., 1999).

Meloxicam effects extended to oxylipids derived from the other oxygenation pathways, with the effects largely related to alterations in redox state and inflammatory status. Isoprostanes are prostaglandin-like compounds that are only produced when there is a significant shift in redox state (Mavangira and Sordillo, 2018). They are considered the gold standard biomarkers of oxidative stress (Kuhn et al., 2018). Therefore, the observed decrease in 8-iso-PGE_2 indicates MEL reduced oxidative stress.

Oxylipids formed by NE oxidation also serve as indicators of oxidative stress and include 9-HETE, 11-HETE, and 9-HODE. Both 9-HETE and 11-HETE were decreased in MEL ewes with greater degrees of initial inflammation, indicating improved redox status. As both are predominately derived by NE oxidation of ArA, reduced substrate availability could partially explain the reductions in 9- and 11-HETE. Kuhn et al. (2017) reported a similar scenario in which milk concentration of 11-HETE was elevated in early lactation; however, the significant correlation with milk ArA ($r = 0.60$) only partially explained the elevation. The greater oxidative environment of the mammary gland also contributed. Since the LA-derived 9-HODE can be produced both enzymatically via LOX or through NE oxidation, it can also serve as a marker of oxidative status (Kuhn et al., 2017). Similar to 9 and 11-HETE, 9-HODE concentration was less in MEL ewes with greater degrees of initial inflammation, but since LA concentration was not

different with MEL, this result can be more confidently attributed to the presence of fewer oxidants.

Decreased concentrations of oxylipids that contribute to a prooxidant environment would also indicate improved oxidative status. 20-HETE is a CYP derived oxylipid that is not only a prooxidant itself, but also indirectly exacerbates oxidative stress via stimulation of mitochondrial reactive oxygen species production and activation of NADPH oxidase enzymes (Han et al., 2013; Waldman et al., 2016). Meloxicam decreased 20-HETE concentration in ewes with greater initial inflammation, which suggests decreased presence of reactive metabolites and thus improved redox status.

The initial products from 15-LOX oxidation of ArA and LA, 15-HPETE and 13-HPODE, respectively, are also highly reactive and greatly contribute to oxidative stress (Mattmiller et al., 2013). Prior to LC-MS quantification, 15-HPETE and 13-HPODE had to be reduced to their hydroxyl and hydroperoxy derivatives. Thus, values for 15-HETE and 13-HODE represent the combined concentrations of the hydroperoxides and their hydroxyl derivatives. MEL decreased concentration of 15-HETE in ewes with greater initial inflammation. Although relative contributions of 15-HPETE and 15-HETE cannot be teased apart in our data, our observed decrease in 15-HETE could have been driven by reductions in 15-HPETE. This alteration would further support improved oxidative stress through decreased reactive metabolite availability.

Soluble epoxide hydrolase (**sEH**), the enzyme that catalyzes the further metabolism of EETs to DHETs and EpHOMEs to DiHOMEs, is upregulated by prooxidant status (Abdelhamid and El-kadi, 2015). Evaluation of ratios between these oxylipids and their downstream metabolites can provide some insight into sEH activity. The tendency for MEL to increase 9,10-EpOME/9,10-DiHOME could be the result of decreased sEH activity because of improved redox

status. As DiHOME are more toxic than EpOME (Slim et al., 2001), the greater ratio in MEL ewes is preferable. Ratios of metabolites from CYP oxidation, EETs to DHETs, could not be evaluated. Although detected in some samples, EET concentrations were not statistically analyzed due to low concentrations with little variability. MEL did decrease 8,9-DHET overall and decreased 12,15-DHET in ewes with greater initial inflammation. Again, these reductions could indicate decreased sEH activity, but without knowing concentrations of their substrate precursors, we couldn't differentiate between MEL shifting whole pathway flux vs. progression of oxidation within a pathway.

The progressive metabolism of LA-derived oxylipids through the LOX pathway was also shifted. The ratios of 13-HODE:13-oxoODE and 9-HODE:9-oxoODE indicated further metabolism of 13-HODE to 13-oxoODE and less oxidation of 9-HODE to 9-oxoODE for MEL ewes compared to control. The further progression to 13-oxoODE is favorable because of its anti-inflammatory properties as a PPAR gamma ligand (Altmann et al., 2007). The increase in 13-HODE:9-HODE for MEL ewes with greater degrees of initial inflammation indicates a preference for LA oxidation through the 15-LOX pathway over 5-LOX or NE pathways.

Oxylipids largely regulate inflammation by influencing the development of oxidative stress (Mavangira and Sordillo, 2018). Our data reveals improved oxidant status for ewes treated with MEL, especially for those with greater initial degrees of inflammation. The haptoglobin covariate × treatment interaction was significant for 9 oxylipids with an additional 2 tending to be significant. The interaction for each of these were in the same direction: decreased oxylipid concentrations for MEL ewes with greater inflammation that were otherwise increased for control ewes. The fact that characteristically pro- and anti-inflammatory oxylipids were both altered in a similar manner demonstrates the natural balance within the complex oxylipid

network. Not only does it seem oxylipids balance each other, but individual oxylipids can have different effects based on the receptor present on target cells or stage of inflammation. For example, 8-iso-PGE_2 promotes vasoconstriction when working through the thromboxane receptor, but vasodilation through the PGE_2 prostanoid receptor (Milne et al., 2015). Overall, our results demonstrate MEL decreased systemic inflammation in ewes with greater degrees of initial inflammation, in part because of alterations to oxylipid biosynthesis across multiple oxidation pathways.

The many interactions we observed with initial inflammatory status could be a possible explanation to the inconsistency of NSAID response in transition dairy cattle studies. For example, Carpenter et al. (Carpenter et al., 2016) reported substantial whole-lactation milk yield responses after oral administration of sodium salicylate to dairy cows for 3 d after calving; however, when the study was later replicated, no difference in milk production through 120 d was observed. (Carpenter et al., 2018). As plasma haptoglobin concentrations were nearly 3-fold greater in the former cohort (~600 vs. 200 μg/mL), authors speculated a milk response to NSAID treatment could be dependent on baseline inflammation (Carpenter et al., 2018). As discussed throughout the paper, our data supports the notion that response to postpartum NSAID treatment in ruminants is dependent on initial inflammatory status.

Despite MEL decreasing inflammation, no treatment difference was observed for milk G6P, an indirect indicator of energy balance. Zachut and others (Zachut et al., 2016) have demonstrated a negative linear correlation between milk G6P concentration and energy balance ($r = -0.45$). As systemic inflammation is correlated with decreased feed intake, we hypothesized alleviation of inflammation would promote feed intake and greater energy balance. Feed intake and milk yield data were not available, therefore analysis of milk G6P was employed to gain

some insight into energy balance. To our knowledge, this is the first experiment to report milk G6P concentrations in sheep milk; values for controls were only slightly lower than concentrations reported for dairy cattle on d 3 of lactation (200– 350 μM; (Olagaray et al., 2019)).

Postpartum meloxicam treatment of ewes did not affect lamb weight produced per ewe (a proxy of lamb growth); however, our small sample size likely limited our ability to detect a statistical difference. Future studies should reevaluate the hypothesis that postpartum MEL might increase ewe milk production and thereby increase lamb growth, especially in regards to ewes bearing multiple offspring. Even though ewes rearing twin lambs produce 17-61% more milk than ewes suckling single lambs (Cardellino and Benson, 2002), the nutrient supply is split between the 2 lambs, resulting in 60-80% of the nutrients; 20-40% less compared to a singleton. Increasing nutrient supply via increased milk production could increase pre-weaning lamb growth, thereby increasing profit potential for sheep producers.

CONCLUSIONS

Postpartum meloxicam administration decreased ewe inflammatory status as measured by plasma haptoglobin, with reductions greatest for those with greater initial haptoglobin concentrations. MEL increased plasma ArA concentrations in ewes bearing singletons, but decreased ArA in ewes with greater initial inflammation. Meloxicam also decreased plasma concentrations of an array of oxylipids extending across different PUFA substrates and oxidation pathways, and altered their progressive metabolism. Many of the oxylipid MEL effects pointed to improved redox state that paralled the reductions in inflammation. No differences in lamb growth were detected, but future research with a larger sample size is warranted.

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LIST OF ABBREVIATIONS

ArA = arachidonic acid; COX = cyclooxygenase; CYP = cytochrome P450; DiHDoHE = dihydroxydocosahexaenoic acid; DiHDPA = dihydroxydocosapentaenoic acid; DiHETE = dihyroxy-eicosatetraenoic acid; DiHOME = dihydroxyoctadecenoic acid; DHET = dihydroxyeicosatetraenoic acid; EET = epoxy-eicosatrienoic acid; EpDPE = epoxydocosapentaenoic acid; EpOME = epoxyoctadecenoic acid; FA = fatty acid; HDoHE = hydroxyl-docosahexaenoic acid; HETE = hydroxyeicosatetraenoic acid; HHTrE= hydroxyeicosatrienoic acid; HODE = hydroxyoctadecadienoic acid; HOTrE = hydroxyocctadecatrienoic acid; LnA = linoleic acid; LOX = lipoxygenase; LXA_4 = lipoxin A_4; MEL = meloxicam; NE = nonenzymatic oxidation; OxoODE = oxooctadecadienoic acid; PG = prostaglandin; PUFA = polyunsaturated fatty acid; ReD = resolvin; sEH: soluble epoxide hydrolase; TXB2 = thromboxane B2

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TABLES AND FIGURES

Table 4.1. Descriptive statistics for ewes, control or treated with meloxicam on d 1 and 4 after lambing, and lambs.

	Control		Meloxicam	
	Average ± sd	**Range**	**Average ± sd**	**Range**
Ewe data				
n	17		19	
Breed				
Hampshire	12 (71%)		16 (84%)	
Hampshire x Suffolk	5 (29%)		3 (16%)	
Parity	4.2 ± 2.2	1-8	3.2 ± 2.1	1-7
Weight, kg	97.5 ± 15.1	73.5 – 122.0	90.4 ± 17.3	64.9 – 142.4
Total no. lambs born/trt	17		19	
No. lambs born/ewe		1-2		1-3
1	6 (33%)		6 (32%)	
2	11 (56%)		12 (63%)	
3	0		1 (5%)	
No. lambs reared		1-2		1-2
Total no. lambs reared	27		31	
1	7 (41%)		7 (37%)	
2	10 (59%)		12 (63%)	
Sire Breed				
Hampshire	13 (76%)		16 (84%)	
Hampshire x Suffolk	4 (24%)		3 (16%)	
Lamb data				
Sex of lamb				
Male	16(59%)		16 (48%)	
Female	11 (41%)		15 (52%)	
Birthweight, kg	6.7 ± 0.7	5.2-8.4	6.4 ± 1.0	4.5-8.6

Table 4.2. Plasma biomarkers of inflammation (haptoglobin) and antioxidant capacity (TEAC) and milk markers of energy balance (G6P) in ewes treated with meloxicam at d 1 and 4 after lambing.

				P-values[1]		
	CON	**MEL**	**SEM**	**Trt**	**Cov**	**Cov x Trt**
Plasma						
Haptoglobin, μg/mL	2063	1713	275	< 0.05	NS	0.04
TEAC, mM	1.00	1.00	0.02	NS	NS	NS
Milk						
G6P[3], μM	190.4	218.0	13.8	NS	0.01	0.09
G6P[4], % of glucose	76.2	82.6	2.80	NS	NS	NS

[1]NS: $P > 0.10$; Cov: effect of d 1 covariate
[2]Cov × cov: $P = 0.01$; Cov × cov × trt: $P = 0.08$
[3]Effect of haptoglobin covariate: $P = 0.05$

Table 4.3 Plasma total fatty acid concentrations (μM) in control ewes and ewes treated with 90 mg meloxicam on d 1 and 4 after lambing.

					P-values[1]		
Fatty acid		**CON**	**MEL**	**SEM**	**Trt**	**Cov**	**Lambs**
Lauric acid	C12:0	17.74	16.55	2.19	NS	NS	NS
Palmitic acid	C16:0	35.22	38.61	7.63	NS	NS	< 0.05
Stearic acid	C18:0	22.11	16.19	6.57	NS	NS	NS
Oleic acid	C18:1	6.73	9.22	0.70	NS	0.09	< 0.01
Linoleic acid[2]	C18:2 (n-6)	97.61	86.50	16.07	NS	< 0.05	< 0.01
α-linolenic acid	C18:3 (n-3)	60.50	61.20	14.27	0.16	NS	< 0.01
Arachidonic acid[3]	C20:4 (n-6)	4.81	10.15	1.66	< 0.01	0.01	< 0.01
Eicosapentaenoic acid	C20:5 (n-3)	1.13	0.94	0.20	NS	NS	0.06
Dihomo-linolenic acid	C20:6 (n-6)	0.33	2.22	0.34	NS	0.03	NS
Adrenic acid	C22:4	0.016	0.013	0.002	NS	0.09	NS
Docosahexaenoic acid	C22:6 (n-3)	4.11	3.55	0.70	0.15	0.11	0.02
Total		263.6	289.6	40.4	NS	NS	< 0.01

[1]NS: $P > 0.20$; Cov: effect of d 1 covariate
[2]loghapto x trt: $P = 0.03$.
[3]Cov × trt: $P = 0.01$; cov x cov: $P = 0.04$; cov x cov x trt: $P = 0.01$; Trt × lambs: $P < 0.01$; loghaptocov: $P < 0.05$; loghaptocov x trt: $P < 0.01$.

Table 4.4. Individual fatty acids as a percent of total plasma fatty acid concentrations in control ewes and ewes treated with meloxicam on d 1 and 4 after lambing.

					P-values[1]			
Fatty acid, % of total		**CON**	**MEL**	**SEM**	**Trt**	**Cov**	**Cov x Trt**	**Lambs**
Lauric acid	C12:0	6.41	6.02	0.97	NS	NS	NS	< 0.01
Palmitic acid[2]	C16:0	14.3	12.2	1.68	NS	< 0.05	NS	NS
Stearic acid	C18:0	7.08	5.00	2.08	NS	NS	NS	NS
Oleic acid[3]	C18:1	2.81	3.46	0.32	0.17	NS	< 0.01	NS
Linoleic acid	C18:2 (n-6)	33.34	32.09	3.05	NS	0.01	NS	NS
α-linolenic acid	C18:3 (n-3)	25.79	24.64	3.44	NS	NS	NS	NS
Arachidonic acid	C20:4 (n-6)	2.65	2.54	0.33	NS	0.04	NS	0.02
Eicosapentaenoic acid	C20:5 (n-3)	0.39	0.32	0.05	NS	NS	NS	NS
Dihomo-linolenic acid	C20:6 (n-6)	0.16	0.16	0.03	NS	0.03	NS	NS
Adrenic acid	C22:4	0.004	0.004	0.001	NS	NS	NS	NS
Docosahexaenoic acid	C22:6 (n-3)	1.38	1.36	0.18	NS	0.07	NS	NS

[1]NS: $P > 0.10$; Cov = d 1 covariate values
[2]Loghaptocov × trt: $P = 0.05$
[3]Oleiccov x oleiccov x trt: $P = 0.01$.

Table 4.5. Cyclooxygenase-derived oxylipids in plasma in control ewes and ewes treated with 90 mg meloxicam on d 1 and 4 after lambing (mean ± SEM; μM)

					P-values[1]			
Oxylipid[2]	**Substrate[3]**	**CON**	**MEL**	**SEM**	**Trt**	**Cov**	**Cov × cov**	**Hpcov x trt**
PGE_2	ArA	0.19	0.15	0.03	NS	0.10	NS	NS
$PGF_{2\alpha}$	ArA	0.21	0.09	0.03	< 0.01	< 0.01	< 0.01	0.10
12-HHTrE	ArA	0.79	0.98	0.11	NS	NS	NS	0.04
TXB_2	ArA	1.67	2.12	0.78	NS	NS	NS	NS

[1]NS: $P > 0.10$; Cov = d 1 covariate values
[2]PG = prostaglandin; HHTrE = hydroxyeicosatrienoic acid; TXB_2 = thromboxane B_2; HETE = hydroxyeicosatetraenoic acid.
[3]ArA = arachidonic acid.

Table 4.6. Lipoxygenase-derived oxylipids in plasma of control ewes and ewes treated with 90 mg meloxicam on d 1 and 4 after lambing (mean ± SEM; µM)

					P-values				
Oxylipid[2]	Substrate[3]	CON	MEL	SEM	Trt	Cov	Cov × trt	Lambs	Hpcov x trt
9-HODE	LA	52.5	44.9	4.2	NS	0.01	0.07	0.03	< 0.01
9-oxoODE	LA	13.99	12.37	1.24	NS	NS	NS	0.01	0.02
13-HODE	LA	133.0	124.8	8.45	NS	NS	< 0.01	NS	NS
13-oxoODE	LA	1.90	2.55	0.31	NS	NS	NS	NS	NS
5-HETE	ArA	0.10	0.29	0.22	NS	NS	NS	0.07	NS
15-HETE	ArA	2.03	1.96	0.16	NS	0.02	NS	NS	0.02
5,6-LXA_4	ArA	0.13	0.16	0.03	NS	NS	NS	NS	NS
13(S)-HOTrE[4]	ALA	130.8	100.3	28.3	NS	0.08	0.08	NS	NS
17-HDoHE[5]	DHA	1.29	0.99	0.22	NS	< 0.01	NS	< 0.05	NS
RvD_2	DHA	0.30	0.17	0.06	NS	NS	NS	NS	NS

[1]NS = $P > 0.10$; Cov = d 1 covariate values

[2]HODE = hydroxyoctadecadienoic acid; OxoODE = oxooctadecadienoic acid; HETE = hydroxyeicosatetraenoic acid; LXA_4 = lipoxin A_4; HOTrE = hydroxyocctadecatrienoic acid; HOTrE = hydroxyoctadecatrienoic acid; HDoHE = hydroxyl-docosahexaenoic acid; RvD_2 = resolvin D_2.

[3]LA = linoleic acid; ArA = arachidonic acid; ALA = α-Linolenic acid; DHA = docosahexaenoic acid.

[4]Cov × cov: $P = 0.08$; cov × cov × trt: $P = 0.08$.

[5]Cov × cov: $P = 0.02$; trt × lambs: $P = 0.07$.

Table 4.7. Cytochrome P450-derived oxylipids in plasma in control ewes and ewes treated with 90 mg meloxicam on d 1 and 4 after lambing (mean ± SEM; µM)

					P-values[1]					
Oxylipid[2]	Substrate [3]	CON	MEL	SEM	Trt	Cov	Cov × cov	Cov × trt	Lambs	Hpcov x trt
9,10-EpOME	LA	8.20	8.12	0.52	NS	< 0.01	NS	NS	NS	0.03
9,10-DiHOME	LA	22.13	17.55	1.05	< 0.01	< 0.10	NS	0.03	0.02	NS
12,13-EpOME	LA	24.30	24.09	1.68	NS	-	-	-	-	-
20-HETE	ArA	5.15	5.26	0.48	NS	NS	NS	NS	NS	0.04
8,9-DHET[4]	ArA	1.11	0.36	0.19	0.04	0.10	0.04	NS	0.04	0.07
11,12-DHET	ArA	1.48	1.48	0.13	NS	0.10	NS	NS	NS	NS
14,15-DHET	ArA	2.72	2.47	0.15	NS	< 0.001	< 0.01	NS	0.07	0.03
14,15-DiHETE	EPA	5.64	5.06	0.38	NS	< 0.01	< 0.01	0.10	0.07	NS
17,18-DiHETE	EPA	45.16	39.07	2.35	0.07	< 0.001	< 0.001	NS	< 0.01	NS
19,20-EpDPE	DHA	3.29	4.06	0.51	NS	NS	-	-	-	-
19,20-DiHDPA	DHA	2.31	2.47	2.39	NS	< 0.01	0.02	NS	< 0.01	0.09

[1]NS = $P > 0.10$; Cov = d 1 covariate values

[2]EpOME = epoxyoctadecenoic acid; DiHOME = dihydroxyoctadecenoic acid; HETE = hydroxyeicosatetraenoic acid; DHET = dihydroxyeicosatetraenoic acid; DiHETE = dihyroxy-eicosatetraenoic acid; EpDPE = epoxydocosapentaenoic acid; DiHDPA = dihydroxydocosapentaenoic acid.

[3]LA = linoleic acid; ArA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

[4]Cov × cov × trt: $P = 0.05$.

Table 4.8. Nonenzymatic-derived oxylipids in plasma in control ewes and ewes treated with 90 mg meloxicam on d 1 and 4 after lambing (mean ± SEM; µM).

					P-values[1]				
Oxylipid[2]	Substrate[3]	CON	MEL	SEM	Trt	Cov × trt	Lambs	Trt x lambs	Hpcov x trt
5-iso-$PGF_{2\alpha}$-VI	ArA	0.39	0.45	0.04	NS	-	-	-	-
8-iso-PGA_2	ArA	0.40	0.45	0.06	NS	NS	NS	0.07	NS
8-iso-PGE_2[4]	ArA	0.70	0.16	0.12	< 0.01	NS	< 0.01	0.08	NS
8,12-iso-$PGF_{2\alpha}$-VI	ArA	0.40	0.42	0.04	NS	NS	NS	NS	NS
9-HETE[5]	ArA	0.06	0.11	0.04	NS	0.04	NS	NS	0.03
11-HETE	ArA	1.07	1.03	0.12	NS	NS	NS	NS	< 0.01

[1]NS = $P > 0.10$.
[2]PG = prostaglandin; HETE = hydroxyeicosatetraenoic acid.
[3]ArA = arachidonic acid.
[4]Haptocov: $P = 0.06$.
[5]Cov × cov: $P = 0.06$.

Table 4.9. Linoleic acid-derived oxylipids in plasma in control ewes and ewes treated with 90 mg meloxicam on d 1 and 4 after lambing (mean ± SEM; µM) expressed as ratios of select upstream:downstream metabolites.

				***P*-values[1]**				
Ratio[2]	**CON**	**MEL**	**SEM**	**Trt**	**Cov**	**Cov × trt**	**Lambs**	**Hpcov × trt**
13-HODE[3]:9-HODE[4]	2.79	2.57	0.14	NS	NS	NS	0.02	0.08
9-HODE:9-oxoODE[5]	3.35	3.59	0.17	NS	NS	0.06	0.06	< 0.10
13-HODE:13-oxoODE[6]	62.3	51.1	5.4	NS	NS	0.04	NS	NS
9,10-EpOME:9,10-DiHOME	0.37	0.46	0.03	0.06	-	-	-	-

[1]NS = $P > 0.10$.
[2]HODE = hydroxyoctadecadienoic acid; oxoODE = oxooctadecadienoic acid; EpOME = epoxyoctadecenoic acid; DiHOME = dihydroxyoctadecenoic acid.
[3]Predominantely enzyme derived.
[4]Both enzymatic and nonenzymatic derived.
[5]Cov × cov × trt: $P = 0.06$.
[6]Cov × cov: $P = 0.09$; Cov × cov × trt: $P = 0.04$.

Table 4.10. Amount of lamb produced per ewe (kg) at approximately 30, 60, 90, and 120 d after lambing for control ewes and ewes treated with meloxicam on d 1 and 4 after lambing. To obtain weights on an individual lamb basis for each of these time points, divide by 1.58 (the average number of lambs reared per ewe) and add 6.3 kg (average birth weight).

				P–values[1]	
Age, d	**CON**	**MEL**	**SEM**	**Trt**	**TOR[2]**
30	14.9	15.6	0.6	NS	< 0.001
60	35.3	36.1	1.3	NS	< 0.001
90	55.0	55.7	1.7	NS	< 0.001
120	72.1	75.9	2.2	NS	< 0.001

[1]NS = $P > 0.10$.

[2]TOR: Type of rearing (single, twin). TOR × trt was tested but not significant.

Figure 4.1. Log haptoglobin concentration × treatment interaction (*P* = 0.04). Control ewes that had greater plasma haptoglobin concentration on d 1 had greater haptoglobin concentration on d 4, whereas initial plasma haptoglobin concentration on d 1 (before meloxicam) of treated ewes was not related to their d 4 values.

Day 4 Haptoglobin concentration (log) = intercept + log haptoglobin covariate +treatment + log haptoglobin covariate × treatment = 8.10 -0.09 + (-5.80 × treatment) + (0.78 × treatment). Shaded areas represent 95% confidence intervals.

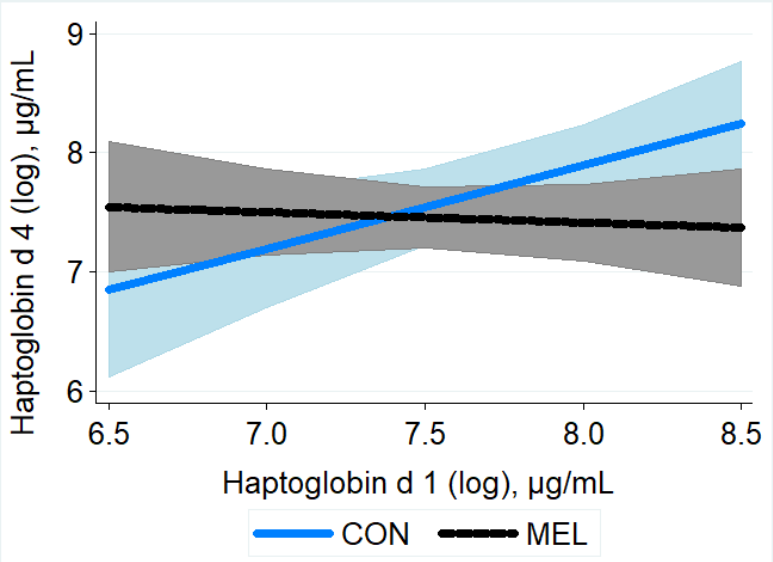
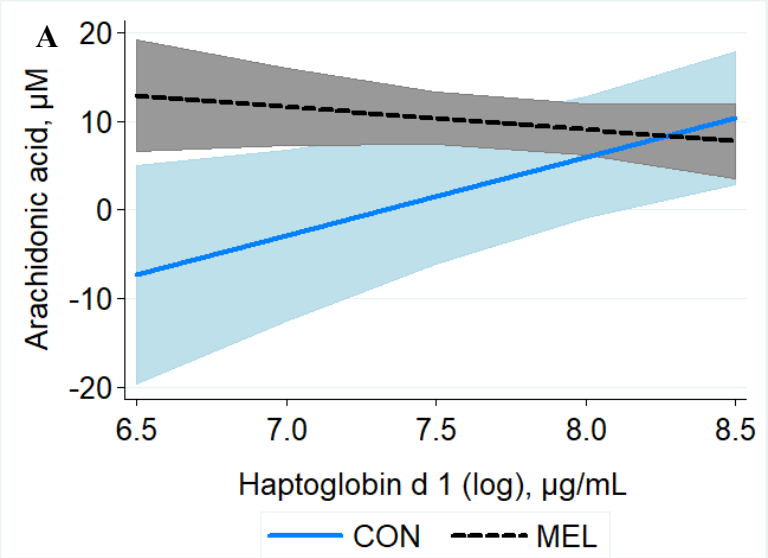


Figure 4.2. A)The treatment × haptoglobin covariate interaction (*P* = 0.01) for plasma arachidonic acid (ArA) concentration in control ewes (CON) and ewes treated with 90 mg meloxicam (MEL) on d 1 and 4 after lambing. Day 4 ArA concentrations was dependent on d 1 haptoglobin concentrations (inflammation), with greater initial haptoglobin related to greater ArA in control ewes, but lesser ArA in ewes receiving MEL. Day 4 ArA = intercept+covariate +(covariate ×covariate) + treatment + (covariate × treatment) + (covariate × covariate × treatment) + lambs + (treatment × lambs) + haptoglobin covariate + (haptoglobin covariate × treatment) = 36.50 – 1.21 + 0.02 +(-97.67 × treatment) + (2.90 × treatment) + (-0.08 × treatment) + 1.47 + (-10.31 × treatment) -2.57 + (11.42 × treatment) where treatment = 0 for MEL. Shaded areas represent 95% confidence intervals. B) Plasma ArA was less in control ewes rearing single lambs compared to MEL ewes rearing either single lambs or twins and control ewes rearing twins (Trt × lambs: *P* < 0.001).



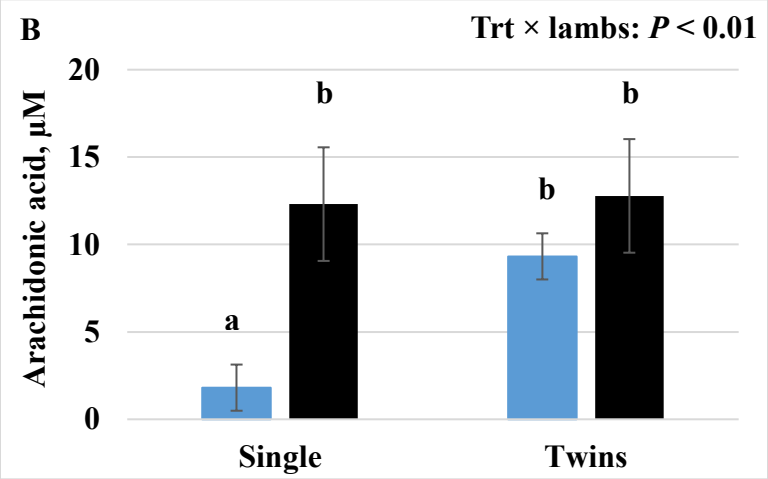


Figure 4.3. Oxylipid biosynthesis by fatty acid substrate and pathway. The effect of MEL administration to ewes on d 1 and 4 after lambing on oxylipid concentrations are shown with decreases represented in red text with a *, treatment interactions with d 1 haptoglobin shown in blue and with #. Oxylipids that were not detected are shown in gray. •Oxylipids can also be derived via non-enzymatic oxidation. sEH = soluble epoxide hydrolase

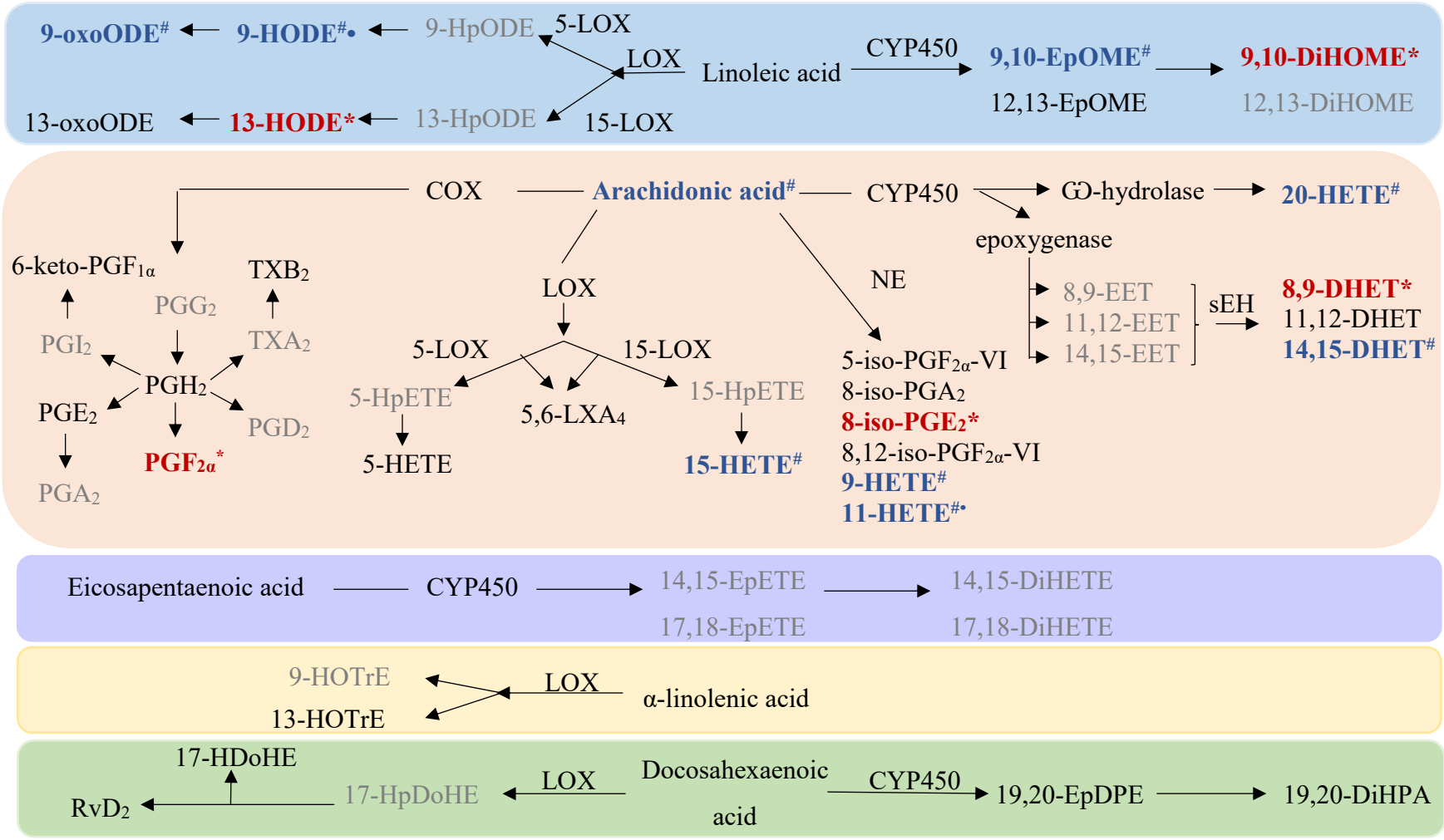
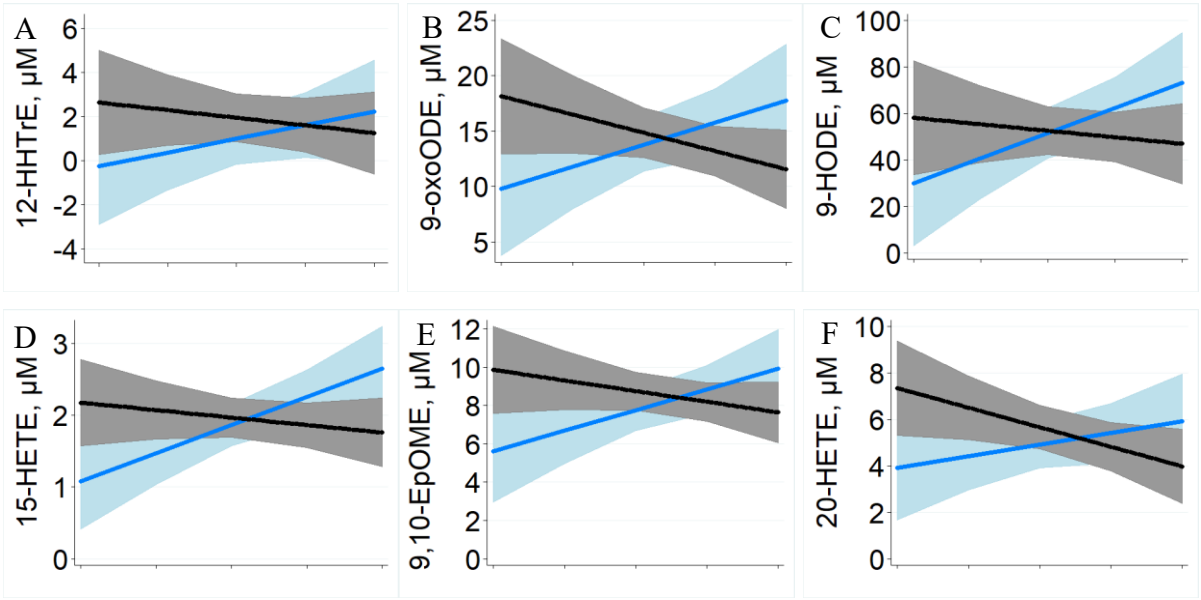
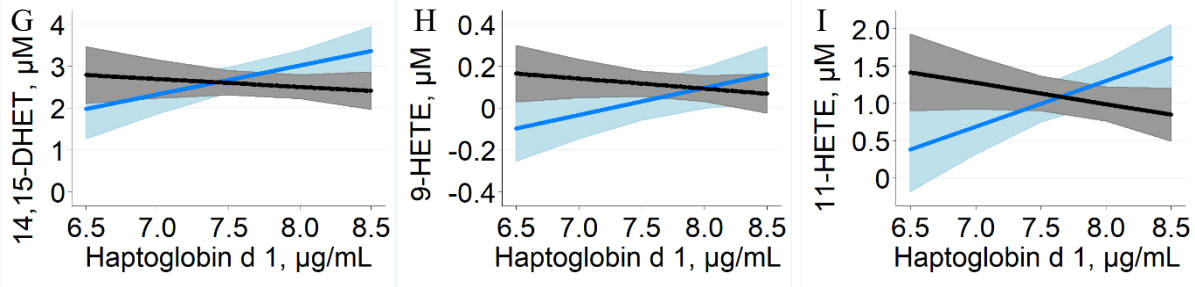


Figure 4.4. Treatment (trt) × haptoglobin covariate (log; loghaptocov) interactions for oxylipid concentrations in control ewes (CON) and ewes treated with 90 mg meloxicam (MEL) on d 1 and 4 after lambing.

Shaded areas represent 95% confidence intervals and trt is equal to 0 for MEL in the following equations. A) cycloxygenase-derived oxylipids: A) 12-HHTrE = intercept + covariate + trt + loghaptocov + (loghaptocov × trt) = ; B-D) lipoxygenase-derived oxylipids: B) 9-HODE = intercept + covariate + (covariate × trt) + trt + lambs + loghaptocov + (loghaptocov × trt) = 163.59 + 0.1248 + (0.4331 × trt) + (-295.14 × trt) - 17.4499 -15.2301 +(36.2586 × trt), C) 9-oxoODE = intercept + covariate + trt + lambs + loghaptocov + (loghaptocov × trt) = 37.07 + 0.27 + (-55.58 × trt) – 5.72 – 3.29 + (7.27 × trt), D) 15-HETE = intercept + covariate + trt + loghaptocov + (loghaptocov × trt) = 3.65 + 0.15 + (–8.24 × trt) -0.28 + (1.08 × trt); E-G) Cytochrome P450-derived oxylipids: E) 9,10-EpOME = intercept + covariate + trt + lambs + loghaptocov + (loghaptocov × trt) = 13.35 + 0.48 + (-26.29 × trt) -2.33 -1.11 + (3.27 × trt), F) 20-HETE = intercept + covariate + trt + loghaptocov + (loghaptocov × trt) = 16.73 + 0.10 + (-20.75 × trt) -1.61 + (2.68 × trt 14,15-DHET, G) 14,15-DHET = intercept + covariate + (covariate × covariate) + trt + lambs + loghaptocov + (loghaptocov × trt) = 2.27 + 0.71 -0.04 (-6.54 × trt) -0.51 -0.19 + (0.88 × trt); H-I) nonenzymatically derived oxylipids: H) 9-HETE = intercept + covariate + (covariate × covariate) + (covariate × trt) + trt + (loghaptcov × trt) = 0.4245+ 0.5025 -0.4603 + (-0.6999 × trt) + (-1.2598 × trt) -0.04793 + (0.1780 × trt), I) 11-HETE = intercept + covariate + trt + lambs + loghaptocov + (loghaptocov × trt) = 0.30810 + 0.1204 + (-6.8635 × trt) – 0.2688 – 0.2827 + (0.8967 × trt).




G
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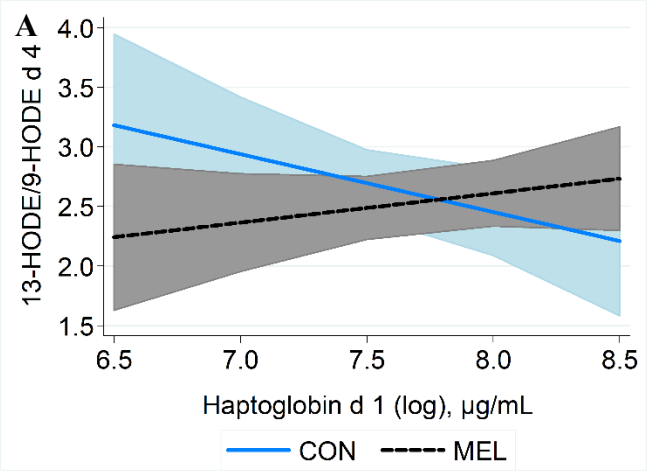
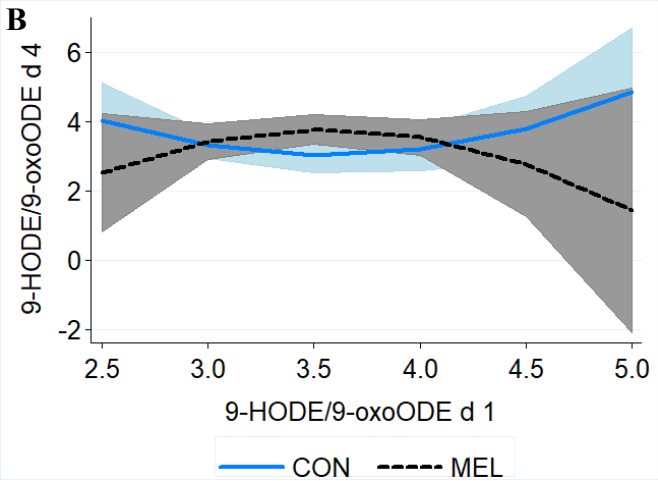
Haptoglobin d 1, µg/mL

Figure 4.5. Linoleic acid-derived oxylipids in plasma in control ewes and ewes treated with 90 mg meloxicam on d 1 and 4 after lambing (mean ± SEM; µM) expressed as ratios of select upstream:downstream metabolites.

Shaded areas represent 95% confidence intervals and treatment (trt) is equal to 0 for MEL in the following equations. A) There was a tendency for the 13-HODE/13-oxoODE ratio to have a trt × haptoglobin covarite (*P* = 0.08). For ewes with greater initial haptoglobin, the d 4 ratio was greater for MEL ewes and less for control ewes. Day 4 13-HODE/9-HODE = intercept + covariate +(covariate × covariate) + trt + (covariate × trt) + (covariate × covariate × trt) + lambs + (trt × lambs) + haptoglobin covariate (log) + [haptoglobin covariate (log) × trt] = 1.63 – 0.68 + 0.10 + (0.08 × trt) + (3.80 × trt) + (-0.64 × trt) + 0.20 + (0.85 × trt) + 0.25 + (-0.73 × trt). B) The 9-HODE/9-oxoODE ratio tended to have a treatment × covariate interaction (*P* = 0.06). Ewes with relatively greater proportion of 9-HODE at d 1 had a greater ratio on d 4 for MEL, but control ewes with a greater ratio at d 1 had a relatively lesser ratio at d 4. Day 4 9-HODE/9-oxoODE = intercept + covariate + (covariate × covariate) + trt + (covariate × trt) +(covariate × covariate × trt) + lambs + haptoglobin covariate (log) + [treatment × haptoglobin covariate (log)] = -12.10 + 7.96 – 1.12 + 30.61 +(-14.24 × trt) + (2.00 × trt) +0.53 + 0.20 + (-0.78 × trt). C) The 13-HODE/13-oxoODE ratio had a treatment × covariate interaction (*P* = 0.06). Progressive metabolism from 13-HODE to 13-oxoODE was greater for MEL with greater initial ratio, but there was less progressive metabolism for control ewes with greater initial ratios. Day 4 13-Hode/13-oxoODE = intercept + covariate + (covariate × covariate) + trt + (covariate × trt) + (covariate × covariate × trt) + haptoglobin covariate (log) = -20.26 – 0.25 + 0.0007 + (-66.97 × trt) + (1.77 × trt) + (-0.005 × trt) + 10.76.

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-2
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3.5
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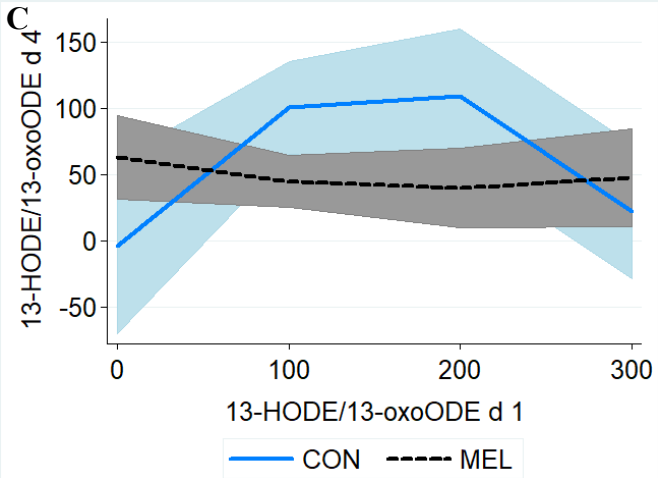
9-HODE/9-oxoODE d 1
CON
MEL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13-HODE/13-oxoODE d 4
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Appendix A - Chapter 2 Supplemental Material

Table A.1. Descriptive statistics of dairies included in the dataset.

Herd	Region	Herd size[1]	% 1st lactation	Herd turnover[2]	305ME, kg	Avg. fat %	Avg. protein %	Avg. LSC
A	West	3350	53.3	45.8	11,843.5	3.49	3.12	3.05
B	Midwest	1305	43.0	30.1	13,701.6	4.06	3.12	2.40
C	Midwest	3211	49.5	53.4	11,702.6	3.46	3.11	2.14
D	Midwest	2210	40.0	51.0	11,174.9	3.39	3.16	3.87
E	West	928	38.9	44.6	13,614.9	3.51	3.09	2.21
F	West	2203	43.8	44.8	10,098.2	.	.	.
H	West	1139	41.5	43.9	14,097.3	3.77	3.21	2.29
I	West	2327	33.9	39.4	13,812.7	3.98	3.01	2.63
J	Midwest	1211	37.2	50.1	15,100.8	3.61	3.21	1.87
K	West	10047	41.7	41.2	11,852.8	3.58	3.17	2.80
M	West	4539	38.6	44.9	12,418.1	3.69	3.03	2.57
N	West	2266	32.2	29.1	12,663.0	3.65	3.05	2.59
O	Midwest	3366	39.3	41.0	12,137.4	3.33	3.06	2.52
P	Midwest	4383	36.7	36.6	13,100.5	3.56	2.90	2.30
Q	Midwest	3374	37.6	37.1	11,588.6	4.07	3.18	1.50
R	Midwest	4157	45.5	46.0	15,236.6	.	.	.
Median		2,769	39.7	44.3	12,540.6	3.60	3.12	2.46
IQR[3]		1979-3570	37.5-43.2	38.8-45.9	11808-13729	3.50-3.75	3.05-3.17	2.23-2.62

[1]Herd size includes both milking and dry cows
[2]Calculated as: number of cows sold or died during 2016 / cows milking or dry during 2016
[3]IQR = 25th percentile, 75th percentile

Table A.2. Evaluation of 305-d ME milk projection bias by stage of lactation. Cows had to have a 9th test to be included. The data are not susceptible to culling bias because this evaluation only contains cows that had a 9th test (n = 21,153).

Test number	305-d ME milk, kg	SD
2	11,951.7	11.4
3	12,105.7	11.9
4	12,241.8	12.7
5	12,429.6	13.6
6	12,627.5	14.5
7	12,795.1	15.1
8	12,882.4	15.6
9	12,890.9	15.6

Table A.3. Odds ratios for significant predictors of treatment group by dairy.

	Study group[1]							
Predictor[2]	**SDSG**	**SDAG**	**ADSG**	**ADAG**	**ADLG**	**LDAG**	**LDLG**	***P*-value**
Dairy A								
Parity								< 0.001
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	5.82	18.14	0.18	Ref	3.10	1.37	1.37	
Calf description				Ref				< 0.001
Female	ref	ref	ref	Ref	ref	ref	ref	
Male	0.70	1.02	0.57	Ref	1.97	0.81	1.75	
Twin	8.36	4.77	3.43	Ref	0.59	1.60	2.68	
LMILK, kg	0.96	1.00	1.02	Ref	1.01	0.93	0.96	< 0.001
PDOPN	0.96	1.00	1.02	Ref	1.01	0.93	0.96	0.02
Dairy B								
Parity								< 0.001
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	4.67	8.34	0.16	Ref	5.63	0.12	0.34	
Calf description								< 0.01
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	1.55	1.36	1.23	Ref	1.26	2.51	2.24	
Twin	25.57	7.52	23.95	Ref	< 0.001	< 0.001	4.30	
LMILK, kg	0.98	1.00	0.98	Ref	1.00	0.89	1.01	< 0.001
PDCC	0.89	0.88	0.91	Ref	1.02	0.99	1.12	< 0.001
PDOPN	1.00	1.00	1.00	Ref	0.99	0.99	1.00	< 0.05
PRVME	1.00	1.00	1.00	Ref	1.00	1.00	1.00	< 0.01
Dairy C								
Parity								< 0.01
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	1.447	7.902	1.00	Ref	1.23	2.357	2.459	
Fresh month								< 0.001
Jan	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Feb	5.43	>999.9	1.05	Ref	0.90	1.78	2.60	
Mar	2.04	1.37	0.62	Ref	0.64	1.51	0.73	
Apr	1.92	1.32	1.37	Ref	1.13	1.98	< 0.001	
May	1.66	>999.9	1.04	Ref	0.44	14.99	4.20	
June	2.19	0.18	1.09	Ref	0.58	6.70	< 0.001	
July	4.07	>999.9	1.5	Ref	0.42	1.83	0.62	
Aug	5.94	0.52	1.84	Ref	0.55	0.27	0.57	
Sept	5.10	0.38	0.92	Ref	0.84	0.17	< 0.001	
Oct	5.52	0.38	1.13	Ref	1.64	0.39	0.18	
Nov				Ref			0.200.0	
	2.63	>999.9	0.87		0.77	0.15	02	
Dec	1.15	0.55	0.95	Ref	0.75	0.16	< 0.001	
Calf description				Ref				< 0.001
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	1.40	2.80	0.74	Ref	1.87	1.20	0.83	

Twin	12.80	< 0.001	5.19	Ref	< 0.001	2.30	< 0.001	
Calf DOA								< 0.01
No	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Yes	4.35	< 0.001	1.03	Ref	1.23	1.85	9.09	
LMILK, kg	0.99	0.91	0.99	Ref	1.01	0.93	0.94	< 0.001
PDOPN	1.00	1.00	1.00	Ref	1.00	1.01	1.00	< 0.001
Dairy D								
Parity								0.002
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	1.27	0.62	1.29	Ref	1.30	2.27	2.85	
Fresh month								0.01
Jan	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Feb	7.54	>999.9	3.34	Ref	0.87	0.45	0.67	
Mar	12.00	>999.9	0.42	Ref	0.47	0.66	0.29	
Apr	13.82	>999.9	3.16	Ref	0.26	0.95	0.12	
May	4.91	>999.9	3.04	Ref	0.49	0.96	< 0.001	
June	13.33	>999.9	3.20	Ref	0.44	1.39	1.39	
July	8.92	>999.9	2.66	Ref	0.96	0.97	0.14	
Aug	13.41	>999.9	4.14	Ref	0.95	0.63	0.25	
Sept	19.81	>999.9	5.16	Ref	0.70	0.64	0.10	
Oct	8.49	>999.9	1.26	Ref	0.57	0.43	0.31	
Nov	4.70	>999.9	1.68	Ref	1.08	0.25	0.53	
Dec	11.49	>999.9	2.18	Ref	1.30	1.36	0.93	
Calf description								< 0.001
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	0.97	0.49	0.65	Ref	1.71	1.08	1.92	
Twin	12.59	1.20	7.26	Ref	< 0.001	1.09	0.69	
LMILK, kg	1.06	1.03	0.94	Ref	1.01	0.94	0.95	< 0.001
PDCC	0.96	0.97	0.98	Ref	1.07	0.99	1.08	< 0.001
PDOPN	1.00	1.00	1.00	Ref	1.00	1.01	1.01	0.001
PRVME	1.00	1.00	1.00	Ref	1.00	1.00	1.00	< 0.001
Dairy E[3]								
-								
Dairy F								
Fresh month								< 0.001
Jan	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Feb	8.135	9.903	0.625	Ref	0.861	0.148	0.299	
Mar	10.828	1.279	2.454	Ref	1.415	< 0.001	0.058	
Apr	3.292	0.344	2.564	Ref	1.452	0.033	0.09	
May	0.786	0.36	3.737	Ref	0.704	0.25	< 0.001	
June	4.702	0.486	4.277	Ref	1.08	0.995	0.3	
July	9.025	1.788	1.728	Ref	1.065	1.034	0.032	
Aug	10.451	1.118	1.505	Ref	0.679	0.477	0.028	
Sept	6.823	1.717	1.61	Ref	0.565	0.052	< 0.001	
Oct	3.212	< 0.001	2.105	Ref	0.76	0.023	< 0.001	
Nov	1.198	0.735	2.01	Ref	1.535	0.035	0.024	

Dec	0.962	1.447	1.928	Ref	1.355	0.156	0.057	
Calf description								
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	< 0.001
Male	0.65	1.01	0.82	Ref	2.37	0.92	1.32	
Twin	5.86	2.47	4.46	Ref	0.27	0.47	< 0.001	
PDOPN	1.00	1.00	1.00	Ref	1.00	1.01	1.01	< 0.001
PRVME	1.00	1.00	1.00	Ref	1.00	1.00	1.00	< 0.001
Dairy H								
Calf description								< 0.001
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	0.76	0.79	0.79	Ref	2.36	1.65	1.81	
Twin	0.667	0.511	14.35	Ref	< 0.001	5.92	< 0.001	
Calf DOA								0.03
No	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Yes	8.25	< 0.001	3.25	Ref	0.58	3.74	< 0.001	
LMILK, kg	0.97	1.03	0.93	Ref	1.02	0.83	0.87	< 0.001
PDCC	0.97	1.01	0.95	Ref	1.03	1.03	1.03	< 0.01
PDOPN	1.00	0.98	1.01	Ref	1.00	1.01	1.01	< 0.01
Dairy I								
Parity								< 0.001
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	0.77	0.65	0.71	Ref	1.87	3.06	5.23	
LMILK, kg	1.06	1.02	0.93	Ref	1.06	0.93	0.96	< 0.001
PDCC	0.96	1.00	0.96	Ref	1.02	0.98	1.03	< 0.001
PDOPN	0.99	0.99	1.01	Ref	1.01	1.02	1.01	< 0.001
PRVME	1.00	1.00	1.00	Ref	1.00	1.00	1.00	< 0.01
Dairy J								
Parity								< 0.001
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	1.67	21.74	0.25	Ref	1.21	0.76	>999.9	
Calf description								< 0.001
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	0.67	1.30	0.21	Ref	1.98	0.82	1.938	
Twin	2.07	< 0.001	12.11	Ref	< 0.001	2.31	< 0.001	
LMILK, kg	1.04	1.02	0.97	Ref	0.99	0.86	0.91	< 0.001
PDCC	0.99	0.98	0.98	Ref	1.13	1.01	1.17	< 0.001
PDOPN	1.00	0.99	1.01	Ref	1.00	1.01	1.00	< 0.001
PRVME	1.00	1.00	1.00	Ref	1.00	1.00	1.00	0.04
Dairy K								
Parity								< 0.001
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	4.88	11.59	0.15	Ref	7.00	0.40	0.29	
Fresh month								< 0.001
Jan	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Feb	1.73	0.92	1.36	Ref	0.87	0.68	1.25	
Mar	1.22	1.32	1.21	Ref	1.61	0.73	1.08	

Apr	2.66	3.13	2.57	Ref	0.84	0.62	0.44	
May	2.44	1.16	2.33	Ref	0.49	0.56	0.36	
June	4.67	1.67	3.39	Ref	0.38	0.52	0.37	
July	6.69	3.82	2.24	Ref	1.00	0.22	0.36	
Aug	8.45	2.44	2.25	Ref	0.53	0.31	0.46	
Sept	2.72	0.99	1.62	Ref	1.14	0.14	0.80	
Oct	2.00	0.82	1.36	Ref	1.26	0.34	1.05	
Nov	1.59	0.91	1.12	Ref	1.12	0.47	1.04	
Dec	1.57	0.56	1.16	Ref	1.32	0.56	1.36	
Calf description								< 0.001
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	0.67	0.66	0.50	Ref	1.68	1.00	1.85	
Twin	5.89	1.40	2.90	Ref	0.22	0.77	0.15	
LMILK, kg	1.01	1.00	0.98	Ref	1.01	0.94	0.99	< 0.001
PDCC	0.97	0.99	0.98	Ref	1.04	1.01	1.05	< 0.001
PDOPN	1.00	1.00	1.00	Ref	1.00	1.00	1.00	< 0.001
Dairy M								
Parity								< 0.001
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	1.94	4.67	0.51	Ref	2.16	2.44	0.53	
Fresh month								< 0.001
Jan	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Feb	0.92	0.72	2.17	Ref	0.83	0.83	0.64	
Mar	0.39	0.84	1.30	Ref	0.69	0.64	1.12	
Apr	1.30	1.47	2.50	Ref	0.75	0.71	0.51	
May	0.79	0.74	3.03	Ref	0.66	0.64	0.48	
June	0.78	0.13	3.68	Ref	1.02	0.58	0.44	
July	0.99	0.32	4.00	Ref	0.93	1.78	1.11	
Aug	0.77	0.30	4.00	Ref	0.55	0.92	0.97	
Sept	0.92	0.70	2.80	Ref	1.05	1.01	1.82	
Oct	0.38	0.41	2.03	Ref	0.58	0.84	1.02	
Nov	0.33	0.31	1.26	Ref	0.85	1.11	1.31	
Dec	1.94	4.67	0.51	Ref	2.16	2.44	0.53	
Calf description								< 0.001
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	0.74	0.77	0.62	Ref	1.09	0.98	1.42	
Twin	3.25	0.83	4.91	Ref	0.22	2.20	2.01	
Calf DOA								< 0.01
No	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Yes	4.00	1.38	1.84	Ref	2.35	1.29	0.86	
LMILK, kg	0.99	0.96	0.99	Ref	1.02	0.94	0.95	< 0.001
PDCC	0.96	0.98	0.96	Ref	1.05	0.99	1.07	< 0.001
PDOPN	1.00	1.00	1.00	Ref	1.01	1.01	1.02	< 0.001
PRVME	1.00	1.00	1.00	Ref	1.00	1.00	1.00	< 0.001
Dairy N								
Parity								< 0.001

2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	0.72	0.16	1.24	Ref	0.71	3.98	1.17	
Fresh month								< 0.001
Jan	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Feb	1.34	< 0.001	>999.9	Ref	0.75	3.59	0.85	
Mar	2.06	< 0.001	>999.9	Ref	0.15	11.68	0.80	
Apr	4.02	1.06	>999.9	Ref	0.69	2.63	0.29	
May	5.60	0.65	>999.9	Ref	< 0.001	1.65	0.36	
June	3.94	0.56	>999.9	Ref	0.30	1.67	0.33	
July	7.96	1.06	>999.9	Ref	< 0.001	3.42	0.72	
Aug	4.83	0.324	>999.9	Ref	0.30	1.50	0.42	
Sept	3.85	1.881	>999.9	Ref	0.61	3.36	1.76	
Oct	2.59	0.663	>999.9	Ref	0.33	1.77	1.68	
Nov	3.19	6.494	>999.9	Ref	0.79	1.17	0.51	
Dec	2.87	0.937	>999.9	Ref	1.22	0.73	0.78	
Calf description								< 0.001
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	0.49	0.67	0.39	Ref	1.76	1.60	2.09	
Twin				Ref			<	
	4.28	1.50	2.06		< 0.001	0.60	0.001	
LMILK, kg	1.02	1.01	1.02	Ref	0.97	0.91	0.92	0.002
PDCC	0.98	1.00	0.93	Ref	1.04	1.03	1.12	< 0.001
PDOPN	1.00	1.00	1.01	Ref	1.00	1.00	0.99	0.03
PRVME	1.00	1.00	1.00	Ref	1.00	1.00	1.00	0.005
Dairy O								
Parity								< 0.001
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	0.77	1.22	0.851	Ref	1.775	3.558	8.18	
Fresh month								< 0.001
Jan	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Feb	0.69	0.97	0.647	Ref	0.839	0.898	1.56	
Mar	0.88	3.82	0.165	Ref	1.39	2.332	1.83	
Apr	1.83	2.83	0.73	Ref	0.854	1.148	1.67	
May	0.89	3.92	0.962	Ref	0.756	1.949	2.38	
June	2.17	2.11	2.119	Ref	0.623	2.019	0.78	
July	2.41	4.06	1.67	Ref	0.559	1.978	0.40	
Aug	1.96	1.44	1.376	Ref	0.331	0.368	0.32	
Sept	1.20	5.19	1.196	Ref	0.521	1.537	0.15	
Oct	1.57	2.32	1.218	Ref	1.197	2.494	2.25	
Nov	1.52	2.23	0.749	Ref	1.354	1.229	0.58	
Dec	0.45	2.45	1.021	Ref	0.672	1.549	1.63	
Calf description								0.01
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	0.62	0.68	0.69	Ref	1.18	0.96	1.36	
Twin								
DOA								< 0.001

No	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Yes				Ref			<	
	6.36	0.92	5.43		1.00	0.50	0.001	
LMILK, kg	1.02	1.03	0.97	Ref	1.00	0.97	0.98	< 0.001
PDCC	0.99	0.99	1.00	Ref	1.01	0.99	1.03	0.03
PDOPN	1.00	1.00	1.00	Ref	1.00	1.01	1.01	< 0.001
PRVME	1.00	1.00	1.00	Ref	1.00	1.00	1.00	< 0.001
Dairy P								
Parity								< 0.001
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	0.85	0.93	1.10	Ref	1.15	2.70	2.31	
Fresh month								< 0.001
Jan	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Feb	1.23	0.25	3.10	Ref	0.77	0.80	0.14	
Mar	0.58	0.23	2.27	Ref	0.54	1.05	0.40	
Apr	0.28	0.33	4.40	Ref	0.49	8.14	1.39	
May	1.12	0.52	3.54	Ref	0.34	3.53	3.14	
June	1.33	0.24	6.22	Ref	0.41	1.37	1.69	
July	1.00	0.18	4.96	Ref	0.82	1.74	0.72	
Aug	1.25	0.21	2.45	Ref	0.64	0.55	1.21	
Sept	0.40	0.20	5.50	Ref	0.87	1.67	1.40	
Oct	0.26	0.11	3.54	Ref	0.74	1.25	1.82	
Nov	0.31	0.09	3.90	Ref	0.79	2.20	1.53	
Dec	0.45	0.66	3.34	Ref	1.02	0.91	0.85	
Calf description								< 0.001
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	0.627	0.67	0.53	Ref	1.50	0.83	1.785	
Twin				Ref			<	
	6.721	1.98	4.44		0.26	0.73	0.001	
DOA								< 0.001
No	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Yes	4.25	1.98	4.47	Ref	1.20	0.51	0.57	
LMILK, kg	1.01	1.04	0.96	Ref	1.01	0.86	0.92	< 0.001
PDCC	0.97	0.98	0.97	Ref	1.05	1.00	1.09	< 0.001
PDOPN	1.00	0.99	1.00	Ref	1.00	1.02	1.01	< 0.001
PRVME	1.00	1.00	1.00	Ref	1.00	1.00	1.00	< 0.001
Dairy Q								
Parity								< 0.001
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	0.48	1.21	1.58	Ref	1.66	4.14	4.72	
Fresh month								< 0.001
Jan	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Feb	0.70	0.92	0.23	Ref	1.27	0.08	0.12	
Mar	1.12	1.20	< 0.001	Ref	2.00	0.05	0.04	
Apr	0.76	1.33	0.31	Ref	1.32	0.03	0.05	
May	0.64	0.63	0.36	Ref	0.85	0.12	0.18	

June	1.43	0.87	0.50	Ref	1.41	0.09	0.10	
July	2.20	0.42	1.06	Ref	0.93	0.12	0.02	
Aug	2.50	0.28	1.82	Ref	0.78	0.16	0.09	
Sept	1.00	0.55	1.41	Ref	0.29	0.18	0.10	
Oct	1.00	< 0.001	2.00	Ref	0.41	0.22	0.15	
Nov	0.75	< 0.001	1.77	Ref	0.79	0.43	0.19	
Dec	0.21	< 0.001	1.81	Ref	0.80	0.80	0.40	
Calf description								< 0.001
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	0.67	0.57	1.08	Ref	1.57	1.09	2.62	
Twin	11.03	1.46	13.83	Ref	0.29	2.12	0.46	
DOA								0.004
No	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Yes	7.58	< 0.001	1.23	Ref	1.78	0.60	< 0.001	
LMILK, kg	1.02	1.02	0.90	Ref	1.05	0.81	0.84	< 0.001
PDCC	0.97	0.99	0.98	Ref	1.08	1.00	1.06	< 0.001
PDOPN	1.00	1.01	1.00	Ref	1.00	1.01	1.02	< 0.001
PRVME	1.00	1.00	1.00	Ref	1.00	1.00	1.00	< 0.001
Dairy R								
Parity								< 0.001
2	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
3+	0.93	0.66	1.30	Ref	1.53	1.70	1.83	
Calf description								< 0.001
Female	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Male	0.81	0.72	0.98	Ref	1.26	1.40	1.29	
Twin	5.67	3.09	4.92	Ref	0.30	0.56	0.27	
DOA								0.03
No	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Yes	2.13	1.55	3.98	Ref	1.06	1.60	1.35	
LMILK, kg	0.99	0.99	0.96	Ref	1.00	0.97	1.01	0.006

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

[2]LMILK = milk yield at last test before dry-off (within 40 d of dry-off); PDCC = previous lactation days carried calf; PDOPN = previous lactation days open ; PRVME = previous lactation 305-d mature equivalent milk yield

[3]No significant variables

Table A.4. Association between study group and milk yield at first test (within 7-35 DIM).

Variable	Coefficient	SE	*P*-value	Mean	SE
Intercept	10.70	1.19			
DIM at first test	1.211	0.046	< 0.001		
DIM at first test squared	-0.017	0.001	< 0.001		
Study group[1]			0.16		
S_DS_G	-1.82	1.94		39.53[d]	1.00
S_DA_G	2.42	2.08		41.50[ab]	1.02
A_DS_G	1.26	1.64		40.80[a]	1.01
A_DA_G	Ref	Ref		42.46[bc]	0.96
A_DL_G	-2.46	1.34		42.93[c]	0.99
L_DA_G	1.58	1.33		43.21[c]	0.99
L_DL_G	-1.65	2.05		42.66[bc]	1.02
Parity group			< 0.001		
2	Ref	Ref		40.67[a]	0.96
3+	2.61	0.14		43.40[b]	0.96
Fresh month			< 0.001		
Jan	Ref	Ref		43.33[ef]	0.98
Feb	0.30	0.31		43.69[f]	0.99
Mar	-0.58	0.33		42.90[def]	0.99
Apr	-0.15	0.34		43.18[def]	0.99
May	-0.64	0.33		42.67[def]	0.99
June	-1.21	0.32		42.32[cd]	0.99
July	-1.93	0.33		41.27[abc]	0.99
Aug	-1.03	0.31		41.33[cde]	0.99
Sept	-2.99	0.29		40.20[a]	0.98
Oct	-2.71	0.29		41.14[ab]	0.98
Nov	-2.72	0.29		41.61[ab]	0.98
Dec	-1.89	0.32		41.88[bc]	0.99
Calf dead on arrival			< 0.001		
No	Ref	Ref		42.32[a]	0.92
Yes	-2.28	0.42		40.04[b]	1.01
Calf sex			< 0.001		
Female	Ref	Ref		42.23[a]	0.96
Male	0.04	0.14		42.24[a]	0.96
Twin	-2.39	0.32		39.48[b]	1.05
Mastitis at first test			< 0.001		
No	Ref	Ref		42.67[a]	0.96
Yes	-1.802	0.175		41.10[b]	0.97
PTA milk, kg *1000	1.39	0.47	0.003		
Previous lactation 305ME milk, per 1000 kg	1.00	0.05	< 0.001		
Study group[1] × previous lactation 305 ME milk per 1000 kg			< 0.001		
S_DS_G	0.05	0.15			
S_DA_G	0.19	0.15			

A_DS_G	-0.26	0.12	
A_DA_G	Ref	Ref	
A_DL_G	0.35	0.10	
L_DA_G	-0.14	0.12	
L_DL_G	0.29	0.18	
Milk at last test before dry off, kg	0.08	0.01	< 0.001
Study group[1] × milk at last test before dry off, kg			< 0.001
S_DS_G	-0.07	0.04	
S_DA_G	-0.12	0.04	
A_DS_G	0.01	0.03	
A_DA_G	Ref	Ref	
A_DL_G	-0.03	0.03	
L_DA_G	0.06	0.03	
L_DL_G	-0.08	0.04	
Previous lactation days open	0.004	0.001	0.003
Study group[1] × previous lactation days open			< 0.001
S_DS_G	0.0022	0.006	
S_DA_G	-0.0217	0.005	
A_DS_G	0.0003	0.004	
A_DA_G	Ref	Ref	
A_DL_G	-0.0071	0.003	
L_DA_G	-0.0054	0.003	
L_DL_G	0.0026	0.004	

Random-effects parameters	Estimate	SE	95% confidence interval	
Dairy variance (_cons)	13.17	5.29	6.00	28.93
Var(residual)	80.05	0.83	78.43	81.70
Intra-cluster correlation coefficient	0.141	0.049	0.070	0.266

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.5. Association between study group and milk fat concentration at first test of lactation (7-35 DIM).

	Coefficient	SE	*P* -value	Mean	SE
Intercept	6.00	0.13	< 0.001		
DIM at first test	-0.081	0.004	< 0.001		
DIM at first test, sq	0.0011	0.0001	< 0.001		
Study group[1]			0.002		
S_DS_G	-0.18	0.11		4.04	0.10
S_DA_G	-0.07	0.13		4.10	0.10
A_DS_G	0.11	0.10		4.08	0.10
A_DA_G	Ref	Ref		4.05	0.10
A_DL_G	-0.08	0.09		4.07	0.10
L_DA_G	0.30	0.10		4.08	0.10
L_DL_G	-0.24	0.12		4.04	0.10
Parity			< 0.001		
2	Ref	Ref		4.01^{a}	0.10
3+	0.11	0.01		4.09^{b}	0.10
DIM at first test x parity group	-0.005	0.002			
Fresh month			< 0.001		
Jan	Ref	Ref		4.16^{efg}	0.10
Feb	-0.13	0.03		4.01^{bcd}	0.10
Mar	-0.04	0.03		4.07^{cde}	0.10
Apr	-0.01	0.03		4.12^{def}	0.10
May	-0.11	0.03		4.02^{bcd}	0.10
June	-0.27	0.03		3.81^{a}	0.10
July	-0.31	0.03		3.81^{a}	0.10
Aug	-0.13	0.03		3.90^{ab}	0.10
Sept	-0.10	0.03		3.99^{bc}	0.10
Oct	0.10	0.03		4.19^{fg}	0.10
Nov	0.16	0.03		4.24^{g}	0.10
Dec	0.20	0.03		4.24^{fg}	0.10
Calf description			< 0.001		
Female	Ref	Ref		4.07^{a}	0.10
Male	-0.04	0.01		4.06^{a}	0.10
Twin	-0.16	0.03		3.90^{b}	0.10
Mastitis at first test			< 0.001		
No	Ref	Ref		4.03^{a}	0.10
Yes	0.12	0.02		4.15^{b}	0.10
Milk at first test, kg	-0.016	0.003	< 0.001		
Milk at first test sq, kg	0.0001	3.18E-05	0.002		
Study group[1] × milk at first test, kg			< 0.001		
S_DS_G	0.0012	0.0023			
S_DA_G	0.0020	0.0027			
A_DS_G	-0.0014	0.0023			
A_DA_G	Ref	Ref			

A_DL_G	0.0004	0.0018	
L_DA_G	-0.0111	0.0021	
L_DL_G	0.0030	0.0026	
PTA fat yield, kg	0.005	0.001	< 0.001
PTA milk, per 1000 kg	-0.3364	0.0444	< 0.001
Previous lactation 305ME milk, per 1000 kg	-0.033	0.003	< 0.001
Previous lactation days open	0.0004	0.0001	0.001
Study group[1] × previous lactation days open			< 0.001
S_DS_G	0.0012	0.0023	
S_DA_G	0.0020	0.0027	
A_DS_G	-0.0014	0.0023	
A_DA_G	Ref	Ref	
A_DL_G	0.0004	0.0018	
L_DA_G	-0.0111	0.0021	
L_DL_G	0.0030	0.0026	

Random-effects parameters	Estimate	SE	95 % confidence interval	
Dairy variance (_cons)	0.149	0.059	0.068	0.324
Var(residual)	0.561	0.006	0.548	0.573
Intra-cluster correlation coefficient	0.210	0.066	0.108	0.366

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.6. Milk fat yield at first test was associated with study group ($P < 0.001$).

	Coefficient	SE	*P* -value	Mean	SE
Intercept	1.060	0.066	< 0.001		
DIM at first test	0.020	0.002	< 0.001		
DIM at first test squared	0.000	5.48E-05	< 0.001		
Study group[1]			< 0.001		
S_DS_G	-0.136	0.015		1.61_c	0.06
S_DA_G	-0.028	0.015		1.72^{ab}	0.06
A_DS_G	-0.060	0.015		1.69^{a}	0.06
A_DA_G	Ref	Ref		1.75^{b}	0.06
A_DL_G	0.020	0.011		1.77^{b}	0.06
L_DA_G	-0.008	0.013		1.74^{ab}	0.06
L_DL_G	-0.002	0.015		1.75^{ab}	0.06
Parity			< 0.001		
2				1.66^{a}	0.10
3+	0.193	0.018		1.81^{b}	0.10
DIM at first test x parity group	-0.002	0.001	0.01		
Fresh month			< 0.001		
Jan	Ref	Ref		1.80^{d}	0.06
Feb	-0.027	0.016		1.77^{bcd}	0.06
Mar	-0.041	0.017		1.76^{bcd}	0.06
Apr	-0.019	0.018		1.78^{cd}	0.06
May	-0.087	0.017		1.71^{ab}	0.06
June	-0.138	0.017		1.66^{a}	0.06
July	-0.209	0.017		1.59^{e}	0.06
Aug	-0.069	0.015		1.73^{bc}	0.06
Sept	-0.137	0.015		1.66^{a}	0.06
Oct	-0.053	0.014		1.75^{bc}	0.06
Nov	-0.022	0.015		1.78^{cd}	0.06
Dec	0.014	0.016		1.81^{d}	0.06
Calf description			< 0.001		
Female	Ref	Ref		1.75^{a}	0.06
Male	-0.004	0.007		1.74^{a}	0.06
Twin	-0.157	0.015		1.59^{b}	0.06
Calf dead on arrival			< 0.001		
No	Ref	Ref		1.74^{a}	0.06
Yes	-0.093	0.021		1.65^{b}	0.06
Mastitis at first test			< 0.001		
No	Ref	Ref		1.75^{a}	0.06
Yes	-0.037	0.009		1.71^{b}	0.06
Milk yield at last test before dry off, kg	0.0022	0.0004	< 0.001		
Previous lactation 305ME milk, per 1000 kg	0.025	0.002	< 0.001		
Previous lactation days open	0.0007	0.0001	< 0.001		

Previous lactation days open squared	-1.12E-06	2.97E-07	< 0.001	
Random-effects parameters	Estimate	SE	95% confidence interval	
Var (_cons)	0.042	0.017	0.019	0.092
Var (residual)	0.205	0.002	0.201	0.209
Intracluster correlation coefficient	0.171	0.056	0.087	0.309

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.7. Association between study group and milk protein concentration at first test of lactation (between 7-35 DIM).

Protein concentration at first test – 15,626 observations, 14 herds					
Variable	**Coefficient**	**SE**	***P*-value**	**Mean**	**SE**
Intercept	4.200	0.036	< 0.001		
DIM at first test	-0.063	0.002	< 0.001		
DIM at first test, sq	0.001	3.88E-05	< 0.001		
Study group[1]			< 0.001		
S_DS_G	0.065	0.010		3.17^{d}	0.02
S_DA_G	0.035	0.011		3.14^{cd}	0.02
A_DS_G	0.014	0.010		3.12^{bc}	0.02
A_DA_G	Ref	Ref		3.10^{b}	0.02
A_DL_G	-0.011	0.008		3.09^{b}	0.02
L_DA_G	-0.054	0.009		3.05^{a}	0.02
L_DL_G	-0.069	0.011		3.03^{a}	0.02
Parity group			< 0.001		
2	Ref	Ref		3.11^{a}	0.02
3+	-0.027	0.005		3.09^{b}	0.02
Fresh month			< 0.001		
Jan	Ref	Ref		3.14^{ef}	0.02
Feb	-0.049	0.011		3.09^{cd}	0.02
Mar	-0.012	0.012		3.13^{def}	0.02
Apr	-0.079	0.013		3.06^{bc}	0.02
May	-0.127	0.012		3.01^{a}	0.02
June	-0.123	0.012		3.02^{ab}	0.02
July	-0.155	0.012		2.99^{a}	0.02
Aug	-0.070	0.011		3.07^{c}	0.02
Sept	-0.021	0.010		3.12^{de}	0.02
Oct	-0.017	0.010		3.12^{de}	0.02
Nov	0.020	0.010		3.16^{f}	0.02
Dec	0.013	0.011		3.15^{ef}	0.02
Dead on arrival			0.002		
No	Ref	Ref		3.10^{a}	0.02
Yes	0.047	0.015		3.14^{b}	0.03
Mastitis at first test			< 0.001		
No	Ref	Ref		3.08^{a}	0.02
Yes	0.089	0.006		3.17^{b}	0.02
Milk yield at first test, kg	-0.0052	0.0003	< 0.001		
PTA protein percent, kg	0.016	0.001	< 0.001		
PTA protein yield, kg	0.003	0.001	< 0.001		
Previous lactation 305 ME per 1000 kg	-6.64E-03	1.44E-03	< 0.001		
Milk yield at last test before dry off, kg	0.004	0.001	< 0.001		
Milk yield at last test before dry off sq., kg	-4.8E-05	1.88E-05	0.01		
Previous lactation days open	-0.0006	9.37E-05	< 0.001		

Previous lactation days open squared	6.75E-07	2.08E-07	0.001	
Random-effects parameters	Estimate	SE	95% confidence interval	
Var (_cons)	0.006	0.003	0.003	0.014
Var (residual)	0.084	0.001	0.082	0.086
Intracluster correlation coefficient	0.069	0.026	0.033	0.142

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.8. Association between study group and milk protein yield at first test of lactation (between 7-35 DIM).

Variable	Coefficient	SE	*P*-value	Mean	SE
Intercept	0.575	0.052	< 0.001		
DIM at first test	0.016	0.002	< 0.001		
DIM at first test, sq	-0.0002	3.69E-05	< 0.001		
Study group[1]			< 0.001		
S_DS_G	-0.040	0.068		1.27^{a}	0.03
S_DA_G	0.004	0.070		1.31^{ab}	0.03
A_DS_G	0.049	0.056		1.29^{a}	0.03
A_DA_G	Ref	Ref		1.33^{b}	0.02
A_DL_G	-0.032	0.046		1.34^{b}	0.02
L_DA_G	0.056	0.047		1.34^{b}	0.03
L_DL_G	-0.054	0.068		1.31^{ab}	0.03
Parity group			< 0.001		
2	Ref	Ref		1.28^{a}	0.02
3+	0.140	0.012		1.36^{b}	0.02
Parity group x dim at test 1	-0.003	0.001	< 0.001		
Fresh month			< 0.001		
Jan	Ref	Ref		1.39^{h}	0.02
Feb	-0.009	0.011		1.38^{gh}	0.02
Mar	-0.034	0.011		1.36^{fgh}	0.02
Apr	-0.049	0.012		1.34^{defg}	0.03
May	-0.093	0.012		1.30^{abc}	0.03
June	-0.092	0.011		1.30^{abcd}	0.02
July	-0.128	0.012		1.26^{a}	0.03
Aug	-0.048	0.010		1.34^{ef}	0.02
Sept	-0.098	0.010		1.29^{ab}	0.02
Oct	-0.091	0.010		1.30^{abc}	0.02
Nov	-0.074	0.010		1.32^{bcde}	0.02
Dec	-0.058	0.011		1.33^{cdef}	0.02
Calf description			< 0.001		
Female	Ref	Ref		1.33^{a}	0.02
Male	0.001	0.005		1.33^{a}	0.02
Twins	-0.079	0.010		1.25^{b}	0.03
Dead on arrival			0.004		
No	Ref	Ref		1.33^{a}	0.02
Yes	-0.042	0.014		1.28^{b}	0.03
Mastitis at first test			< 0.001		
No	Ref	Ref		1.33^{a}	0.02
Yes	-0.025	0.006		1.31^{b}	0.02
PTA protein yield, kg	0.007	0.001	< 0.001		
Study group[1] × PTA protein yield, kg	Ref	Ref	0.03		
S_DS_G	0.0137	0.0039			
S_DA_G	0.0037	0.0042			
A_DS_G	-0.0003	0.0038			

A_DA_G	Ref	Ref	
A_DL_G	0.0014	0.0028	
L_DA_G	0.0047	0.0033	
L_DL_G	-0.0002	0.0043	
PTA milk, kg	-8E-05	3.37E-05	0.02
PTA milk squared, kg	-1.40E-07	7.00E-08	< 0.05
Study group[1] × PTA milk, kg			< 0.001
S_DS_G	-0.0004	0.0001	
S_DA_G	-5.1E-05	0.0001	
A_DS_G	6.87E-05	9.99E-05	
A_DA_G	Ref	Ref	
A_DL_G	-6.4E-05	7.25E-05	
L_DA_G	-0.00016	8.39E-05	
L_DL_G	6.35E-05	0.0001	
Previous lactation 305 ME per 1000 kg	0.045	0.006	< 0.001
Previous lactation 305 ME squared per 1000 kg	-6.35E-07	1.99E-07	0.001
Study group[1] × previous lactation 305 ME per 1000 kg			< 0.001
S_DS_G	-6.66E-04	5.29E-03	
S_DA_G	1.25E-02	5.10E-03	
A_DS_G	-7.66E-03	4.20E-03	
A_DA_G	Ref	Ref	
A_DL_G	8.32E-03	3.37E-03	
L_DA_G	-9.15E-03	4.04E-03	
L_DL_G	3.63E-03	5.97E-03	
Milk yield at last test before dry off, kg	0.0031	0.0004	< 0.001
Comcat x milk yield at last test before dry off, kg			
S_DS_G	-0.001	0.001	
S_DA_G	-0.005	0.001	
A_DS_G	-2.2E-05	0.001	
A_DA_G	Ref	Ref	
A_DL_G	-0.002	0.001	
L_DA_G	0.003	0.001	
L_DL_G	-0.002	0.001	
Previous lactation days open	-4.7E-05	4.68E-05	0.32
Study group[1] × previous lactation days open			0.02
S_DS_G	7.15E-05	0.0002	
S_DA_G	-0.0006	0.0002	
A_DS_G	3.82E-05	0.0001	
A_DA_G	Ref	Ref	
A_DL_G	-0.0001	0.0001	
L_DA_G	-6E-05	0.0001	
L_DL_G	0.0001	0.0001	

Random-effects parameters	Estimate	SE	95% confidence interval	
Var (_cons)	0.007	0.003	0.003	0.018
Var (residual)	0.077	0.001	0.075	0.078
Intracluster correlation coefficient	0.088	0.036	0.038	0.189

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.9. Association between study group and LSC at first test of lactation (between 7-35 DIM).

Somatic cell linear score at first test. 12,646. 13 herds					
Variable	**Coefficient**	**SE**	***P*-value**	**Mean**	**SE**
Intercept	4.325	0.232	< 0.001		
DIM at first test	-0.012	0.002	< 0.001		
Study group[1]			0.08		
S_DS_G	-0.024	0.071		2.37	0.18
S_DA_G	0.237	0.077		2.59	0.18
A_DS_G	0.028	0.071		2.39	0.18
A_DA_G	Ref	Ref		2.38	0.17
A_DL_G	0.067	0.054		2.47	0.17
L_DA_G	0.058	0.059		2.41	0.17
L_DL_G	0.051	0.078		2.41	0.18
Parity group			< 0.001		
2	Ref	Ref		2.06[a]	0.17
3+	0.628	0.033		2.69[b]	0.17
Fresh month			0.004		
Jan	Ref	Ref		2.55[b]	0.17
Feb	-0.050	0.065		2.49[ab]	0.17
Mar	-0.241	0.072		2.30[ab]	0.18
Apr	-0.061	0.075		2.48[ab]	0.18
May	-0.098	0.078		2.45[ab]	0.18
June	-0.179	0.077		2.37[ab]	0.18
July	-0.173	0.077		2.37[ab]	0.18
Aug	-0.149	0.073		2.40[ab]	0.17
Sept	-0.184	0.073		2.36[ab]	0.17
Oct	-0.293	0.070		2.25[a]	0.17
Nov	-0.156	0.072		2.39[ab]	0.17
Dec	-0.248	0.078		2.30[ab]	0.18
Calving difficulty			< 0.05		
No	Ref	Ref		2.38[a]	0.17
Yes	0.088	0.044		2.46[b]	0.17
Dead on arrival			0.007		
No	Ref	Ref		2.41[a]	0.17
Yes	-0.291	0.107		2.12[b]	0.20
Milk yield at first test, kg	-0.072	0.007	< 0.001		
Milk yield at first test squared, kg	0.001	8.39E-05	< 0.001		
Somatic cell count linear score at last test before dryoff	0.0034	0.0008	< 0.001		
Somatic cell count linear score at last test before dry off squared	-1.10E-06	2.61E-07	< 0.001		
Study group[1] × somatic cell count linear score at last test before dry off			0.004		
S_DS_G	0.002	0.001			
S_DA_G	-0.003	0.001			

A_DS_G	-0.002	0.001		
A_DA_G	Ref	Ref		
A_DL_G	0.003	0.003		
L_DA_G	-0.002	0.002		
L_DL_G	-0.002	0.001		
Previous lactation days open	0.0007	0.0002	0.001	

Random-effects parameters	Estimate	SE	95% confidence interval	
Car(_cons)	0.337	0.143	0.146	0.775
Var(residual)	3.100	0.039	3.025	3.178
Intra-cluster correlation coefficient	0.098	0.038	0.045	0.200

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.10. Association between study group and ratio of fat to protein concentration at first test of lactation (between 7-35 DIM).

	Coefficient	SE	*P*-value	Mean	SE
Intercept	1.514	0.044	< 0.001		
Study group[1]			0.54		
S_DS_G	-0.015	0.061		1.29	0.03
S_DA_G	0.078	0.065		1.32	0.03
A_DS_G	-0.003	0.050		1.32	0.03
A_DA_G	Ref	Ref		1.32	0.03
A_DL_G	-0.016	0.042		1.33	0.03
L_DA_G	0.074	0.044		1.34	0.03
L_DL_G	-0.028	0.063		1.34	0.03
Parity			< 0.001		
2	Ref	Ref		1.30[a]	0.03
3+	0.047	0.004		1.35[b]	0.03
Fresh month			< 0.001		
Jan	Ref	Ref		1.31b[cd]	0.03
Feb	-0.024	0.010		1.29[ab]	0.03
Mar	-0.006	0.010		1.31[abc]	0.03
Apr	0.036	0.011		1.35[def]	0.03
May	0.021	0.010		1.33[cde]	0.03
June	-0.028	0.010		1.28[ab]	0.03
July	-0.037	0.010		1.28[a]	0.03
Aug	-0.006	0.009		1.31[abc]	0.03
Sept	-0.015	0.009		1.30[ab]	0.03
Oct	0.047	0.009		1.36[ef]	0.03
Nov	0.048	0.009		1.36[ef]	0.03
Dec	0.062	0.010		1.37[f]	0.03
Calf description			< 0.001		
Female	Ref	Ref		1.33[a]	0.03
Male	-0.008	0.004		1.32[a]	0.03
Twin	-0.046	0.009		1.28[b]	0.03
Milk yield at first test, kg					
Study group[1] × milk yield at first test, kg			< 0.001		
S_DS_G	0.001	0.001			
S_DA_G	0.002	0.001			
A_DS_G	-0.001	0.001			
A_DA_G	Ref	Ref			
A_DL_G	0.001	0.001			
L_DA_G	-0.004	0.001			
L_DL_G	0.000	0.001			
PTA milk, kg	-0.00003	0.00001	0.03		
Previous lactation 305 ME milk per 1000 kg	-0.007	0.001	< 0.001		

Study group[1] × previous lactation 305 ME milk, kg			
S_DS_G	-0.006	0.005	
S_DA_G	-0.014	0.004	
A_DS_G	0.003	0.004	
A_DA_G	Ref	Ref	
A_DL_G	-0.005	0.003	
L_DA_G	0.005	0.004	
L_DL_G	0.001	0.005	
Milk yield at last test before dry off, kg	-0.004	0.001	< 0.001
Milk yield at last test before dry off squared, kg	0.00005	0.00002	0.002
Previous lactation days open per 1000	0.511	0.085	< 0.001
Previous lactation days open squared, kg	-0.0006	0.0002	0.002
Study group[1] × previous lactation days open, per 1000			0.01
S_DS_G	0.2264	0.1683	
S_DA_G	0.0129	0.1461	
A_DS_G	-0.0835	0.1158	
A_DA_G	Ref	Ref	
A_DL_G	0.2563	0.0986	
L_DA_G	0.2845	0.0986	
L_DL_G	0.1796	0.1184	
DIM at first test	0.0013	0.0014	0.37
DIM at first test squared	-0.00008	0.00003	0.02

Random- effects parameters	**Estimate**	**SE**	**95% confidence interval**	
Var(_cons)	0.013	0.005	0.006	0.029
Var(residual)	0.062	0.001	0.061	0.063
ICC	0.175	0.058	0.088	0.316

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.11. Association between study group and 305-d mature equivalent milk production predicted from third test during lactation.

	Coefficient	SE	*P*-value	Mean	SE
Intercept	9332.53	269.28			
Study group			< 0.001		
S_DS_G	-222.30	340.74		12024.2[a]	256.2
S_DA_G	239.60	333.76		12526.9[b]	256.7
A_DS_G	578.13	311.46		12408.0[b]	256.5
A_DA_G	Ref	Ref		12537.2[b]	249.5
A_DL_G	-706.73	223.63		12504.8[b]	253.3
L_DA_G	-88.90	230.26		12296.4[b]	256.3
L_DL_G	-464.11	336.45		12541.9[b]	257.1
Parity group			< 0.001		
2	Ref	Ref		12045.6 [a]	250.1
3+	-366.82	22.31		11833.0 [b]	250.2
Fresh month			< 0.001		
Jan	Ref	Ref		11977.7[abc]	251.9
Feb	77.78	62.96		12103.5[cd]	252.3
Mar	-144.81	66.14		11866.0[ab]	252.9
Apr	-45.66	68.62		12006.6[abcd]	253.2
May	147.52	66.31		12155.4[de]	252.9
June	138.70	65.02		12127.8[cde]	252.6
July	160.34	68.51		12185.4[de]	253.0
Aug	222.66	61.24		12276.9[e]	252.2
Sept	0.63	59.65		12010.9[bcd]	251.8
Oct	-202.07	58.65		11852.1[a]	251.7
Nov	-235.87	59.48		11850.6[a]	251.8
Dec	-265.83	64.53		11824.2[a]	252.3
Study group × fresh month			0.02		
S_DS_G × Jan	Ref	Ref		11409.6	301.8
S_DS_G × Feb	246.33	251.98		11733.7	306.8
S_DS_G × Mar	42.82	265.75		11307.6	317.6
S_DS_G × Apr	491.77	251.62		11855.7	305.6
S_DS_G × May	344.57	243.90		11901.7	299.5
S_DS_G × June	-98.09	222.44		11450.2	282.8
S_DS_G × July	536.41	229.04		12106.4	286.7
S_DS_G × Aug	335.85	211.31		11968.1	274.4
S_DS_G × Sept	82.23	223.24		11492.5	283.8
S_DS_G × Oct	310.72	225.53		11518.3	286.2
S_DS_G × Nov	389.90	243.88		11563.6	300.2
S_DS_G × Dec	482.31	271.48		11626.1	321.9
S_DA_G × Jan	Ref	Ref		11869.6	286.0
S_DA_G × Feb	344.00	218.80		12291.4	295.4
S_DA_G × Mar	175.33	242.25		11900.1	312.0
S_DA_G × Apr	88.80	215.43		11912.7	292.0

S_DA_G × May	370.28	235.55	12387.4	307.4
S_DA_G × June	136.02	234.44	12144.3	306.8
S_DA_G × July	167.57	233.18	12197.5	304.9
S_DA_G × Aug	166.17	217.11	12258.4	294.6
S_DA_G × Sept	-148.88	210.46	11721.3	290.1
S_DA_G × Oct	80.18	211.53	11747.7	290.7
S_DA_G × Nov	194.20	219.08	11827.9	296.2
S_DA_G × Dec	640.85	242.86	12244.6	313.5
A_DS_G × Jan	Ref	Ref	11811.5	314.4
A_DS_G × Feb	57.23	255.63	11946.5	298.3
A_DS_G × Mar	-56.47	303.38	11610.2	338.9
A_DS_G × Apr	9.52	263.31	11775.3	302.2
A_DS_G × May	169.57	260.71	12128.6	301.8
A_DS_G × June	297.66	241.82	12247.8	285.3
A_DS_G × July	9.74	262.75	11981.5	301.3
A_DS_G × Aug	262.54	235.48	12296.7	280.6
A_DS_G × Sept	27.08	231.02	11839.2	277.6
A_DS_G × Oct	53.17	243.10	11662.6	287.6
A_DS_G × Nov	421.31	248.70	11996.9	292.8
A_DS_G × Dec	165.99	253.44	11711.6	295.9
A_DA_G × Jan	Ref	Ref	12071.5	253.3
A_DA_G × Feb	Ref	Ref	12149.3	253.9
A_DA_G × Mar	Ref	Ref	11926.7	254.7
A_DA_G × Apr	Ref	Ref	12025.8	255.3
A_DA_G × May	Ref	Ref	12219.0	254.7
A_DA_G × June	Ref	Ref	12210.2	254.4
A_DA_G × July	Ref	Ref	12231.8	255.3
A_DA_G × Aug	Ref	Ref	12294.1	253.4
A_DA_G × Sept	Ref	Ref	12072.1	253.1
A_DA_G × Oct	Ref	Ref	11869.4	252.9
A_DA_G × Nov	Ref	Ref	11835.6	253.0
A_DA_G × Dec	Ref	Ref	11805.6	254.1
A_DL_G × Jan	Ref	Ref	11971.1	267.1
A_DL_G × Feb	72.17	153.14	12121.1	270.9
A_DL_G × Mar	135.39	159.70	11961.7	273.9
A_DL_G × Apr	166.00	178.51	12091.5	285.0
A_DL_G × May	111.49	183.39	12230.1	288.5
A_DL_G × June	-226.13	177.79	11883.7	285.2
A_DL_G × July	106.99	172.48	12238.5	281.7
A_DL_G × Aug	113.26	166.10	12307.0	279.1
A_DL_G × Sept	241.19	146.87	12212.9	268.4
A_DL_G × Oct	149.93	144.36	11919.0	267.2
A_DL_G × Nov	154.09	140.09	11889.3	264.9
A_DL_G × Dec	296.06	154.83	12001.3	271.5
L_DA_G × Jan	Ref	Ref	11938.9	271.0
L_DA_G × Feb	19.75	178.57	12036.5	284.1

L_DA_G × Mar	-167.46	187.45		11626.7	288.8
L_DA_G × Apr	72.80	192.49		11966.1	291.2
L_DA_G × May	-117.65	172.64		11968.8	279.3
L_DA_G × June	16.18	176.81		12093.8	282.3
L_DA_G × July	-101.32	178.07		11998.0	282.4
L_DA_G × Aug	93.51	188.10		12255.1	290.3
L_DA_G × Sept	-25.24	172.35		11914.3	280.7
L_DA_G × Oct	299.45	167.19		12036.3	277.1
L_DA_G × Nov	458.99	164.13		12162.1	275.4
L_DA_G × Dec	168.37	174.22		11841.5	280.6
L_DL_G × Jan	Ref	Ref		11840.5	279.6
L_DL_G × Feb	101.53	201.30		12019.8	290.8
L_DL_G × Mar	472.60	230.53		12168.3	309.4
L_DL_G × Apr	370.45	271.26		12165.3	341.6
L_DL_G × May	-367.06	243.86		11621.0	321.3
L_DL_G × June	333.87	247.01		12313.1	322.1
L_DL_G × July	110.73	258.71		12111.6	331.2
L_DL_G × Aug	313.46	233.70		12376.6	313.6
L_DL_G × Sept	194.62	196.58		12035.7	287.1
L_DL_G × Oct	244.82	184.12		11883.2	279.8
L_DL_G × Nov	31.90	194.93		11636.5	286.3
L_DL_G × Dec	1.27	197.33		11575.9	287.1
Calf description			0.02		
Female	Ref	Ref		12534.5[a]	249.7
Male	110.04	26.93		12446.5[b]	249.7
Twin	28.35	73.93		12404.5[ab]	262.2
Study group × calf description			0.003		
S_DS_G × female	Ref	Ref		11888.5	266.6
S_DS_G × male	-381.17	102.19		12141.7	262.6
S_DS_G × twin	-77.25	140.61		12238.8	279.6
S_DA_G × female	Ref	Ref		12716.6	266.9
S_DA_G × male	198.85	101.49		12336.7	262.8
S_DA_G × twin	-7.96	207.79		12461.8	342.8
A_DS_G × female	Ref	Ref		12369.0	269.0
A_DS_G × male	-49.98	104.35		12440.6	260.9
A_DS_G × twin	-112.89	145.54		12480.9	284.5
A_DA_G × female	Ref	Ref		12606.7	250.1
A_DA_G × male	Ref	Ref		12470.5	250.1
A_DS_G × twin	Ref	Ref		12486.1	265.4
A_DL_G × female	Ref	Ref		12541.4	254.5
A_DL_G × male	-138.96	69.79		12529.6	257.3
A_DL_G × twin	-470.90	371.66		11936.4	523.6
L_DA_G × female	Ref	Ref		12270.8	260.4
L_DA_G × male	-192.90	79.43		12316.8	262.0
L_DA_G × twin	-160.69	186.57		12354.0	328.8
L_DL_G × female	Ref	Ref		12629.9	260.2

L_DL_G × male	15.06	99.52		12467.7	268.1
L_DL_G × twin	-271.03	368.16		12386.2	510.0
Dead on arrival			< 0.001		
No	Ref	Ref		12502.3[a]	249.3
Yes	-223.81	66.39		12159.8[b]	262.6
Mastitis at first test			< 0.001		
No	Ref	Ref		12604.1 [a]	249.5
Yes	-121.78	27.77		12196.7 [b]	250.4
PTA milk, kg	0.82	0.11	< 0.001		
PTA milk sq., kg	-0.00086	0.0003	0.008		
Previous lactation 305ME milk, kg	0.231	0.007	< 0.001		
Study group × Previous lactation 305ME milk, kg			< 0.001		
S_DS_G	-0.021	0.022			
S_DA_G	-0.019	0.022			
A_DS_G	-0.056	0.019			
A_DA_G	Ref	Ref			
A_DL_G	0.054	0.015			
L_DA_G	0.020	0.018			
L_DL_G	0.012	0.026			
Milk at last test before dry off, kg	6.4037	1.3913	< 0.001		
Previous lactation days open	-3.2915	0.4422			
Previous lactation days open sq.	0.0047	0.0010			
Study group × previous days open			< 0.001		
S_DS_G	0.174	0.865			
S_DA_G	-2.464	0.780			
A_DS_G	-0.784	0.630			
A_DA_G	Ref	Ref			
A_DL_G	-0.016	0.522			
L_DA_G	-1.711	0.518			
L_DL_G	0.690	0.644			

Random-effects parameters	Estimate	SE	95% confidence interval	
Dairy variance (_cons)	929398.7	358179.8	436675.3	1978088
Var(residual)	1807330	19553.5	1769409	1846063
Intra-cluster correlation coefficient	0.340	0.086	0.195	0.523

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.12. Association between study group and average fat concentration throughout lactation.

Variable	Coefficient	SE	*P*-value	Mean	SE
Intercept	4.026	0.080			
Study group			0.005		
S_DS_G	0.13	0.09		3.68	0.07
S_DA_G	-0.10	0.10		3.69	0.07
A_DS_G	-0.08	0.07		3.66	0.07
A_DA_G	Ref	Ref		3.67	0.07
A_DL_G	0.16	0.06		3.70	0.07
L_DA_G	-0.13	0.06		3.65	0.07
L_DL_G	-0.10	0.10		3.67	0.07
Parity group			< 0.001		
2	Ref	Ref		3.66^{a}	0.07
3+	0.04	0.01		3.68^{b}	0.07
Parity group × study group			< 0.001		
2 × S_DS_G	Ref	Ref		3.69^{ab}	0.07
2 × S_DA_G	Ref	Ref		3.67^{ab}	0.07
2 × A_DS_G	Ref	Ref		3.69^{ab}	0.07
2 × A_DA_G	Ref	Ref		3.69^{ab}	0.07
2 × A_DL_G	Ref	Ref		3.68^{ab}	0.07
2 × L_DA_G	Ref	Ref		3.65^{ab}	0.07
2 × L_DL_G	Ref	Ref		3.66^{ab}	0.07
3+ × S_DS_G	-0.054	0.028		3.69^{b}	0.07
3+ × S_DA_G	-0.040	0.033		3.67^{ab}	0.07
3+ × A_DS_G	-0.069	0.029		3.72^{b}	0.07
3+ × A_DA_G	Ref	Ref		3.68^{ab}	0.07
3+ × A_DL_G	0.014	0.023		3.62^{a}	0.07
3+ × L_DA_G	-0.095	0.025		3.64^{ab}	0.07
3+ × L_DL_G	0.016	0.030		3.70^{ab}	0.07
Fresh month			< 0.001		
Jan	Ref	Ref		3.61^{a}	0.07
Feb	0.0003	0.016		3.61^{a}	0.07
Mar	-0.003	0.017		3.60^{a}	0.07
Apr	0.031	0.017		3.64^{ab}	0.07
May	0.055	0.017		3.66^{abcd}	0.07
June	0.084	0.016		3.69^{bcd}	0.07
July	0.093	0.016		3.70^{cd}	0.07
Aug	0.109	0.015		3.72^{de}	0.07
Sept	0.155	0.015		3.76^{e}	0.07
Oct	0.097	0.015		3.70^{d}	0.07
Nov	0.068	0.015		3.68^{bcd}	0.07
Dec	0.047	0.015		3.65^{ab}	0.07
PTA fat yield, kg	0.016	0.001	< 0.001		
PTA milk, kg	-0.0005	2.39E-05	< 0.001		

Previous lactation 305 ME milk per 1000 kg	-0.044	0.002	< 0.001
Study group × prvme			0.002
S_DS_G	-7.51E-03	6.66E-03	
S_DA_G	1.06E-02	7.03E-03	
A_DS_G	8.29E-03	5.73E-03	
A_DA_G	Ref	Ref	
A_DL_G	-1.17E-02	4.66E-03	
L_DA_G	1.21E-02	5.09E-03	
L_DL_G	6.96E-03	7.71E-03	
Milk yield at last test before dry off, kg	0.003	0.001	0.026
Milk yield at last test before dry off squared, kg	-7.3E-05	2.56E-05	0.004
DIM when left	0.00023	0.00004	< 0.001

Random-effects parameters	**Estimate**	**SE**	**95% confidence interval**	
Var(_cons)	0.068	0.027	0.031	0.148
Var(residual)	0.206	0.002	0.202	0.210
Intra cluster correlation coefficient	0.249	0.074	0.132	0.418

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.13. Association between study group and whole lactation milk fat yield.

Variable	**Coefficient**	**SE**	***P*-value**	**Means**	**SE**
Intercept	284.11	20.68			
Study group			0.03		
S_DS_G	19.39	18.33		436.2^{a}	14.5
S_DA_G	-2.30	19.56		451.0^{b}	14.6
A_DS_G	34.95	14.85		446.2^{ab}	14.6
A_DA_G	Ref	Ref		452.5^{b}	14.3
A_DL_G	7.77	11.83		455.4^{b}	14.4
L_DA_G	-27.91	12.41		448.0^{b}	14.5
L_DL_G	-2.11	20.85		453.8^{b}	14.6
Parity group			< 0.001		
2	Ref	Ref		456.7^{a}	14.3
3+	-11.03	1.45		445.7^{b}	14.3
Fresh month			< 0.001		
Jan	Ref	Ref		434.0^{a}	14.4
Feb	12.95	2.82		447.0^{bc}	14.4
Mar	5.02	2.98		439.0^{ab}	14.5
Apr	11.68	3.15		445.7^{bc}	14.5
May	9.63	3.25		443.6^{abc}	14.5
June	26.27	3.15		460.3^{de}	14.5

July	32.46	3.23		466.5[e]	14.5
Aug	31.63	3.03		465.6[e]	14.4
Sept	28.57	3.02		462.6[de]	14.4
Oct	20.34	3.05		454.4[cd]	14.5
Nov	19.45	3.01		453.5[cd]	14.4
Dec	12.26	3.28		446.3[bc]	14.5
Dead on arrival			0.03		
No	Ref	Ref		451.2[a]	14.3
Yes	-9.25	4.36		441.9[b]	14.9
Mastitis at first test			0.001		
No	Ref	Ref		452.1[a]	14.3
Yes	-5.96	1.78		446.2[b]	14.4
PTA fat yield, kg	2.10	0.13	< 0.001		
PTA milk yield, kg	-0.038	0.005	< 0.001		
Previous 305 ME milk per 1000 kg	0.82	0.17	< 0.001		
Previous 305 ME milk sq. per 1000 kg	-2.07E-04	5.64E-05	< 0.001		
Study group × previous lactation 305 ME milk per 1000 kg			0.02		
S_DS_G	-2.61	1.37			
S_DA_G	0.57	1.37			
A_DS_G	-2.44	1.10			
A_DA_G	Ref	Ref			
A_DL_G	0.005	0.86			
L_DA_G	2.18	1.00			
L_DL_G	0.71	1.59			
Milk yield at last test before dry off, kg	0.28	0.09	< 0.001		
Somatic cell linear score at last test before dry off, kg	-0.04	0.11	0.72		
Study group × somatic cell linear score at last test			0.04		
S_DS_G	-0.60	0.83			
S_DA_G	-2.22	1.64			
A_DS_G	-3.20	1.75			
A_DA_G	Ref	Ref			
A_DL_G	-1.65	0.78			
L_DA_G	-1.67	0.98			
L_DL_G	-1.93	1.77			
Previous lactation days open	-0.108	0.026	< 0.001		
Previous lactation days open squared	0.0001	0.0001	0.007		
DIM left herd	0.48	0.07	< 0.001		
DIM left her squared	-0.0007	0.0001	< 0.001		

Random-effects parameters	Estimate	SE	95% confidence interval	
Var (_cons)	2793.7	1141.7	1254.1	6223.4
Var (residual)	4580.7	62.1	4460.5	4704.1
Intra-cluster correlation coefficient	0.379	0.096	0.215	0.576

[1]Study groups: S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.14. Association between study group and average protein concentration throughout lactation.

Variable	Coefficient	SE	*P*-value	Means	SE
Intercept	3.019	0.035			
Study group			< 0.001		
S_DS_G	0.046	0.006		3.16[d]	0.03
S_DA_G	0.016	0.007		3.13[c]	0.03
A_DS_G	-0.001	0.006		3.11[bc]	0.03
A_DA_G	Ref	Ref		3.11[bc]	0.03
A_DL_G	-0.008	0.005		3.10[b]	0.03
L_DA_G	-0.033	0.005		3.08[a]	0.03
L_DL_G	-0.036	0.006		3.07[a]	0.03
Parity group			< 0.001		
2	Ref	Ref		3.12[a]	0.03
3+	-0.016	0.003		3.10[b]	0.03
Calf description			0.001		
Female	Ref	Ref		3.11[a]	0.03
Male	-0.006	0.003		3.10[a]	0.03
Twin	0.016	0.007		3.13[b]	0.03
Fresh month			< 0.001		
Jan	Ref	Ref		3.07[a]	0.03
Feb	0.025	0.007		3.09[bc]	0.03
Mar	0.015	0.007		3.08[ab]	0.03
Apr	0.028	0.007		3.09[bc]	0.03
May	0.032	0.007		3.10[bc]	0.03
June	0.070	0.007		3.13[d]	0.03
July	0.071	0.007		3.14[d]	0.03
Aug	0.082	0.007		3.15[d]	0.03
Sept	0.070	0.006		3.13[d]	0.03
Oct	0.041	0.006		3.11[c]	0.03
Nov	0.036	0.006		3.10[bc]	0.03
Dec	0.036	0.007		3.10[bc]	0.03
Dead on arrival			0.006		
No	Ref	Ref		3.11[a]	0.03
Yes	0.024	0.009		3.13[b]	0.03
PTA protein percent	0.019	0.002	< 0.001		
PTA protein yield, kg	0.005	0.001	< 0.001		
PTA milk, kg	-0.0001	3.53E-05	0.003		
Previous 305 ME milk, kg	-0.021	0.001	< 0.001		
LMILK, kg	0.003	0.001	< 0.001		
LMILK sq., kg	-5.5E-05	1.09E-05	< 0.001		
Previous lactation days open	-0.00021	5.58E-05	< 0.001		
Previous lactation days open squared	3.80E-07	1.26E-07	0.003		

Random-effects parameters	Estimate	SE	95% confidence interval	
Var (_cons)	0.012	0.005	0.005	0.026

Var (residual)	0.037	0.0004	0.036	0.038
Intra-cluster correlation coefficient	0.241	0.073	0.127	0.408

S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.15. Association between study group and whole lactation protein yield.

Variable	Coefficient	SE	*P*-value	Means	SE
Intercept	233.49	9.33	<0.001		
Comcat			<0.001		
S_DS_G	-6.69	1.44		371.90[a]	7.88
S_DA_G	-0.44	1.55		378.15[bc]	7.90
A_DS_G	-4.11	1.46		374.48[abc]	7.89
A_DA_G	Ref	Ref		378.60[c]	7.77
A_DL_G	1.13	1.11		379.73[c]	7.83
L_DA_G	-6.70	1.29		371.89[a]	7.85
L_DL_G	-5.97	1.53		372.63[ab]	7.90
Parity group			< 0.001		
2	Ref	Ref		383.20[a]	7.78
3+	-11.44	0.72		371.77[b]	7.78
Fresh month			<0.001		
Jan	Ref	Ref		371.4[a]	7.8
Feb	7.42	1.57		378.8[b]	7.8
Mar	-1.35	1.65		370.1[a]	7.9
Apr	6.64	1.71		378.1[b]	7.9
May	9.68	1.66		381.1[bc]	7.9
June	14.10	1.61		385.5[cd]	7.9
July	16.50	1.68		387.9[d]	7.9
Aug	18.35	1.53		389.8[d]	7.8
Sept	7.45	1.47		378.9[b]	7.8
Oct	0.32	1.45		371.7[a]	7.8
Nov	-1.40	1.47		370.0[a]	7.8
Dec	-2.58	1.59		368.8[a]	7.8
Mastitis at first test			0.02		
No	Ref	Ref		377.76[a]	7.77
Yes	-1.96	0.87		375.80[b]	7.79
PTA protein concentration, %	1.73	0.17	< 0.001		
PTA protein yield, kg	0.77	0.09	< 0.001		
Prvme per 1000 kg	4.61	0.20	< 0.001		
Milk at last test before dry off, kg	0.14	0.04	0.002		
Previous lactation days open	-0.13	0.01	< 0.001		
Previous lactation days open squared	0.00019	0.00003	< 0.001		
DIM when left herd	0.51	0.04	< 0.001		
DIM when left herd squared	-0.0007	7.27E-05	< 0.001		

Random-effects parameters	Estimate	SE	95% confidence interval	
Var (_cons)	8375238	3399611	3779933	1.86E+07
Var (Residual)	1.80E+07	196817.9	1.76E+07	1.84E+07
Intra-class correlation coefficient	0.318	0.088	0.174	0.508

S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average

gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.16. Association between study group and average LSC throughout lactation.

Variable	Coefficient	SE	*P*-value	Means	SE
Intercept	2.864	0.178			
Study group[1]			0.17		
S_DS_G	-0.394	0.176		2.43	0.17
S_DA_G	-0.107	0.147		2.29	0.17
A_DS_G	-0.044	0.199		2.41	0.17
A_DA_G	Ref	Ref		2.36	0.16
A_DL_G	-0.169	0.106		2.39	0.16
L_DA_G	0.002	0.114		2.43	0.17
L_DL_G	-0.219	0.129		2.33	0.17
Parity group			< 0.001		
2	Ref	Ref		2.10^{a}	0.16
3+	0.505	0.025		2.61^{b}	0.16
Fresh month			0.84		
Jan	Ref	Ref		2.39^{ab}	0.16
Feb	-0.025	0.066		2.36^{ab}	0.16
Mar	-0.076	0.068		2.37^{ab}	0.16
Apr	-0.057	0.076		2.36^{ab}	0.17
May	-0.098	0.076		2.34^{ab}	0.17
June	-0.081	0.072		2.42^{ab}	0.17
July	-0.137	0.076		2.23^{a}	0.17
Aug	-0.093	0.069		2.32^{ab}	0.16
Sept	-0.130	0.071		2.39^{ab}	0.16
Oct	-0.091	0.069		2.40^{ab}	0.16
Nov	-0.086	0.070		2.39^{ab}	0.16
Dec	-0.032	0.076		2.46^{b}	0.17
Study group[1] × fresh month			0.002		
Jan					
S_DS_G	Ref	Ref			
S_DA_G	Ref	Ref			
A_DS_G	Ref	Ref			
A_DA_G	Ref	Ref			
A_DL_G	Ref	Ref			
L_DA_G	Ref	Ref			
L_DL_G	Ref	Ref			
Feb					
S_DS_G	0.456	0.242			
S_DA_G	-0.284	0.218			
A_DS_G	-0.100	0.257			
A_DA_G	Ref	Ref			
A_DL_G	-0.228	0.160			
L_DA_G	0.205	0.181			
L_DL_G	0.069	0.199			
March					
S_DS_G	0.156	0.283			

S_DA_G	-0.117	0.234
A_DS_G	0.752	0.295
A_DA_G	Ref	Ref
A_DL_G	0.160	0.160
L_DA_G	-0.141	0.195
L_DL_G	0.341	0.249
April		
S_DS_G	0.575	0.270
S_DA_G	0.199	0.221
A_DS_G	0.085	0.268
A_DA_G	Ref	Ref
A_DL_G	-0.245	0.196
L_DA_G	-0.238	0.195
L_DL_G	0.274	0.264
May		
S_DS_G	0.443	0.256
S_DA_G	-0.284	0.264
A_DS_G	0.052	0.288
A_DA_G	Ref	Ref
A_DL_G	0.001	0.206
L_DA_G	0.278	0.193
L_DL_G	0.065	0.265
June		
S_DS_G	0.587	0.238
S_DA_G	0.319	0.305
A_DS_G	-0.148	0.259
A_DA_G	Ref	Ref
A_DL_G	0.274	0.188
L_DA_G	0.415	0.204
L_DL_G	0.540	0.275
July		
S_DS_G	0.389	0.243
S_DA_G	-0.129	0.271
A_DS_G	-0.040	0.279
A_DA_G	Ref	Ref
A_DL_G	0.044	0.185
L_DA_G	-0.334	0.188
L_DL_G	-0.136	0.287
Aug		
S_DS_G	0.338	0.227
S_DA_G	0.157	0.255
A_DS_G	-0.182	0.252
A_DA_G	Ref	Ref
A_DL_G	0.121	0.170
L_DA_G	0.081	0.205
L_DL_G	0.067	0.260

Sept					
S_DS_G	0.715	0.245			
S_DA_G	0.044	0.237			
A_DS_G	0.186	0.253			
A_DA_G	Ref	Ref			
A_DL_G	0.476	0.166			
L_DA_G	0.079	0.179			
L_DL_G	0.358	0.246			
Oct					
S_DS_G	0.646	0.247			
S_DA_G	0.556	0.267			
A_DS_G	0.188	0.264			
A_DA_G	Ref	Ref			
A_DL_G	0.169	0.169			
L_DA_G	0.033	0.187			
L_DL_G	0.276	0.244			
Nov					
S_DS_G	0.902	0.263			
S_DA_G	-0.118	0.280			
A_DS_G	0.165	0.274			
A_DA_G	Ref	Ref			
A_DL_G	0.181	0.155			
L_DA_G	0.086	0.181			
L_DL_G	0.055	0.238			
Dec					
S_DS_G	0.709	0.326			
S_DA_G	0.071	0.285			
A_DS_G	-0.180	0.293			
A_DA_G	Ref	Ref			
A_DL_G	0.121	0.173			
L_DA_G	0.269	0.193			
L_DL_G	0.595	0.273			
Mastitis at first test			< 0.001		
No	Ref	Ref		2.15^{a}	0.16
Yes	1.027	0.040		3.26^{b}	0.16
Study group[1] × mastitis at first test			< 0.001		
S_DS_G × no mastitis	Ref	Ref		2.24^{a}	0.17
S_DA_G × no mastitis	Ref	Ref		2.10^{a}	0.17
A_DS_G × no mastitis	Ref	Ref		2.15^{a}	0.17
A_DA_G × no mastitis	Ref	Ref		2.16^{a}	0.16
A_DL_G × no mastitis	Ref	Ref		2.11^{a}	0.17
L_DA_G × no mastitis	Ref	Ref		2.19^{a}	0.17
L_DL_G × no mastitis	Ref	Ref		2.12^{a}	0.17
S_DS_G × mastitis	-0.034	0.125		3.24^{bc}	0.19
S_DA_G × mastitis	0.008	0.143		3.10^{bc}	0.20
A_DS_G × mastitis	0.321	0.139		3.49^{bc}	0.20

A_DA_G × mastitis	Ref	Ref		3.19[b]	0.16
A_DL_G × mastitis	0.417	0.098		3.54[c]	0.18
L_DA_G × mastitis	0.231	0.108		3.42[bc]	0.18
L_DL_G × mastitis	0.081	0.143		3.24[bc]	0.20
Previous lactation 305 ME milk, kg	-1.1E-05	2.85E-06	< 0.001		
Milk at last test before dry off, kg	-0.004	0.001	0.01		
Somatic cell linear score at last test	0.003	0.001	< 0.001		
Somatic cell linear score at last test, sq.	-7.03E-07	2.06E-07	< 0.001		
Study group[1] × Somatic cell linear score at last test			0.001		
S_DS_G	-0.0006	0.0006			
S_DA_G	0.0002	0.0010			
A_DS_G	-0.0017	0.0010			
A_DA_G	Ref	Ref			
A_DL_G	0.0029	0.0021			
L_DA_G	-0.0047	0.0012			
L_DL_G	-0.0012	0.0008			

Random-effects parameters	Estimate	SE	95% confidence interval	
Var (_cons)	0.351	0.142	0.158	0.777
Var (Residual)	1.866	0.023	1.822	1.911
Intra-class correlation coefficient	0.158	0.054	0.078	0.294

[1]S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Table A.17. Association between study group and first service conception risk.

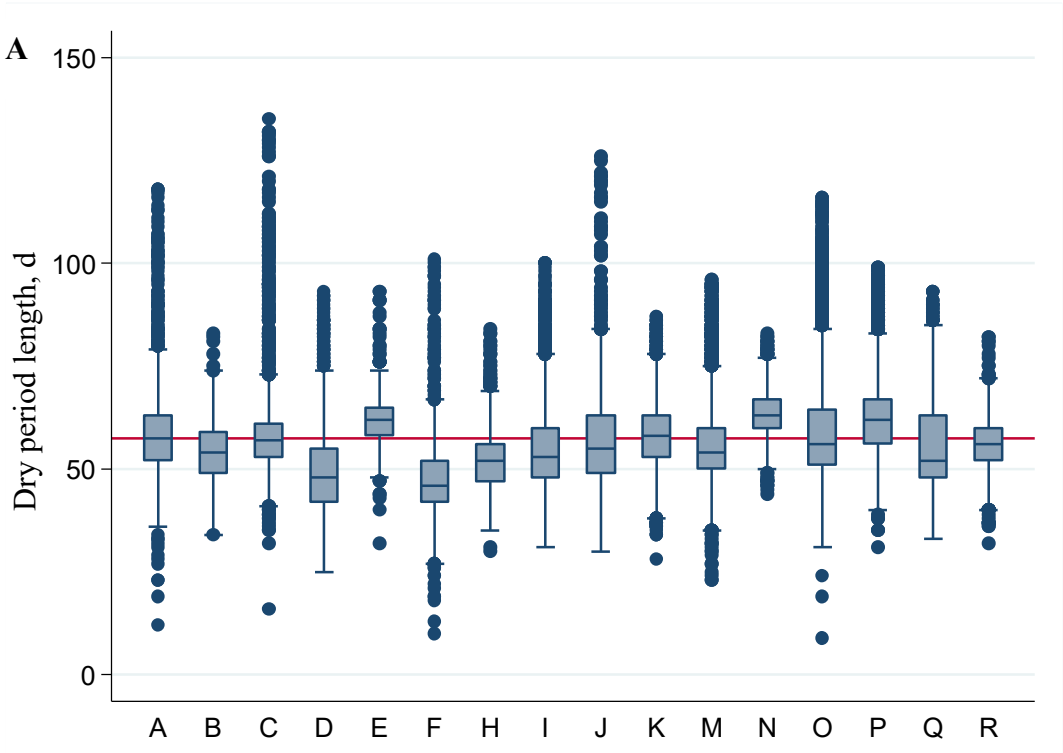
Variable	Coefficient	SE	95% CI	Odds ratio	SE	95% CI	*P*-value
Intercept	-0.820	0.140	-1.094, -0.546	0.44	0.06	0.335, 0.580	< 0.001
Study group[1]							0.06
S_DS_G	-0.040	0.062	-0.162, 0.081	0.96	0.06	0.851, 1.085	0.52
S_DA_G	0.132	0.067	0.0003, 0.263	1.14	0.08	1.000, 1.301	0.05
A_DS_G	0.034	0.066	-0.095, 0.163	1.04	0.07	0.909, 1.177	0.61
A_DA_G	Ref	Ref	Ref	Ref	Ref	Ref	
A_DL_G	-0.058	0.048	-0.152, 0.036	0.94	0.05	0.859, 1.037	0.23
L_DA_G	0.051	0.057	-0.061, 0.164	1.05	0.06	0.941, 1.178	0.37
L_DL_G	-0.138	0.066	-0.267, -0.009	0.87	0.06	0.766, 0.991	0.04
Parity							< 0.001
2	Ref	Ref	Ref	Ref	Ref	Ref	
3+	-0.164	0.029	-0.221, -0.106	0.85	0.03	0.802, 0.899	
Fresh month							< 0.001
Jan	Ref	Ref	Ref	Ref	Ref	Ref	
Feb	0.192	0.066	0.063, 0.32	1.21	0.08	1.065, 1.378	0.004
Mar	0.057	0.071	-0.082, 0.195	1.06	0.08	0.921, 1.216	0.42
Apr	-0.060	0.074	-0.206, 0.086	0.94	0.07	0.814, 1.089	0.42
May	-0.297	0.074	-0.443, -0.151	0.74	0.06	0.642, 0.860	< 0.001
June	-0.177	0.071	-0.317, -0.038	0.84	0.06	0.728, 0.963	0.01
July	-0.101	0.069	-0.236, 0.034	0.90	0.06	0.790, 1.035	0.14
Aug	0.003	0.066	-0.126, 0.133	1.00	0.07	0.882, 1.142	0.96
Sept	0.029	0.064	-0.096, 0.154	1.03	0.07	0.909, 1.167	0.65
Oct	0.011	0.064	-0.114, 0.135	1.01	0.06	0.892, 1.145	0.87
Nov	0.018	0.064	-0.107, 0.143	1.02	0.07	0.898, 1.154	0.78
Dec	0.070	0.067	-0.061, 0.201	1.07	0.07	0.941, 1.223	0.30
Calf description							< 0.001
Female	Ref	Ref	Ref	Ref	Ref	Ref	
Male	-0.080	0.029	-0.137, -0.024	0.92	0.03	0.872, 0.977	0.005
Twin	-0.422	0.073	-0.565, -0.279	0.66	0.05	0.568, 0.756	< 0.001
Dead on arrival							< 0.001
No	Ref	Ref	Ref	Ref	Ref	Ref	
Yes	-0.435	0.099	-0.629, -0.242	0.65	0.06	0.533, 0.785	
Milk1, kg	0.004	0.001	0.002, 0.007	1.00	0.001	1.002, 1.007	0.002
Lmilk, kg	-0.006	0.002	-0.009, -0.002	0.99	0.002	0.991, 0.998	0.001
pdopn	-0.003	0.0002	-0.003, -0.003	0.997	0.0002	0.997, 0.997	< 0.001
DIM at first service	0.006	0.001	0.003, 0.009	1.01	0.001	1.003, 1.009	< 0.001

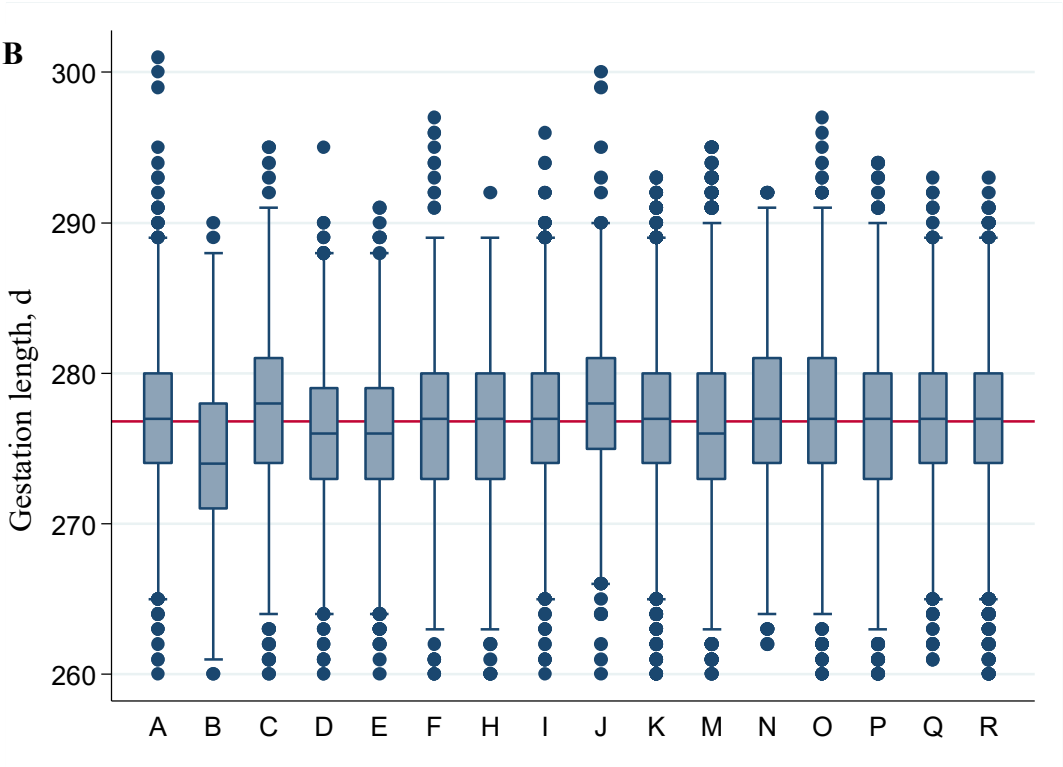
Random-effects parameters	Estimate	SE	95% CI
sd(_cons)	0.186	0.036	0.127, 0.273
ICC	0.054		

[1]S_DS_G = short dry period, short gestation length; S_DA_G = short dry period, average gestation length; A_DS_G= average dry period, short gestation length; A_DA_G = average dry period, average gestation

length; A_DL_G = average dry period, long gestation length; L_DA_G = long dry period, average gestation length; L_DL_G = long dry period, long gestation length

Figure A.1. Box and whiskers plots of A) dry period length and B) gestation length by dairy. The outer boundaries of the box plots closest to and farthest from the horizontal axis indicate the 25th and 75th percentiles, respectively. The line within the box indicates the median; the whiskers above and below the box indicate the upper adjacent value (quartile 3 +1.5[interquartile range]) and the lower adjacent value (quartile 1 -1.5[interquartile range]), and the dots above and below the whiskers designate outliers. The red line across the chart marks the overall average for each (dry period length = 57.6 d; gestation length = 276.9 d).




B
Gestation length, d
300
290
280
270
260
A
B
C
D
E
F
H
I
J
K
M
N
O
P
Q
R