

**EARLY LIFE EVENTS ALTER FUTURE HOLSTEINHEIFER GROWTH,
SURVIVABILITY, REPRODUCTION, AND FIRST LACTATION MILK
PRODUCTION**

by

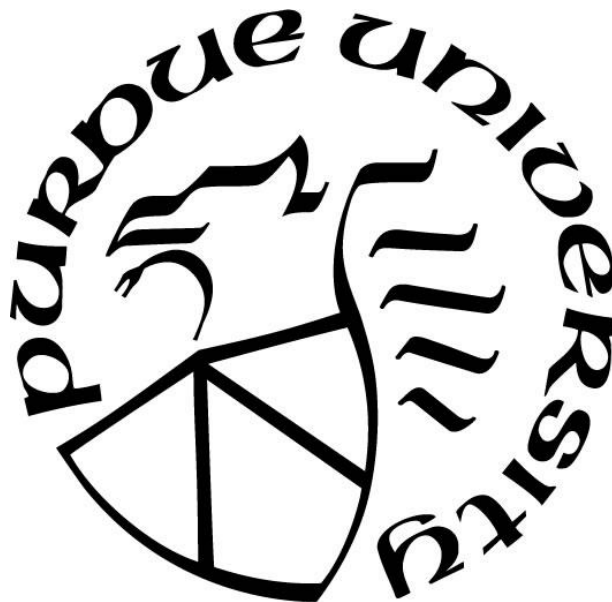
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This thesis is dedicated to my dad, Paul Steckler and my husband Bryan Hurst. To my dad for instilling in me a love of agriculture, stringent work ethic, and a curious mind! To Bryan, without your support and encouragement throughout this process, I would not be where I am today!

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ABSTRACT

The objective of this study was to evaluate the long-term effects that early life events have on heifer growth up to 400 d of age, heifer conception rate, survivability through first lactation, and first lactation milk production of calves raised in automatic calf feeders. Chapter one evaluates possible early life variables that would affect heifer growth and lifetime production as well as research that has been done to predict future growth. The major points discussed include pre-weaning feeding strategies, automatic calf feeding systems, respiratory disease and ways to diagnose cattle with this disease, and the impact of early life growth on the future productivity of the dairy cow.

The second chapter discusses in detail the process of creating a predictive equation using significant early life variables that affect Holstein heifer growth up to 400 d of age. Variables collected for the growth analysis included sixty d cumulative milk consumption (MC), serum total protein values, respiratory disease and scours incidences, genetic body size, birthweights, and incremental body weight variables on a commercial dairy farm from October 1, 2015 to January 1, 2019. Calves were fed pasteurized whole milk through an automated calf feeding system (feeders = 8) for 60 d (range: 48 – 126d), with a 30% Crude Protein (CP) and 5% Crude Fat enhancer added at 20 g/L of milk. Calves were weighed at birth and several other times prior to calving. Average birth weight of calves was 40.6 ± 4.9 kg (mean \pm SD), serum total protein was 6.7 ± 0.63 g/dL, and cumulative 60 d MC was 508.1 ± 67.3 L with a range of 179.9 to 785.1 L. Daily body weights were predicted for individual animals using a third order orthogonal polynomial to model growth curves. The linear and quadratic effects of cumulative 60 d milk consumption, birthweight, feeder, yr born, season born, respiratory incidence, and genetic body size score were significant ($P < 0.0001$) when predicting heifer body weight at 400 d (pBW₄₀₀) of age ($R^2 = 0.31$). There was up to a 263 kg difference in pBW₄₀₀ between the heaviest and lightest animal. Birthweight had a significant effect on predicted weights up to 400 d ($P < 0.0001$), and for every 1 kg increase in birthweight, there was a 2.5 kg increase in pBW₄₀₀. The quadratic effect of cumulative 60 d MC was significant for pBW₄₀₀ ($P < 0.0001$). When 60 d MC was divided into quartiles, heifers had the highest pBW₄₀₀ in the third quartile, when 60 d MC was between 507.8 and 552.5 L. Body size composite (genomic index) showed a 21.5 kg difference in pBW₄₀₀ between the top and bottom 25th percentile of heifers. Heifers were 4.2 kg lighter at 400 d if treated

for respiratory disease 3+ times during the first 60 d of life, compared to heifers not treated for respiratory disease.

The third chapter utilizes the data described in chapter two and followed those heifers through breeding and first lactation. Heifer conception age and 280 d first lactation milk production (280M) were collected. Average age at conception was 437.5 ± 45.0 d; range of 308 to 631 d ($n=5,193$), and average 280M was $9,305 \pm 1,371.8$ kg; range of 712-13,358 kg ($n=1,324$). Heifer conception age was impacted by season, yr, and the quadratic effects of predicted bodyweight at 300 d of age (pBW_{300}) and ADG (0-400; all $P < 0.05$; total model $R^2 = 0.08$). Season born, ADG (0 - 400 d), genomic milk, and the linear effect of heifer conception age had a significant impact on 280M (all $P < 0.05$; $R^2 = 0.28$). For every 1 kg increase in genomic milk value there is 1.42 kg increase in first lactation 280M. Calves not diagnosed with bovine respiratory disease (BRD) from 60-120 d old had a significantly higher chance for survival to first lactation than animals treated three or more times for BRD (hazard ratio = 0.71, 95% CI = 0.574 to 0.886, $P = 0.0023$, Table 3.3). Heifers treated twice or more for BRD had reduced likelihood to become pregnant than heifers not treated for BRD from 60-120 d (twice $P = 0.02$; three or more $P = 0.05$).

In conclusion, the results from this thesis support that early life events in Holstein heifers continue to influence future growth and productivity. Future research aims to validate the predictive equation generated in chapter two on farm as well as adapt the equation to other farms allowing them to utilize it as well. The goal is to have farms utilize this tool to aid in their replacement heifer management decisions and to select the most productive heifers for the future of their herds.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Dairy replacement heifers represent the next generation of cows entering the herd making their management important for their performance as heifers and future performance as a dairy cow. From birth to first parturition it takes an approximate two yr investment and requires on average \$2,510 per heifer before she generates any income for the farm (Akins and Hagedorn., 2015). This literature review will investigate the heifer research done during the pre-weaning period for feeding strategies, the incidence of respiratory disease, the use of automatic calf feeders, and the impact of early life growth on the future productivity of the dairy cow. There is a gap in our knowledge related to the direct effect of early life health and management interactions on the productivity of the future dairy cow. This thesis aims to identify early-life factors on a commercial dairy farm will affect growth, survivability, reproductive performance, and milk production.

1.2 Feeding Strategies

1.2.1 Conventional, Accelerated, and *Ad Libitum* Feeding

A recent qualitative study done in the United Kingdom was conducted to evaluate why farmers use different pre-weaning feeding strategies (Palczynski et al., 2020). Palczynski et al. 2020 observed that farms will feed calves based on the perception of maximizing growth while implementing the most simplistic and cost/time effective feeding strategy in comparison to another strategy. The pre-weaning phase is the most expensive time in rearing the replacement heifer with Wisconsin dairy farm costs averaging \$5.84/hd/d when housed individually and \$6.35/hd/d when housed in automatic calf feeders (Akins et al., 2018). Even though the cost associated with the pre-weaning phase is extremely important to dairy producers, Palczynski et al. (2020) observed that the majority of the 26 farms interviewed could not recall basic aspects of their feeding program such as fat and protein content of the milk replacer they were feeding. Farms can feed different types of milk (whole milk, waste milk, milk replacer), different amounts of milk, different milk replacers with alternative protein sources, different protein and fat ratios, and different solids concentrations. Therefore, to obtain the highest return on investment during the pre-weaning period, it is imperative for each farm to know exactly why and how they are feeding their calves.

Producers can successfully raise replacement heifers using several different feeding strategies. The different feeding strategies are separated into three programs: *ad libitum*, accelerated (intensified) and conventional (limit-fed). Traditionally, conventional feeding programs offer calves milk or milk replacer at 10% of their body weight (BW) (Jasper and Weary, 2002; Khan et al., 2007; Drackley, 2008) or a milk replacer containing approximately 20% CP and 20% fat and fed at 0.53 to 0.56 kg/d DM milk replacer (Cowles et al., 2006; Raeth-Knight et al., 2009). In 2011, the USDA reported that the majority of calves fed in the U.S. are offered 4 to 5 quarts per day in two meals; therefore, many producers follow this conventional system (USDA, 2012). However, the accelerated feeding strategy is not as well defined. Raeth-Knight et al. (2009) fed calves an intensified diet that consisted of a 0.68 kg of milk replacer with 28% CP and 18% fat. In contrast to a conventionally fed diet, Davis Rincker et al. (2011) fed an accelerated/intensified diet of a high protein milk replacer (30.6% CP, 16.1% fat) at a 2.1% of BW on a DM basis. Kiezebrink et al. (2015) fed animals either 4 L/d or 8 L/d and the researchers considered 8 L/d to be an enhanced feeding program. *Ad libitum* feeding will allow the calves to drink as much milk as they desire, and Jasper and Weary (2002) accomplished this by filling a 23 L bucket with milk twice a day allowing the calf continuous access. Von Keyserlingk et al. (2004) allowed calves access to milk from an artificial teat 24 h/d. Milk or milk replacer can also be offered *ad libitum* through an automatic calf feedings system as well (Welboren et al., 2019).

Calves fed on a conventional system have been shown to eat significantly more starter during the pre-weaning phase than calves that were offered more milk (Jasper and Weary, 2002; Richard et al., 2010; Dennis et al., 2018). For example, when calves were offered 0.66 kg of DM/d versus 1.09 kg of DM/d of milk replacer powder, calves that drank the decreased amount of milk ate 0.35 kg more starter per day until 56 d old (Dennis et al., 2018). Supplying starter in addition to milk or milk replacer instead of hay during the pre-weaning phase increases the volatile fatty acid supply, specifically butyrate, which in turn increases calf rumen fermentation (Laarman et al., 2012). Butyrate increases rumen fermentation by stimulating the development of rumen papillae (Flatt et al., 1958). This increased rumen fermentation from greater starter grain intake was found in Dennis et al. (2018) when calves fed the lower milk allowance had higher DM, OM, CP, NDF, ADF, and fat digestibility at d 84. Even though there is an increase in starter intake and a potential for improved rumen development when calves are fed on the conventional system, there are some

concerns with this approach such as reduced rate of gains, decreased health, and abnormal calf behavior.

The current recommendation for calf growth is to double the birthweight by weaning (ex. Birthweight = 36.3 kg, weaning weight = 72.6 kg) and to obtain this goal by 56 d the calf needs to gain 0.64 kg/d (DCHA, 2016). According to the NRC 2001 a 30 to 60 kg calf needs to consume 0.32 to 0.54 kg of dry matter (DM) to maintain BW, and to gain 0.4 kg/d a 30-60 kg calf will need to consume 0.56 to 0.84 kg of DM (National Research Council, 2001). Dennis et al. (2018) fed calves 0.66 kg of DM/d of milk replacer and offered *ad libitum* starter and obtained a 0.588 kg/d average daily gain (ADG) from 0-42 d of age. In this conventional feeding example calves did not double their average birthweight (initial: 43.3 kg; final: 76.2 kg) or obtain adequate ADG. Another conventional feeding program (85% of calves fed 20% CP and 20% fat milk replacer at 0.57 kg/calf/d) reported a six wk ADG of 0.54 kg/d which is lower than the current recommendation (Rauba et al., 2019).

Different feeding strategies can also influence calf behavior, and this can be observed from data of calves in an automatic calf feeder. De Passillé et al. (2011) reported an increase in unrewarded visits to the automated calf feeder when fed a lower amount of milk indicating a change in normal behavior. An unrewarded visit is identified by the automatic calf feeder when calves enter the feeder to drink milk before their next allotted feeding time. The feeder does not dispense any milk for the calves and it is recorded as an unrewarded visit. Sutherland et al. (2018) reported that calves had on average between 2.61 and 3.94 unrewarded visits before any treatments were applied and when a stressful event such as dehorning occurred, unrewarded visits decreased indicating a change in normal behavior. Health status of animals fed on the conventional system is not well understood and several studies have reported no difference in disease incidence from calves fed differing amounts of milk; therefore, more research is needed to understand if conventional systems negatively affect health status (Raeth-Knight et al., 2009; Bach et al., 2013).

The accelerated feeding strategy allows the calf to consume an increased amount of milk during the pre-weaning phase. This type of feeding system is highly variable and encompasses a wide variety of programs. The fundamental idea behind increasing the amount of milk fed is to increase the growth of calves during the pre-weaning period. Multiple studies have evaluated the influence of milk consumption on growth, and many have reported an increased growth rate of calves from the increase in milk offered (Davis Rincker et al., 2011; Drackley, 2008; Jasper and

Weary, 2002). Calves had increased birthweight (BW) and ADG at 56 d of age when offered an increased amount of milk replacer in an incremental feeding program (454, 681, 908, and 454 g/d on d 0 to 7, 8 to 14, 15 to 31, and 32 to 41, respectively) at a higher CP amount (28% CP, 17% fat) in comparison to a conventional program (454 g/d at a 20% CP and 20% Fat) (Quigley et al., 2010). Jensen (2006) reported an increase in ADG from d 12-54 in calves fed 8 L/d versus 4 L/d until weaned at 54 d; however, no difference in ADG was observed at 82 d of age.

Research has also been conducted on calves offered *ad libitum* milk consumption (Jasper and Weary, 2002; Khan et al., 2007; Welboren et al., 2019). *Ad libitum* milk consumption is often included in accelerated feeding systems although there is typically an even larger increase in milk offered when compared to the two other feeding systems and simulates the amount of milk calves would receive if left with their dams. De Passillé et al. (2008) found that calves reared by their dams drank 6.5 L/d during the first wk of life and on average 12.5 L/d after nine wks of age. In one study using the *ad libitum* feeding rate, calves drank 316 L in 42 d, which amounts to approximately 7.5 L per day (Jasper and Weary, 2002). Similarly, calves feed *ad libitum* semi-acidified milk replacer drank 8.2 L/d on average during a 35 d pre-weaning period (Welboren et al., 2019). When calves were offered up to 12 L/d in an automatic calf feeder, the average milk take was 9.6 L/d which aligns with other studies offering calves *ad libitum* milk consumption (Rosenberger et al., 2017).

The accelerated and *ad libitum* milk feeding strategies however raise concerns because they increase the cost of the pre-weaning period and may delay starter intake and rumen development of calves. Quigley et al. (2010) reported a \$27.22 difference in milk replacer cost per animal when feeding a conventional milk replacer fed at 454 g/d (20% CP, 20% fat) versus an accelerated milk replacer fed at varying rates with a maximum of 908 g/d from d 15-31 (28% CP, 16% fat). When the milk replacer cost and starter costs were combined, the total feed cost for the accelerated calves was \$18.30 more than conventionally fed calves, even though accelerated calves ate 22% less starter (Quigley et al., 2010). Research done on *ad libitum* feeding has confirmed the results found when feeding a conventional program in regards to rumen development. When calves were slaughtered after a five wk trial of feeding different levels of milk replacer (ranging from 3.10 to 8.34 kg/d as fed), those calves offered an accelerated amount had a reduced rumen and reticulum weight than those fed less milk replacer. However, there was no difference in papillae length in the atrium or ventral rumen sac (Kristensen et al., 2007).

1.2.2 Milk or Milk Replacer Options

Once a producer decides which type of feeding program works best for their operation, they must decide the source of the nutrients fed to calves. According to the National Animal Health Monitoring System (NAHMS) survey conducted in 2010, milk replacer was fed on 85.9% of the farms in the United States, and one third of farms (33.4%) were feeding waste or non-salable milk (USDA, 2012). Milk replacer protein type is an aspect to consider if the farm decides to feed a powdered milk replacer. Milk replacers can be derived from animal-based proteins including dried whey protein concentrate, dried whey, dried whey product, skim milk, casein, and sodium or calcium caseinate. Alternatively, milk replacers can be derived from plant based proteins such as soy protein isolate, protein modified soy flour, soy protein concentrate, soy flour, animal plasma, wheat gluten or isolate (BAMN, 2003). The use of alternative milk replacer proteins has seen mixed effects on calf growth and performance. Quigley, (2002) reported a 184 g/d reduction in ADG, lower starter intake and lower feed efficiency during the pre-weaning period when substituting 20% of whey milk replacer protein with spray-dried whole egg powder. In contrast, Huang et al. (2015) tested the effects of five alternative milk replacer protein sources included at 70% of the total protein (30% whole milk protein) and found no significant difference in ADG and feed to gain ratio between calves fed whole milk protein (775.6 g/d), soybean protein concentrate (698.2 g/d), or rice protein isolate (711.6 g/d). Calves were all fed at a 22% CP, 14.43-15.15% fat concentration and offered 100 g/kg of BW per day in milk replacer. ADG for the other protein sources used in the study from 22 to 63 d of age was 626.7 and 554.2 g/d for hydrolyzed wheat protein and peanut protein concentrate.

Waste or non-salable milk feeding programs feed milk from fresh cows and high somatic cells cows that would otherwise be discarded. James and Scott (2005) observed that three North Carolina herds discarded on average 2.8, 10.3, and 4.4 kg of nonsalable milk/cow/d. Therefore, farms have found ways to utilize the non-salable milk and reduce the cost of purchased milk replacer. Of operations feeding waste milk, approximately 70% of farms will pasteurize it before feeding to calves (USDA, 2012). There are two pasteurization methods used on farms: high temperature, short time and low temperature, long time. The high temperature, short time method heats milk to 71.6°C for 15 s. Low temperature, long time pasteurization on farm is done by heating milk to 62.7°C for 30 min. Farms will utilize pasteurization when feeding waste milk to decrease the bacterial counts. Elizondo-Salazar et al. (2010) compared two different pasteurization methods

on six different dairy farms and observed that bacteria counts measured from standard plate counts, environmental streptococci, and coliforms can be reduced by over 99% after both the pasteurization processes.

Bacterial counts in waste milk are decreased with pasteurization, but other research has focused on the long term effects of feeding calves discarded milk from cows treated with antibiotics. Keith et al. (2010) showed no detrimental growth effects of calves fed waste milk from antibiotic treated cows, but was unsure of the long-term effects that cow antibiotic treatment would have on the calves. Maynou et al. (2017) fed pasteurized waste milk to calves and collected fecal samples to analyze antibiotic resistance exhibited in *Escherichia coli*. They reported that *Escherichia coli* isolated from feces had increased phenotypic resistance to ampicillin, cephalotin, ceftiofur, and florfenicol, common antibiotics used in dairy production, compared to calves fed with milk replacer. However, other antibiotic resistance genes (ex. Tetracyclines) were found in calves fed milk replacer; this suggested that there are other potential factors such as genetic resistance or resistance to antibiotics passed on from cows to their offspring that play a role in antibiotic resistance (Maynou et al. 2017).

Since waste milk is collected from fresh cows and cows treated with antibiotics for mastitis or other diseases, research has been done to compare the composition of waste milk and fresh whole milk. Zhang et al. (2019) reported that whole milk (saleable milk) contained $3.3 \pm 0.27\%$ CP, $4.25 \pm 0.25\%$ crude fat, and $4.47 \pm 0.27\%$ lactose. In this study, the composition of waste milk was $4.29 \pm 0.61\%$ CP, $3.91 \pm 0.56\%$ crude fat, and $3.85 \pm 0.52\%$ lactose. Tempini et al. (2018) collected waste milk from fresh and antibiotic treated cows on 25 California dairies and reported an average of 3.74% CP, 4.24% crude fat, and 4.4% lactose. Transition cow milk has a different composition when compared to cows in established lactation which would account for the differences in composition of waste milk. Andrew (2001) reported that transition milk had $4.17 \pm 0.61\%$ CP and $5.01 \pm 1.27\%$ fat from Holstein dairy cows. Additionally, cows with mastitis have a different milk composition. Ogola et al. (2007) reported that mastitis milk had higher concentrations of non-casein fractions, sodium, chloride, and free fatty acids in comparison to milk from cows not treated for mastitis. This difference could also account for some of the differences observed in the composition of waste milk. Composition of waste milk on farms can be highly variable, especially on smaller farms, due to the variation in number of fresh cows, high somatic cell cow, and cows being treated with antibiotics (Drackley, 2008).

The amount of waste milk produced was also shown to be highly variable and often farms would not have enough waste milk to feed their calves (James and Scott, 2005). To alleviate some inconsistency of the waste milk being fed to calves, some farms will add a milk replacer balancer or fortifier. Milk replacer balancers are concentrated products to add when feeding whole or non-salable milk to calves and they are fed when a producer needs to extend the milk supply or make it more consistent. Adding balancers to whole or nonsalable milk have been shown to increase ADG, BW, and feed efficiency of pre-weaned dairy calves in comparison to feeding just waste milk and are a way to increase the nutrient content fed to calves without increasing the volume of milk fed (James and Scott, 2005). The farms using balancers have the ability to feed their waste milk even when they have a fluctuating amount of non-salable milk available (Glosson et al., 2015).

1.2.3 Weaning Procedures

The weaning period is an extremely stressful time in a calf's life due to the change in nutrition, group moves, new housing, etc. Due to the stress associated with weaning, different protocols to mitigate stressful events have been shown to effect growth of animals post-weaning (Sweeney et al., 2010; Steele et al., 2017). Therefore, finding the best strategy to reduce stress is important for future growth. Many weaning strategies have been practiced, such as abrupt and gradual/step-down weaning. Accelerated feeding programs have introduced gradual weaning to their standard operating procedures in attempts to increase the calf's starter intake prior to weaning and minimize the reduction in growth after weaning (De Passillé et al., 2011; Klopp et al., 2019). When comparing four different weaning strategies (22 d weaning, 10 d weaning, 4 d weaning, and abrupt weaning), with complete weaning at 41 days old, the abrupt weaning calves had the higher total milk consumption and lower starter intake than other calves. This increase in milk offered resulted in the highest BW gains, but post-weaning, abruptly weaned calves lost weight for three d. It was concluded that calves perform the best on a 10 d gradual step-down weaning period because calves had the highest BW at 49 d of age (Sweeney et al., 2010). When feeding larger amounts of milk, a step-down weaning method allows calves to increase starter intake sooner and maintain growth rates post-weaning (Khan et al., 2007). When comparing a conventional feeding system (4 L/d and wean by decreasing milk allowance to 2 L/d) to an accelerated program with a weaning program that incrementally increased and decreased milk allowance, calves had increased starter intake, total dry matter intake, and higher average daily gain (ADG) through 70 d of age

(Omidi-Mirzaei et al., 2015). However, others have shown that starter intake did not differ when implementing a step-down weaning strategy using an accelerated feeding method (Dennis et al., 2018). Steele et al. (2017) reported that calves fed using a 12 d step down weaning protocol ate 1.8 times more starter during the weaning period than calves fed using an abrupt weaning protocol, but there was no difference in starter intake after weaning (d 48). This same study revealed that calves had similar rumen papillae development at d 55 and calves fed on the abruptly weaned treatment adapted from the decreased starter intake relatively quickly post weaning. Similarly, a study that gradually weaned for 18 d found that calves ate more starter compared to abruptly weaned calves during the pre-weaning period. Calves abruptly weaned had a higher BW pre-weaning but during the 56 d grower phase this growth advantage did not remain and calves had similar BW at 112 d of age (Klopp et al., 2019).

Another suggested weaning approach is based on the amount of starter intake they consume. Roth et al. (2009) used automatic calf feeders for milk and starter grain feed to wean calves based on the starter intake, and as soon as a calf consumed 700 g/d of starter for 4 consecutive d, the weaning process began. Calves in this study were allotted 6 L of milk at the beginning of the study and as starter increased, milk consumption decreased linearly (ex. if a calf consumed 1,500 g starter, the calf was offered 3 L of milk). Milk allowance was cut off when the calf consumed 2,000 g/d of starter for 4 consecutive d. From this study it was concluded that calves were weaned on average by 76 d of age, and this weaning process does not have any negative effects on rumen development, BW, or health compared to weaning calves based on d of age. Benetton et al. (2019) also weaned calves in an automatic feeding system based on starter intake. In contrast to Roth et al. (2009), this study offered calves 12 L of milk and this allotment was reduced to 75% of each calf's previous 3 day average milk consumption to begin weaning on day 31. From day 31, calves' milk allowance was reduced after they reached the starter intake targets of 225, 675, and 1,300 g/d; however, if calves did not reach these targets by d 84, they were automatically weaned. The authors reported that 67.4% of animals were weaned by 63 d of age, but concluded that starter intake for animals is highly variable and certain calves need more time on milk. The contrasting results from these weaning method studies suggest further research is need to understand how fast weaning should occur to ensure calves are still offered optimal amounts of milk without altering their ability to incrementally increase starter intake.

1.3 Automated calf feeders

The idea of using an automated machine to feed dairy calves has been around for nearly 50 yrs. According to Bentley and Paulson, (2012), automatic calf feeders are self-contained units that will mix milk replacer and heated water based on a computer programmed amount and will then dispense the milk or milk replacer from a nipple feeding station when a calf enters for a meal. Currently, there are several companies selling automatic calf feeders in the United States including GEA Farm Technologies (Bönen, Germany), Calf Star LLC (New Franken, WI), DeLaval (Tumba, Sweden), Rombouts Ag Service (Salford, Canada), and Lely (Maassluis, Netherlands). The premise behind automatic milk feeders (AMF) is that they allow calves the ability to interact socially with others in a group housing system (Costa et al., 2014), easily allow farms to increase nutrients given to calves during the pre-weaning period (Cantor et al., 2019), and allow for reallocation of labor with a potential decrease in employees needed on the farm (Sinnott et al., 2019). When producers were asked what motivates them to use automatic calf feeding systems, they stated that they wanted to allow for increased milk consumption of calves while still reducing labor, and they also strived to improve working conditions for themselves and their employees while raising better calves (Medrano-Galarza et al., 2018a).

In addition to allowing more milk consumption per day, automatic milk feeding systems (AMS) allow calves to drink the milk offered in multiple meals per day (James, 2015). Medrano-Galarza et al. (2018a) offered *ad libitum* (36 L/d) milk replacer up to 32 days and allowed calves 3 L every 2 hours. Several other studies offered calves 12 L per day of milk replacer when researching different aspects of autofeeders (de Passillé and Rushen, 2016; Fujiwara, Rushen et al., 2014; Rosenberger et al., 2017). Rosenberger et al. (2017) evaluated the effect of milk allowance (6, 8, 10, or 12 L/day) on weight gains in autofeeders, and reported that calves drinking more milk maintained an increase in weight gain throughout the entire 10-wk study. Seventeen farms with automatic calf feeders had a median maximum milk allowance of 10 L/d (range: 6-15 L/day; Medrano-Galarza et al., 2018b). There is still a large variety of milk allowances specified in AMF, and the ability to easily customize the program is often an advantage to automatic milk feeders.

A disadvantage of AMF systems is that since increased milk consumption is typically offered, starter intake could be delayed, especially before weaning (Dennis et al., 2018; Khan et al., 2007). In contrast, Costa et al. (2014) found that housing calves in a social environment

experienced decreased neophobia or the fear of newness associated with unknown objects such as grain; therefore, calves grouped together ate more of the new feed than calves housed individually. Tapki (2007) compared individual versus group housing calves and found that those housed in a group (3 calves) ate more starter than individually housed calves even when they were offered the same amount of milk during the pre-weaning period due to the increased activity of calves in the AMF systems. Automatic milk feeders offer farms the ability to house their calves in a group system. A feeding station with a single nipple can feed between 20-30 calves on the system at a time (Shulte, 2013). The number of calves per pen depends on the farm's management strategies, number of feeders, farm size, and if farms have a seasonal or continuous calving system. A survey of 38 farms with automatic calf feeders reported the average pen size was approximately 18 calves and a maximum number of 26 calves per pen (Jorgensen et al., 2017). When housed in a pen containing 12 to 18 calves, calves had a higher risk of clinical respiratory disease and grew less than calves housed in groups of 6 to 9 calves per pen (Svensson and Liberg, 2006). Fujiwara et al. (2014) never had groups larger than 9 calves per pen, and in this study there was up to a 34 day age difference between the oldest and youngest calf in the pen. Jensen, (2007) found that calves housed in larger group pens took longer to train to drink from the autofeeder than calves housed in smaller pens. For many farms, the price of the AMF is a factor to consider when deciding the optimal pen size on a farm.

1.3.1 Costs Associated with Automated Calf Feeders

In 2013, the average AMF system cost \$18,000 with an additional \$4,000 for the computer software (Shulte, 2013). University of Wisconsin Extension highlighted that calves housed in AMF were fed increased amounts of milk compared to individually housed calves (60.8 lbs. versus 36.3 lbs. of milk replacer), farms had lower labor costs but similar management costs (labor costs: \$64 versus \$104/calf), and housing costs were higher for farms using AMF (\$55.44 versus \$26.41/calf; Akins et al., 2018). Sinnott et al. (2019) reported that labor was decreased in AMF by 1:28 (mm:ss)/calf/day when compared to labor needed for individually housed calves. Gleeson et al. (2008) surveyed 57 different dairy herds to understand the differences in labor associated with different management systems implemented on calves between eight d and eight wks old. This research revealed that total calf care time was not different between calves fed through an AMF, fed once using teats, fed twice with teats or buckets, or twice through a trough. When broken down

to labor tasks associated with different feeding systems, it took longer to clean the feeding equipment and bed/clean calf pens in AMF compared to the other feeding systems.

1.3.2 AMF and Disease Incidences

Since calves are housed in a group setting in the AMF, there is a concern of increase disease transference between animals in a pen. However, there is not a consensus in disease risk associated with AMF represented in the literature. Medrano-Galarza et al. (2018a) reported a 23% diarrhea and 17% incidence rate of calf respiratory disease on 17 dairy farms with AMF. Calves were only assessed at a single timepoint; therefore, disease rate could be higher than reported. When researching four different housing systems: individually housed, bottle fed; individually housed, bucket fed; group housed, bottle fed; group housed, trough fed, Bernal-Rigoli et al. (2012) reported that there was no effect of housing system on calf health or fecal consistency. Curtis et al. (2016) found a 19% higher incidence rate of respiratory disease in calves fed *ad libitum* milk in AMF than calves individually housed for 21 d before group housing. Currently, the use of data generated from automatic calf feeders is being evaluated to diagnose diseases before calves present clinical signs. Svensson and Jensen (2007) found that unrewarded visits to the autofeeder were significantly reduced before calves were diagnosed with disease. Drinking speed, a measurement calculated by AMF every time a calf goes to the feeder, can also be an indication of calf illness. In a study detecting illness via measurements taken by AMF systems, they reported that sick calves drank slower (183 ± 27 mL/min less), drank less milk (1.2 ± 0.6 L/d less), and had fewer unrewarded visits (3.1 ± 0.7 fewer) to the autofeeder on the day of illness treatment (Knauer et al., 2017). Even though calves experience a change in behavior around an illness event, exact timing and severity of these changes will vary between calves.

1.3.3 Training

Utilizing an AMF system requires longer training times and one study reported that it took on average six minutes and 29 seconds longer/d to train calves in an automated feeding system compared to manual feeding systems (Sinnott et al., 2019). Fujiwara et al. (2014) found that only 27% of calves voluntarily drank milk from the AMF during the first 24 h of being placed in a pen with an AMF system. Only 16% of calves in another study successfully learned to use the AMF

after the first training, but after the third training 71% of calves were drinking from the feeder on their own with the average number of retraining sessions for calves being 2.27 sessions (Wilson et al., 2018). The age in which calves are introduced to the AMF feeder may affect the amount of trainings calves need. Medrano-Galarza et al. (2018a) introduced calves to the automated system within the first 24 h of life or after five d of individual feeding and found that it took longer for calves introduced within the first 24 h to successfully drink from the autfeeder on their own. By d six on the feeder, only 6.5% of calves needed assistance drinking in the AMF after being introduced to the system after six d of individual feeding (Fujiwara et al., 2014). Another suggestion is to introduce calves on the automatic calf feeder between one and seven d of age (Ziemerink, 2015). Even though calves took longer to train in the AMF, calves introduced at 24 h compared to five d of age had an overall reduction in milk feeding labor for calves introduced early (Medrano-Galarza et al., 2018b). Jensen, (2007) found that there was no difference in BW at 40 d of age when calves were introduced to AMF at either six or 14 d. Calves introduced to the autfeeder that were less than eight d old had a reduced prevalence of respiratory disease (within pen) than calves introduced to the feeder greater than eight d old (Medrano-Galarza et al., 2018c).

1.3.4 Age of Introduction into AMF

Range of age at introduction into the automatic calf feeder can be influence the success rate of these systems. Jorgensen et al. (2017) found that farms using AMF systems had on average 3.1 ± 2.0 wk. difference between the oldest and youngest calf in the pen. Manufacturer recommendations suggest that AMF systems can manage between 25-30 calves in each feeder, but for smaller farms, the age variation between calves becomes too large to manage the needs of the differing ages. When grouping calves of different ages, Færevik et al. (2010) reported that older calves were more dominant at the feeding station and overall more active than the younger calves in the group. The difference in behavior suggested that performance of younger calves may be impaired due to the dominance of older calves in this system. It is recommended that farms group their calves by age, and to maintain optimal growth, groups should not have more than two wks difference in age (Bentley et al., n.d.). Even though age variation is a key aspect in managing automatic calf feeding systems, the overall performance effects from calves raised in groups with large variation of age are not well understood.

1.3.5 Social Interaction in AMF

Another advantage to the automated systems is that they allow calves to live in an environment with increased social interaction between calves. When assessing behavior of calves housed individually or in a group setting, Tapki (2007) observed that calves housed in groups had an increased amount of walking, playing, and grooming when compared to individually housed calves. It was also found that calves housed individually had increased amount of time licking objects, idle standing, lying, and restlessness which are signs of decreased welfare (Tapki, 2007). Babu et al. (2004) also found increased lying, increased idle standing, and decreased play behavior in individually housed calves compared to group housed calves. However, calves housed in a group spent more time cross-suckling in the pre and post weaning periods than individually housed calves (2:14 vs. 3:36 pre-weaning, 4:02 vs. 6:30 post-weaning; mm:ss/d; Babu et al., 2004). The individually housed calves cross-suckling events were defined when they reached out of the pen for adjacent calves nearest to them. The number of calves housed in a group pen can influence their behavior, and one study reported that calves housed in groups of 24 had an increased rate of milk ingestion than calves housed in groups of 12 (Jensen, 2004). Jensen (2004) also found that there was no effect of group size on live weight gain or incidence of cross suckling. Calves that were weaned earlier on automatic calf feeders made more visits to the aut feeder during weaning than calves later weaned or weaned by starter intake (de Passillé and Rushen, 2016b). Therefore, many factors influence calf behavior, but overall, housing calves in groups increases behaviors associated with improved welfare.

1.3.6 Sanitation Practices

When using AMF systems, following recommended sanitation procedures is important to consider when raising healthy calves. However, 23% of producers using AMF who provided additional information (n=5 out of 22) stated that maintaining adequate sanitation of the feeders was a disadvantage to owning them (Medrano-Galarza et al., 2018a). Even though maintaining sanitation of aut feeders is a disadvantage to some producers, Sinnott et al. (2019) reported that it takes longer to perform cleaning tasks in individually housed systems than it does in automated systems. Automated calf feeder sanitation management can differ between farms. Dietrich (2015) observed that farms ranged between two to four mixer and heat exchanger cleanings per d and

between one and seven circuit cleanings per wk. These ten farms also used a variety of cleaning detergents in their sanitation protocols: chlorinated alkaline detergent, alkaline detergent, peroxide/acid mix, or acid detergent. On average, farms with AMF in the Midwest (n=38) performed automatic cleaning 2.5 times/d, circuit cleaning 3.4 times/wk, nipple cleaning 6.1 times/wk, and manual tube cleaning 1.9 times/wk (Jorgensen et al., 2017). Jorgensen et al. (2017) also found that 37.5% of farms did not monitor sanitation practices and 31.3% of farms only monitored sanitation visually. Without proper management of sanitation, calves may not perform as well in AMF systems. Dietrich (2015) reported performing mixer cleanliness scoring (measurement of AMF cleanliness ranging from 0 = dirtiest to 3 = cleanest) that for each point of increase in this score, coliform count petrifilms decreased by 0.46 log₁₀ cfu/ml. Medrano-Galarza et al. (2018c) found that there was an increased pen prevalence of calf diarrhea in the summer if the total bacteria count of milk samples were higher than 100,000 cfu/ml. Therefore, it is imperative to maintain proper sanitation of automated calf feeders to ensure their success in feeding calves.

1.4 Bovine respiratory disease

Bovine Respiratory Disease (BRD) is a clinical disease of the respiratory tract affecting cattle that is caused by a bacterial, mycoplasmal, viral infection, or a combination (Van Der Fels-Klerx et al., 2002). It is one of the leading causes of morbidity and mortality in pre-weaned dairy calves. The National Health Monitoring System performed a studying using heifers raised on heifer grower operations to document different management practices, the survey reported that more than 11.4% of pre-weaned and 5.1% of weaned dairy heifers raised on these farms were treated for some form of respiratory disease (USDA, 2018). Respiratory disease rates vary; however, and Heins et al. (2014) reported a 61% treatment rate (range 20.7 to 89.9% treatment) when researching respiratory treatment rate on four different farms. Overton (2019) reported a 36.6% average treatment rate on heifer calves up to 120 d of age (n= 104,100). For producers and their employees, identifying and diagnosing calves with respiratory disease is a challenge the industry is currently facing.

1.4.1 Causes of BRD

The bovine lung consists of eight different lobes: right cranial lobe, right cardiac lobe, right diaphragmatic lobe, accessory lobe, left cranial lobe, left cardiac lobe, left diaphragmatic lobe. Pathogens causing BRD affect different lobes of the lung and the cattle may exhibit different symptoms when presented with this disease challenge. *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*, *Arcanobacterium poygenes*, and *Bibersteinia trehalosi* are common bacterial pathogens that will affect calves with BRD (Panciera and Confer, 2010). Fulton et al. (2002) took nasal swabs on incoming feedlot cattle and tested animals for *M. haemolytica*, *P. multocida*, and *H. somni*. They reported that the most commonly found bacteria was *M. haemolytica* although many nasal swabs or animals were identified with more than one bacterium. Cattle identified with *M. haemolytica* bacteria will exhibit respiratory symptoms quickly and typically die within three to seven d if left untreated. In contrast, cattle inoculated with *P. multocida* will develop respiratory symptoms slower and are typically those cattle identified as chronically ill (Montgomery, 2009).

Viral pneumonia in cattle can destroy the epithelium in the respiratory tract and animals have trouble expelling particles from their airways. Because of this damage, a secondary infection commonly arises from pathogens typically located in the nasal cavity (Ollivett, 2014). Fulton et al. (2002) similarly described that viruses are a pre-disposing agent to bacterial infections, which lead to bovine respiratory disease. Viruses affect the immune system's ability to react to bacterial infections invading the cattle's body as well as damage parts of the upper respiratory track which leads to the translocation of the virus from the sinuses to the lung tissue (Fulton et al., 2002). When comparing viral versus bacterial respiratory agents associated with fatal BRD in beef feedlot steers, Booker et al. (2008) found that the bacterial agents (*M. Haemolytica* and *M. bovis*) were more common in animals than bovine viral diarrhea virus which is a common virus responsible for respiratory disease.

Booker et al. (2008) demonstrated that causative agents (ex. *M. haemolytica* and *M. bovis*) for respiratory disease will reside in different parts of the lung tissue resulting in cattle displaying varying symptoms. These causative agents result in different forms of respiratory disease such as bronchopneumonia, fibrinous pneumonia, pleuropneumonia, aspiration pneumonia, caseonecrotic pneumonia, interstitial pneumonia, embolic pneumonia, and verminous pneumonia (Panciera and Confer, 2010). For example, weaned beef cattle exhibiting shipping fever, typically present with

fibrinous pneumonia, which is a form of pneumonia in the upper lung lobes produced by *M haemolytic* and *H somni*. In extreme conditions, fibrinous pneumonia will consolidate the upper five lobes of the animal's lung (Panciera and Confer, 2010). Holschbach et al. (2019) found lung consolidation in all the cranioventral lung lobes of weaning aged dairy calves when challenged with *P. multocida*. The location in the lung and causative agent associated with respiratory disease will affect which symptoms an animal will exhibit and how the disease will affect the animal's overall growth and productivity.

1.4.2 Diagnosis

Diagnosis of respiratory disease is subjective because of the different causative agents and symptoms displayed by animals. Each farm typically has different criteria when deciding to treat calves for this disease. One study defined BRD as “dullness, decreased appetite, or listlessness, with at least one of the following signs: rectal temperature $>39.5^{\circ}\text{C}$, nasal discharge, or elevated respiratory effort” (Stanton et al., 2012). Curtis et al. (2016), diagnosed calves with BRD if they had $> 39.4^{\circ}\text{C}$ rectal temperature, coughing, eye discharge or increased respiratory rate. When 21 experts in respiratory disease from the Netherlands were asked in a survey to define BRD, they concluded that this disease is a combination of one or more symptoms of increased coughing, respiratory rate, or nasal discharge. Three criteria were required by participants to be selected for the survey: candidates must have a degree in veterinary medicine, must have on farm experience and training in BRD, and they needed to know about current practices within the Netherlands dairy industry. These experts answered questionnaires and responses were weighted based on the extent of their experience using statistical methods (Delphi and Elicitation techniques) that combine responses to answer a common question. From this analysis, BRD symptoms were divided into either mild or severe categories, with calves exhibiting mild BRD showed one or more symptoms of either nasal discharge, increased respiratory rate, or coughing. Calves with severe BRD, displayed the above symptoms as well as an elevated rectal body temperature greater than or equal to 40°C accompanied by harsh, deep and elevated breathing (Van Der Fels-Klerx et al., 2002). To help with the variation in symptoms calves display when infected by respiratory disease, researchers have developed several methods farms can use to evaluate and properly diagnose them.

1.4.3 Clinical Scoring Systems

Researchers have developed clinical respiratory scoring systems that are simple to implement on farm and increase the accuracy of diagnosing calves with respiratory disease. The California clinical scoring system considers six variables: cough, respiratory rate, nasal discharge, eye discharge, fever (body temperature greater than 39.2°C), and ear/head carriage. Calves are identified as normal or abnormal and if abnormal each symptom receives a different point values: eye discharge = 2, nasal discharge = 4, ear droop/head tilt = 5, cough = 2, respiratory rate = 2, and temperature = 2. This system is designed to handle calves minimally and those calves exhibiting nasal discharge or a combination of two of the other signs should be handled to measure temperature. In the California system, symptoms are added together and a score of five constitutes as a case of clinical respiratory disease (Love et al., 2014). The Wisconsin calf health scoring system utilizes five different symptoms: nasal discharge, eye discharge, ear score, temperature score. Each category is scored between 0-3 (with 0 = normal and 3 = severely abnormal). If the sum of these five categories exceeds five, calves are diagnosed with BRD (McGuirk, 2008). When comparing the two systems, one study found that the California scoring system had a screening sensitivity (used to investigate the prevalence of a disease in a population) of 46.8%, a diagnostic sensitivity (used confirm a diagnosis in animals suspected of the disease) of 72.6%, and specificity of 87.4%. In contrast, the Wisconsin scoring system had a screening sensitivity of 46.0%, a diagnostic sensitivity of 71.1%, and specificity of 91.2%. There was no statistical difference between the scoring systems, and they bring more objectivity to the physical observations of the people who provide calf care (Love et al., 2016).

1.4.4 Thoracic Auscultation

Another way veterinarians and researchers are monitoring respiratory disease is thoracic auscultation. Thoracic auscultation is the action of listening to the lungs using a stethoscope and is widely used in veterinary medicine practice. Lung tissue was assigned abnormal when the auscultation makes crackling, wheezing, pleural friction rubbing, or the absence of normal respiratory sounds (Buczinski et al., 2014). Thoracic auscultation was found to have a relatively low sensitivity of 0 to 16.7% in comparison to lung damage identified via thoracic ultrasonography (described below). The researchers did not include bronchial sounds of the airways when

diagnosing abnormal sounds; therefore, this method for BRD detection could have contributed to the low sensitivity. Based on the low sensitivity and time it takes to learn the auscultation technique, this has not been adopted on many farms.

1.4.5 Thoracic Ultrasonography

Another technique for BRD detection increasing in the United States dairy industry is thoracic ultrasonography. Using thoracic ultrasonography, trained technicians can visually view lung damage or consolidation using an ultrasound machine. Ollivett and Buczinski (2016) developed an on-farm technique to implement thoracic ultrasonography on dairy farms which includes restraint (halter, headlock or another individual), hair clipping for better conductivity (optional), application of transducing agent (70% isopropyl alcohol, coupling gel, or vegetable oil), and scanning of the lung field using probe with visualization on the ultrasound display (Ollivett and Buczinski, 2016). Different brands of ultrasound machines as well as different shaped probes were studied to find the most successful combination to accurately identify lung consolidation. The most common machine type used for lung ultrasonography is the IBEX medical imaging ultrasound machine (Adams and Buczinski, 2015; Cramer and Ollivett, 2019), but there has been a variety of probes used: 8.5 MHz linear probe (Buczinski et al., 2014; Adams and Buczinski, 2015), 7.5 MHz sector scanner (Rabeling et al., 1998), 6.2 MHz linear rectal scanner (Ollivett, 2014). Transrectal probes are often used by veterinarians, and allow for easier observation of the lungs behind the shoulder blade and upper thorax compared to other probes (Ollivett and Buczinski, 2016).

In preparation of scanning, some technicians will clip both sides of the thorax to provide a better contact surface for the probe (Flöck, 2004; Reinhold et al., 2002; Ollivett and Buczinski, 2016). Others do not believe hair clipping is necessary and increases scanning time, making it impractical on commercial farms (Buczinski et al., 2013; Cramer and Ollivett, 2019). Different studies have focused their scanning on varying intercostal spaces: 3rd-10th intercostal spaces (Adams and Buczinski, 2015), 4th-8th intercostal spaces (Buczinski et al., 2013), 7th-11th and 3rd-4th intercostal spaces (Flöck, 2004), and 3rd-12th intercostal spaces (Reinhold et al., 2002). The lungs span between the 1st and 10th intercostal spaces with the right cranial lobe located in the first and second intercostal space. For the most accurate score, the entire lung field should be scanned on both sides of the thorax (Ollivett, 2014; Ollivett and Buczinski, 2016). The proper technique to

use when thoracic ultrasound scanning is to begin at the caudal thorax and move forward to the cranial thorax placing the probe between each of the intercostal spaces (10-1). When scanning each intercostal space move the probe dorsal to ventral along the curve of each rib (Ollivett and Buczinski, 2016).

Thoracic ultrasonography was validated by Bayesian latent class analysis (a statistical estimation that uses sensitivity and specificity as a reference for success) with a sensitivity of 79.4% and specificity of 93.9% (Buczinski et al., 2015). This validated technique scanned the lung field from the 3rd-11th intercostal spaces using a linear rectal probe. A calf was positive for BRD when greater than or equal to one centimeter of consolidation was found in the lung tissue (Buczinski et al., 2015). Lung consolidation determined via thoracic ultrasonography has been highly correlated to necropsy results of lung tissue (Flöck, 2004; Reinhold et al., 2002). Buczinski et al. (2014) compared the Wisconsin calf health monitoring system, thoracic auscultation, and thoracic ultrasonography to evaluate which method was better at detecting respiratory disease. Thoracic ultrasonography was suggested to be the gold standard in identifying lung damage when compared to scanning, thoracic auscultation and health scoring charts.

When using lung ultrasonography, Ollivett and Buczinski (2016) suggest to use a six-point scale ranging from zero to five. According to this technique, if a calf scores a zero there is no visual lung damage and very little to no comet tail artifacts (“diffuse pleural roughening”) which appear as small white lines lying vertically to the white horizontal line (pleural surface) on the ultrasound scan (Steckler and Boerman, 2019). Comet tail artifacts are extremely common when using thoracic ultrasonography; one study found that 104 out to 106 calves scanned had at least one comet tail (Buczinski et al., 2014). Since comet tails are so commonly seen on animals when scanned, this slight disruption in the pleural space is not considered lung consolidation. A score of one would reveal a large presence of comet tails, but no consolidation. When a calf is scored a two, they have lobular pneumonia or lung consolidation on different spots of the lung lobes. A score of three, four, or five indicates a calf with lobar pneumonia in either one, two, or three or more lung lobes, respectively. They defined calves as normal if their ultrasound score was either one or two (Ollivett and Buczinski, 2016).

Others used a one to four scoring system where a score of one showed no abnormalities, two showed comet tails, and three and four showed varying sizes of lung consolidation. Cramer and Ollivett (2019) simplified the original six level ultrasound scoring system suggested by

Ollivett and Buczinski (2016) into a two level scoring system where animals either have lung consolidation or they do not (≥ 1 cm of consolidation). In a study completed by Cramer and Ollivett (2019) reported that calves with a positive clinical respiratory score (positive score when calves score greater than or equal to 2 on at least of two symptoms: nose, eyes, ears, cough, or rectal temperature) had a 0.10 kg/d reduction in ADG from calves that did not have a positive clinical respiratory score. In the same study, a two point scoring system for thoracic ultrasonography (positive when there was one centimeter or greater lung consolidation) was also used and calves that had lung consolidation had 0.11 kg/d less ADG than those that did not have consolidation. Health measurements were taken every two wk over the study period. ADG analysis was calculated based on the first respiratory event identified during the study period (21 to 50 d) for both the clinical scoring system and thoracic ultrasonography. Based on the information found in this paper, lung damage causes a decrease in growth. If calves were treated for BRD in the following 60 days after first moved to group housing, calves were 14.4 kg lighter and 1.7 cm shorter at 13 months of age than calves that were not treated during this time (Stanton et al., 2012). Respiratory disease impacts the beef cattle industry as well, and similar reductions in ADG were observed in cattle during the feedlot phase (Thompson et al., 2006). These researchers reported that South African feedlot cattle (137 d on feed) had an overall 24 g/d reduction in ADG and a 5.1 increase in d on feed if they were treated for respiratory disease. This reduced efficiency cost the feedlot on average \$1.79 per animal entering the feedlot.

1.4.6 Effect of BRD on Heifer Production Parameters

Respiratory disease affects other production parameters in addition to growth. Calves that had a history of BRD during the first 60 d after moving to group housing were 18% less likely to survive to first calving. If they survived, calves with BRD were on average 12 d older at first calving compared to calves not treated for BRD (Stanton et al., 2012). Adams and Buczinski (2015) scored calves by the severity of lung consolidation at weaning using a lung scoring system of 1 to 4 (1 = normal lung and 4 = at least 1 location of lung consolidation ≥ 6 cm). They reported that calves with a lung consolidation score of 4 had a significantly reduced survival rate (74%) in comparison to calves with a score of 1, 2, or 3 (99%, 97%, and 95%). This study did not find any association between respiratory disease and age at first calving. However, those calves that left the herd prior to calving were not included in the analysis, which could alter the results and explain

why there was no difference in age at first calving. Another group found that calves with lung consolidation at 60 d old had a lower hazard of pregnancy (0.70) and a lower chance of becoming pregnant compared to those that did not have lung consolidation (Teixeira et al., 2017). Several studies evaluated the effect of respiratory disease on milk production, but did not find a significant difference between animals with or without lung consolidation (Stanton et al., 2012; Teixeira et al., 2017). In contrast, another study found no difference in age at first calving, but reported a 525 kg reduction in first lactation 305 day milk production when calves had at least three centimeters of lung consolidation present during one of the eight times calves were scanned during the first eight wks of life (Dunn et al., 2018).

1.5 Growth and Production

To assess effectiveness of feeding and management strategies, dairy farmers and researchers measure growth over time. Average growth rates in heifers have been recorded since 1920. Eckles (1920) evaluated ways to obtain growth measurements and asked the question: what is normal heifer growth? From this study, which contained limited animal numbers, they reported that Holstein heifers weighed on average 40.8 kg at birth, 158.3 kg at six mo., 277.6 kg at 15 mo, 394.2 kg at 24 mo., and 463.1 kg at 30 mo. of age, providing a reference for average animal weight and growth on farms at this time. Farm managers would then have the opportunity to use these averages to adjust their management strategies according to weights by ages.

Because of the small sample size and limited animal measurements being taken from a single farm in early growth studies, Heinrichs and Losinger (1998) studied heifer growth in the United States using weights collected from 8,565 animals on 659 different dairy farms. This study allowed the researchers to measure average heifer growth over an animal's lifetime. From this larger study, heifers weighed 53.1 ± 8.7 kg at 0.5 months old, 191.0 ± 33.4 kg at 6.5 months, 409.3 ± 61.4 kg at 15.5 months, and 528.9 ± 99.4 kg at 23.5 months old. This study demonstrated that Holstein heifer BW has increased over time.

1.5.1 Estimating Growth

Research has evaluated methods for estimating weight of animals on farms without scales. Davis et al. (1961) verified the correlation between BW and heart girth measurements and was

able to use a heart girth tape to estimate animal weights. Heinrichs et al. (1992) reported that heart girth, wither height, hip width, or body length could be used to accurately predict BW ($R^2 > 0.95$). Heinrichs and Losinger (1998) used the calf heart girth tape to weigh Holstein heifers from 659 dairy farms in the eastern United States. To successfully use the weigh tape, the calf should be standing squarely with all four feet directly under the body. The tape should be wrapped tightly around the barrel of the animal directly behind the shoulders. A farmer can then compare the measurement from the weight tape to a correlated BW. Dingwell et al. (2006) found no overall significant difference between Holstein heifer weights (between 0 to > 24 months of age) collected via an electronic scale or heart girth weigh tape, but the weight tape had significantly lower estimated weights for calves ≤ 3 months old when compared to the electronic scale weights. The authors noted that further research validating the weigh tape needed to be completed due to the small sample size for this age of heifers. Therefore, measuring heart girth can be a way for farms that do not have access to a scale to estimate heifer weights on their farm.

Animal size can vary between farms with average weight on an individual farm differing from the national average. In order for the dairy industry to quantify individual animal growth and evaluate animal variation, large numbers of animals and farms are needed for the analysis. Weighing all animals at the same age and at multiple time points is a time-consuming task that requires extra labor and equipment; therefore, not many farms will weigh their heifers due to the impracticality. The inability to obtain large dataset to study growth has been a struggle because of the failure of farms to consistently weigh heifers. Thus, to combat this issue, researchers have been predicting heifer growth based on observations obtained from many animal observations. Each animal may not have every timepoint measured, but if the dataset is large enough, accurate predicted weights can be derived even with inconsistent timing of actual weights. One way researchers have been able to accomplish these predictions is to generate individual animal regressions. Individual animal regressions allow researchers to use incomplete weight data from animals that do not have weights for each timepoint and they can predict the missing data points based on other animals' weights at those timepoints as well as the animal's other recorded weights.

When measuring and predicting growth, calves do not grow at a constant rate. Cue et al. (2012) took incremental weights over a heifers life from two to 27 months and found that calves had higher ADG during the first yr of life in comparison to when they were nearing mature BW. Suchocki and Szyda (2011) explained that linear and quadratic growth curves do not accurately

depict growth rates over time. Since growth is neither linear nor quadratic, researchers have fit both weights and ADG to third and higher order polynomials to more accurately model growth over time. Orthogonal polynomials are coefficients that have been used to model weight in growing animals. Research has shown that this method better predicts animal growth compared to linear, quadratic, or cubic equations (Kirkpatrick et al., 1990; Coffey et al., 2006; Handcock et al., 2019). Yin and König (2018), reported that third order orthogonal polynomial had the lowest Bayesian information criterion (BIC) value when compared to other tested models: Logistic, Gompertz, Brody, Richards, or first/second Legendre polynomials. Additionally, Handcock et al. (2019) evaluated growth between breeds using a fourth order Legendre polynomial to predict growth in cattle between three and 22 months of age. Cue et al. (2012) used mixed models and random regression equations to predict growth of dairy breeds from zero to 32 months of age. This study started with a linear, quadratic, and cubic regression equations, but found the cubic term was not significant and had a higher BIC value than other models. Authors suggested the cubic term was insignificant in their model in comparison to other models using this term because they included the random effect of animal in their model, which allowed them to account for the covariance (association between variables) between heifer weight observations. The authors concluded that by using the quadratic equations, farms could predict the growth of their cows at 32 months from the weight records recorded during the pre-weaning period.

1.5.2 Average Growth Measurements

Using the predictions reported for BW, Holstein-Friesian cattle recorded by Handcock et al. (2019) were 93.5 ± 0.3 kg at three months, 156.5 ± 0.5 at 6 months, 304.6 ± 0.7 at 15 months, and 430.4 ± 0.7 kg at 22 months. These averages are smaller than presented in Heinrichs and Losinger (1998) possibly due to the number of observations taken (Handcock: 189,936; Heinrichs: 8,565 animals), genetic differences between Holstein and Holstein-Friesian cattle, or the difference in environment (Handcock: New Zealand; Heinrichs: United States). Pietersma et al. (2006) found that calves had the highest ADG from four to ten months (0.89 kg/d) and lowest ADG from 14 months to pre-calving (0.70 kg ADG). Heifers from this study reached 68% of their mature BW at breeding and 89% of mature bodyweight by calving. To compare, Handcock et al. (2019) had the highest ADG from three to five months (0.769 kg/d) and the lowest ADG from 20 to 22 months

(0.205 kg/d). Calves from this study also experienced a lower ADG from five to 12 months (0.465 kg/d) when compared to calves in Pietersma et al. (2006).

1.5.3 Factors that Influence Growth

Average growth in heifers can be estimated based on repeated measurements of BW or predicted BW. Curtis et al. (2018) reported that dam parity, respiratory disease within the first 12 wks of life, and plasma total protein affect BW from zero to 108 wk of life. The incidence of diarrhea, septicemia (28% of cases resulted in death), or respiratory disease, as well as the season of birth, birthweight, farm, and actual age at six mo significantly affected growth and height up to six mo. of age (Donovan et al., 1998). Bach (2011) reported that heifers that experienced four or more cases of bovine respiratory disease before their first calving were 1.87 ± 0.14 times more likely to be culled before completion of their first lactation than calves that did not experience this disease. Similarly, those calves that experienced one to three cases of respiratory disease tended to have a decrease in their likelihood to survive through their first lactation.

Raubal et al. (2018) reported the protein and metabolizable energy (ME) intake from starter, calculated via predetermined equations from the NRC (2001), had a larger impact on growth and ADG than the protein and ME consumed from milk replacer. Calves in the highest ADG bracket (greater than 0.80 kg/d) consumed 153.4 Mcal more ME than calves in the lowest ADG bracket (0.23-0.34 kg/d) meaning that increased starter consumption lead to significantly higher ADG. Long-term effects of ADG and growth impact reproductive performance of heifers. Maternal growth traits passed on to offspring traits also impact animal growth. Yin and König (2018) determined that calf birth weight and weight at first insemination were moderately heritable (0.47 and 0.20) and there was a positive correlation between the two traits (0.31). Arango et al. (2002) also reported that bodyweight was moderately heritable (0.49). Calves with higher birth weight had higher weight at first insemination. Birth season also has been shown to have an effect on growth as well as lactation performance (Chester-Jones et al., 2017). Calves born in the fall and winter had higher eight wk DMI, BW, and ADG than calves born in the summer and spring. When comparing 305 d milk, calves born in the summer produced more milk than calves born in the fall and winter.

1.5.4 Impact of Heifer Growth on Future Productivity

Pietersma et al. (2006), hypothesized that heifer growth impacted age at conception, age at first calving, first lactation milk production, as well as first lactation milk components. They collected 39 different phenotypic traits on 44,489 Holstein heifers including BW, withers height, ADG, age at conception, age at first breeding, body condition score, milk production and components, and calving intervals. BW at 14 mo of age had moderate negative correlation on age at conception; calves with a higher BW at 14 mo had a lower age at conception. Age at first breeding and age at first calving were significantly impacted by BW at 30, 180, and 450 d of age, and the ADG for heifers calving in less than 775 d of age was 0.82 ± 0.01 kg between 30 and 180 d of age (Brickell et al., 2009). When calves were fed to gain 700 g/d versus 1000 g/d starting at 2.5 months old, those fed to have a higher ADG were 12 kg heavier and reached puberty 23 days earlier than calves feed to have a lower ADG (Lammers et al., 1999).

Milk production is also impacted by heifer growth. Schneider and Van Vleck (1986) reported that milk production in Holstein dairy cattle was moderately heritable (0.33). Van der Waajj et al. (1997) reported that the phenotypic correlation between liveweight of heifers at nine mo of age and first lactation milk production was 0.32. Chester-Jones et al. (2017) investigated the effect of ADG on future heifer performance and this study utilized 2,880 calves from three different dairy farms. To obtain this large number of animals, the researchers combined data from 37 different research projects. The majority of calves in these projects were fed a 20% CP and 20% fat milk replacer fed at a 0.57 kg/calf/d; however, some trials evaluated the effect of protein levels or increased feeding rate. The average six wk ADG, corresponding to weaning, for this study was 0.53 kg/d. From this study, the researchers reported that six wk ADG had a significant impact on 305-d milk yield, with every 1 kg increase in ADG, 305 d milk yield increased by 456.25 ± 229 kg/305 d lactation (mean \pm SE). In comparison, Soberon et al. (2012) determined that for every 1 kg increase in ADG pre-weaning lead to a 1,113 kg increase in 395 d first lactation milk production ($n = 623$ first lactation records) from a single commercial dairy in New York. The average ADG on this commercial dairy was 0.66 kg/d, which is higher than ADG reported by Chester-Jones et al. (2017). Pre-weaning ADG alone accounted for 22% of variation in first lactation milk production (Soberon et al., 2012).

Zanton and Heinrichs (2005) analyzed eight different studies ($n = 472$ cows) over a 15 yr time span and reported that pre-pubertal growth had a quadratic effect on 305 d milk production,

with animals with the highest milk production gained approximately 0.80 kg/d. The meta-analysis reported that heifers with pre-pubertal ADG above or below 0.799 kg/d had lower overall 305 d milk production. Another trial utilizing a large data set from 7,768 Holstein heifers reported that calves that survived to second lactation had a 0.10 kg/d higher ADG (0.8 ± 0.04 kg/d compared to 0.7 ± 0.04 kg/d) during the first 65 d of life than cows culled before reaching this period (Bach, 2011). However, the authors did not see a difference in survivability to second lactation for animals with different BW at breeding or first calving. The researchers concluded that ADG between 12 and 65 d better predicted survivability to second lactation than age at first calving.

1.6 Conclusions

Throughout this literature review, the effects of milk feedings strategies, automatic calf feeders, bovine respiratory disease, and the impacts of heifer growth on future cow profitability are discussed. Even though differing milk feeding strategies have been researched, there still is not a consistent recommendation of the best way to feed milk or milk replacer during the pre-weaning period. Automatic calf feeding systems have been around for nearly 50 yrs. and provide insight into calf feeding and health behavior. Bovine respiratory disease has been shown to impact growth and the cow's future profitability. Research has been done to implement better management techniques to identify animals with BRD; however, there needs to be an overall improvement in management to lower the current incidence rates on the farm. Additionally, phenotypic variables such as bodyweights and milk production have been shown to be genetically heritable and could influence the overall profitability of a heifer as well. Finally, the use of statistical models to create regression equations that allow the industry to predict growth of animals over time and the effects of growth on the future productivity of that cow. This research seeks to combine all these variables into one model to identify calves directly after the pre-weaning period that will develop into the most productive cows. Therefore, this research looks at the combination of an accelerated feeding strategy through an automatic calf feeder, health events such as respiratory disease, as well as genetic variables and how they impact growth. This research will also provide predicted BW calculated using methods explained in this review to compare individual animals on farm to one another on a specific d of age.

1.7 References

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CHAPTER 2. PREDICTIVE EQUATIONS FOR EARLY LIFE INDICATORS OF FUTURE GROWTH IN HOLSTEIN DAIRY HEIFERS

2.1 Abstract

It takes an approximate two yr investment to raise a replacement heifer from birth to first calving, and selecting the most productive heifers earlier in life could reduce input costs. Daily milk consumption, serum total protein, pneumonia and scours incidences, body size composite, birthweights, and incremental bodyweights were collected on a commercial dairy farm from October 1, 2015 to January 1, 2019. Holstein calves (n=5,180) were fed whole pasteurized non-salable milk with a 30% protein and 5% fat enhancer added at 20 g/L of milk through a Förster-Technik automated calf feeding system (feeders = 8) for 60 d (range 50 – 126 d). Calves were weighed at birth and several other times prior to calving. Average birth weight of calves was 40.6 ± 4.9 kg (mean \pm SD), serum total protein was 6.7 ± 0.63 g/dL, and cumulative 60 d milk consumption was 508.1 ± 67.3 L with a range of 179.9 to 785.1 L. Daily bodyweights were predicted for individual animals using a third order orthogonal polynomial to model bodyweight curves. The linear and quadratic effects of cumulative 60 d milk consumption, birthweight, feeder, yr born, season born, respiratory incidence, scours incidence and body size composite score were significant when predicting heifer bodyweight at 400 d (pBW₄₀₀) of age ($R^2=0.31$). There was up to a 263 kg difference in pBW₄₀₀ between the heaviest and lightest animal. Birthweight had a significant effect on predicted weights up to 400 d, and for every 1 kg increase in birthweight, there was a 2.5 kg increase in pBW₄₀₀. Quadratic effect of cumulative 60 d milk consumption was significant up to 400 d. Milk consumption 60 d was divided into quartiles and heifers had the highest pBW₄₀₀ in the third quartile, when 60 d consumption was between 507.8 and 552.5 L. Body size composite score showed a 21.5 kg difference in pBW₄₀₀ between the top and bottom 25th percentile of heifers. Heifers were 4.2 kg lighter at 400 d if treated for respiratory disease 3+ times during the first 60 d of life, compared to heifers not treated for respiratory disease. Measurements that can be obtained in early life of dairy calves continue to influence heifer growth up to 400 d of age.

2.2 Introduction

Raising high quality dairy replacement heifers is important to ensure profitable dairy farms. An approximate two yr investment is incurred before replacement heifers have the potential to generate income. Reported costs for raising a replacement heifer were \$2,510 per heifer from birth to first parturition (Akins and Hagedorn 2015). Due to the immense input costs, selecting the most profitable replacement heifers for the herd is more important than ever. Increasing growth rate of heifers early in life may result in earlier age at first calving, increased lifetime milk production (Soberon et al., 2012; Raeth-Knight et al., 2009) and reduction in overall raising costs (Ettema and Santos 2004; Tozer and Heinrichs 2001). Understanding what variables impact bodyweight could help producers better select replacement heifers for their herd. Early life factors that influence growth include proper colostrum management (Renaud et al., 2018), accelerated feeding programs (Davis Rincker et al., 2011), health incidences (Stanton et al., 2012), different housing systems (Costa et al., 2014), and different environmental conditions (Chester-Jones et al., 2017). Many management strategies have focused on obtaining a growth advantage during the pre-weaning phase.

One strategy to influence heifer growth is to feed calves with an accelerated feeding program. A conventional calf feeding program offers calves milk or milk replacer at 10% of bodyweight (Khan et al., 2007; Drackley, 2008; Jasper and Weary, 2002). In contrast, an accelerated feeding program allows the calf to consume increased amounts of milk or milk replacer during the pre-weaning phase, and often these programs increase the concentration of protein offered in the milk replacer (up to 30% CP) (Khan et al., 2011). Another way to offer an accelerated feeding program is to increase the volume of milk supplied to calves. Khan et al. (2011) identified that calves can consume up to 20% of their bodyweight in milk or milk replacer which doubles the amount of milk offered in the conventional feeding systems. An extensive number of publications have reported the effect these two feeding programs have on the growth of heifers without a consensus on the long-term benefits.

Accelerated feeding programs fed to pre-weaned dairy calves, report increases in growth at weaning (Miller-Cushon et al., 2013; Rosenberger et al., 2017; Byrne et al., 2018). Calves fed an increased milk replacer solids with an increased nutrient density milk replacer program calved 27.5 days sooner than calves feed on a conventional feeding program (Raeth-Knight et al. 2009). Soberon et al. (2012) reported the growth of calves during the pre-weaning phase was highly correlated with first

lactation milk production and reported that for every one kg increase in ADG during the pre-weaning phase, first lactation milk production increased by 850 kg. Additionally, other research has shown positive behavioral effects when offering calves an accelerated amount of milk (von Keyserlingk et al., 2009; de Passillé et al., 2011). However, a concern with feeding calves an accelerated amount of milk is the increased cost associated with these programs. Quigley et al. (2010) reported a \$27.22 difference in milk replacer cost per animal when feeding a conventional milk replacer fed at 454 g/d (20% CP, 20% fat) versus an accelerated milk replacer fed at varying rates with a maximum of 908 g/d from d 15-31 (28% CP, 16% fat).

One way to implement the accelerated feeding strategy is to use automatic calf feeders. With the increased acceptance of technology in agriculture industries (Rutten et al., 2013), the use of automatic calf feeders has increased without fully understanding how to best utilize the data generated or how to alter management for calves. Variation in individual milk consumption from automatic calf feeders can provide information on health status of animals. Respiratory disease is one of the leading causes of morbidity in pre-weaned dairy calves. Respiratory disease rates vary from farm to farm and Heins et al. (2014) reported a 61% treatment rate for respiratory disease (range 20.7 to 89.9% treatment) on four different farms. Calves identified with respiratory disease have been shown to have reduced growth rate (Cramer and Ollivett 2019), increased age at first calving (Teixeira et al., 2017) as well as lower total milk production (527.27 kg; Dunn et al., 2018) during first lactation.

Understanding which variables collected during the pre-weaning phase influence future outcomes of heifers may allow farms the ability to make more informed management decisions regarding heifer development. However, data collected on farms is not well integrated and makes the utilization of data time consuming for farmers. Therefore, the objective of this study was to investigate if variables collected during the pre-weaning phase can be used to predict future bodyweight of replacement heifers. We hypothesized that milk consumption and incidences of respiratory disease that occur pre-weaning will influence bodyweight of dairy heifers up to 400 d of age.

2.3 Materials and Methods

All calves were housed at a commercial dairy farm in north-central Indiana.

2.3.1 Protocols followed by farm

At birth, calves were fed 3.8 L of colostrum via an esophageal feeder, and six hrs. following the first colostrum feeding, they received another 1.9 L before being introduced to an automated calf feeder on day one. To measure success of passive transfer of immunity, blood was drawn from the jugular vein of calves between 1-10 d of age and serum total protein values (STP) were measured using a refractometer. The farm determined success of passive transfer if calves had a serum total protein value ≥ 6.0 g/dL, and 95% of calves had a STP of 6.0 g/dL or greater.

Calves were fed with eight automated calf feeders (Förster-Technik, Engen, Germany) located in four calf barns. Each barn contained four pens with two feeding stations per pen, and each feeder supplied milk to two pens. Each pen contained approximately 50-60 calves, fed with two feeding stations, were 9.14 x 24.38 m in size.

All calves could drink up to 24 L of milk per d from d zero to 32; however, the automated calf feeder offered incrementally increased in the amount of milk calves could consume within a 2 h period. Each calf was allocated 2 L of milk every 2 h until d 10. From d 10 to 21 calves could consume 2.5 L every 2 h. Then, calves could drink up to 3 L every two h until d 32. At d 32 the milk step-down phase began and the maximum milk allowance incrementally dropped by 2 L/d until d 39 when calves had a maximum milk allotment of 10 L/d. From d 39 to d 46 calves were offered up to 10 L/d, and at d 46 milk allotment decreased to 8L/d. Milk allotment then continued to be reduced until calves were fully weaned on average 60 ± 4.6 d of age (range 50-126 d), and for the exact step-down procedure refer to Supplemental Figure 1. Additionally, the average milk consumption by can be seen in Supplemental Figure 2. Calves were fed whole pasteurized non-saleable milk with a 30% protein and 5% fat enhancer added at 20 grams per L of milk. Adding balancers to whole or non-salable milk have been shown to increase gain, BW, and feed efficiency of pre-weaned dairy calves in comparison to feeding just waste milk and are a way to increase the nutrient content fed to calves without increasing the volume of milk fed (James and Scott, 2005). The farm utilizes a balancer to have the ability to feed their waste milk even when they have a fluctuating amount of non-salable milk available (Glosson et al. 2015).

Training calves to drink from the automated calf feeder was done two times/d by guiding and familiarizing calves to the feeding stations. Calves who did not drink at least four L from d 1-14 (83% of calves drank ≥ 4 L per d by d 14), or at least 5 L from 15-30 d (81% of calves drank \geq

5 L per d by d 30) were identified and manually fed. Calves were offered *ad libitum* starter (18% CP and 3.5% Fat) and water from d one of age inside the autofeeder pens.

Barns were ventilated with two positive pressure ventilation tubes and run yr-round. When the temperature increased above 15.6°C, all the curtains were open. Below 15.6°C, curtains and side doors were incrementally closed until the temperature reached -6.7°C or below, when all curtains were closed. Weather was monitored morning and evening and adjusted depending on the temperatures.

Calves were diagnosed with bovine respiratory disease (BRD) if they had a drinking speed deviation of 80% compared to the previous d, elevated temperature ($\geq 39.5^\circ\text{C}$), and rapid breathing compared to pen mates. If all symptoms were present, calves were treated with subcutaneous thulathromycin (DRAXXIN[®], Zoetis, Kalamazoo, MI) or intravenous flunixin meglumine (Intervet Inc., Roseland, NJ). After d 4 following treatment, calves were reevaluated and if no improvement, they were retreated with florfenicol and flunixin meglumine (RESFLOR GOLD[®], Intervet Inc., Roseland, NJ) subcutaneously. After d 7 of the first treatment, if symptoms did not resolve themselves calves were retreated with tulathromycin and flunixin meglumine. Calves were treated for scours if they had a drinking speed deviation of 80% compared to the previous d, loose stool, and appeared dehydrated/depressed. Calves that had a lower drinking speed deviation and loose stool were given Bismuth Subsalicylate (Durvet Inc., Blue Springs Missouri). Calves that appeared dehydrated/depress were also given oral electrolytes in addition to Bismuth Subsalicylate. Calves that had blood in the stool were treated with Megalumine (Intervet Inc., Roseland, NJ); however, only those treated with antibiotics were recorded in DairyComp 305 (VAS, Tulare, CA).

Daily milk consumption for calves was collected through the Förster-Technik automated calf feeders from October 1, 2015 to January 1, 2019. Total and 60-d milk consumption was calculated by individual data collected from the automated calf feeders. The value varied from the total days on feeder because calves were kept in the autofeeder longer than they were offered milk. The time in the feeder varied depending on calving frequency of the farm. Each calf was designated to one feeder, and this factor was included in the model. To account for the variation in d on the autofeeder, this variable was added to the analysis. Milk consumption 60 d was used to standardize milk consumption and compare the amount of milk consumed on the average age of weaning.

Health incidences, serum total protein values, bodyweights, and birthdates were obtained from DairyComp 305 (VAS, Tulare, CA). When deciding how to eliminate inaccurate farm collected STP, it was found that Elsohaby et al. (2015) used calves one to eleven d old for a project looking at failure of passive transfer of immunity. Wilm et al. (2018) found that STP values in calves taken up to 9 d old are highly correlated to each other ($r \geq 0.88$) and values taken up to 10 d old ($r = 0.76$) can be positively linked to one another. Higher STP values can be an indication of calf dehydration (Tyler et al. 1996); therefore, we did not utilize STP values collected after 10 d old or if STP was higher than 10 g/dL. Birthdates were extracted from DairyComp 305 (VAS, Tulare, CA) and four birth seasons were defined; calves born between January-March was winter, April-June was spring, July-September was summer, and October-December was fall. Average temperature for each season can be viewed in Supplemental Table 1. Yr of birth was determined from the birth date. Incremental weights were collected on farm at birth (zero d of age), leaving the autfeeder, approximately 3 mo of age, and breeding using an individual weigh scale (Figure 2.1; Tru-Test Limited, Auckland, New Zealand). Health events were obtained via DairyComp 305 (VAS, Tulare, CA). Calf respiratory disease and scours were obtained from DairyComp 305 and an individual event was defined as antibiotic treatment given five days apart from each other.

2.3.2 Animal Data

In total 9,099 calves with automated milk feeder consumption were included in the analysis. Calf milk consumption, birthdate, number of days on feeder, season born, yr born, respiratory incidence, and scours incidence files were combined and all calves had to have milk consumption and birthdate to be retained. Of the 9,099 calves, birthdate was recorded for 8,764 calves. From this data set, calf weights and body size composite values were added, and animals had to have at least one weight to be included. Body size composite score was collected through the Zoetis Clarifide® program and estimates an individual animal's expected differences in overall mature size and compacity by combining estimates of stature, strength, body depth, and width of rump and distributes the data into a single composite index (Zoetis, Kalamazoo, MI). After merging these files, there were 7,792 calves in the data set. Daily bodyweights were predicted for each animal using orthogonal polynomials (explained with more details below). Outliers were identified from predicted bodyweight at each of the eight time points and that individual weights were removed if the weight for that time point was above or below four standard deviations from the

mean (n=45) similar to outlier removal in Pietersma et al. (2006). Similarly, seven birthweights were removed using the same outlier removal process for the predicted bodyweights. After the predicted bodyweights were calculated for each day of age, 6,212 animals remained in the dataset. Lastly, STP values were added to the dataset, and the final analysis dataset included 60 d milk consumption, feeder number, number of d on feeder, yr born, season born, respiratory incidence, scours incidence, body size composite, and STP values from 5, 180 animals. Number of animals included at each step of merging and number of animals included in the final 400 d predicted bodyweight model after all insignificant values were excluded are displayed (Table 2.1). Respiratory treatment rate was categorized into four incidence rates, 0 = animals were not treated for respiratory, 1 = animals were treated once, 2 = animals were treated twice, and 3 = animals were treated three or more times for respiratory disease during the first 60 days of life.

2.3.3 Growth Model Calculation

Calves were not weighed on the same d of life; therefore, individual regressions were used to generate daily bodyweights. Bodyweight curves for the population and each animal were modeled with a random regression model based on a third order Legendre polynomial (Kirkpatrick et al., 1990) using the MIXED procedure of SAS version 9.4 (SAS Institute Inc.). The model was represented as:

$$w_{it} = (\beta_0 p_{0t} + \beta_1 p_{1t} + \beta_2 p_{2t} + \beta_3 p_{3t}) + (\alpha_{0i} p_{0t} + \alpha_{1i} p_{1t} + \alpha_{2i} p_{2t} + \alpha_{3i} p_{3t}) + e_{it}$$

where w_{it} is the weight of the i^{th} animal at day t , β_k is the k^{th} the fixed regression coefficient for the population, α_{ki} is the k^{th} random regression coefficient for animal i^{th} and p_{kt} are the functions normalized to x , which is the standardized unit of time defined as $x = 2 \left(\frac{t - t_{\min}}{t_{\max} - t_{\min}} \right) - 1$, calculated as: $p_0(t) = 1$, $p_1(t) = x$, $p_2(t) = \frac{1}{2}(3x^2 - 1)$, and $p_3(t) = \frac{1}{2}(5x^3 - 3x)$. In the current study, $t_{\min}=1$ d and $t_{\max}=400$ d so that the weight records between 1 to 400 days were converted into the interval of -1 to 1. e_{it} is the random error. The third order orthogonal polynomial was determined as the best fit based on BIC compared to second and fourth order orthogonal polynomial. Other measures of goodness of fit were R^2 and relative prediction error (RPE). The RPE was calculated as

$$\text{RPE} = \left(\frac{\sqrt{\text{MSPE}}}{\bar{A}} \right) \times 100$$

where MSPE is the mean square prediction error calculated as

$$\text{MSPE} = \sum_{i=1}^N (A_i - P_i)^2 / N$$

where A_i is the actual weight, P is the predicted weight, \bar{A} is the mean of the actual weights, and N is the number of records, $i = 1, 2, \dots, N$, and N is the number weight observations.

Bodyweight was only predicted up to 400 d because of the limited number of bodyweights collected between 500 and 600 d and the increased variability of weights around calving due to differences in fetus size and mammary development. Therefore, weights could not be accurately predicted after 400 d with this dataset, so the effect of early life on heifer bodyweight will be studied up to 400 d of age. Certain time points (1, 60, 100, 150, 200, 300, 365 and 400) were used for analysis.

2.3.4 Statistical Analysis

Data was analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The effect of nine factors on bodyweight at the different ages of the animals were evaluated with a linear model using the MIXED procedure. Variables included in the model were the linear and quadratic effect of milk consumption, feeder, number of days in the feeder, yr born, season born, respiratory disease score, birthweight, serum total protein, and body size composite score. Only factors with significant effect ($P < 0.05$) were retained in the final model for weight each age.

Bodyweight curves were modeled for different scenarios of factors affecting weights at different ages to illustrate the effect of these factors on the long-term weight of growing heifers. Average daily gain (ADG) was calculated at each of the selected heifer ages with a cubic polynomial.

2.4 Results

Estimates of the regression coefficients that describe the bodyweight curve of the population are presented in Table 2.2. Measures of goodness of the random regression model were $R^2=0.9956$ and relative prediction error of 4.36%.

Results of analysis of variance of predicted weights at different ages are presented in Table 2.3 showing the number of variables included in the model along with the number of animals and the model's corresponding R^2 values. The final model that explained variation on predicted weight at 400 d (pBW₄₀₀) included the linear and quadratic effects of 60 d milk consumption, birthweight, feeder, yr born, season born, respiratory incidence, scours incidence, and body size composite score (all $P < 0.0001$; Table 2.4). The number of days on feeder and serum total protein value were not significant in the model ($P \geq 0.20$) and were removed from the model. The final equation for pBW₄₀₀ was:

$$\text{pBW}_{400} \text{ (kg)} = 197.37 + 0.48 \times (\text{milk consumption in L}) - 0.0003 \times (\text{milk consumption in L})^2 + 1.60 \times (\text{birthweight in kg}) + \text{feeder} + \text{yr born} + \text{season born} + \text{respiratory incidence} + \text{scours incidence} + 8.89 \times (\text{body size composite score})$$

Feeder number had a significant effect on pBW₄₀₀, and between the eight feeders, there was up to a 17 kg difference in pBW₄₀₀. The pBW₄₀₀ of heifers was highest in 2015 and significantly decreased every yr with calves in 2019 having the lowest pBW₄₀₀. Calves born in the spring (April-June) had the highest pBW₄₀₀. Calves born in the fall (October – December) had the lowest pBW₄₀₀.

Even though feeder, season the calf was born, and yr the calf was born significantly impacted the bodyweight of heifers up to 400 d of age, they were excluded from the final model because they were random factors that cannot be replicated and therefore, would not be able to be determined for a predictive equation which was similar to reports from (Cue et al. 2012). Our objective was to use the significant variables from early life to generate a predictive model that farms could use to select dairy replacement heifers at an earlier age. Therefore, significant and controllable variables were the linear and quadratic effects of milk consumption, birthweight, respiratory incidence, scours incidence and body size composite score (all $P < 0.0001$; Table 2.5). The predictive equation without random effects was:

$$\text{pBW}_{400} \text{ (kg)} = 193.14 + 0.55 \times (\text{milk consumption in L}) - 0.0004 \times (\text{milk consumption in L})^2 + 1.66 \times (\text{birthweight in kg}) + \text{respiratory incidence} + \text{scours incidence} + 8.53 \times (\text{body size composite score})$$

Predicted bodyweight of five individual heifers with different values for milk consumption, season born, respiratory disease, body size composite score, birthweight, serum total protein is displayed in Figure 2.2. A 210 kg difference in pBW_{400} exists between heifer E and C (Figure 2.2). The animals in Figure 2.2 had large variation in the significant variables from the model: milk consumption, birthweight, body size composite score, and respiratory differences. For example, heifer C drank 203.6 less liters of milk, had a higher birth weight, was treated for respiratory disease and had a higher body size composite score than heifer E. The differences in inputs between these two heifers is observed by differences in pBW_{400} . The significant early life predictive variables account for 31% of the variation in bodyweight at 400 d, therefore, other variables contribute to the difference seen at 400 d as well.

The ADG at different ages are showed in Figure 3. The ADG was reduced from 1.00 kg/d at 60 d to 0.91 at 400 d of age (Figure 2.3).

Average milk consumption by animal on this farm was 508.1 ± 67.3 L (mean \pm SD) which averaged 8.5 L per day, with a range from 179.9 to 785.1 L in a 60 d period. Milk consumption averages at varying d of age (10, 21, 32, 39, 46, 53, 60) are shown in Supplemental Figure 2. Increasing total milk consumption increased pBW_{400} (quadratic $P < 0.0001$; Figure 2.4; $R^2 = 0.16$). Higher birthweights resulted in heavier heifers at 400 d of age (Figure 2.5; $R^2 = 0.13$). Average birthweight was 40.6 ± 4.9 kg (mean \pm SD) with a range from 22.7 to 59.42 kg.

Health factors including serum total protein, scours incidence, and respiratory disease during the first 60 days of life were included in the model. The average serum total protein value was 6.68 ± 0.63 g/dL (mean \pm SD) and the values ranged from 4.6 to 9.2 g/dL. Effects of serum total protein were significant on bodyweight up to 100 days of age, but were not significant for time points beyond 100 d. Scours treatment rate did not significantly influence bodyweight up to 365 d of age. At 400 d the variable became significant ($P < 0.05$), and calves diagnosed with scours had significantly heifer bodyweights at 400 d. Respiratory disease also had a significant effect ($P < 0.05$) on predicted bodyweight of heifers up to 400 d. The association between different respiratory incidence rates and predicted bodyweights of heifers is shown in Figure 2.6. For the first 200 d, effect of each respiratory category was significantly different from one another (all P

< 0.05). At 300 d of age, respiratory disease category two and three were not significantly different from one another ($P = 0.07$), but were different from category zero and one ($P < 0.05$). At 400 d, the pBW₄₀₀ for animals without respiratory disease was significantly higher (-1.95 kg for category 1, -2.9 kg for category 2, and -4.2 kg for category 3 compared to animals not diagnosed with respiratory disease between 1 – 60 d of age). The effect of respiratory disease in the first 60 days of life has on predicted bodyweight decreases as heifers get older.

Genetic body size composite score was divided into quartiles and compared to pBW₄₀₀ (Table 2.6). The average body size composite score for quartiles one through 4 were -0.77, -0.12, 0.31, and 0.95. Each quartile was significantly different from the other ($P < 0.001$) with the pBW₄₀₀ for the quartiles were 406.6, 415.4, 421.7, and 428.1 kg. There was a 21.6 kg difference in predicted bodyweight when comparing the top and bottom 25% of heifers.

2.5 Discussion

Milk consumption, birthweight, feeder, yr born, season born, respiratory incidence, scours incidences and body size composite were all significant variables impacting predicted bodyweight of dairy heifers. The milk consumption had a significant linear and quadratic effect on BW, indicating that each additional L of milk does not increase predicted BW as much as the previous L of milk. The quadratic effect may not be seen in conventional diets because of the set amount of milk fed to animals and small variation in calf intake. By feeding intensified diets through an automatic calf feeder, there was variation in milk consumption between calves and we were able to observe the effect of milk consumption on bodyweight. In this study, when comparing a single variable, milk consumption accounted for 16% of the variation in bodyweight at 400 d. Feeding of intensified diets during the pre-weaning phase has affected growth later in life in other studies as well, Davis Rincker et al. (2011) reported heifers on an intensified diet reached the breeding target weight of 397 kg sooner than those on a conventional diet. However, others find no advantage of intensified feeding on BW after weaning (Dennis et al., 2017; Kiezebrink et al., 2015; Morrison et al., 2009). Most research looking into the effect of milk feeding strategies on growth of calves offer a specified amount of milk in their treatments. In automatic calf feeders, calves have the option to choose the amount of milk they consume; therefore, the milk consumption factor could produce a different result on farms that individually house calves and only offer a set amount of milk/milk replacer.

Heifers with higher birthweight had higher pBW₄₀₀. Greenwood et al. (2006) found that finished beef cattle (Piedmontese or Wagyu crossed) with lower birthweights weighed 56 kg less at 30 months old than higher birthweight cattle. In contrast, Donovan et al. (1998) found that birthweight only impacted weight gain up to 6 months of age. Even though higher birthweight results in heavier animals at 400 d, increases in birth weight can also lead to increases in dystocia rate of cows. Johanson and Berger (2003) revealed that dystocia increased 13% per one kg increase in birthweight. Calf birth weight can affect subsequent milk production. Rahbar et al. (2016) saw that cows with lower birthweight calves (20-25 kg) had lower 305 d milk production than cows with 40-45 kg birthweight calves. Therefore, it is important to consider calf birthweight when understanding calf bodyweight.

Respiratory disease has a significant impact on heifer bodyweight through 400 d. If a calf was treated for respiratory disease once there was a 0.71 kg reduction in predicted BW at 60 d of age and nearly 2 kg different at 400 d. At 60 d, each respiratory disease category was significantly different from the other. Over time and by 400 d of age, calves treated once, twice, or three or more times were not statistically different from one another, but if calves were not treated, they were statistically different from calves treated for respiratory disease. Other research reported that pre-weaning respiratory disease negatively impacts bodyweight (Stanton et al., 2012; Van der Fels-Klerx et al., 2001). The objective of this study was to determine which factors in early life impact future bodyweight, therefore pre-weaning respiratory disease was considered in this study; but respiratory disease post-weaning would likely have an effect on BW after the 60 d time point (Adams and Buczinski 2015). Additionally, if calf health scoring charts (McGuirk, 2008) or thoracic ultrasonography (Adams and Buczinski 2015) were used to identify calves with respiratory disease rather than treatment records, the results may have had a larger impact on predicted bodyweight due to increased accuracy of disease detection.

Scours is the most common disease diagnosed in pre-weaned dairy calves, 16% treated for digestive problems (USDA, 2018). Pre-weaning scours incidence was never significant in the model until 400 d of age, and at 400 d calves with scours had higher predicted bodyweights in comparison to calves without scours. We believe that this was due to the relatively low number of incidences on this specific farm recorded in their records. Donovan et al. (1998) predicted a 9.1 kg reduction in 180 d weight gain when calves were treated an average of 3.76 days for scours, but after 6 months, scours incidence did not affect weight gain. However, Curtis et al., (2018) reported

no difference in growth with varying scours incidences. From October 1, 2015- January 1, 2019 there were only 237 animals treated for scours with antibiotics in our analysis. Other scours treatments on this farm included oral electrolytes and Bismuth Subsalicylate (Durvet Inc.; Blue Springs, Missouri), which were not recorded in the farm management software. Therefore, only animals treated with antibiotics for scours were recorded and included in the analysis. If all incidences of scours were recorded, scours incidences might have had a different outcome in the model. Another possible explanation for the unexpected scours results is that the increase in milk consumed could cause a looser stool, leading to treatment for scours, even if the calf is not clinically ill.

Introducing genetic variables improves reliability of the overall predictive equation, body size composite score accounted for 5.7% of bodyweight differences in heifers at 400 d. The body size composite score measurement included in this analysis measures the difference in animal's stature, strength, body depth, and width of rump. A higher body size composite score indicates a larger animal. It was estimated that a one point increase in body size composite showed an 18.2 kg increase in mature bodyweight (Zoetis, Kalamazoo, MI). When body size composite was divided into quartiles, there was a 21.5 kg difference in BW between the top and bottom 25% of animals which had an average 1.72 increase in body size composite score.

The yr the heifer was born was highly significant in the model up to 400 d with a decrease of 40.5 kg, 8.6 kg, 2.6 kg, and 11.1 kg respectively, from 2015 to 2019. Other research has found yr to also be significant when measuring bodyweight over time (Cue et al. 2012; Donovan et al. 1998). Based on evidence seen by Dietrich (2015) looking at standard plate and coliform count in autofeeders pre and post-cleaning, bacteria buildup in the autofeeders as they age could explain some of the differences seen among yrs. We speculate that bacterial load in the autofeeders increased over time, resulting in reduced animal performance.

Eight automatic calf feeders on a single farm is relatively uncommon. Research done on 10 commercial dairy farms with automated calf feeders averaged 1.4 feeders per farm (Dietrich 2015); therefore, not much is known about the affect different feeders on the same farm have on growth. Some variation could be due to factors that we are not controlling for, such as including yr in which feeders were installed (1, 2, 3, 4 = 2015; 7 and 8 = 2016; and 5 and 6 = 2017), barn location, or unforeseen weather events. Because not all autofeeders were installed at the same time or in the same location, this may explain some of the variation seen in bodyweight of animals

raised in different feeders. Regardless, feeder had a significant effect on heifer bodyweight at 400 days.

Similar to what others have reported, season affects animal bodyweight and performance (Handcock et al., 2019; Van der Fels-Klerx et al., 2001; Donovan et al., 1998). Results of seasonal effect may vary due to the months used to define a season. Our study defined winter as January to March; while Van der Fels-Klerx et al. (2001) defined winter as November through January. Seasonal effect was also considered when examining different growth intervals such as season of birth date used, in this study vs. season at weaning, or season at breeding. Because studies define seasons differently and measure its effect at different growth intervals, it often becomes difficult to compare seasonal effect across research findings. For example, in the current study, calves born in winter were the heaviest up to 300 d, but at 400 d those born in the spring (April-June) had the heaviest BWs. Chester-Jones et al. (2017) reported that calves born in the fall and winter had the highest BW at eight wk old. Though they did not measure growth past eight wk, but reported calves born in the summer had higher 305 d milk yield (Chester-Jones et al. 2017).

Nonsignificant variables at 400 d of, serum total protein and number of days on feeder were and removed from the model. Effect of serum total protein was significant up to 100 d of age on heifer bodyweight. The average STP value on this farm was 6.67 g/dL. Weaver et al. (2000) and Renaud et al. (2018) found that calves with ≥ 5.2 g/dL or ≥ 5.1 g/dL STP have successful passive transfer of immunity. The average on the commercial farm in the current study exceeded this target number and 95% of calves had a STP value ≥ 6 g/dL; therefore, this could be one reason serum total protein was not significant in the final 400 d model.

The average number of d animals consumed milk in the automated calf feeder was 60 ± 4.6 (mean \pm SD) with range of 50-126 d. This variable was significant in the model to at least 300 d of age; however, this variable becomes insignificant at timepoints 365 and 400 d of age. This is reasonable because the more time calves spend on the feeder, the more they consume, and since milk consumption impacts bodyweight, the higher their predicted bodyweight will be (De Passillé et al., 2011). In contrast to this finding, the results from timepoints up to 300 d of age indicate that the longer the calves are on the automated calf feeder, the lower their predicted bodyweights were. This finding is not surprising because calves who had higher days on the feeder were held back by the farm due to lower performance in comparison to pen mates.

Of the significant variables in the model, yr born, season born, and the feeder the calf was in are not variables that can be measured and improved on farm. In order to generate an equation to better monitor measurable variables that affect heifer performance, all 3 variables were removed from the model. Cue et al. (2012) removed herd, yr, and month because they wanted to generate a predictive model. Excluding significant variables introduces error, but including them will diminish the usefulness of the model when implementing it on farm. If used in the predictive model future predictions become unimportant because the effects of a different yr born, season born, or feeder are not known. Therefore, the final predictive model on this commercial dairy farm included milk consumption and the quadratic effect, birthweight, respiratory incidence, and body size composite score $R^2 = 0.27$.

There was a 263 kg difference in predicted bodyweight at 400 d from the lightest to the heaviest heifer present in the dataset. Based on the results found in the study, variables collected during the first 60 d of life were able to account for over 30% of the difference in bodyweight up to 400 d of age. The next steps will be to understand how early life impacts other production variables later in life.

Some limitations to this study include the inability to quantify starter intake for calves. With starter intake it would allow a more accurate understanding of how all nutrients during the preweaning period impact bodyweight. Variable respiratory treatment rates are seen on farm, so utilizing a more standardize scale such as thoracic ultrasonography to identify damage from respiratory disease could also help better understand the effect this variable has on growth (Cramer and Ollivett, 2019; Buczinski et al., 2015). Additionally, data generated from this study did not allow for an accurate analysis of the effect of scours on predicted bodyweight due to the inability to account for all types of treatments. Since the farm only recorded antibiotic treatments our analysis could not consider animals treated with non-antibiotic productions. In the future, our analysis would ideally include heifer height and withers width to most accurately identify growth of heifers because the current analysis only accounted for the difference in bodyweights. Future research needs to validate this predictive model and be able to adjust the equation for each farm.

2.6 Conclusions

In conclusion, early life variables collected during the preweaning phase can influence over 25% of the variation in heifer growth at 400 d on a single commercial dairy. Combining phenotypic

and genotypic traits allows the generation of a predictive model that a farm can use to aid in making management decisions. It is important to note that there are influential variables that are out of a farm's control, and this predictive equation will only match findings found on this single dairy farm. Future research is needed to allow an understanding of how to best implement this model based on individual farm's data collection.

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2.8 References

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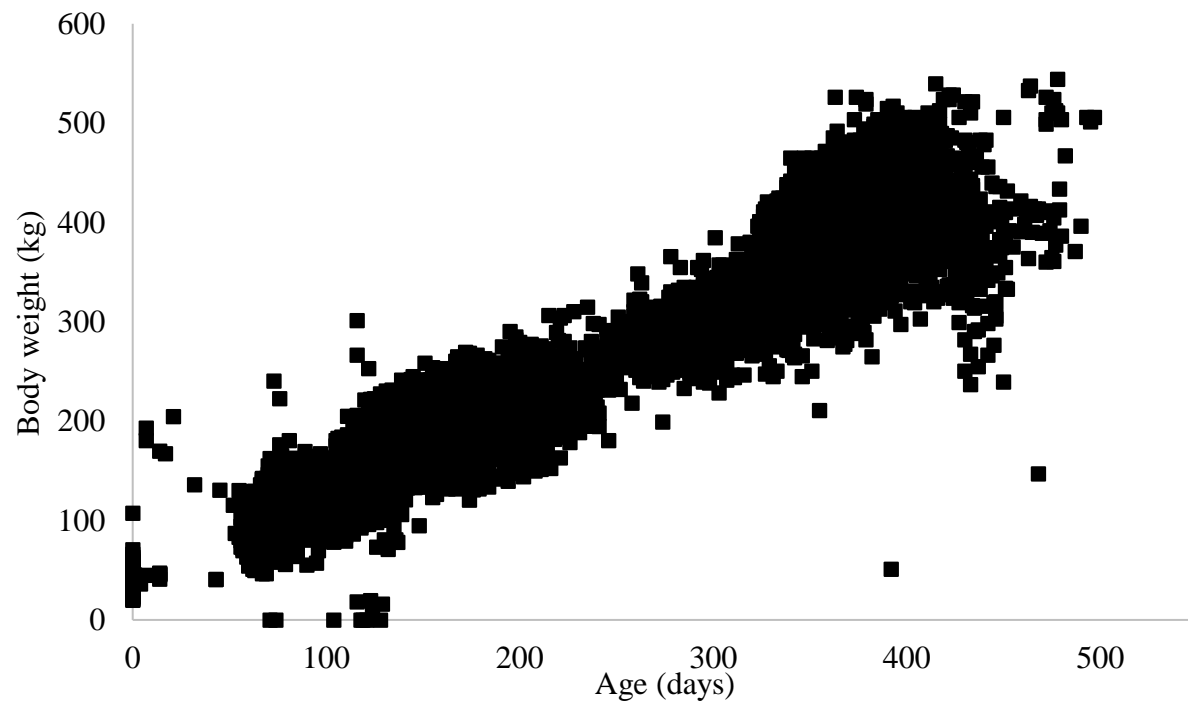


Figure 2.1 Initial, unedited heifer body weights data before predicted body weight analysis was performed and outliers were removed. Body weights were collected from October 1, 2015 to January 1, 2019.

Table 2.1 Data organization and tracking of animal numbers during analysis. All animals including in the final analysis were required to have all variables in the model. Analysis started with 9,099 animals and ended with 5,180 heifers.

Data Cleaning	Number of Animals
Autofeeder milk consumption	9,099
Dataset 1: milk consumption, birthdate, respiratory and scours	8,764
Dataset 2 (dataset 1, feeder number, actual animal weights, genetic body score, birthweight)	7,792
must have dataset 1 and one animal weight	
Dataset 3 (dataset 2, predicted body weights)	6,212
Dataset 4 (dataset 3 with 45 outlying weights removed)	6,206.
Dataset 5 (dataset 3, with STP ¹)	6,206
Predicted body weight model at 400 days	5,180

¹ Serum total protein value

Table 2.2 Estimates of regression coefficients for the third order orthogonal polynomial of the growth curve of dairy heifers in an accelerated feeding programs fed to pre-weaned dairy calves.

Coefficient	Estimate	SE
β_0	232.27	0.32
β_1	187.90	0.33
β_2	-3.32	0.31
β_3	0.87	0.27

Table 2.3 Organization of predicted bodyweight analysis by age of animals. Table displays number of contributing variables in the model, number of animals in the model, and the R^2 associated with each model at each age.

Age (days)	Number of contributing variables	Number of animals in model	R^2
60	9 ¹	4,996	0.58
100	9 ¹	4,997	0.58
150	8 ²	5,183	0.52
200	8 ²	5,182	0.44
300	8 ²	5,183	0.33
365	7 ³	5,182	0.31
400	7 ³	5,180	0.31
Predictive (400)	4 ⁴	5,180	0.27

¹Variables included in the model: milk consumption, feeder, number of days in the feeder, yr born, season born, respiratory disease, birthweight, serum total protein, genetic body size score.

² Variables included in the model: milk consumption, feeder, number of days in the feeder, yr born, season born, respiratory disease, birthweight, genetic body size score.

³Variables included in the model: milk consumption, feeder, yr born, season born, respiratory disease, birthweight, genetic body size score.

⁴Variables included in the model: milk consumption, respiratory disease, birthweight, genetic body size score.

Table 2.4 Parameter estimates of significant variables, calculated by resitricted maximum likelihood using the MIXED procedure of SAS, to predict body weight at 400 d old.

Variable	Estimate	SE	<i>P</i> - value
Intercept	197.37	19.65	<0.0001
Milk consumption	0.48	0.06	<0.0001
Milk consumption x Milk consumption	-0.0003	0.000057	<0.0001
Birthweight	1.60	0.09	<0.0001
Feeder			<0.0001
1	8.51	1.43	<0.0001
2	6.70	1.46	<0.0001
3	-3.13	1.46	0.0327
4	-1.23	1.45	0.3956
5	5.94	2.19	0.0065
6	13.70	1.74	<0.0001
7	-0.75	1.45	0.6056
8	0	-	-
Year born			<0.0001
2015	62.89	28.32	0.0264
2016	22.18	4.71	<0.0001
2017	13.57	4.56	0.0029
2018	11.11	4.48	0.0132
2019	0	-	-
Season born in			<0.0001
Winter	1.53	1.15	0.185
Spring	11.01	1.19	<0.0001
Summer	3.97	1.07	0.0002
Fall	0	-	-
Respiratory incidence			0.0149
0	4.22	1.83	0.0214
1	2.26	1.83	0.2185
2	1.34	1.99	0.4994
3	0	-	-
Scours incidence			0.0178
0	-25.83	12.51	0.039
1	-21.66	12.67	0.0874
2	0	-	-
Body size composite	8.89	0.58	<.0001

Table 2.5 Parameter estimates of significant factors, calculated by restricted maximum likelihood using the MIXED procedure of SAS, to predict bodyweight at 400 d after not significant ($P > 0.05$) variables were removed from the model. This model removes the random effects of year, season and feeder.

Variable	Estimate	SE	<i>P</i> - value
Intercept	193.41	19.72	<0.0001
Milk consumption	0.55	0.06	<0.0001
Milk consumption \times milk consumption	-0.0004	0.00006	<0.0001
Birthweight	1.66	0.09	<0.0001
Respiratory incidence			<0.0001
0	6.18	1.86	0.0009
1	3.42	1.88	0.0688
2	1.94	2.04	0.3436
3	0	-	-
Scours incidence			0.0078
0	-24.03	12.83	0.0611
1	-18.61	13.00	0.1522
2	0	-	-
Genetic body size composite	8.53	0.60	<0.0001

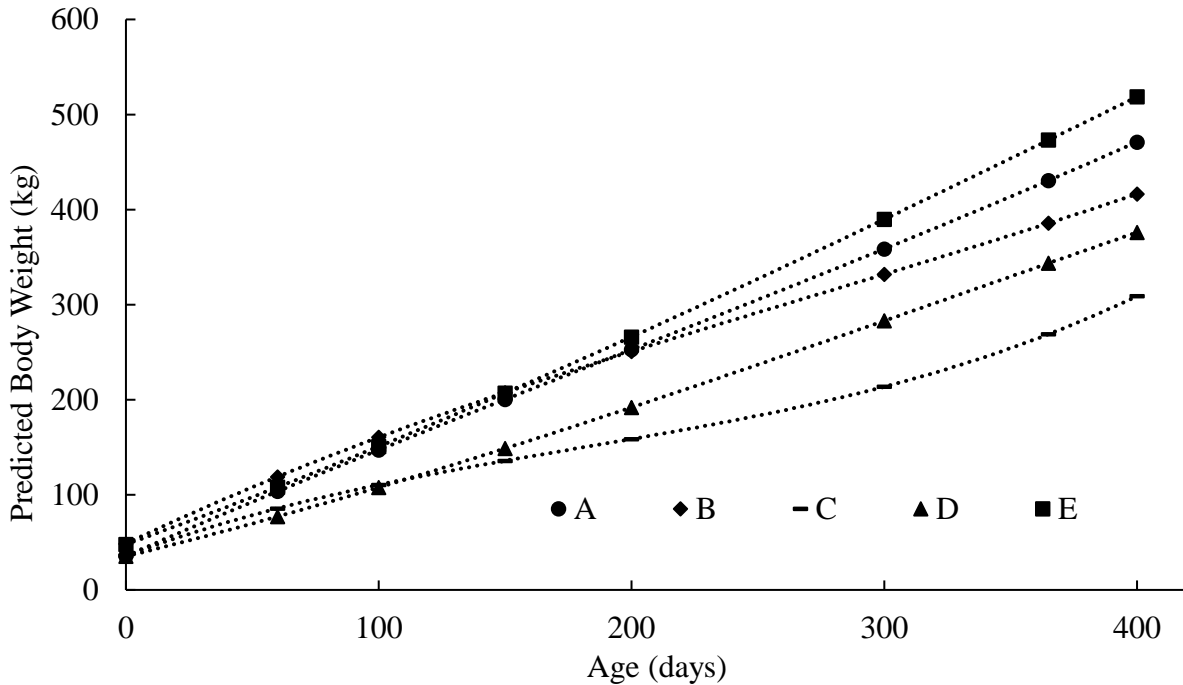


Figure 2.2 Comparison between predicted bodyweights (kg) of individual animals over time (days). Body weight was predicted using a third order orthogonal polynomial. **A**: 607 L intake, born in the summer 2017, zero incidence of pneumonia, 1.18 genetic body size composite score, 48.08 kg at birth, and 6 g/dL serum total protein. **B**: 562.6 L intake, born in the fall 2017, zero incidence of pneumonia, 0.83 genetic body size composite score, 40.82 kg at birth, 6.2 g/dL serum total protein. **C**: 429.6 L intake, born in winter 2018, one pneumonia treatment, 0.48 genetic body size composite score, 38.41 kg at birth, 6.4 g/dL serum total protein. **D**: 370.4 L intake, born summer 2018; one pneumonia treatment, -0.41 genetic body size composite score, 36.74 kg at birth, 6.8 g/dL serum total protein. **E**: 633.2 L intake, born spring 2017; zero incidence of pneumonia, -0.11 genetic body size composite score, 35.83 kg at birth, 6 g/dL serum total protein.

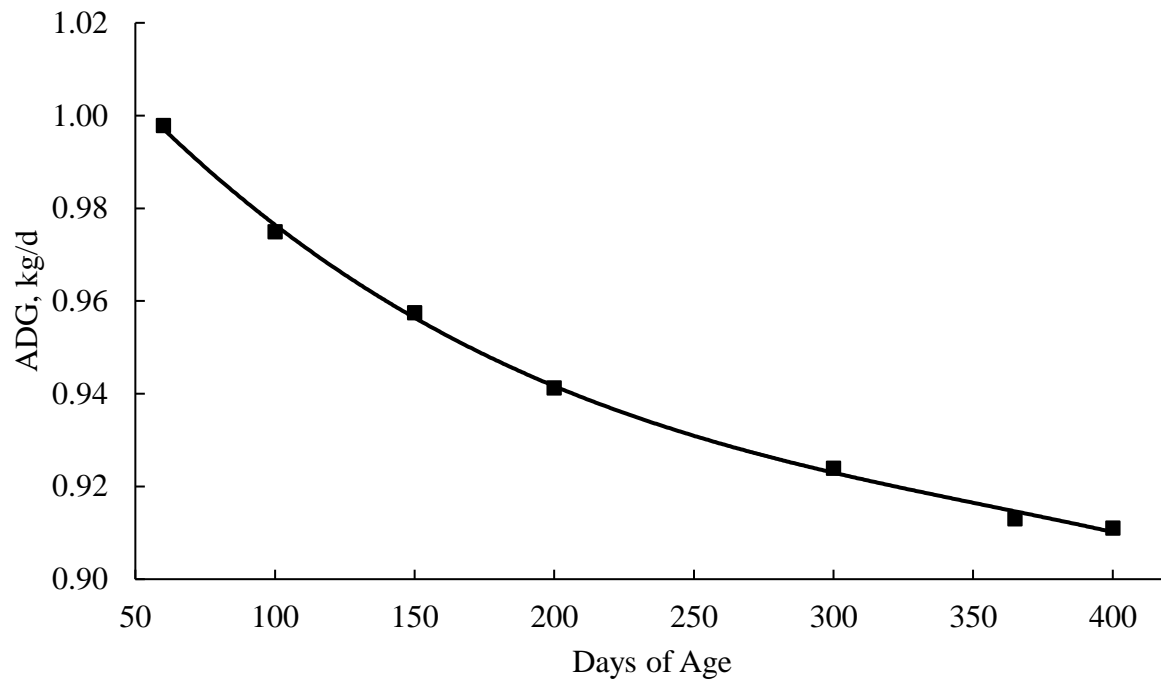


Figure 2.3 Average daily gain of heifers over time. $ADG = -2E^{-09}x^3 + 2E^{-06}x^2 - 0.0008x + 1.0375$, x = age at measurement and $R^2 = 0.9987$

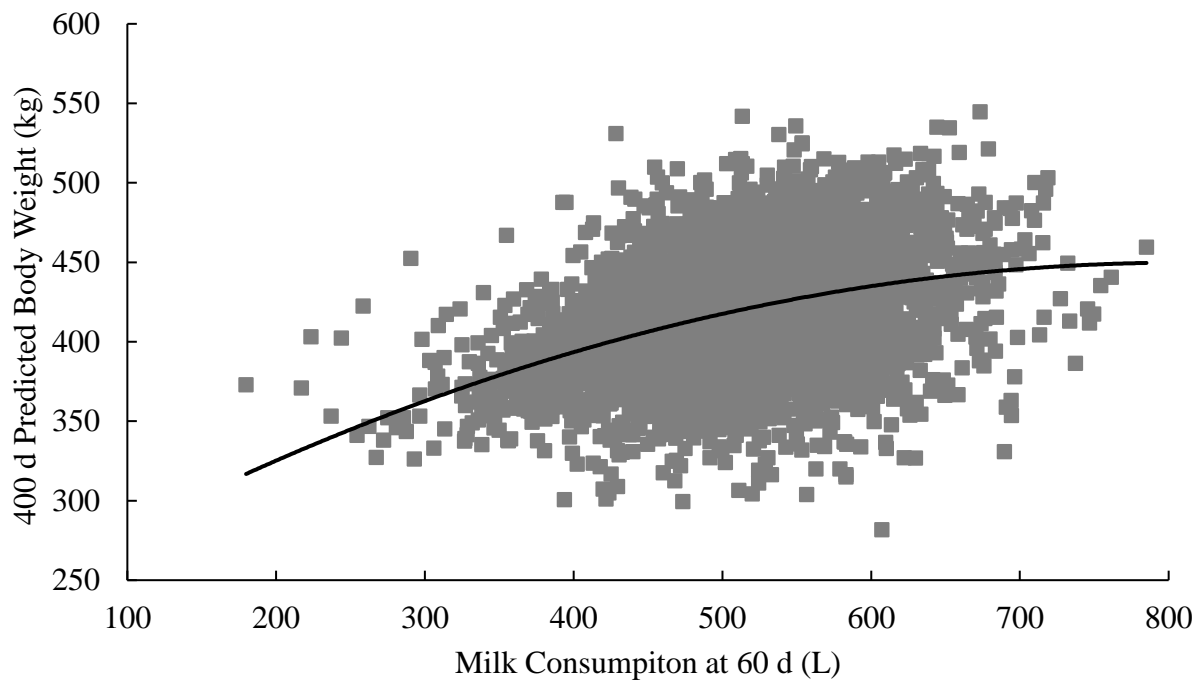


Figure 2.4 The effect 60-day milk consumption has on the predicted bodyweight of animals at 400 days. $p400BW = -0.0003x^2 + 0.5417x + 230.22$, x = milk consumption (L) and $R^2 = 0.16$.

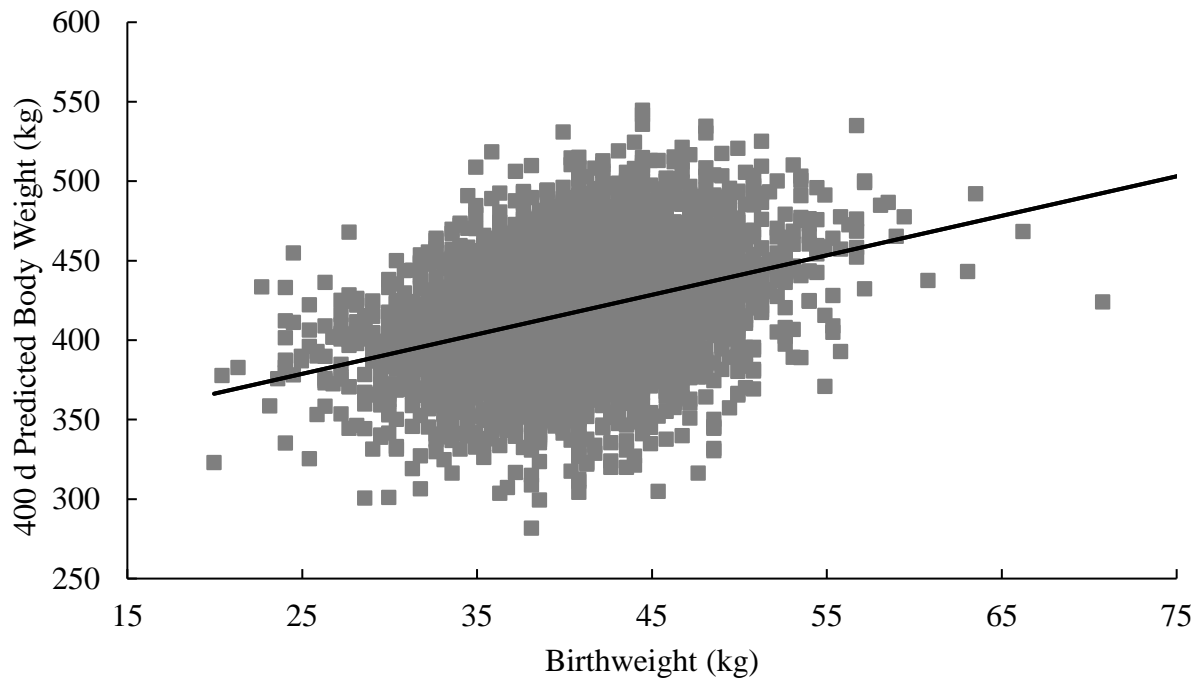


Figure 2.5 The effect of birthweight on the predicted bodyweight of animals at 400 d. $p400BW = 2.5626x + 313.58$, $x = \text{birthweight (kg)}$ and $R^2 = 0.13$.

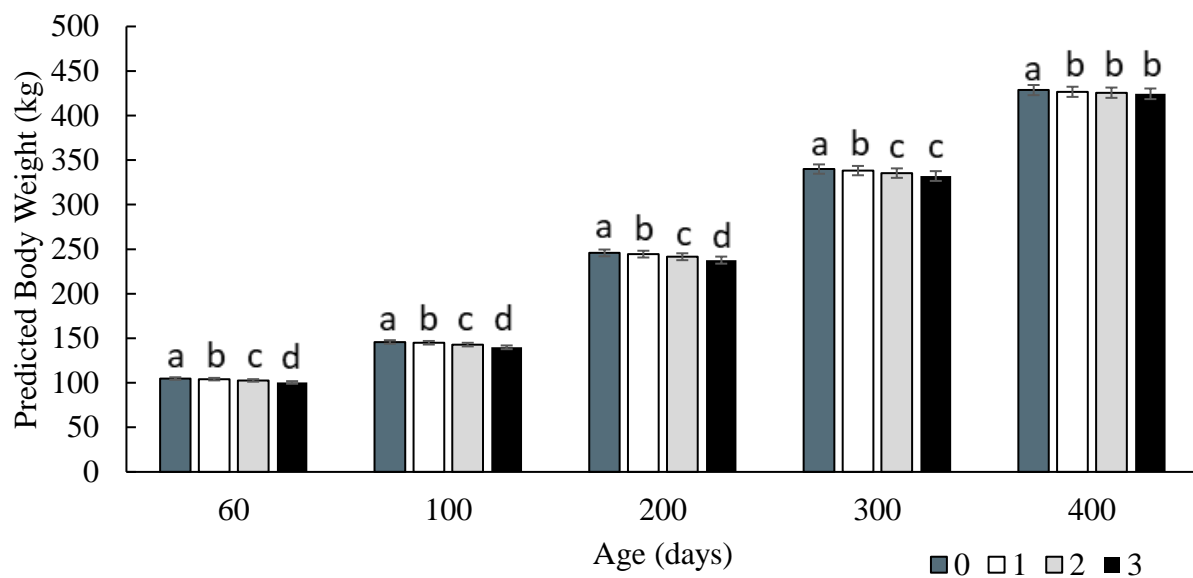
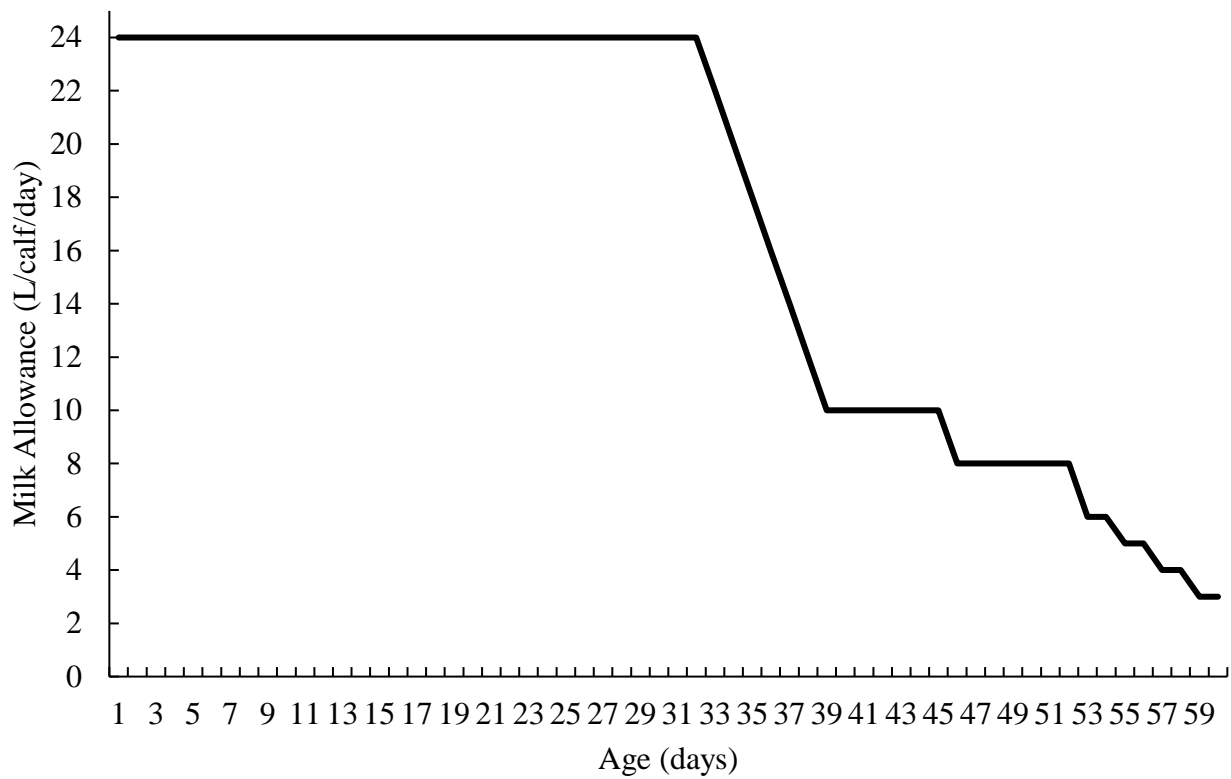


Figure 2.6 The effect of respiratory disease on predicted bodyweight over time (60, 100, 200, 300, 400). 0 = no treatment, 1 = one treatment, 2 = two treatments, 3 = three or more treatments.

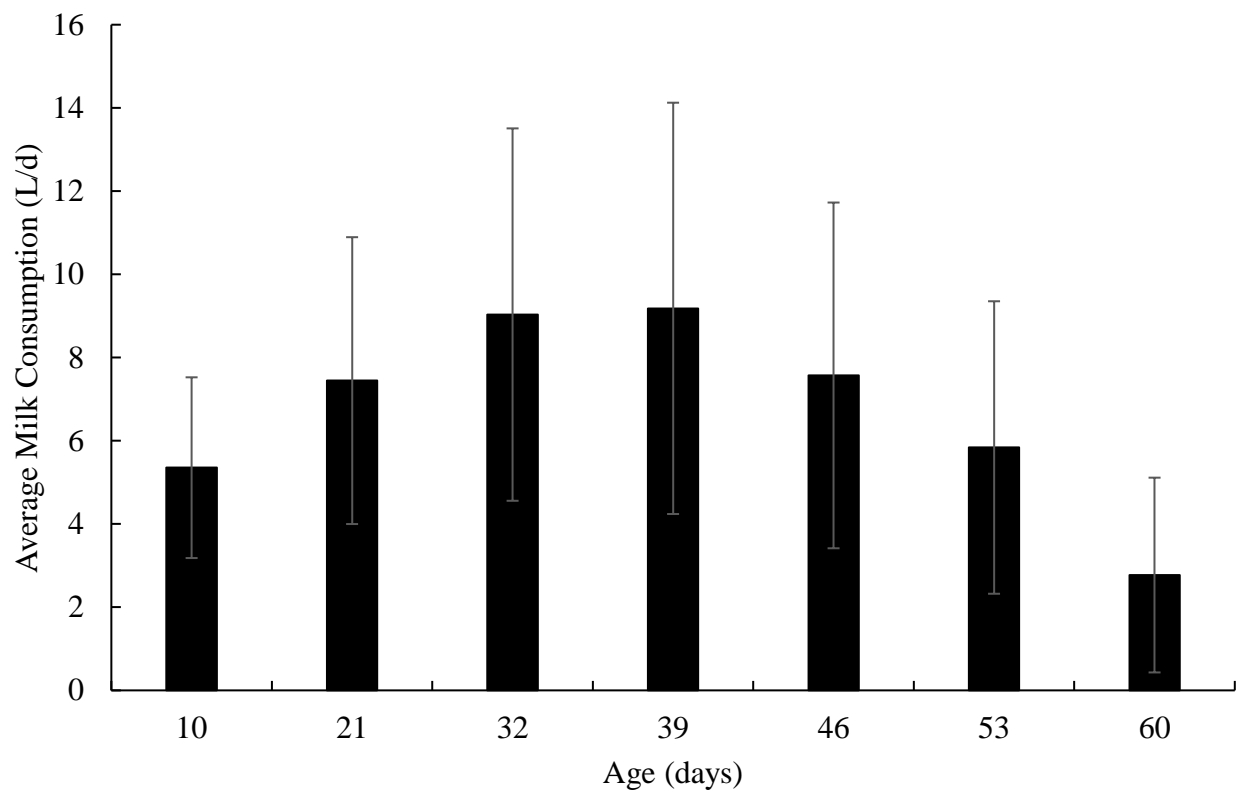
a,b,c and d Means with different letter are significantly different ($P < 0.05$).

Table 2.6 The effect of body size composite score on predicted bodyweight of animals at 400 days. Body size composite score was divided into quartiles with the average and range presented for each quartile.

Quartiles	Average body size composite	Range body size composite	Predicted body weight mean	SEM
1	-0.77	-2.62 to -0.36	406.6	0.90
2	-0.12	-0.36 to 0.09	415.4	0.90
3	0.31	0.09 to 0.54	421.7	0.90
4	0.95	0.54 to 3.05	428.1	0.90



Supplemental Figure 2.1 Milk allotment (L/calf/d) offered to calves in the automated calf feeder. This figure displays the incremental weaning process that the feeder does automatically for each individual animal in the pen.



Supplemental Figure 2.2 Average milk consumption (L/d) of calves in the automated calf feeder.

Supplemental Table 2.1 Daily Average, average daily minimum, and maximum temperature, and overall minimum and maximum for each season from October 1, 2015 to January 31, 2020. Data collected from NOAA: National Centers for Environmental Information.

Season	Temperature ¹				
	Daily Average	Average Daily Maximum ⁶	Average Daily Minimum ⁷	Maximum Reported	Minimum Reported
Winter ²	-2	4	-5	-29	26
Spring ³	11	21	9	-8	35
Summer ⁴	17	28	15	3	35
Fall ⁵	3	10	1	-27	32

¹Degrees Celsius

²January-March

³April-June

⁴July-September

⁵October-December

⁶Average of recorded daily maximum temperatures for each of the seasons

⁷Average of recorded daily minimum temperatures for each of the seasons

CHAPTER 3. THE EFFECT OF EARLY LIFE INDICATORS ON FUTURE HOLSTEIN HEIFER SURVIVABILITY, REPRODUCTIVE, AND FIRST LACTATION MILK PRODUCTION PERFORMANCE

3.1 Abstract

The objective of this study was to evaluate the long-term effects of early life events on heifer conception rate, survivability through first lactation, and first lactation milk production of heifer calves raised in automatic calf feeders. Sixty d cumulative milk consumption (MC), 400 d predicted body weight (BW), average daily gain from zero to 60 d (ADG), ADG from zero to 400 d, heifer conception age (d), and 280 d first lactation milk production (280M) were collected between Oct. 1, 2015 to Jan. 31, 2020. Calves were fed pasteurized whole milk through an automated calf feeding system (feeders = 8) for 60 d (range: 48 – 126d), with a 30% CP and 5% Crude Fat enhancer added at 20 g/L of milk. Calves were weighed at birth and several other time periods prior to calving. Daily BW predictions were calculated for individual animals using third order orthogonal polynomials and 300 d (421.0 ± 34.1 kg; mean \pm SD) weights (pBW300) were used for this model. ADG was calculated based on predicted BW between 0-60 d and 0-400 d. Cumulative 60 d MC was 508.1 ± 67.3 L (range 179.9-785.1 L). Average age at conception was 437.5 ± 45.0 d (range 308 to 631 d; n=5,193), and average 280M was $9,305 \pm 1,371.8$ kg (range of 4,631 to 13,358 kg; n=1,324). Heifer conception age was impacted by season, yr, and the quadratic effects of pBW300 and ADG (0-400; all $P < 0.05$; total model $R^2 = 0.08$). Season born, ADG (0 - 400 d), genomic milk, and the linear effect of heifer conception age had a significant impact on 280 d first lactation milk production (all $P < 0.05$; $R^2 = 0.28$). For every 1 kg increase in genomic milk value there was a 1.42 kg increase in first lactation 280M. Calves not diagnosed with bovine respiratory disease (BRD) from 61-120 d old had a significantly higher chance for survival to first lactation than animals treated three or more times for BRD (hazard ratio = 0.65, 95% CI = 0.52 to 0.81, $P = 0.002$). Heifers treated twice or more for BRD had reduced likelihood to become pregnant than heifers not treated for BRD from 61-120 d (twice $P = 0.02$; three or more $P = 0.05$). Early life events continue to influence heifer reproduction, survivability and milk production through first lactation.

3.2 Introduction

Selecting replacement heifers is important to maximize farm profitability. Due to the increased use of data collection and technology on farms, farms often have the ability to collect several measurements during the early life period to aid in selecting replacement heifers. If farmers can utilize the data they collect to make culling decision sooner, heifer rearing costs which average \$2,510 could be allocated to the most profitable heifers (Akins and Hagedorn, 2015), and heifers selected may remain on the farm longer (Bach, 2011; Teixeira et al., 2017). The collection of data such as milk consumption (Davis Rincker et al., 2011), starter intake (Benetton et al., 2019), growth measurements (Raeth-Knight et al., 2009), health incidences (Stanton et al., 2012), genetic information and other early life measurements could be utilized by farms to improve decision making related to replacements.

Early life feeding strategies have been studied to determine the effects of nutrition on growth of dairy heifers. One strategy is to adopt an enhanced milk feeding program, during the pre-weaning period, by feeding an increased amount of milk or milk replacer to calves. Some research reported that increased early life nutrition, above the conventional 10% of body weight milk allotment, resulted in increased heifer growth at weaning (Jasper and Weary, 2002; Drackley, 2008; Davis Rincker et al., 2011). Previous research in our lab from Hurst et al. submitted found that the amount of milk consumed from calves in automatic calf feeders during the first 60 d of life influenced growth through 400 d old. This increased growth during early life has been reported to impact heifer conception rate (Pietersma et al., 2006), first lactation milk production (Soberon et al., 2012), and the ability to remain in the herd to second lactation (Bach, 2011). However, others have found little long term effects of increased growth from an accelerated feeding program (Morrison et al., 2009; Kiezebrink et al., 2015).

The incidence of early life diseases has been shown to affect future growth (Cramer and Ollivett, 2019). Some reported a decreased survival rate in heifers identified with early life respiratory disease (Adams and Buczinski, 2015). Stanton et al. (2012) reported that calves with a history of BRD during the first 60 d after moving to group housing were 18% less likely to survive to first calving and if they survived, calves with BRD were on average 12 d older at first calving compared to calves not treated for BRD. Dunn et al. (2018) also reported a decrease in total first lactation milk production if calves were diagnosed with BRD early in life. However not all research has reported consistent results on the overall effect of BRD on future heifer productivity.

There is not a consensus on the effect that early life has on the future productivity of the dairy cow or which commonly available variables/information in progressive herds influence future production to the highest extent. It was hypothesized that since there was a significant increase in BW for heifers with increased milk consumption (Hurst et al., submitted), increased growth would also positively influence future reproductive performance and first lactation milk production. Other variables hypothesized to influence future productivity included disease incidences and genetic traits. Therefore, the objective of this study was to identify the combination of early life factors that affect heifer survivability to first lactation, heifer reproductive performance, and first lactation milk production on a commercial dairy farm with the intention of being able to identify the most productive heifers at an earlier stage of development to reduce development costs.

3.3 Materials and Methods

All calves were housed at a commercial dairy farm in north-central Indiana.

3.3.1 On-farm Protocols

At birth, calves were fed 3.8 L of colostrum via an esophageal feeder, and six hrs. following the first colostrum feeding, they received another 1.9 L before being introduced to an automated calf feeder on day one. Calves were fed with eight automated calf feeders (Förster-Technik, Engen, Germany) located in four calf barns. Each barn contained four pens with two feeding stations per pen, and each feeder supplied milk to two pens. Each pen contained approximately 50-60 calves, fed with two feeding stations, were 9.14 x 24.38 m in size.

All calves could drink up to 24 L of milk per d from d zero to 32; however, the automated calf feeder offered incrementally increased in the amount of milk calves could consume within a 2 h period. Each calf was allocated 2 L of milk every 2 h until d 10. From d 10 to 21 calves could consume 2.5 L every 2 h. Then, calves could drink up to 3 L every two h until d 32. At d 32 the milk step-down phase began and the maximum milk allowance incrementally dropped by 2 L/d until d 39 when calves had a maximum milk allotment of 10 L/d. From d 39 to d 46 calves were offered up to 10 L/d, and at d 46 milk allotment decreased to 8L/d. Milk allotment then continued to be reduced until calves were fully weaned on average 60 ± 4.6 d of age (range 50-126 d Calves

were fed whole pasteurized non-saleable milk with a 30% protein and 5% fat enhancer added at 20 grams per L of milk. Adding balancers to whole or non-saleable milk have been shown to increase gain, BW, and feed efficiency of pre-weaned dairy calves in comparison to feeding just waste milk and are a way to increase the nutrient content fed to calves without increasing the volume of milk fed (James and Scott, 2005). The farm utilizes a balancer to have the ability to feed their waste milk even when they have a fluctuating amount of non-saleable milk available (Glosson et al. 2015).

Training calves to drink from the automated calf feeder was done two times/d by guiding and familiarizing calves to the feeding stations. Calves who did not drink at least four L from d 1-14 (83% of calves drank ≥ 4 L per d by d 14), or at least 5 L from 15-30 d (81% of calves drank ≥ 5 L per d by d 30) were identified and manually fed. Calves were offered *ad libitum* starter (18% CP and 3.5% Fat) and water from d one of age inside the autofeeder pens.

Barns were ventilated with two positive pressure ventilation tubes and run yr-round. When the temperature increased above 15.6°C, all the curtains were open. Below 15.6°C, curtains and side doors were incrementally closed until the temperature reached -6.7°C or below, when all curtains were closed. Weather was monitored morning and evening and adjusted depending on the temperatures.

Calves were diagnosed with bovine respiratory disease (BRD) if they had a drinking speed deviation of 80% compared to the previous d, elevated temperature ($\geq 39.5^\circ\text{C}$), and rapid breathing compared to pen mates. If all symptoms were present, calves were treated with subcutaneous thulathromycin (DRAXXIN[®], Zoetis, Kalamazoo, MI) or intravenous flunixin meglumine (Intervet Inc., Roseland, NJ). After d 4 of treatment, calves were reevaluated and if no improvement, they were retreated with florfenicol and flunixin meglumine (RESFLOR GOLD[®], Intervet Inc., Roseland, NJ) subcutaneously. After d 7 of the first treatment, if symptoms did not resolve themselves calves were retreated with tulathromycin and flunixin meglumine.

Daily milk consumption for calves was collected through the Förster-Technik automated calf feeders from October 1, 2015 to January 1, 2019. Total and 60-d milk consumption was calculated by individual data collected from the automated calf feeders. The value varied from the total days on feeder because calves were kept in the autofeeder longer than they were offered milk. The time in the feeder varied depending on calving frequency of the farm. Each calf was designated to one feeder, and this factor was included in the model. To account for the variation

in d on the autfeeder we add this variable into the analysis. We used 60 d milk consumption to standardize milk consumption and compare the amount of milk consumed on the average age of weaning.

Genomic body size composite and genomic milk indexes were added to the analyses; body size composite is a value collected through the Zoetis Clarifide® program (Zoetis, Kalamazoo, MI) that provides a prediction of an individual animals' stature, strength, body depth, and rump and compiles the data into a single composite index. Genomic milk displays the difference in total kg of milk produced during a 305 d lactation period.

Heifer conception age was collected from October 2016 through January of 2020 to correspond with animals that were fed through the automatic calf feeder. The farm protocols for heifer reproduction changed during the duration of the study (Supplemental Table 1); however, all animals in the same cohort were treated similarly. First lactation milk production was collected and combined by day in DairyComp305 (Valley Agriculture Software, Tulare, CA) from calves that were fed from the automatic calf feeders and data was collected from September 2019 through January 2020.

Birthdates, body weights, health events, animals sold for productivity reasons, died events, heifer conception dates, and first lactation milk production information were obtained from DairyComp 305. Birthdates were used to define the season the calf was born in. The four birth seasons were then defined as: calves born between January-March was winter, April-June was spring, July-September was summer, and October-December was fall. Average temperature for each season can be viewed in Supplemental Table 3.1. Yr of birth was determined from the birth date. Incremental weights were collected on farm at birth (zero d of age), leaving the autfeeder, approximately 3 mo of age, and breeding using an individual weigh scale (Tru-Test Limited, Auckland, New Zealand). Health events were obtained via DairyComp 305 (VAS, Tulare, CA). Calf respiratory disease were obtained and an individual event was defined as antibiotic treatment given five days apart from each other. Respiratory disease was divided into two different categories: treatment between zero and 60 d old and treatment between 61 and 120 d old. Survivability to first lactation was defined as animals that reached 850 d of age. Heifer conception age in d was calculated based on the date the heifer was bred based on a confirmed pregnancy. Total first lactation milk production was retrieved up to 280 DIM.

3.3.2 Animal Data

Early life variables including birthweight, year born, season born, feeder, 60 d milk consumption, pBW300 (predicted body weight at 300 d), pBW400 (predicted body weight at 400 d), zero to 60 d respiratory disease incidence, and genetic body size score were used for heifers as described by Hurst et al. submitted. The number of animals used as well as the mean and standard deviation of variables are shown in Table 3.1. Heifers were followed through conception (n=5,187) and first lactation (n=1,324). First lactation milk production had fewer animals because they had to complete at least 280 d of lactation by January 2020 to be included in the study. Outliers were identified from 280 d milk production and were removed if the production was above or below four standard deviations from the mean (n=2) similar to outlier removal in Pietersma et al. (2006). Survivability to 850 d of age of animals in the herd was collected for 12,459 animals over the study period to analyze if BRD treatment influenced survival (i.e., culling or death). In each time period, respiratory treatment was categorized to represent four groups; 0 = animals were not treated for BRD, 1 = animals were treated once, 2 = animals were treated twice, and 3 = animals were treated three or more times for BRD during 0 to 60 d and 61 to 120d. Finally, 5,193 heifers were analyzed to study the association of BRD on time to pregnancy.

3.3.3 Statistical analysis

Kaplan-Meier survival analysis was performed to assess the effect of calves diagnosed with BRD from 0 to 60 d and 61 to 120 d of age on the survival rate to first lactation and ability to conceive as a heifer. Data was analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC) using the LIFETEST procedure. Cox proportional hazard models using the PHREG function were utilized to evaluate the association of BRD with the pregnancy hazard. Significance was declared at $P < 0.05$, and tendencies are discussed if $0.05 \leq P \leq 0.1$.

Age of conception and 280 d first lactation milk production data were analyzed using linear mixed models with the MIXED procedure. Twelve early life variables were considered as potential predictors. Class effects included season born, year born, automatic calf feeder number, BRD incidence between zero to 60 d and 61 to 120 d. Linear effects included genetic body size score and genomic milk. Linear and quadratic effects included birthweight, 60 d MC, ADG (0-60 d), ADG (0-400d), and pBW₃₀₀. To analyze 280 d milk production all variables included in the

heifer conception model were included in addition to heifer conception age with a linear and quadratic effect. Only factors with a significant effect ($P < 0.05$) were retained in the final model for both age of conception and first lactation milk production. Final models were tested for collinearity using the REG procedure of SAS and any variables with a variance inflation factor (VIF) above 10 were removed from the model.

PBW300 and pBW400 for each heifer were predicted using third order Legendre polynomial (Kirkpatrick et al., 1990). Due to data collect originating on a commercial dairy farm and not all heifers weighed on the same d of age, predicted weights were generated to better compare growth at 300 and 400 d of age. The third order polynomial was determined as the best fit based on Bayesian Information Criterion (BIC) compared to second and fourth order polynomial. This prediction was done using the MIXED procedure of SAS version 9.4. ADG for each animal was calculated based on the predicted body weights. The complete model and analysis can be seen in Hurst et al. submitted. Our analysis included the predicted body weights calculated for 300 and 400 d of age. (ADG) was calculated based on the daily predicted body weights from (0 – 60 d) and (0 – 400 d).

3.4 Results

Sixty d MC, ADG (0-60 d), ADG (0 – 400 d), birthweight, pBW300, pBW400, genetic body size index, genomic milk, incidence of BRD between (0 – 60 d) and (61 – 120 d), age of conception, and 280M were analyzed for their correlations to each other in Table 3.2. ADG (0 – 60 d) and (0 – 400 d) was significantly correlated to milk consumption ($P \leq 0.0001$; $r=0.52$; $r=0.33$). ADG from zero to 400 d and pBW300 was moderately negatively correlated to heifer conception ($P \leq 0.0001$; $r=-0.18$). Heifer conception age had a significant negative correlation with milk consumption ($P \leq 0.0001$; $r=-0.11$). However, there was not a significant correlation between milk consumption and first lactation milk production ($P = 0.62$; $r=0.01$). There was also a positive correlation between genomic milk and 280 d milk production ($P \leq 0.0001$; $r=0.52$). When comparing to other variables, BRD had a moderate negative correlation to ADG from zero to 60 d, zero to 400 d, and pBW300 ($P \leq 0.0001$; $r=-0.11$; $r=-0.15$; $r=-0.13$).

A Kaplan-Meier analysis was performed to identify survival to first lactation (850 d of age) for heifers identified with BRD between 61 and 120 d of age (Figure 3.1). There was not a significant difference between BRD categories of calves diagnosed between 0 and 60 d of age on

the ability to survive to first lactation or their ability to become pregnant. Calves treated for BRD 0, 1, or 2 times during this period had a significantly higher chance for survival to first lactation than animals treated 3+ times for BRD (hazard ratio 0 vs. 1 = 0.92: $P = 0.0014$, hazard ratio 0 vs. 2 = 0.80: $P = 0.0002$, hazard ratio 0 vs. 3 = 0.65: $P = 0.0002$, Table 3.3). There was a significant difference in the ability of heifers to survive to first lactation if treated 1 vs. 2 ($P = 0.0317$) and 1 vs. 3 ($P = 0.003$) time for BRD as well. The hazard of pregnancy or the ability of a heifer to become pregnant by 550 of age is shown in Figure 3.2. While there was no difference in hazard of pregnancy between heifers treated 1 vs. 0 times ($P = 0.16$) heifers treated 2 or more for BRD between 61 – 120 d had reduced likelihood of pregnancy (0 vs. 2 treatments of BRD, $P = 0.02$; 0 vs. ≤ 3 treatments for BRD, $P = 0.05$; Table 3.4). No difference was observed between animals treated once, twice, or 3 or more times for BRD ($P > 0.05$).

Significant variables that influenced heifer conception age included a quadratic effect of pBW300 and ADG (0-400 d) as well as season and year born (all $P < 0.05$; Table 3.5). The effect of pBW300 on heifer conception age is displayed in Figure 3.3 ($R^2 = 0.0508$). Predicted BW300 had a significant quadratic relationship with heifer conception age showing that there is an inflection point (~375 kg) where another kg increase in body weight does not continue to decrease conception age. ADG from (0-400 d) had a significant quadratic effect on heifer conception age (Figure 3.4; $R^2 = 0.0398$). As ADG during this period increases, heifer conception age decreases until heifers gain over a 1.09 kg/d. If heifers gained more than this, conception age increased. Animals born in the summer (July through September) had the lowest age at conception followed by animals born in the fall, and spring.

A similar analysis was performed to evaluate which early life variables influence 280 d milk in first lactation. Season born, ADG between zero and 400 d, genomic milk, and heifer conception age had a significant impact on 280M (all $P < 0.05$; Table 3.6). Heifers born in the winter had the highest 280 d milk production followed by animals born in the fall, summer, and spring. Genomic milk had a linear effect on milk production and accounted for over a quarter of the variation between cows ($R^2 = 0.27$; Figure 3.5). For every 1 kg increase in genomic milk value there is 1.42 kg increase in 280 first lactation milk production. ADG (0-400 d) had a linear effect on first lactation milk production and for every one kg of ADG increase in the first 400 d of age, cows produced 1,299 kg more milk (Figure 3.6). Heifer conception age had a linear effect on milk

production and for every d increase in age at conception 4.0 kg more milk was produced (Figure 3.7).

3.5 Discussion

We observed that pre-weaning respiratory disease or respiratory disease between zero to 60 d did not have a significant effect on heifer conception age or first lactation milk production. However, a risk of pregnancy and survivability analysis revealed that there was a significant difference in animals diagnosed with BRD between 61 and 120 d of age and their ability to become pregnant and survive to first lactation. Our analysis did not see a difference in survival rates or percent pregnant for calves diagnosed with respiratory disease during the pre-weaning period. This observation was unexpected because in our previous study, Hurst et al. (submitted) we reported that pre-weaning respiratory disease affected growth of heifers up to 400 d of age, and it was hypothesized that this effect would negatively impact reproductive performance and milk production in the first lactation. We believe that we did not see a significant difference in animals diagnosed with BRD from 61 to 120 d in our heifer conception age model and first lactation production model because this factor impacted the heifer's ability to survive to those periods and those heifers were already removed from the herd. Similar to our findings, a study utilizing thoracic ultrasonography to identify calves diagnosed with lung lesions at weaning, reported that animals with lung consolidation have a significant decrease in survival to 750 d of age. Animals identified with lung consolidation were culled at 15.6% in comparison to those without lung consolidation at 3.5%. Heifers with lung consolidation had a lower hazard of pregnancy and higher age at first calving than animals without lung consolidation (Teixeira et al., 2017). Zhang et al. (2019) reported that most common reasons that affected survival rates in heifers were health incidences including digestive, respiratory, and circulatory diseases. They also reported that birth year, birth season, and dam parity significantly affected survival rates as well. These findings suggest that early life respiratory disease has long term effects on the growth and survival of heifers, and that farms may experience the disease at different stages of heifer development dependent on their management strategies. McCorquodale et al. (2013) reported that other early life factors such as low serum total protein values (<5.0 g/dL were 2.4 times more likely to not survive) and low body weight during the first week of life also influence heifer survival rates.

Genomic milk had the largest impact on first lactation milk production accounting for 27% of the variation. Dairy cattle have been selected for milk production since early 1900s. In the 1920's the average cow produced ~2,000 kg of milk in a 305-d lactation period, and a 100 years later, the average north American Holstein cow will produce over 10,000 kg of milk (Miglior et al., 2017). Suzuki and van Vleck (1994) also reported that the average heritability for milk production in Japanese Holsteins was 0.30. Due to these findings, the impact of genetic milk as the variable with the largest impact was expected. Future research should look at the impact of other commonly utilized traits on farm such as longevity, fertility, and health on the future production of dairy heifers as well as how phenotypic values will influence the heifer's genetic potential.

Season born, ADG from zero to 400 d of age, and heifer conception age were the other significant early life factors that impacted first lactation milk production. These variables combined account for 4% of the variation in first lactation milk production. In comparison, Soberon et al. (2012) reported that early life ADG alone accounted for 22% of the variation in first lactation milk production and reported that for every one kg increase of pre-weaning ADG a heifer on a commercial dairy farm would produce 1,113 kg more milk during her first lactation. Our study found a similar estimate when looking at the effect of ADG from zero to 400 d on milk production. For every one kg of ADG increased during the first 400 day of life the heifer would produce 1,299 kg more milk. The low amount of variation these variables account for suggest that there is a large number of influential environmental variables that affect milk production between the first few months of life and first calving. Rauba et al. (2019) reported that protein and metabolizable energy consumed from starter intake impacted 305-d milk production. Therefore, obtaining starter intake from individual calves could have helped explain more variation in first lactation.

Season and year born, ADG from zero to 400 d of age, and predicted body weight at 300 d of age were all early life factors that impacted heifer conception age. Heifers had a lower conception age as their pBW_{300} increased; however, once heifers were reaching approximately 375 kg at 300 d of age their conception age began to increase. ADG also had a significant quadratic effect on ADG (0-400) and if heifers exceeded 1.1 kg/d, their conception age began to increase. Pietersma et al. (2006) reported that the highest period of ADG for Holstein heifers was from weaning to puberty (average: 0.89 kg/d). The highest point of pre-weaning ADG described in Kiezebrink et al. (2015) when feeding 8 L of milk replacer/d was 0.96 kg/d between 43-56 d of age. These reported ADG's are smaller in comparison to the average in our study, and could

explain why our body weight and ADG results did not explain a larger variation in conception age. Brickell et al. (2009) reported that age at first breeding and age at first calving were significantly impacted by body weight at 30, 180, and 450 d old. Similarly, to first lactation milk production, the combination of significant early life variables only accounted for 8% of the variation in heifer conception age. The cohort study performed before this, Hurst et al. submitted, reported that growth up to 400 d was impacted by the quadratic effect of 60 d milk consumption, birthweight, feeder, year born, season born, respiratory incidence, and genetic body size score. We hypothesized that since these variables from the previous study were significant and accounted for 31% of variation in growth between animals at 400 d, that the variables would also significantly impact conception age, especially since average conception age was 437 d. One explanation could be that for most of the period data was collected, the farm's protocol was to begin breeding heifers at 400 d instead of by weight.

A limitation from data collected in this study was that all variables were sourced from a single commercial dairy farm. Due to the variation in standard operating procedures on farm, the results may not be representative of farms across the United States or worldwide. For example, the dairy from our study had higher pre-weaning ADG compared to other reported data (Brown et al., 2010; Kiezebrink et al., 2015; Omid-Mirzaei et al., 2015). These calves were also fed through an automatic calf feeder and given the opportunity to drink up to 24 L of milk until 32 d of age. Other studies have shown an increased age in first calving (Stanton et al., 2012) as well as reduction in first lactation milk production (Dunn et al., 2018) from animals diagnosed with respiratory disease which is contrasting to our results. Since all data collected for health incidences were based on observations and recorded in a farm management system, our study may not represent actual disease incidence. We also believe that we did not see a significant difference in pre-weaning respiratory disease on heifer conception age or first lactation milk production because of the large treatment rate on the farm (55% treated at least once). USDA, (2018) reported that only 11.4% of pre-weaned heifers raised in heifer raising operations were treated for respiratory disease while Overton (2019) reported an average treatment rate of 36.6% from over 100,000 animals. These treatment rates are lower than our reported treatment rate and could have altered our survival analysis for pre-weaning animals. Another limitation to using a commercial dairy is that farm protocols change and heifers move to different farms as they transition through the different stages

of development. We did not have the capability to control for the variation in changes in protocols or the different locations heifers were raised.

Overall, we still observed that heifer development and overall performance is impacted by events taking place during the early pre-weaning phase. This study was able to combine phenotypic and genotypic data for a large number of heifers over an extended period of time. Many studies do not have the ability to collect this large number of individual data points, therefore, this study allows the industry a better insight into single heifers as compared to an average. The results provide valuable insight on the possibility to utilize early life indicators to better select future replacement heifers. Next steps will be to utilize the predictive equations from Hurst et al. submitted along with these results to aid in replacement decisions.

3.6 Conclusions

In conclusion, early life variables collected influence survivability to first lactation, ability to become pregnant, conception age, and first lactation milk production. Respiratory treatment between 60 and 120 d significantly influenced the heifer's ability to become pregnant and survive to first lactation. Genetic traits such as genomic milk play a large role in the productivity of heifers as well. Finally growth measurements provide a valuable insight into the overall performance of the heifer later in life. The ability to utilize these variables to identify the most productivity heifers at a younger age could be a valuable asset on farms and aid in reducing heifer raising costs.

3.7 Acknowledgements

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Table 3.1. Descriptive results of variables measured in analysis. Characterization of variables measured on a commercial dairy farm from October 1, 2015 to January 31, 2020 for Holstein heifers.

Variable	No.	Mean	SD
60 d milk consumption, (L)	5,193	516.50	62.41
Heifer conception age	5,193	437.51	44.94
ADG (0-60 d)	5,193	1.02	0.14
ADG (0-400 d)	5,193	0.95	0.08
Birthweight	5,193	40.76	4.81
pBW ₃₀₀	5,193	330.16	29.25
280 d milk production	1,324	9,291	1,378

¹Predicted body weight of heifers at 300 d of age reported in Hurst et al. 2020.

Table 3.2 Correlation coefficients for tested early life variables collected. Variables were measured on a commercial dairy farm from October 1, 2015 to January 31, 2020 for Holstein heifers.

*() are the corresponding p-values to the correlation coefficients

	60 d Milk Consumption (L)	ADG 0 to 60 (kg/d)	ADG 0 to 400 (kg/d)	Birthweight (kg)	pBW300 (kg)	pBW400 (kg)	Genetic Body Size	Genomic Milk (kg)	BRD (0-60 d)	BRD (60- 120 d)	Conception Age (d)	280 d Milk Production (kg)
60 d Milk Consumption (L)	1 ---											
ADG 0 to 60 (kg/d)	0.5 (<0.0001)*	1 ---										
ADG 0 to 400 (kg/d)	0.3269 (< 0.0001)	0.43 (<0.0001)	1 ---									
Birthweight (kg)	0.3 (<0.0001)	0.73 (<0.0001)	0.41 (<0.0001)	1 ---								
pBW300 (kg)	0.3561 (< 0.0001)	0.56 (<0.0001)	0.82 (<0.0001)	0.27 (<0.0001)	1 ---							
pBW400 (kg)	0.34 (<0.0001)	0.42 (<0.0001)	0.99 (<0.0001)	0.33 (<0.0001)	0.84 (<0.0001)	1 ---						
Genetic Body Size	0.11 (<0.0001)	0.22 (<0.0001)	0.25 (<0.0001)	0.16 (<0.0001)	0.25 (<0.0001)	0.25 (<0.0001)	1 ---					
Genomic Milk (kg)	0.02 (0.29)	0.08 (<0.0001)	0.11 (<0.0001)	0.07 (<0.0001)	0.11 (<0.0001)	0.1 (<0.0001)	-0.09 (<0.0001)	1 ---				
BRD (0-60 d)	-0.11 (<0.0001)	-0.15 (<0.0001)	-0.07 (<0.0001)	-0.009 (0.50)	-0.13 (<0.0001)	-0.09 (<0.0001)	-0.01 (0.49)	0.04 (0.02)	1 ---			
BRD (60-120 d)	-0.08 (<0.0001)	-0.08 (<0.0001)	-0.11 (<0.0001)	0.02 (0.21)	-0.13 (<0.0001)	-0.12 (<0.0001)	-0.001 (0.92)	-0.01 (0.48)	0.11 (<0.0001)	1 ---		
Conception Age (d)	-0.11 (< 0.0001)	-0.11 (< 0.0001)	-0.18 (<0.0001)	-0.06 (<0.0001)	-0.21 (< 0.0001)	-0.18 (< 0.0001)	-0.03 (0.05)	-0.06 (0.0002)	0.03 (0.04)	0.05 (0.0001)	1 ---	
280 d Milk Production (kg)	0.01 (0.620)	-0.08 (0.006)	0.08 (0.006)	0.07 (0.010)	0.09 (0.001)	0.08 (0.005)	-0.05 (0.100)	0.52 (< 0.0001)	0.01 (0.520)	-0.01 (0.650)	0.07 (0.001)	1 ---

Table 3.3 Hazard ratios of Holstein heifer calves reaching first lactation (850 d) if being treated for respiratory disease between 61 and 120 d of age as compared to not being treated for respiratory disease.

Number of treated respiratory incidences	Hazard Ratio	95% Confidence Intervals	SE	<i>P</i> -value
0	1.0	---	---	---
1	0.93	0.93 to 1.03	0.03	0.41
2	0.89	0.79 to 0.99	0.06	0.04
3+	0.71	0.57 to 0.89	0.11	0.002

Table 3.4 Hazard ratios of Holstein heifers not conceiving by 550 d in function of being treated for respiratory disease between 61 and 120 d of age as compared to not being treated for respiratory disease.

Number of treated respiratory incidences	Hazard Ratio	95% Confidence Intervals	SEM	<i>P</i> -value
0	1.0	---	---	---
1	0.95	0.89 to 1.02	0.04	0.16
2	0.81	0.66 to 0.97	0.1	0.02
3+	0.66	0.43 to 1.01	0.22	0.05

Table 3.5 Parameter estimates of significant factors, calculated by resitricted maximum likelihood using the MIXED procedure of SAS, used to predict heifer conception age.

Variable	Estimate	SE	<i>P</i> – value
Intercept	1001.68	59.56	<0.0001
Season ¹	Category		
Winter	8.96	2.01	<0.0001
Spring	7.41	1.96	0.0002
Summer	-2.37	1.71	0.1669
Fall	0	-	-
Year ²	Category		
2015	-10.13	30.61	0.7406
2016	21.53	2.17	<0.0001
2017	2.21	1.57	0.1593
2018	0	-	-
ADG 0-400 d,(kg) ³	-5.14.28	147.3	0.0005
ADG 0-400 d × ADG 0-400 d	266.68	77.72	0.0006
pBW ₃₀₀ ⁴	-1.68	0.42	<0.0001
pBW ₃₀₀ × pBW ₃₀₀	0.002	0.00065	0.0015

¹Season the heifer was born in: winter (January-March), spring (April-June), summer (July-September), fall (October-December).

²Yr the Heifer was born in.

³Calculated based on the predicted body weight of heifers at birth (0 d) and 400 d reported in Hurst et. al., 2020.

⁴Predicted body weight of heifers at 300 d of age reported in Hurst et al. 2020.

Table 3.6 Parameter estimates of significant factors, calculated by resitricted maximum likelihood using the MIXED procedure of SAS, to predict 280 d first lactation milk production.

Variable	Estimate	SE	<i>P</i> - value
Intercept	4,901.10	638.24	< 0.0001
Season born ¹	Category		
Winter	235.15	87.37	0.0072
Spring	-217.58	99.47	0.0289
Summer	-107.38	98.37	0.2752
Fall	0	-	-
ADG 0-400 d ²	1,299.20	478.34	0.0067
Genomic Milk, (kg) ³	1.42	0.07	< 0.0001
Heifer Conception Age, (d)	4.001	0.86	< 0.0001

¹Season the heifer was born in: winter (January-March), spring (April-June), summer (July-September), fall (October-December).

² Calculated based on the predicted body weight of heifers at birth (0 d) and 400 d reported in Hurst et. al., 2020.

³ Index created by Zoetis Clarifide® which explains the genetic differences in total kg of milk produced during a 305 d lactation period.

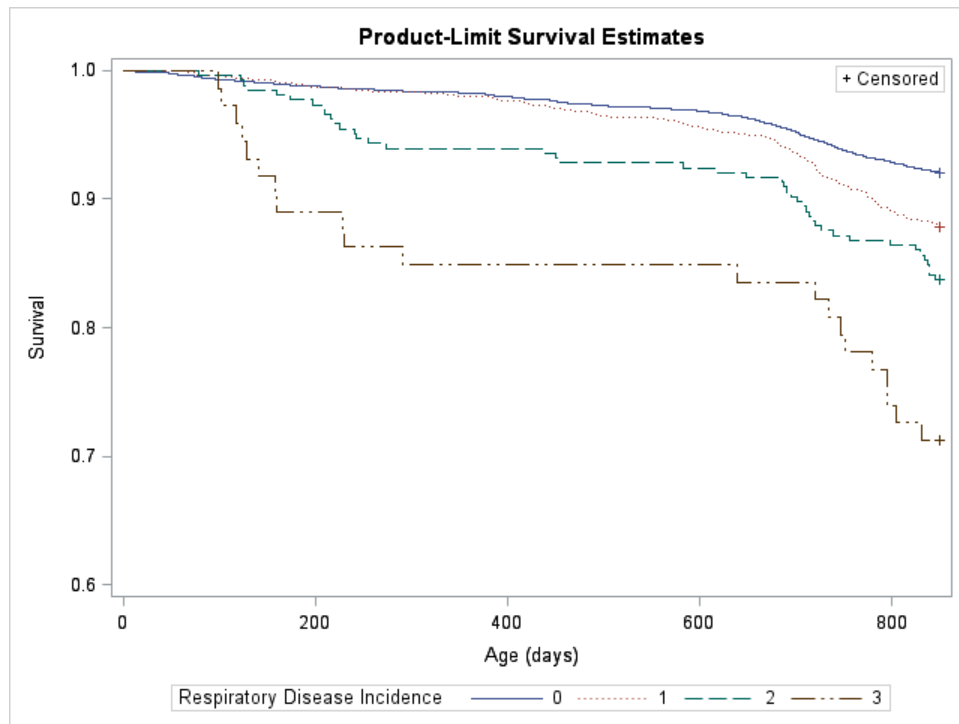


Figure 3.1 Kaplan-Meier analysis of time to culling/death of Holstein dairy heifers who were analyzed for incidence of respiratory disease between 61 to 120 d of age. Respiratory disease incidence 0 = no treatment, solid line; 1 = one treatment, dotted line; 2 = two treatments, dashed line; 3 = three or more treatments, dashed/dotted/dashed line. A respiratory disease greater than one was identified if the heifer was treated \geq five days after the previous treatment.

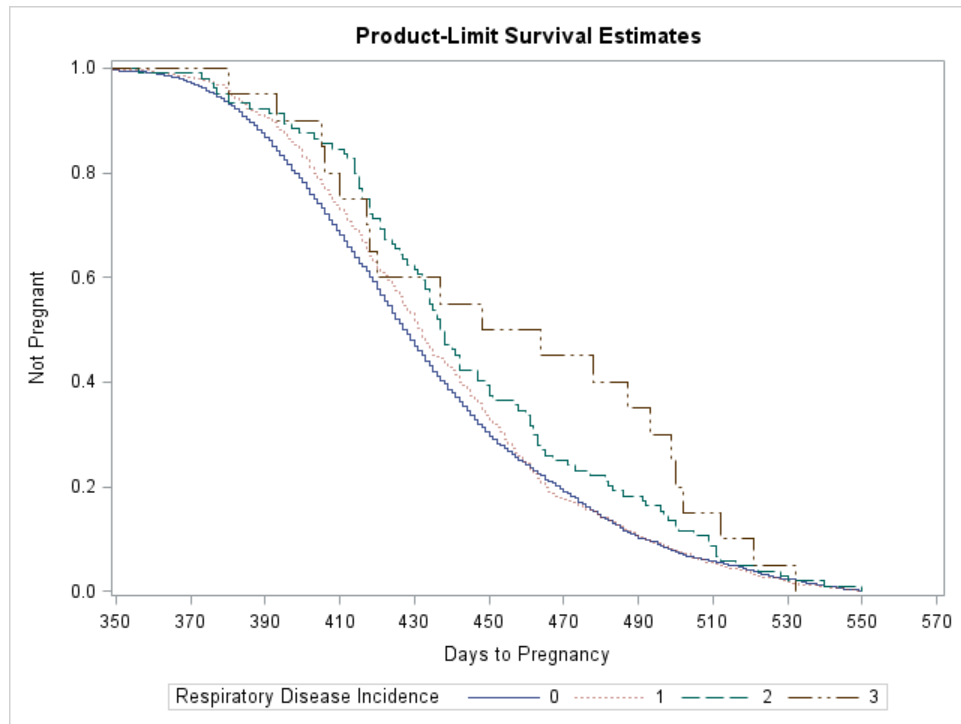


Figure 3.2 Kaplan-Meier analysis of days to pregnancy of Holstein dairy heifers who were analyzed for incidence of respiratory disease between 61 to 120 d of age. Respiratory disease incidence 0 = no treatment, solid line; 1 = one treatment, dotted line; 2 = two treatments, dashed line; 3 = three or more treatments, dashed/dotted/dashed line. A respiratory disease greater than one was identified if the heifer was treated \geq five days after the previous treatment.

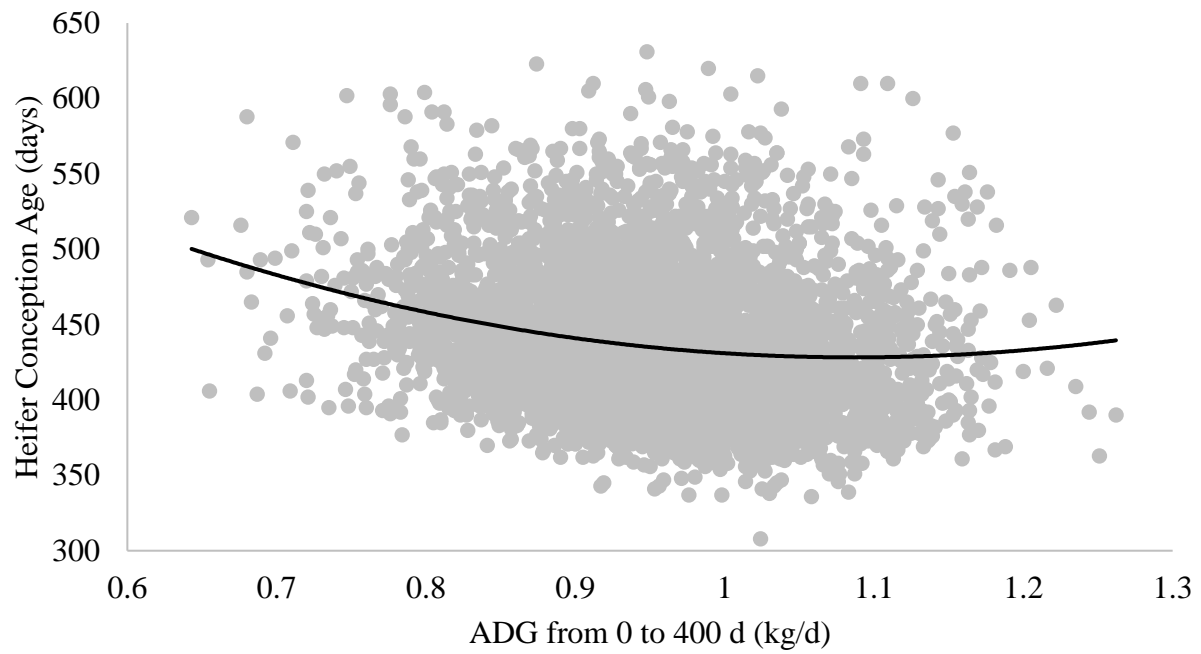


Figure 3.3 The effect ADG from zero to 400 d has on heifer conception age. Heifer conception age = $365.72x^2 - 794.65x + 859.91$, x = ADG (0 to 400 d), $P = <0.0001$, $R^2 = 0.0398$.

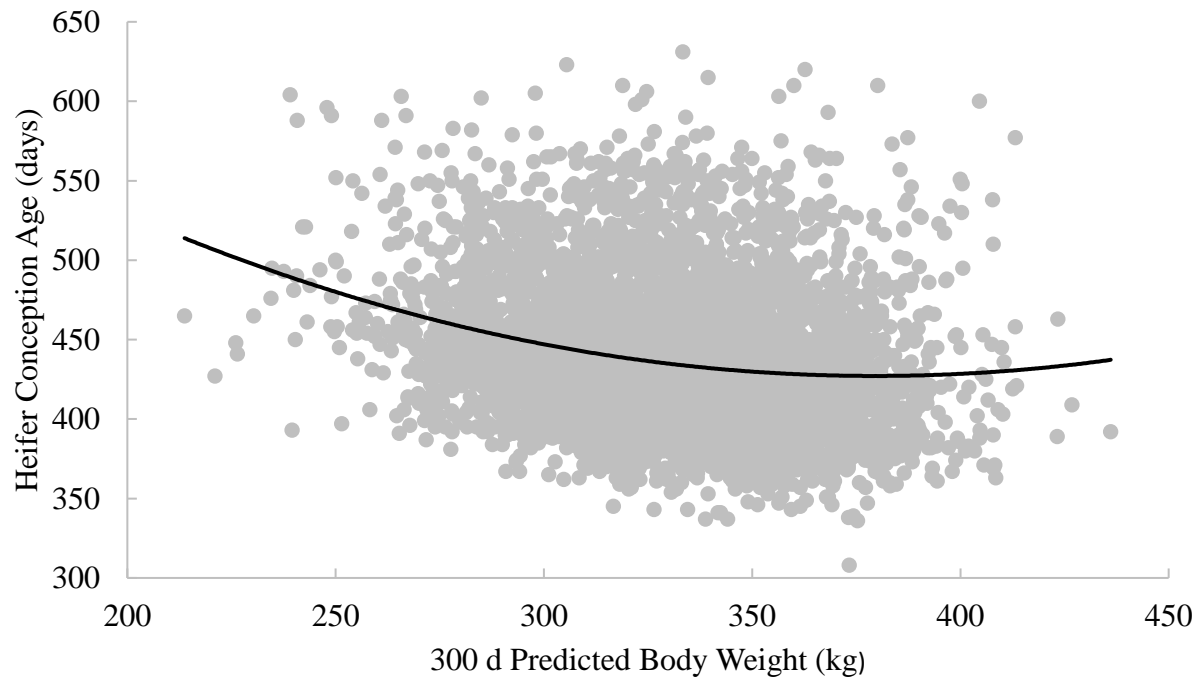


Figure 3.4 The effect 300 d predicted body weight has on heifer conception age.
Heifer conception age = $0.0032x^2 - 2.3952x + 881.62$, $x = \text{pBW}_{300}$, $P = <0.0001$, $R^2 = 0.0508$.

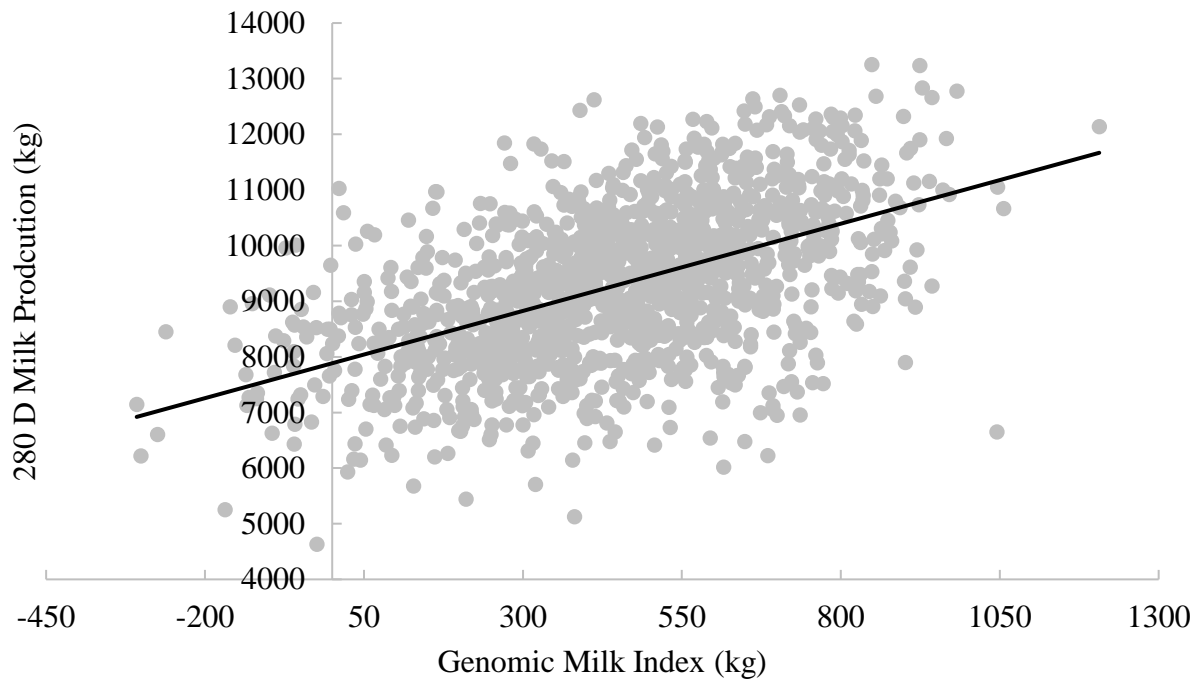


Figure 3.5 The effect genomic milk index has on the 280 d first lactation milk production. Genomic milk index explains the genetic differences in total kg of milk produced during a 305 d lactation period. $280DM = 1.4222x + 7,884.7$, x = genomic milk index, $P = <0.0001$, $R^2 = 0.27$.

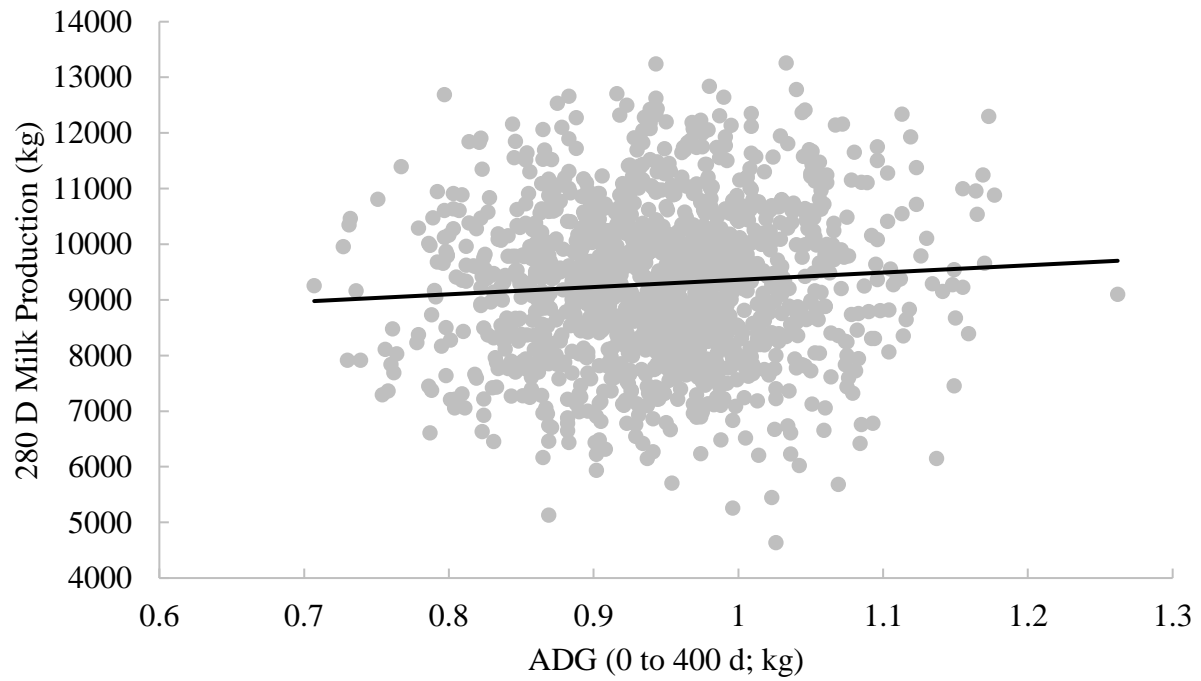


Figure 3.6 The effect of ADG from zero to 400 d ADG age has on 280 d first lactation milk production. $280DM = 1304.1x + 8,056.6$, $x = \text{ADG (0 to 400 d; kg/d)}$, $P = 0.0058$, $R^2 = 0.0053$.

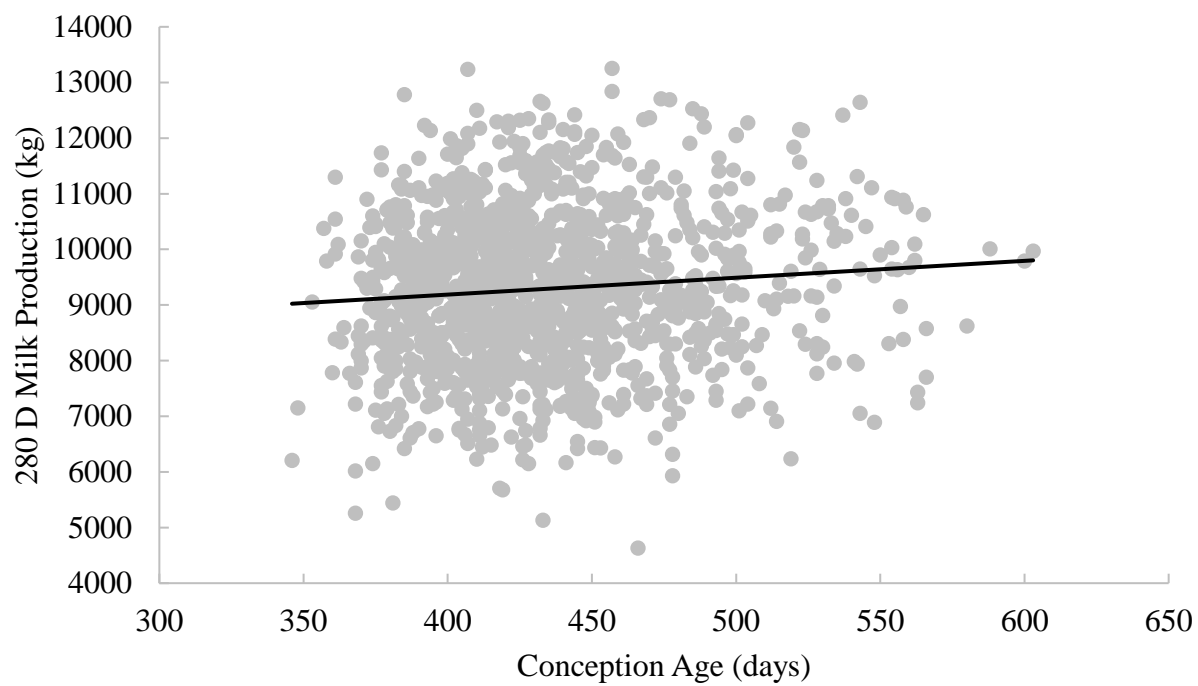


Figure 3.7 The effect heifer conception age has on 280 d first lactation milk production.
 $280DM = 3.0334x + 7,973.5$, x = Heifer Conception Age (days), $P = 0.001$, $R^2 = 0.0085$.

CHAPTER 4. CONCLUSIONS

Throughout this project we were able to identify early life variables that influence heifer growth, survivability, and production parameters. The second chapter reports that heifer growth was highly variable on this commercial dairy and there could be up to a 263 kg difference in body weight at 400 d of age. From our predictive equation that utilized the significant early life variables of milk consumption, respiratory disease incidence, birthweight, and genetic body size score we were able to account for 27 percent of the variation seen between heifer growth rates. Milk consumption from the automatic calf feeders was the largest contributing variable and had a significant quadratic effect on future growth. This result suggested that there is an inflection point in which drinking more milk pre-weaning will not increase growth as much as drinking or offering less during this period. Results from this chapter also showed that combining phenotypic variables with genetic values provides more insight into understanding the large variation in heifer growth. Additionally, this chapter provided insight on average heifer growth and ADG throughout the different growth phases. These results also provide the industry with a Holstein growth comparison of today's animals with past and future animal growth rates.

Chapter three further emphasizes the importance of increased growth earlier in life on heifer conception age and first lactation milk production. Results from heifer conception age analysis revealed that extreme low and high growth rates negatively impact the age in which heifers conceive; however, this quadratic relationship was not seen for first lactation milk production. Even though significant, growth rates did not explain a large variation in production parameters, and we believe these findings could be due to other variables during the raising period that we could not control for in this study. Incorporating genomic milk in the analysis allowed us to control for over a quarter of the variation in first lactation milk production. Furthermore, diagnosis of respiratory disease between 60 and 120 d of age had a significant influence on the ability to survive to first lactation as well as the ability for heifers to become pregnant.

From this research we were able to collect real time data from a commercial dairy farm and follow the life cycle of Holstein heifers through first lactation to observe the impact early life raising has on the future outcome of individual heifers. Using the impactful early life variables, we were able to generate a predictive equation with the goal to provide helpful insight into which

heifers farmers should select for future replacements. This research also highlights the importance of individual record taking on farm and how beneficial the results from record keeping can be.

Future research will utilize the predictive equation on the dairy it was developed from to validate how actual heifer performance comes to predicted performance. After validation, the goal is to adapt the predictive equation for different farms as well as integrating the equation in an already used data management software. From incorporating this management tool farmers will be able to select the most productive replacement heifers sooner and reduce overall heifer rearing costs.