ABSTRACT

FAULKNER, BROOKLYN. Development of an Objective Body Condition Scoring (BCS) System to Assess Energy Status in Dairy Cattle. (Under the direction of Dr. Stephanie Ward).

The objectives of this work were to 1) investigate a prototype caliper to objectively quantify body condition in dairy cows and 2) assess the relationship between body condition and energy status in early lactation cows. A prototype caliper was developed with the assumption that as an animal loses condition, the topline becomes more angular, therefore, body condition score (BCS) could be determined through change in angularity. The arms of the calipers were 3.8 cm tall and the calipers were adjustable to widths of 11.6, 16.5, 21.5, and 26.7 cm. Data were collected in 2017 from Holstein cows (n = 29) at the Lake Wheeler Dairy Field Lab (LWD, Raleigh, NC) and in 2021 at the N.C. Department of Agriculture and Consumer Services (NCDA&CS) Piedmont Research Station (PRS, n = 53). Cows in various days in milk (DIM) were subjected to a single collection of visual body condition scoring (BCS), measurements of subcutaneous rump fat (RF) and back fat (BF) thickness (cm) via ultrasonography images (Aloka SSD-500V with 3.5MHz probe), and caliper measurements at the withers, 12^{th} rib, short rib, and tailhead areas using calipers set at 11.6, 16.5, 21.5, and 26.7cm widths. In a companion study assessing the relationship between BCS and energy status in early lactation, Holstein (n = 25) and Jersey (n = 15) dairy cows were subjected to the same measurements as described above. Calipers used were either 11.6 cm or 16.5 cm and data was collected weekly across the transition from dry (week 0) to week 8 in lactation. Caliper and visual scores were collected weekly and ultrasound measurements were collected bi-weekly (week 0, 2, 4, 6, and 8). In addition to body measurements, blood samples were collected via coccygeal vein weekly into a 10mL serum separator vacuum tube (Fisher, Suwanee, GA) and placed on ice. Later, serum was separated after centrifugation for 30 min at 3,000 × *g* and frozen at -18°C within 3 hours of collection.

Data were analyzed using the CORR, REG, and MIXED procedures in SAS (SAS® v.-9.4, Cary, NC). Significance was declared when $P < 0.05$. The first set of collection showed the 16.5-cm caliper at the withers had the best correlation followed by the tailhead location ($r = 0.71$, 0.67, respectively) as compared to the calipers set at the 21.5 and 26.7 cm widths at the withers and tailhead ($r = 0.42$, 0.58, 0.64, 0.62, respectively). A smaller caliper width was developed for testing. In the second set of collects with the calipers, 11.6 and 16.5 cm caliper widths were used at the withers (W) and tailhead (T) locations (11.6W, 11.6T, 16.5W, 16.5T). Both the 11.6 and 16.5 cm calipers had a similar relationship to BCS ($r = 0.61$ and 0.62, respectively, $P < 0.01$) when used at the withers. In the companion study to assess the relationship between body condition and energy status in early lactation cows, the 16.5cm caliper at the withers again showed the greatest correlation to BCS ($r = 0.35$). No differences were noted, however, at the tail head between caliper sizes or at either location using the small calipers between breeds. Non-esterified fatty acids (NEFA) concentrations were negatively correlated to BCS ($r = -0.25$, $P < 0.01$) and NEFA concentrations were greater in Holstein cows (0.56 mmol/L) compared to Jersey cows (0.44 mmol/L). Concentrations were lowest in the dry period (0.16 mmol/L), increasing during weeks 1 through 4 of lactation then decreasing after week 5 ($P < 0.01$). Body condition calipers could potentially be a useful tool in managing BCS in dairy cows, particularly when using the 16.5cm width at the withers location. When using the tool as a predictor of BCS on cows in various stages of lactation, the correlation between the caliper and BCS was greater than when using the tool to track weekly changes in early lactation. Metabolic and genetic information pertaining to the different breeds could influence the differences observed in NEFA concentrations and body measurements and should be considered in further research investigating this tool.

Development of an Objective Body Condition Scoring (BCS) System to Assess Energy Status in Dairy Cattle

by
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DEDICATION

Mom, you have taught me more about strength than you will ever realize. Thank you for raising me to be strong and independent, and for a childhood full of great memories on our farm. You instilled the love for animals in me at a young age and it is the reason I am here today. Dad, thank you teaching me the meaning of hard work and dedication, for always supporting me and reassuring me that I can do anything I put my mind to. You have always been my biggest supporters and I love you both, this work is dedicated to you.

BIOGRAPHY

Brooklyn Elizabeth Faulkner was born on July 13th, 1997 to Scott and Joni Faulkner. Brooklyn grew up in Lexington, NC and graduated from Central Davidson High School. Growing up, Brooklyn always had an undeniable love for animals and caring for them. As an NCSU Animal Science alumni's child, Brooklyn became a Wolfpack fan at a young age and knew she wanted to go to college there. Brooklyn completed her Bachelor of Science in Animal Science with a minor in nutrition from NCSU in May of 2019. During her last year of undergraduate studies, Brooklyn decided she wanted to apply to graduate school and study dairy nutrition and management. During the summer of her sophomore year of undergraduate, she started working as a student employee at the Lake Wheeler dairy field lab in Raleigh, NC. She had shown and judged dairy in high school and always had a passion for cattle and gaining more experience at the Lake Wheeler farm encouraged her interests in pursuing graduate school. During her time at NC State, Brooklyn has had the opportunity to work in extension, research, and teaching. While in graduate school, she realized her passion for not only dairy management, but also teaching and interacting with students and dairy producers. Since, Brooklyn has remained an employee on the Lake Wheeler Field Lab Dairy Unit and Howling Cow Creamery in a supervising role where she continues to interact with students.

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CHAPTER 1

Literature Review

Introduction

All lactating mammals are designed to convert body stores of energy (lipids), into nutrient rich milk for their offspring when production demands cannot be met via dietary intake. This functionality allows the mother to provide for the offspring, even when food may not be available, but it happens at the cost of her own body condition. Body reserves are responsible for up to one-third of the milk solids produced by dairy cows in early lactation (Bauman and Currie, 1980) and the modern dairy cow produces milk in quantities beyond the requirements of offspring. Dairy cows can experience a period of negative energy balance (NEB) during early lactation when the energy demand of lactation cannot be met by dietary intake alone (Figure 1.1). Body condition scoring (BCS) is a method of visually assessing the body condition (fat and muscle) of an animal. In dairy cows, a scale of 1-5 with quarter point intervals is used (Wildman et al., 1981). The "ideal" BCS in dairy cows is between 3.0 and 3.25. Condition scores at calving lower than 3.0 were associated with reduced reproductive performance, and a calving BCS greater than 3.5 was associated with reduced milk production and increased risk of metabolic disorders (Roche et. al, 2009). Monitoring individual cow body fat and maintaining a desirable body condition is essential to maintaining productive animals that have appropriate nutrition and fertility while producing high volumes of milk. Holstein dairy cows are known for producing high volumes of milk, but they are also characterized by having lower body condition score (BCS), and reduced fertility and survival compared to other breeds (Dillon et al., 2006). Both low (< 3) and high (> 3.5) BCS were related to greater incidences of metritis, retained placenta, milk fever, lameness, cystic ovaries, dystocia, displaced abomasum, and mastitis (Bewley and

Schutz, 2008). Although BCS is useful if assessed properly, the subjective nature of visual assessment resulted in BCS used inconsistently as a management tool.

Methods of Measuring Body Reserves

Body weight (BW), heart girth (HG), and fat thickness

One way of assessing body condition changes in dairy cows is monitoring BW. This could be done by sending cows across a scale each day and collect BW data using an automated system and database to store and assess individual cow changes throughout lactation. Since BW can influence milk yield, reproduction, and overall farm profitability, monitoring BW changes may aid in preventing issues that are associated with drastic weight loss. Calving BW and age at first calving (AFC) can also impact milk yield in first lactation. Low production is a driver of culling rate, which along with AFC, was reported as a primary factor affecting dairy heifer rearing costs, and accounts for 15-20% of total farm expenses (Tozer and Heinrichs, 2001). Han et al. (2020) conducted a large study and found heavier heifers produced more milk in first lactation than lighter heifers but lost more BW, faced a higher risk of being culled, and did not produce more milk in the long term. Thus, monitoring BW is important considering the investment in raising replacement heifers. Collecting a body weight at calving and monitoring frequently to evaluate changes could aid in identifying issues before they become clinical. There are several determinates of BW such as size (skeletal development), fatness, and gut fill (Enevoldsen and Kristensen, 1997), and in lactating cows, udder weight. For several management and research applications, these components of BW should be measured separately. Automated weighing systems exist and new technology performing semi-automated body condition scoring has emerged, so frequent BW and BCS measurements are feasible with appropriate equipment (Halachmi et al., 2008; Bewley and Schutz, 2009; Azzaro et al., 2011).

There are several technologies recently developed to predict body condition via 3-dimensional (3- D) vision systems that can assess the influence of various sources of uncertainty on BW prediction (Song et. al, 2018) such as BW fluctuation from milk production, fat and muscle, and frame size. Accuracy in these image systems still needs improvement and more research is needed on integration of these systems into daily operations of the dairy farm. Although measuring BW has become more feasible in practice, these methods have not been very suitable for detecting short-term changes because infrequent measurements make it difficult to detect rapid changes occurring in early lactation (Thorup et al., 2011).

Heart girth (HG) has been found to be highly correlated with BW (Heinrichs et al., 1992; Veerkamp and Brotherstone, 1997; Koenen and Groen, 1998) and is easier to be measured routinely than BW. Heart girth has been found to have the highest correlation with body weight ($r = 0.99$) and is used as a predictor of body weight, along with wither height, hip width, and body length ($R^2 = 0.99$) (Heinrichs et. al, 1992). Although HG may be easier to collect, HG measurements can be difficult to perform uniformly because the positioning of the cow during measurement can easily affect the results. Wither height, hip height, and hip width are indicators of skeletal development (body size) that are relatively easy to obtain precisely because the locations for measurement can be easily identified (Enevoldsen and Kristensen, 1997).

Fat thickness along the back and rump areas are commonly evaluated via ultrasonography. Previously used methods with skin-penetrating needles are now obsolete. Two methods with a mechanical and electrical needle probe have been described (Staufenbiel, 1992). Ultrasound equipment transfers electrical pulses into high-frequency sound waves by piezoelectric crystals. The image is generated by the sound waves being reflected from boundaries between different tissue densities (Houghton and Turlington, 1992) between adipose tissue, fascia, and muscle. Table 1 represents back fat thickness (BFT) measurements (mm) and total body fat content (TBF) (kg) with body condition scores ranging from 1 to 5 (Staufenbiel, 1997). Staufenbiel (1997) accounted for skin thickness of 5 to 6 mm which was included in the ultrasound measurement, so that the actual subcutaneous fat thickness is less by that amount. A value of 6 mm of BFT consequently means an almost complete loss of body fat reserves. Backfat is positioned between the skin and the profound fascia. In most cows, the superficial fascia is present in the adipose tissue and appears as a thinner white line and separates the backfat into subcutaneous fat and interfascial fat (Staufenbiel, 1992). Staufenbiel and Klawuhn (1992) examined the entire back region of the dairy cow from the thoracic spine to the tail and found the sacral examination site to be most suitable for assessing BFT. The sacral region has the largest amount of adipose tissue in the back and high correlations ($r = 0.90$) exist between body fat content and BFT.

Body Condition Scoring

Body condition scoring (BCS) is a subjective method of visually assessing the body reserves (amount of metabolizable energy stored in fat and muscle) in a live animal. Techniques and methods of BCS differ across livestock species. The initial BCS system was developed for ewes by Jefferies (1961) and involved palpating the lumbar and backbone region to feel for covering of fat over the bones and sharpness. This technique scored ewes on a 0 to 5 scale, where 0 was emaciated and 5 was obese. Jefferies technique was adopted as a scoring system for beef cattle and was modified by Lowman et al. (1976) to incorporate intermediate values to adjust for those animals whose condition was in between, functioning as an 11-point scale. Since then, two systems for BCS in dairy cattle have been developed in the United States. Wildman et al. (1981)

developed a system assigning dairy cows a BCS ranging from 1 to 5, focusing on a subjective visual evaluation of the tailhead and lumbar regions of the cow. Results from that work presented a correlation between BCS and weight gain, but correlation between frame size and BCS was not significant. Body condition decreased sharply in early lactation, and increased from peak lactation to late lactation, remaining constant during prepartum period. Edmonson et al. (1989) proposed a BCS system in which cows were visually scored on a 1 to 5 scale based on evaluation at the lumbar, thurl and tailhead regions. Edmonson et al. (1989) created a chart for BCS in freely moving Holsteins, using literature from previous BCS procedures in the United Kingdom, Australia, New Zealand, and the US (Earle, 1976; Jefferies, 1961; Lowman et al.1976; Mulvany, 1981; and Wildman et al., 1981). The chart produced consistent scores using a wide range of body conditions, with the overall score most closely related to the condition of the tailhead and pelvic areas of the cow. Wildman et al. (1981) suggested the lumbar, thurl, and tailhead regions to be the primary focus when assessing dairy cows, which has been adopted as the modern scoring system assessing body condition on a 1 to 5 point scale with quarter point intervals.

Management of BCS on dairy farms has implications for nutritional practices, herd health, milk yield, reproductive performance, farm profitability, and overall animal well-being (Bewley and Shultz, 2008). BCS fluctuation naturally occurs at different stages of lactation, particularly in early lactation cows. Some loss in BCS in early lactation is expected; however, managing and minimizing such loss could influence performance. Difficulties with transition cow disorders, reproduction, animal well-being, and genetic differences in cows' ability to manage body reserves have increased the interest in monitoring BCS. Unfortunately, due the subjective nature of current BCS methods, results are not consistent when used as an on-farm assessment tool. Thus, validity, subjectivity, and repeatability of BCS systems have been questioned, regardless of the scoring system. New technologies developed for BCS management may facilitate collection and use of BCS data if objectivity can be increased.

BCS Calipers

Knauer and Baitinger (2015) developed a prototype caliper to quantify BCS by measuring the angularity of the topline in sows (Figure 1.2). The BCS caliper was designed to measure angularity of the topline from the spinous process to the transverse process in sows and is based on the concept that as an animal loses back fat and muscle it becomes more angular. This tool was developed for the use in sows with the goal of predicting BCS for managing feeding amounts, as sows are commonly fed based on a target body condition (Young et al., 2004). The arms of the caliper were 3.8 cm tall and the caliper could be adjusted to 16.5, 21.5, 26.7, 31.7-cm widths. Knauer and Baitinger (2015) collected caliper measurements on sows (n = 242) behind the shoulder at the 21.5-cm caliper width, in the middle of the back at the 26.7-cm width and across the last rib at the 16.5, 21.5, 26.7, 31.7-cm widths. Backfat and loin depth (cm) were measured from a cross sectional 10th rib image using an Aloka 500V SSD ultrasound machine (Corometrics Medical Systems Inc., Wallingford, Conn.). Weight, backfat, loin depth, and BCS were used to determine optimal caliper width and location because body condition is a composite trait of weight, backfat, and muscling (Wright and Russel, 1984). Knauer and Baitinger (2015) found that backfat, loin depth, and BCS were correlated with calipers ($r = 0.50$ to 0.60, 0.40 to 0.47, and 0.60 to 0.77, respectively) set a several widths but showed the optimal caliper measure in sows, when considering weight, BF, loin depth, and BCS, to be at the last rib using the 26.7 cm caliper ($P < 0.05$). When comparing fat and muscling between meat vs. dairy animals, meat animals typically have excess musculature and fat covering the entire body, while dairy animals are usually lean, with visibility of most of the bony structures. When using the caliper to predict BCS in meat animals, measuring muscling and fat thickness to assess the proportions of fat and muscling contributing to the overall BCS could provide explanation to caliper measurements observed.

Metabolic Changes in Early Lactation

Adipose tissue metabolism changes into a catabolic state in early lactation as more nutrients and energy are used to produce milk. Several metabolic adaptations occur during early lactation as cows enter negative energy balance (NEB) (Table 2.2). Adipose tissue functions to provide energy in the form of fatty acids and glycerol to other organs. These metabolic changes are controlled by endocrine regulations and enzyme hormone-sensitive lipase is responsible for the release of free fatty acids from triacylglycerols in the adipose tissue. Both homeostatic and homeorhetic mechanisms are responsible for these changes in lipid metabolism during early lactation. Homeostatic control implies that if the nutritional environment is adequate, the lactating dairy cow can meet its energy demands from DMI and tissue mobilization, but early lactation lipolysis has been found to be largely genetically controlled (homeorhetic), whereas enzymes involved in lipogenesis were primarily regulated by energy intake (homeostatic) (McNamara and Hillers, 1986; Smith and McNamara, 1990; Roche et al., 2006). Lipogenesis is generally slow during early lactation and increases during late lactation, whereas lipolysis is more prominent in early lactation and the diameter and cell volume of adipocytes decrease while the animal is in negative energy balance (NEB) (Reid et al., 1986). Lipogenesis occurs in ruminant adipocytes via de novo synthesis and uptake of preformed fatty acids from circulation (Bauman and Currie, 1980). During the uptake of fatty acids from circulating lipids, lipoprotein lipase (LPL) hydrolyses plasma triacylglycerides (TAG), producing NEFA and monoacylglycerides (Fielding and Frayn, 1998). During lipolysis, hormone-sensitive lipase (HSL) acts as a catalyst at the lipid droplet surface in the adipocyte to hydrolyze fatty acids at the sn-1 and sn-3 positions, then monoacylglycerol lipase hydrolyses the remaining fatty acid at the sn-2 position, generating 3 NEFA and a glycerol backbone (Stipanuk, 2000). As dairy cows transition from the dry period into lactation, the prevalence and risk of metabolic diseases are higher during these first several weeks of lactation as metabolic changes occur due the sudden increase of nutritional requirements needed to support milk production (Goff and Hurst, 1997). Treacher et al. (1986) suggested that significantly more cases of disease (mastitis, retained placenta, ketosis, milk fever, and lameness) occurred after calving in over-conditioned cows than in the under-conditioned cows. Over-conditioned cattle with inadequate feed intake in early lactation had more problems dealing with the increased tissue mobilization.

Implications of non-esterified fatty acids (NEFA) concentrations

During times of energy deficiency, animals break down triglycerides stored in adipose tissue. In result, nonesterified fatty acids (NEFA) enter the blood stream to be transported to organs and tissues throughout the body and can be used directly for energy by many tissues especially the peripheral tissues such as muscle (Bell, 1995). Normal NEFA concentrations for cows in positive energy status are estimated at equal to or less than 0.2 mmol/L and cows with severe NEB ranged from 0.7 to 1.45 mmol/L (Drackley, 2000). The concentration of NEFA measured in blood has been shown to reflect the amount of fat being mobilized from body fat reserves. Circulating NEFA can be oxidized by the liver and skeletal muscle as an energy source; re-esterified to triglyceride in the liver; or used by the mammary gland as a source of milk fat

(Drackley, 1999). Prolonged elevated NEFA concentrations indicate that dietary energy intake is insufficient for the needs of milk production or fetal growth and that body fat is being broken down to supply the energy deficit. Bell (1995) suggests four possible explanations for elevated NEFA concentrations in periparturient cows: 1) suppression of the de novo synthesis or uptake and esterification of fatty acids, 2) promotion of lipolysis, 3) reduction of the intracellular re-esterification of fatty acids released by lipolysis, 4) some combination of all. Once mobilized, NEFA attach to serum albumin for transport to various tissues. During increased rates of lipolysis, the ratio of NEFA to albumin increases and favors delivery and uptake of NEFA by energy and lipid requiring tissues (Stipanuk, 2000). The NEFA that do not undergo hepatic β-oxidation are re-esterified to triglyceride and released into the circulation as very low density lipoproteins (VLDL). During periods of negative energy balance, hepatic capacity for fatty acid re-esterification increases, but VLDL export rate from the liver remains low (Bauchart, 1993). Increased NEFA uptake and re-esterification combined with inefficient VLDL release can result in hepatocyte triglyceride accumulation, known as "fatty liver". The liver has a limited capacity to metabolize NEFA into triacylglycerol and once the limit is reached, triacylglycerol accumulates in the liver, and acetyl CoA (from oxidation of fatty acids) that is not utilized in the tricarboxylic acid cycle (TCA) is converted into ketone bodies, such as acetone, acetoacetate and beta-hydroxybutyrate (BHB) (Nelson and Cox, 2005) and could appear in the blood, urine and milk (Goff and Hurst, 1997). During the close-up period, NEFA concentration increased slowly as the cow approached calving, and usually ranged between 0.5 and 1.0 mmol/L during the last week before calving, but this increase usually happens very close to calving due to hormonal changes. Prolonged NEFA values above 0.4 mmol/L during the prepartum period are associated with negative outcomes such as ketosis, reduced reproductive performance and reduced milk yield during the subsequent lactation (Bell, 1995). When measured during the postpartum period, the NEFA threshold value used to predict negative energy balance is >0.7 mmol/L (Whitaker, 2004; McArt et al., 2015). Hyperketonemia (HYK), ketosis, is one of the more frequent and costly metabolic disorders in dairy cows and its diagnosis is based on β-hydroxybutyrate (BHB) concentration in blood. Most studies apply the threshold of 1.2 mmol/l of BHB concentration in blood to indicate HYK, with BHB concentrations between 1.2 and 2.9 mmol/l as subclinical ketosis, and values ≥3.0 mmol/l as clinical ketosis (McArt et al., 2015; Benedet et al.2019). Mantysaari et al. (2019) investigated relationships between body traits and NEFAs, BW had the highest correlation with plasma NEFA concentration (r = −0.51), whereas the correlation between BCS and plasma NEFA was lower (r = −0.29). A moderate correlation between plasma NEFA concentration and milk fat concentration (r = 0.47) was also found.

Comparing differences in early lactation Holstein and Jersey cows

Breed differences have been observed in frame size, body composition, maintenance requirements, milk production, and milk composition for Jersey and Holstein cows (Solis et al., 1988; Beaulieu and Palmquist, 1995; Bitman et al., 1996; Enevoldsen and Kristensen, 1997). Brown et al. (2012) investigated genetic differences in NEFA between Holstein and Jersey cows and Jersey*Angus crossbred. Purebred Holsteins had the highest average NEFA concentration (0.52 mmol/L) and the highest days open (169) as compared to the other genetic groups. Concentrations of NEFA were higher in second lactation for all genetic groups compared with first lactation and gradually declined throughout lactation (Brown et al., 2012). Oikonomou et al. (2008) investigated genetic variances for BCS, energy content, blood concentrations of glucose, BHB and NEFA, and found that estimated genetic variance of serum NEFA was different from zero between breeds until week 24 of lactation and that there is a significant genetic variation in body energy and metabolic traits throughout lactation, specifically in early lactation. Rastani et al. (2001) investigated body composition and energy balance differences in transition Holstein and Jersey cows (n = 26) that were allotted by breed, parity, and calving date, and were fed a total mixed ration (TMR) individually for 120 days in milk. Body weight, visual BCS, subcutaneous fat depth (between the 12th and 13th rib, and 10 cm posterior to the tuber coxae (thurl), and NEFA concentrations were collected biweekly, and feed intake and milk production were recorded daily. Holsteins had greater net energy intake (NEI) during early lactation compared to Jerseys, but fat depth and BCS were not affected by breed. A positive correlation was found between fat depth and BCS for Jersey cows, however this was not observed in the Holstein cows. Net energy intake and efficiency of milk energy production (calculated using an equation with milk production, and fat; $NE_L = MP \times (0.3512 + [0.0962 \times F])$ was greater in Holsteins, but in contrast, gross efficiency as a proportion of metabolic BW was greater in Jerseys. Non-esterified fatty acid concentrations were also found to be greater overall in Holsteins compared to Jerseys, which supports majority of NEFA literature. A vast amount of literature is available describing the energy balance change in early lactation Holstein cows (Coppock, 1985; Flatt et al., 1967; Moe, 1981, Sieber, 1987) but information regarding these changes in Jerseys is lacking.

Since BCS and existing methods of monitoring body condition have not been widely adopted of frequent on-farm assessment, the interest in developing other body condition technologies to quantify BCS has increased in attempt to remove subjectivity. Comparison of energy efficiency between Holsteins and Jersey cows in early lactation has not been widely researched, and evidence that does exist is outdated. The following studies investigate the use of a BCS caliper that has been successful in measuring angularity and predicting BCS in another species, while also investigating body condition and energy status in Holstein and Jersey cows during early lactation. The objectives of this work were to 1) investigate a prototype caliper to objectively quantify body condition in dairy cows and 2) assess the relationship between body condition and energy status in early lactation cows.

CHAPTER 2

Investigation of a prototype caliper to objectively quantify body condition in dairy cows

Abstract

The objective of this work was to investigate a prototype caliper to objectively quantify body condition in dairy cows. A prototype caliper was developed with the assumption that as an animal loses condition, the topline becomes more angular, therefore, body condition score (BCS) could be determined through change in angularity. The arms of the calipers were 3.8 cm tall and the calipers were adjustable to widths of 11.6, 16.5, 21.5, and 26.7 cm. Data were collected in 2017 from Holstein cows (n=29) at the Lake Wheeler Dairy Field Lab Unit (LWD, Raleigh, NC) and in 2021 at the NCDA&CS Piedmont Research Station (PRS, n=53). Cows in various days in milk (DIM) were subjected to a single collection of visual body condition scoring (BCS), measurements of subcutaneous rumpfat (RF) and backfat (BF) thickness (cm) via ultrasonography images (Aloka SSD-500V with 3.5MHz probe), and caliper measurements at the withers, 12th rib, short rib, and tailhead areas using calipers set at 11.6, 16.5, 21.5, and 26.7cm widths. Data were analyzed using the CORR, REG, and MIXED procedures in SAS (SAS® v.-9.4, Cary, NC). Significance was declared when $P < 0.05$. In study 1, caliper measurements were positively correlated to BCS with the 16.5cm caliper at the withers location being best correlated to BCS ($r = 0.73$, respectively, $P <0.01$). The percent of variation in BCS explained by the 16.5 caliper at the withers location was 79%. In study 2, the 11.6cm caliper was introduced and caliper measurements using the 11.6 and 16.5 cm widths at the withers and tailhead were collected. Caliper measurements were positively correlated to BCS ($r= 0.62$, 0.55, 0.61, 0.43, respectively, $P <0.01$), with the 16.5cm at the withers being best correlated to BCS. The percent of variation explained by BCS in caliper measurements with the 16.5cm at the

withers is 44%. Body condition calipers could potentially be a useful tool in managing BCS in dairy cows, particularly when using the 16.5cm width at the withers location.

Introduction

Monitoring individual cow body fat and maintaining a desirable body condition is essential to maintaining productive animals that have appropriate nutrition and fertility while producing high volumes of milk. Although body condition scoring (BCS) is useful if assessed properly, the subjective nature of visual assessment resulted in BCS used inconsistently as a management tool. Since BCS and existing methods of monitoring body condition have not been widely adopted of frequent on-farm assessment, the interest in developing other body condition technologies to quantify BCS has increased in attempt to remove subjectivity. A prototype BCS caliper was designed to measure angularity of the topline from the spinous process to the transverse process in sows and is based on the concept that as an animal loses back fat and muscle it becomes more angular (Knauer and Baitinger, 2015) and was successful in predicting BCS in swine (Figure 1.2). The following studies investigate in use of this prototype caliper in dairy cows.

Materials and Methods

Data was collected in 2017 with lactating Holstein cows (n=29) in various DIM (173 ± 39 d) at the Lake Wheeler Field Lab Dairy Unit (LWD) herd in Raleigh, NC. This study was conducted with the approval of the North Carolina State University Institutional animal care and use committee (IACUC #: 17-167-A). For each cow, a visual BCS, heart girth (HG), and caliper measurements were collected by the same technician. Calipers were set at different widths (16.5, 21.5, 26.7 cm) and measurements taken at the withers (W), short rib (SHrib), 12th rib (rib), and tailhead (T) to determine the best size and location for collection. Ultrasonography images of rump fat (RF; between hook and pin), back fat (BF; between 12^{th} and 13^{th} rib), and a loin muscle area (cm) between the 12-13^{th} rib (Aloka SSD-500V with 3.5MHz probe) were collected in duplicates for each cow. A second set of data was collected using the smaller caliper widths (11.6 and 16.5cm) at the NCDA&CS Piedmont Research Station (PRS) in 2021. Holstein cows (n = 53) in various DIM (193 ± 33 d) were subjected to a single collection of visual BCS, body weight (BW), caliper measurements at the withers and tailhead, and ultrasonography images of RF and BF thickness (Aloka SSD-500V with 3.5MHz probe). Back fat and rump fat thickness were measured to the nearest 0.1 cm. In previous studies using ultrasound to measure fat thickness, the entire skin layer was included along with the subcutaneous fat layer (Staufenbiel, 1997). In the present studies the skin layer was not included, so ultrasound measurements represent the true thickness of the subcutaneous fat layer.

Statistical Analysis

Data were analyzed using the CORR procedure of SAS (SAS v. 9.4, Cary, NC). Correlations (r) were evaluated between BCS, BW, HG, caliper scores, rump fat and back fat thickness, and DIM Stepwise regression models were created with BCS and with the caliper size and location as the dependent variables and RF, BF, loin area, and HG as independent variable. The MIXED procedure (SAS v. 9.4, Cary, NC) was also used to determine the relationship between rump fat and back fat thickness and BCS. This data is reported as least squares means. Results were considered significant if $P < 0.05$ and declared a trend if $0.05 < P < 0.10$.

Results and Discussion

Correlation values between BCS and caliper measurements in the LWD data showed the 16.5-cm caliper at the withers had the best correlation followed by the tailhead location (r = 0.71, 0.67, respectively) as compared to the calipers set at the 21.5 and 26.7 cm widths (r = 0.42, 0.58, 0.64, 0.62, respectively; -Table 2.1). The short rib and 12th rib locations showed the lowest correlations with BCS and were physically hardest to collect, so the withers and tailhead locations were chosen for further studies. The 16.5 cm caliper used at the withers explained more variation in BCS (R^2 = 0.79) compared to smaller calipers at other locations (R^2 = 0.72, 0.70, 0.70, 0.74, 0.73, 0.71, 0.78.). Backfat consistently explained 60% of the variation associated with BCS, which is expected since the rump and back area are the common sites for visual scoring. When visually assessing BCS in dairy cows, heart girth and caliper measurements, alone, explained less than 10% of the variation associated with BCS. In the second data set collected at PRS, both the 16.5cm and 11.6cm calipers had a similar relationship to BCS (r = 0.62 and 0.61, respectively, P <0.01, Table 2.4) when used at the withers. Overall, measurements taken at the withers had a better relationship to BCS compared to those taken at the tail head, but the 11.6cm caliper used at the tail head was more strongly correlated to BCS than the 16.5cm (r = 0.55 vs. 0.43, respectively, P <0.01). RF and BF thickness were also positively correlated to BCS (r = 0.67, 0.74, respectively, P <0.01) in this data set. The percent of variation explained by RF (between hooks and pins) for the tailhead location caliper measurements was 39% for the 11.6cm and 25% for the 16.5cm caliper, and the percent of variation explained by BCS for the withers location caliper measurements was 33% for the 11.6cm and 36% for the 16.5cm caliper (Table 2.5). Back fat was removed from the regression model based on lack of significance (P <0.15). Klawuhn and Staufenbiel (1997) examined the entire back region of the dairy cow from the thoracic spine to the tail and found the sacral examination site (between hooks and pins) to be most suitable for assessing BF thickness.

Conclusion

This prototype caliper has been found useful in predicting BCS in swine and aids in removing subjectively from visual BCS. The prototype BCS calipers were found to be useful in dairy cows at the withers location with the 16.5 cm caliper, and potentially at the tailhead with the 11.6 cm. Based on these two studies investigating the validity of this tool in dairy cattle, the smaller calipers (11.6 and 16.5 cm widths) at the withers and tailhead locations were chosen for further investigation. A companion study was designed to assess the relationship between body condition and energy status in early lactation cows of different breeds.

Chapter 3

Development of an objective body condition scoring (BCS) system to assess energy status in dairy cattle.

Abstract

The objective of this study was to assess the relationship between body condition and energy status in early lactation cows. Holstein (n = 25) and Jersey (n = 15) dairy cows were subjected to body measurements including visual BCS, caliper measurements, and ultrasound measurements of subcutaneous fat thickness weekly from -15 ±4.7 (dry period collection; D) to 54 ±2.23 (week 8 of lactation) DIM. A prototype caliper was developed with the assumption that as an animal loses condition, the topline becomes more angular, therefore, BCS could be determined through change in angularity. The arms of the calipers were 3.8 cm tall, the calipers were either 11.6cm or 16.5cm wide. Caliper measurements were taken weekly at the withers (11.6W; 16.5W) and tailhead (11.6T, 16.5T). Rump fat (RF) and back fat (BF) thickness (cm) were collected via ultrasonography (Aloka SSD-500V with 3.5MHz probe) biweekly along with body weight (BW). In addition to body measurements, blood samples were collected via coccygeal vein weekly into a 10mL serum separator tube (Fisher, Suwanee, GA) and placed on ice. Serum was separated using a centrifuge for 30 min at 3,000 × g and frozen at -18°C within 3 hours of collection. Data were analyzed using the CORR, REG, and MIXED procedures in SAS (SAS® v. 9.4, Cary, NC). Significance was declared when $P < 0.05$. Rump and BF thickness and caliper measurements (11.6W, 11.6T, 16.5W, 16.5T) had a weak positive correlation to visual BCS (r = 0.65, 0.59, 0.27, 0.30, 0.42, 0.22, respectively, $P < 0.01$). The 11.6T and 16.5W were significant by BCS ($P < 0.01$). Holstein cows had a greater caliper score ($P < 0.01$) with the large caliper at the withers, compared to Jersey cows. No differences were detected between tailhead caliper measurements or at either location using the small calipers between breeds. Non-esterified fatty acids (NEFA) concentrations were negatively correlated to BCS ($r = -0.25$, $P < 0.01$). Non-esterified fatty acid concentrations were greater in Holstein cows (0.56 mmol/L) compared to Jersey cows (0.44 mmol/L) and were lowest in the dry period (0.16 mmol/L), increasing through weeks 1 through 4 of lactation then decreasing after week 5 ($P < 0.01$). BCS calipers could potentially be a useful tool in managing BCS in dairy cows particularly when using the 16.5cm width at the withers location. Investigation of metabolic and genetic information pertaining to the different breeds that may be responsible for these differences observed in NEFA concentrations should also be considered in further research investigating this tool.

Keywords: body condition score, negative energy balance, NEFA, Holstein, Jersey

Introduction

Dairy cows can experience a period of negative energy balance (NEB) during early lactation when the energy demand of lactation cannot be met by dietary intake alone (Figure 1.1). Therefore, body reserves become responsible for up to one-third of the milk solids produced in early lactation (Bauman and Currie, 1980). Monitoring individual cow body fat and maintaining a desirable body condition is essential to maintaining productive animals that have appropriate nutrition and fertility while producing high amounts of milk. Body condition scoring (BCS) is a method of visually assessing the body reserves of an animal. A scoring scale of 1 to 5 with quarter point intervals was developed for use in dairy cows (Wildman et al. 1981; Edmonson et al. 1989), and the “ideal” BCS at calving in dairy cows is reported to be between 3.0 and 3.25, with < 3.0. BCS at calving being associated with reduced reproductive performance, and a calving BCS >3.5 associated with reduced milk production and increased risk of metabolic disorders (Roche et al., 2009). Although BCS has been found to be useful if assessed properly, the subjective nature of visual assessment has resulted in BCS not being used as a frequent management tool. The development of an objective BCS tool may increase accuracy and application of BCS in dairy cattle to improve milk production, fertility, and cow welfare while decreasing metabolic disorders.

Materials and Methods

Experimental Design and Animal Management

Forty cows (both primiparous and multiparous) were utilized in the study (Jerseys, n = 15; Holsteins, n = 25). This study was conducted with the approval of the North Carolina State University Institutional Animal Care and Use Committee (IACUC #: 17-167-A). A prototype caliper was developed to quantify the angularity of the topline and is based on the concept that as an animal loses fat and muscle, the topline becomes more angular (Knauer and Baitinger 2015; Figure 1.1). The arms of the calipers used in this study were 3.8 cm tall and were either 11.6 cm or 16.5 cm wide. Caliper measurements were taken at the withers (11.6W; 16.5W) and tailhead (11.6T; 16.5T). Data collection started at approximately 2 weeks (15.4 ±4.7d) prior to calving (dry period, D) and continued through 8 weeks of lactation, approximately 54 DIM (54.54±2.2). Caliper measurements, serum samples and visual BCS were collected weekly. Biweekly collection of body weights (BW) and duplicate images of rumpfat (RF, between hips and tailhead area) and backfat (BF, between 12th and 13th rib) thickness (cm) were measured via ultrasonography (Aloka SSD-500V with 3.5MHz probe). Two visual BCS scores were collected from the same trained technicians, using the 1 to 5 BCS scale (Wildman et al., 1981) with quarter point intervals traditionally used in dairy cows.

Sample collection and analysis

Blood samples were collected via coccygeal vein into a 10mL serum separator tube (Fisher, Suwanee, GA) and placed on ice. Serum was separated using a centrifuge for 30 min at 3,000 × g and frozen at -18°C within 3 hours of collection. Serum samples were later thawed and NEFA concentrations were analyzed with an commercial enzymatic assay kit (HR Series NEFA-HR (2), Fujifilm Medical Systems U.S.A., Inc., Atlanta, GA) and a colorimetric procedure using BioTek Synergy HT plate reader at 550 nm with KC4 software. A pooled cow sample and a human serum control sample were used as the control, each serum sample was analyzed in duplicates, and the intra- and inter- assay CV were both less than 5%. Individual cow milk yields (kg) were recorded from daily milking sessions and milk component yields (kg) were calculated based on daily milk weights and test results (milk fat and protein, %) from monthly Dairy Herd Improvement Association milk testing services (DHIA). Energy corrected milk (ECM) was calculated using fat and protein percentages (DHIA) according to the equation below:

ECM = (0.327 x milk lbs.) + (12.95 x fat lbs.) + (7.65 x protein lbs.)

Average dry matter intake (DMI), net energy intake (NEI), protein intake, and fat intake were calculated based on nutrient values obtained from herd rations and estimates of DM offered, per group, from TMR Tracker™. Holstein and Jersey cows were housed separately, but study cows were housed in the same pens as other cows, within breed. Holsteins and Jerseys were fed different rations, but their ration was consistent over the study and cows were fed for no refusals.

Statistical Analysis

Data were analyzed using the CORR procedure of SAS (SAS v. 9.4, Cary, NC). Correlations (r) were evaluated between BCS, caliper scores, body measurements, and DIM. The MIXED procedure (SAS v. 9.4, Cary, NC) was also used to determine the relationship between caliper scores and NEFA concentrations. Breed, week, and BCS were independent variables and NEFA concentrations were the dependent variable. Week was also used as a repeated measure in the mixed model. This data is reported as least squares means. Results were considered significant if $P < 0.05$ and declared a trend if $0.05 < P < 0.10$.

Results

Caliper measurements (11.6W, ST, LW, LT) were positively correlated with visual BCS ($r = 0.24$,0.31, 0.35, 0.21 respectively, $P < 0.01$) where the 16.5W and 11.6T size and location combinations showed the greatest correlation with BCS (Table 3.1). Where BCS increased from a 2.5 to a 3.5, the small caliper score at the tailhead increased from 35.6 to 37.6 ($P < 0.01$) and the large caliper score at the withers increased from 27.9 to 29.5 ($P < 0.01$) (Table 3.2). Holsteins had a greater score with the large caliper at the withers, compared to Jerseys, but tailhead and small caliper measurements were not different between breeds (Table 3.2).

Serum NEFA concentration was negatively correlated to visual BCS ($R^2 = -0.25$, $P < 0.01$) (Table 3.1). Mean NEFA concentrations over the first 8 weeks of lactation were greater in Holsteins (0.56 mmol/L) than Jerseys (0.44 mmol/L) and were lowest in the dry period (0.16 mmol/L) for both breeds ($P < 0.01$). Concentrations increased throughout weeks 1-4 and then began decreasing after week 5 (Figure 3.1). Holstein cows experienced a greater spike in NEFA concentrations between the dry period collection (15.4 days ±4.7 prior to calving) and the wk1 (5.2±2 days in milk) when compared to Jerseys (Figure 3.1). Cows categorized with a greater BCS (3.3+) during the second and third weeks of lactation had greater NEFA concentrations (LSmeans, proc MIXED, SAS) than the cows categorized with a lesser BCS (2.5-3), although these lesser BCS cows had higher NEFA concentration starting off in the dry period (15.4 days ±4.7 prior to calving) (Figure 3.2). The change in NEFA concentration was greatest between the dry period collection and the week 1 collection for both breeds, with the mean increase of 0.377 mmol/L (Figure 3.3), with the most change in BCS also occurring between the dry period collection and the wk1 collection with a mean decrease of -0.15 BCS points (Figure 3.4). More cows were categorized with a BCS greater than 3.3 during the dry period collection, but as lactation progressed and NEFA concentrations increased, the prevalence of cows with a BCS of 3.3+ decreased. During the sixth week of lactation there were no Jersey cows categorized with a BCS greater than 3.3+ so data within the high BCS category may lack variation. Body weight and DIM were not significantly correlated to BCS or NEFA in this set. When estimating DM offered and NEI, Jersey were offered less than Holsteins (Table 3.4). When analyzing milk component data (proc MIXED, LSmeans, SAS), the average milk fat and protein percentages were higher in Jerseys (Fat %: 4.93, Protein %: 3.58) when compared to Holsteins (Fat %: 4.25, Protein %: 3.12), but the Jersey ration had a higher fat percent (Table 3.5) which could have impacted result observed in the NEFA data. The MIXED procedure (SAS v. 9.4, Cary, NC) was used to analyze intake and milk data comparing them with NEFA concentrations. Between week 1 and 2 of lactation, both breeds increased in milk yield, potential NEI (Mcal) and NEFA (mmol/L) concentration (Figures 3.5 and 3.6). Once potential NEI increased and plateaued, NEFA concentration began to decrease back below the threshold of suggesting NEB, between weeks 2 and 4.

Discussion

When designing this project and understanding that BCS was a subjective assessment, NEFA concentrations and ultrasonography data were used to better support what is being reflected by the caliper measurements and visual BCS scores. Body weight and DIM were also included in the model to further support these finding, although not significantly correlated to caliper measurements, visual BCS or NEFA concentrations. This could be potentially explained by the lack of variation in this data set due to repeated measures on the same animal and that all cows were within the same DIM (between 3 and 54.54±2.23 days). Body condition score is highly influenced by DIM (Waltner et al., 1993) as condition loss is observed during the transition period. Monitoring BCS becomes even more important later in lactation to separate fat from thin cows to ensure an adequate replenishment of body tissue reserves to prepare for the subsequent lactation (Schroder and Staufenbiel, 2006). Additionally, BW was only collected biweekly as they came through the chute area for ultrasounds. Other measurements were easily collected in the free stall barn while cows were in headlocks, but the scale was located in a chute area that only allowed for a few cows at a time. Thus, we decided to collect BW biweekly as cows come in for ultrasounds. Body weight may have not appeared significant in the model as BW can fluctuate quickly and frequently during lactation as milk volume fluctuates. The 16.5cm at the withers (r = 0.35) and the 11.6cm at the tailhead (r = 0.31) were the highest correlated to BCS (Table 3.1). The tailhead of a dairy cow has very little fat variation and is particularly bony which could potentially explain why it was not as strongly correlated to BCS at the withers location. The withers location in both sizes were significant by breed, which could be explained by frame size difference between Holsteins and Jerseys but could also suggest they carry condition around the withers differently.

Brown et al. (2012) discussed the breed differences in NEFA between Holsteins and Jerseys and their crossbreeds. Results found in the NEFA data showed that Holsteins had higher NEFA concentrations compared to the Jersey cows and concentrations were highest during week 1 of lactation and gradually decreased over 10 weeks of lactation. The results of this current study support these findings as Holsteins had overall higher NEFA concentrations (0.56 mmol/L) compared to the Jerseys (0.44 mmol/L) and reached a higher concentration before the Jersey cows did. Body condition score change and NEFA change were both highest between weeks 1 and 2 of lactation. Holstein NEFA concentrations reached potential NEB (≥0.7 mmol/L) during weeks 2 and 3 of lactation, while Jersey cows reached their highest concentration during these weeks as well, they did not reach the threshold of NEB. When categorizing BCS and comparing NEFA concentrations across the different categories, the cows that had a greater BCS (BCS 3.3+) reached the threshold of greater NEFA concentrations that suggests NEB (≥0.7 mmol/L) during the second and third week of lactation. The lower BCS cows (BCS 2.5-3) during weeks 2 and 3 of lactation did not experience >0.7 mmol/L NEFA concentrations as compared to the greater BCS cows, but the lower BCS cows started with a greater NEFA concentration in the dry period (D).

In attempt to further investigate nutrient distribution and differences between the breeds, we later analyzed milk yield and milk component data, as well as estimated DMI with data collected from TMR Tracker and ration analyses. Between the first and second week of lactation, both breeds increased milk yield, potential NEI (Mcal) and NEFA (mmol/L) concentration. This could suggest during the first 2 weeks of lactation, both breeds are expending more energy than they are receiving from NEI which is to be expected during the transition period. Once NEI increased and plateaued, NEFA concentration began to decrease between weeks 2 and 4. The average milk fat and protein percentages were higher in Jerseys (Fat %: 4.93, Protein %: 3.58) when compared to Holsteins (Fat %: 4.25, Protein %: 3.12), but the Jersey ration had a higher fat % (Table 3.5) which could have impacted result observed in the NEFA data. When estimating DM offered and NEI, Jersey were offered less than Holsteins (Table 3.5), as expected. Fat intake and protein intake (kg) from the diet were higher in the Jerseys, which could also influence milk fat and protein (%). This difference in available fat from the diet could influence NEFA concentrations and may decrease the risk of NEB. Holsteins produced more yield, with an average milk yield of 41.29 kg per day during the first 8 weeks of lactation and an average daily ECM of 46.32 kg. Interestingly, when comparing Jersey ECM to their milk yields, this increase was not observed (milk yield: 28.57 kg, ECM: 28.74 kg) (Figure 3.7). When comparing NEFA concentration with estimated NEI and milk component yields, Jersey cows did not experience as high of NEFA concentrations as Holsteins, but Jerseys produced less volume of milk and were offered less DM, resulting in a lower NEI than the Holsteins. These findings support those of Rastani et al. (2001) that investigated body composition and tissue energy balance (TEB) in early lactation Holstein and Jerseys (n=26) that were paired by breed, parity, and calving date. Feed intake and milk production were measured daily and body measurements (BW, BCS, subcutaneous fat depth, milk composition, and NEFA concentrations) were measured biweekly. Rasanti et al. (2001) found that Jerseys mobilized body tissue for a shorter period as a result of their lower production (28 kg of 4% fat corrected milk: FCM) when compared to Holsteins (42 kg of 4% FCM), though NEI was higher in Holsteins compared to Jerseys (37.8 and 28.2 Mcal, respectively). Energy balance was lower for Holsteins from 1 wk postpartum through the 7th week of lactation. Fat depth and BCS did not differ between breeds, however a positive relationship existed between BCS and fat depth for Jerseys but was not observed in Holsteins. The best-fit regression model for their study for predicting TEB included week of lactation, milk composition, and BCS. Erdman and Andrew (1989) initially investigated TEB and also found that lower producing cows remain in a state of negative TEB for a shorter period, experiencing lower concentrations than higher production cows. Blake et al. (1986) studied comparative feed efficiency of Holstein and Jersey cows and at the time found that energy efficiency of Holsteins and Jerseys did not differ (P>.2) in first trimester of lactation regardless of the statistical model (accounting or ignoring regressions on milk, DM intake, and N balance), although Holsteins exceeded Jerseys (P<0.01) in second trimester when DM intake was considered. In contrast, the results of this study suggests differences in energy efficiency among these two breeds during early lactation, but several factors such as genetic merit, milk volume, DMI, nutrient availability in the diet, and body condition at calving are major influences of energy status.

Conclusion

In conclusion, the BCS calipers were found to be useful at the withers location with the 16.5 cm caliper, and potentially at the tailhead with the 11.6 cm. Correlations were lower between BCS and caliper measurements potentially due to lack of variation in the data as these cows were all in early lactation. Further investigation of this tool is needed before implementing this as a useful BCS tool in dairy animals. Differences in energy status were observed between Holsteins and Jerseys, but there are many factors influences energy status in early lactation. Changes in body lipid content during early lactation are influenced by genetics to a large extent (Schroder and Staufenbiel, 2006). The NEFA concentrations in Holstein cattle peaked above the threshold indicating a NEB (≥0.7 mmol/L), while the Jersey cattle did not. The only significant difference measured between the breeds when comparing milk components and feed intake were estimated DMI, in which the Jerseys consumed less by volume, and milk fat and protein %, in which the Jerseys averaged higher in both. The Jersey cows had a higher fat % in their diet which could have impacted the findings in the NEFA data predicting energy status. Considering genetic variables such as sire and breed, as well as measuring true individual cow DMI would potentially explain differences seen in NEFA concentrations and caliper measurements between the breeds. Larger sample size offering more variation is needed to further validate the calipers ability to accurately predict BCS in dairy cows. Individual cow DMI and genetic variances should be measured in subsequent studies to account for true NEI and how it affects energy status and body condition.

Chapter 4

Conclusion and Implications

In conclusion, the prototype BCS calipers were found to be useful at the withers location with the 16.5 cm caliper, and potentially at the tailhead with the 11.6 cm. Correlations with BCS calipers were higher in data collections investigating the caliper that contained cows in various stages of lactation, offering more variation in the data (LWD 2017 and PRS 2021). As a single measurement, the calipers had higher correlation with BCS compared to when using the caliper over period of time during early lactation to track BCS changes. In the set of 40 early lactation cows that contained both Holstein and Jersey breeds at the Lake Wheeler Dairy Field Lab Unit, correlations were lower potentially due to lack of variation in the data. All cows in this set were within 54.54±2.2 DIM, narrowing the potential for variety in the data. Significant breed differences in caliper measurements were observed at the withers location and could suggest that breed and frame size could influence which caliper size is best suited for individual breeds. The NEFA data shows that Jerseys did not experience concentrations suggesting potential NEB (≥0.7mmol/L) as Holsteins did, but the reasoning behind these differences could be several factors. The only significant difference between the breeds in this set were estimated DMI (Jersey: 21.8 kg, Holstein: 24.3 kg) (LSmeans, P = 0.02), in which the Jerseys consumed less by volume, and milk fat and protein % (LSmeans, P <0.01), in which the Jerseys averaged higher in both. Since intake was only an estimate and individual cow intake could not be collected at these facilities, accurate intake and metabolism variables that may have contributed to the differences observed between the two breeds could not be accounted for in this set. This prototype BCS could potentially be a useful tool in dairy, although larger sample sizes are needed for validity of the BCS caliper before implementing it as an on-farm assessment tool. Deeper investigation of metabolic and genetic information pertaining to the different breeds that may be responsible for these differences observed should be considered in further research. Considering genetic variables such as sire and breed, as well as measuring individual cow DMI would potentially explain differences observed in NEFA concentrations and caliper measurements between the breeds and offer more insight on which size is best suited for each breed.

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TABLES

Table 1.1 Assessment of body condition (BCS), back fat thickness (BFT) and total fat content (TBF)[1].

Descripton	BCS	BFT (mm)	TBF (kg)
Emaciated	1.0	<5	<50
Very poor	1.5	5	50
Poor	2.0	10	76
Moderate	2.5	15	98
Good	3.0	20	122
Very good	3.5	25	146
Fat	4.0	30	170
Adipose	4.5	35	194
Obese	5.0	35	194

[1]Adapted from Staufenbiel (1997).

Table 1.2: Metabolic adaptations associated with negative energy balance in dairy cows[1].

Physiological function	*Metabolic changes*	*Tissues involved*
Milk synthesis	Increased use of nutrients (particularly glucose)	Mammary gland
Lipid metabolism	Increased lipolysis	Adipose tissue
	Decreased lipogenesis	
Glucose metabolism	Increased gluconeogenesis	Liver
	Increased glycogenolysis	Body tissue
	Decreased glucose oxidation	
	Increased lipid β-oxidation	
Protein metabolism	Mobilization of protein reserves	Muscle and other body tissue

[1]Adapted from Bauman and Currie (1980).

Table 2.1. Correlation (r) between body condition score, heart girth, rump fat thickness, and back fat thickness with caliper scores of three different widths[1] at four different body locations[2] with Holstein cows (n=29) at the Lake Wheeler Field Lab Dairy Unit.

Caliper	16.5 rib	16.5 SHrb	16.5 T	21.5 W	21.5 rib	21.5 SHrb	21.5 T	26.7 W	26.7 rib	26.7 SHrb	26.7 T	DIM	BCS	HG	RF	BF	loin area
16.5W	**0.49**	**0.11**	**0.06**	**0.73**	**0.33**	**0.19**	**0.09**	**0.80**	**0.39**	**-0.15**	**-0.05**	**-0.01**	**0.73**	**-0.37**	**0.22**	**0.16**	**0.21**
P	0.01	0.59	0.77	<.01	0.08	0.33	0.64	<.01	0.04	0.46	0.79	0.98	<.01	0.05	0.26	0.42	0.28
16.5rib		**0.47**	**0.16**	**0.29**	**0.56**	**0.45**	**0.14**	**0.27**	**0.64**	**0.31**	**0.21**	**0.03**	**0.64**	**0.06**	**0.36**	**0.47**	**0.36**
P		0.01	0.40	0.13	<.01	0.01	0.46	0.17	<.01	0.11	0.27	0.86	<.01	0.77	0.06	0.01	0.06
16.5SH rib			**0.60**	**0.03**	**0.30**	**0.86**	**0.69**	**-0.33**	**0.10**	**0.75**	**0.78**	**0.15**	**0.66**	**0.70**	**0.66**	**0.68**	**0.55**
P			<.01	0.90	0.11	<.01	<.01	0.08	0.62	<.01	<.01	0.44	0.00	<.01	0.01	0.01	<.01
16.5T				**0.14**	**0.01**	**0.49**	**0.88**	**-0.13**	**0.01**	**0.37**	**0.68**	**0.22**	**0.42**	**0.53**	**0.52**	**0.40**	**0.34**
P				0.48	0.95	0.01	<.01	0.50	0.95	0.05	<.01	0.26	0.03	<.01	<.01	0.04	0.08
21.5W					**0.39**	**0.17**	**0.12**	**0.58**	**0.07**	**-0.09**	**-0.06**	**-0.25**	**0.33**	**-0.43**	**0.05**	**-0.03**	**0.02**
P					0.03	0.39	0.52	<.01	0.72	0.66	0.77	0.21	0.08	0.02	0.81	0.89	0.91
21.5rib						**0.47**	**0.07**	**0.23**	**0.47**	**0.38**	**0.01**	**-0.15**	**0.29**	**-0.11**	**0.26**	**0.18**	**0.12**
P						0.01	0.73	0.23	0.01	0.04	0.97	0.46	0.13	0.57	0.19	0.36	0.54
21.5SH rib							**0.49**	**-0.16**	**0.22**	**0.86**	**0.57**	**0.04**	**0.39**	**0.50**	**0.47**	**0.43**	**0.40**
P							0.01	0.43	0.25	<.01	<.01	0.86	0.04	0.01	0.01	0.02	0.03
21.5T								**-0.15**	**-0.03**	**0.42**	**0.78**	**0.24**	**0.52**	**0.55**	**0.53**	**0.47**	**0.34**
P								0.45	0.87	0.03	<.01	0.22	<.01	<.01	<.01	0.01	0.08
26.7W									**0.54**	**-0.32**	**-0.33**	**-0.03**	**0.65**	**-0.67**	**0.13**	**-0.02**	**0.05**
P									<.01	0.10	0.08	0.88	<.01	<.01	0.51	0.93	0.80
26.7rib										**0.24**	**-0.07**	**0.15**	**0.44**	**-0.29**	**0.26**	**0.17**	**0.02**
P										0.23	0.73	0.46	0.02	0.14	0.17	0.37	0.95
26.7SH rib											**0.53**	**0.24**	**0.09**	**0.49**	**0.39**	**0.38**	**0.26**
P											<.01	0.24	0.65	0.01	0.04	0.05	0.17
26.7T												**0.10**	**0.59**	**0.75**	**0.46**	**0.57**	**0.43**
P												0.63	<.01	<.01	0.01	<.01	0.02
DIM													**0.25**	**0.20**	**0.29**	**0.25**	**0.31**

Table 2.1 (continued)

P	0.21	0.30	0.14	0.22	0.11
BCS		**0.68**	**0.67**	**0.72**	**0.58**
P		<.01	<.01	<.01	0.01
HG			**0.47**	**0.60**	**0.34**
P			0.01	<.01	<.01
RF				**0.87**	**0.65**
P				<.01	0.01
BF					**0.67**
P					<.01

Table 2.2. Stepwise regression analysis for body condition score, and caliper measurements and back fat depth ultrasonography data from the initial caliper data with Holstein cows in various days in milk (n=29) at the Lake Wheeler dairy field lab (Raleigh, NC, 2017).

Caliper Size	Location	Regression Equation[1]	Partial R-Squared			Model R-Squared (HG+BF+Caliper)
			Heart girth	Back fat	Caliper	
16.5cm	Withers	Y= -1.37 + 0.07*HG + -0.04*6.5W	0.08	0.61	0.10	0.79
	Rib	Y= -0.67 + 0.04*HG + 1.05*BF+ -0.03*6.5R	0.06	0.60	0.05	0.72
	Tailhead	Y= -0.30 + 0.03*HG + 1.29*BF+ -0.02*6.5TH	0.05	0.61	0.06	0.72
21.5cm	Withers	Y= -1.61 + 0.06*HG + 0.97*BF + -0.02*8.5W	0.05	0.60	0.04	0.70
	Rib	Y= -0.92 + 0.05*HG + 0.91*BF+ -0.04*8.5R	0.05	0.60	0.04	0.70
	Tailhead	Y= -0.80 + 0.05*HG + 1.13*BF+ -0.02*8.5TH	0.06	0.60	0.07	0.74
26.7cm	Withers	Y= 0.06 + 0.04*HG + 0.97*BF + -0.04*10.5W	0.06	0.60	0.07	0.73
	Rib	Y= -1.33 + 0.05*HG + 1.16*BF+ -0.03*10.5R	0.06	0.60	0.05	0.71
	Tailhead	Y= -0.18 + 0.04*HG + 1.07*BF+ -0.03*10.5TH	0.06	0.60	0.10	0.78

[1]HG = heart girth, BF = backfat, R = Rib, TH = Tailhead, W= Withers. BCS as the dependent variable. Variables were included in the model at P<0.15. Loin area was not significant at P<0.15.

Table 2.3. Stepwise regression analysis for caliper measurements and fat depth ultrasonography data, heart girth, and body condition score from the initial caliper data with Holstein cows in various days in milk (n=29) at the Lake Wheeler dairy field lab.

Caliper Size	Location	Regression Equation[1]	Partial R-squared					Model R-squared (RF+BCS+HG)
			RF	Loin area	BF	BCS	HG	
16.5cm	Withers	Y= 27.24 + 9.83*BCS + -0.41*HG	-	-	-	0.44	0.05	0.49
	12th Rib	Y= 9.45 + 8.06*BCS	-	-	-	0.32	-	0.32
	Short Rib	Y= 29.44 + 11.96*RF	0.39	-	-	-	-	0.39
	Tailhead	Y= 20.24 + 12.55-RF	0.25	-	-	-	-	0.25
21.5cm	Withers	Y= 87.21 + 10.33*RF + 7.33*BCS + - 1.14*HG	0.07	-	-	0.10	0.20	0.37
	12th Rib	93.16 + 19.05*RF + - 0.88*HG	0.10	-	-		0.19	0.29
	Short Rib	Y= 99.09 + 14.25*RF + 5.48*BCS + - 1.07*HG	0.13	-	-	0.08	0.16	0.37
	Tailhead	Y= 65.98 + 11.41*RF + 7.68*BCS + - 0.82*HG	0.09	-	-	0.29	0.08	0.45
26.7cm	Withers	Y= 32.33 + 8.93*RF + 5.29*BCS + - 0.45*HG	0.05	-	-	0.40	0.08	0.53
	12th Rib			-	-	-	-	-
	Short Rib			-	-	-	-	-
	Tailhead	Y= 37.46 + 10.83*BCS + -0.55*HG		-	-	0.40	0.08	0.48

[1]HG = heart girth, BF = backfat, R = Rib, TH = Tailhead, W= Withers.
Caliper as the dependent variable. If variable was not significant at P<0.15, it was left blank in the table and not included in the model.

Table 2.4. Correlation (r) between body condition score, rump fat thickness, and back fat thickness and caliper scores using two caliper widths[1] at two locations[2] with Holstein cows (n=53) from the NCDA Piedmont research station (Salisbury, NC, 2021).

Measures[3]	*DIM* [3]	*BW*[3]	*11.6W*	*11.6T*	*16.5W*	*16.5T*	*BCS*[3]	*RF*[3]	*BF*[3]
DIM [3]		**0.33**	**0.26**	**0.38**	**0.31**	**0.35**	**0.36**	**0.30**	**0.27**
p		0.23	0.07	0.01	0.03	0.01	0.01	0.06	0.09
BW[3]			**0.39**	**0.23**	**0.22**	**-0.12**	**0.27**	**0.06**	**0.15**
p			0.15	0.41	0.43	0.68	0.32	0.82	0.60
11.6W				**0.42**	**0.70**	**0.19**	**0.61**	**0.53**	**0.60**
p				<.01	<.01	0.18	<.01	0.00	<.01
11.6T					**0.36**	**0.40**	**0.55**	**0.50**	**0.42**
p					0.01	0.00	<.01	0.00	0.01
16.5W						**0.52**	**0.62**	**0.45**	**0.57**
p						<.01	<.01	0.01	0.01
16.5T							**0.43**	**0.34**	**0.41**
p							0.01	0.03	0.01
BCS[3]								**0.68**	**0.74**
p								<.01	<.01
RF[3]									**0.74**
p									<.01
BF[3]									
p									

[1] Caliper widths = 11.6 and 16.5 cm
[2] Locations = W =Withers; T = tailhead
[3]BCS = Body Condition Score; BW = body weight; RF = Rump fat thickness; BF = back fat thickness, DIM= days in milk

Table 2.5. Stepwise regression analysis for caliper measurements using two widths[1] at two locations[2], and fat depth ultrasonography data, body weight and body condition score in Holstein cows in various days in milk (n=53) at the NCDA Piedmont research station (Salisbury, NC, 2021).

Caliper Size	**Location**	**Regression Equation[3]**	**Partial R-Squared**				**Model R-Squared (RF+BF+BW+BCS)**
			Rump fat	**Back fat**	**BW**	**BCS**	
11.6cm	Withers	Y= 13.45 + 8.92*BCS		-	-	0.33	0.33
	Tailhead	Y= 26.08 + 34.38*RF	0.39	-	-		0.39
16.5cm	Withers	Y= 1.97 + 9.30*BCS		-	-	0.36	0.36
	Tailhead	Y= 25.24 + 19.73*RF	0.25	-	-	-	0.25

[1]Caliper widths = 11.6 and 16.5 cm
[2] Locations = W =Withers; T = tailhead
[3]BCS = Body Condition Score; BW = body weight; RF = Rump Fat Thickness; BF = back fat thickness.
Calipers were used as the dependent variable in this model. If variable was not significant at P<0.15, it was left blank in the table and not included in the model.

Table 2.5b Stepwise regression analysis for caliper measurements using two widths[1] at two locations[2], and fat depth ultrasonography data, body weight and body condition score in Holstein cows in various days in milk (n=53) at the NCDA Piedmont research station (Salisbury, NC, 2021).

Caliper Size	**Location**	**Regression Equation[3]**	**Partial R-Squared**				**Model R-Squared (RF+BF+BW+Caliper)**
			Rump fat	**Back fat**	**BW**	**Caliper**	
11.6cm	Withers	Y= 1.73 + 4.52*BF		0.79	-	-	0.79
	Tailhead	Y= 1.73 + 4.52*BF		0.79	-	-	0.79
16.5cm	Withers	Y= 1.73 + 4.52*BF		0.79	-	-	0.79
	Tailhead	Y= 1.73 + 4.52*BF		0.79	-	-	0.79

[1]Caliper widths = 11.6 and 16.5 cm
[2] Locations = W =Withers; T = tailhead
[3]BCS = Body Condition Score; BW = body weight; RF = Rump Fat Thickness; BF = back fat thickness.
BCS used as the dependent variable in this model. If variable was not significant at P<0.15, it was left blank in the table and not included in the model.

Table 2.6. Least-squares means for rump fat and back fat thickness (cm) with body condition score (BCS) from data collected in Holsteins at the Lake Wheeler dairy field lab (LWD) (Raleigh, NC, 2017) (n=29) and the NCDA Piedmont research station (PRS) (Salisbury, NC, 2021) (n=53).

Lake Wheeler Dairy field lab 2017	*BCS*	RF[1] (cm)	BF[1] (cm)	SE	P
2.75		0.30	0.30	0.09	<.01
3		0.43	0.44	0.06	<.01
3.25		0.58	0.48	0.06	<.01
3.5		0.65	0.57	0.05	<.01
3.75		0.70	0.70	0.06	<.01
NCDA&CS Piedmont research station 2021	*BCS*				
2.75		0.20	0.20	0.06	<.01
3		0.35	0.30	0.06	<.01
3.25		0.39	0.25	0.06	<.01
3.5		0.41	0.35	0.05	<.01
3.75		0.45	0.41	0.06	<.01

[1]RF= rump fat (sacral area, between hook and pin bones), BF= Back fat (between 12th and 13th rib), BCS=body condition score

Table 3.1. Correlation (r) between body measurements[1], days in milk (DIM), non-esterified fatty acid concentration (NEFA), and caliper scores using two widths[2] at two locations[3] with early lactation Holstein (n=25) and Jersey (n=15) cows at the Lake Wheeler dairy field lab (Raleigh, NC, 2020).

	DIM	11.6W	11.6T	16.5W	16.5T	NEFA
BCS[1]	**-0.38**	**0.24**	**0.32**	**0.36**	**0.21**	**-0.25**
P	<.0001	<.0001	<.0001	<.0001	<.0001	0.0046
RF[1]	**-0.49**	**0.27**	**0.33**	**0.39**	**0.27**	**-0.10**
P	<.0001	0.0002	<.0001	<.0001	0.0002	0.1558
BF[1]	**-0.44**	**0.32**	**0.40**	**0.43**	**0.30**	**-0.09**
P	<.0001	<.0001	<.0001	<.0001	<.0001	0.2241
BW[1]	**-0.001**	**0.006**	**0.23**	**0.14**	**0.40**	**0.33**
P	0.991	0.948	0.0059	0.1036	<.0001	0.0001

[1]body measurements= body condition score (BCS), body weight (BW), rump fat (RF) and back fat (BF) thickness
[2] Caliper widths= 11.6 and 16.5 cm
[3] Locations= Withers (W), and Tailhead (T)

Table 3.2. Least-squares means for caliper measurements using two widths[1] at two locations[2] by breed and body condition score (BCS) category[3] with early lactation Holsteins (n=25) and Jerseys (n=15) from the Lake Wheeler dairy field lab (LWD) (Raleigh, NC, 2020).

	Breed				**BCS Category**				
Caliper	Holstein	Jersey	SE	*P*<	Small[3] (>3)	Medium[3] (3-3.25)	Large[3] (>3.25)	SE	*P*<
11.6W	38.99	36.85	0.87	0.04	37.38	37.80	38.61	0.88	0.47
11.6T	36.65	36.44	0.72	0.80	35.64	36.37	37.63	0.60	<0.01
16.5W	30.07	26.94	0.79	0.01	27.94	28.06	29.51	0.73	0.01
16.5T	29.34	27.55	0.79	0.07	27.97	28.25	29.11	0.63	0.11

[1]Caliper widths= 11.6 and 16.5 cm
[2] Locations= Withers (W) and tailhead (T)
[3]BCS categories= Small (BCS less than 3), Medium (BCS of 3 to 3.25), and Large (BCS greater than 3.25).

Table 3.3. Least-squares means of milk yield and components[1] and estimated intake data[2] in early lactation Holstein (n=25) and Jersey (n=15) cows at the Lake Wheeler dairy field lab (Raleigh, NC, 2020).

	Holsteins	**Jerseys**	**SE**	**P**
Milk Yield (kg)	41.28	28.57	0.89	<.0001
Energy Corrected Milk (ECM) (kg)	46.32	28.74	1.40	<.0001
Fat, %	4.25	4.93	0.13	<.0001
Fat Yield (kg)	1.75	1.42	0.04	<.0001
Protein, %	3.12	3.58	0.06	<.0001
Protein Yield (kg)	1.28	1.01	0.02	<.0001
Estimated DMI (kg)	24.31	21.8	0.31	<.0001
Estimated NE (Mcal)	53.56	43.74	0.57	<.0001
Estimated CP Intake (kg)	3.65	4.05	0.04	<.0001
Estimated Fat Intake (kg)	2.09	1.50	0.01	<.0001

[1]Milk components= fat and protein
[2]Intake data= estimated dry matter intake (DMI), estimated net energy (NE), estimated crude protein intake (CP), and estimated fat intake.

Table 3.4. Ration formulations for the Holstein and Jersey herds at the Lake Wheeler dairy field lab (Raleigh, NC, 2020)

Ingredient (lbs.)	Holstein	Jersey
Citrus pulp	2.73	2.55
Soybean hulls	4.09	3.41
Ground corn	6.16	7.04
Soybean meal, 48%	6.41	6.41
Megalac/fusion	0	0.49
Jordan trace pack	0.13	0.11
Limestone	0.49	0.54
Salt	0.13	0.11
Mag oxide	0.12	0.12
Dynamate	0.13	0.15
biotin 640	0	0.03
rumensin 90	0.004	0.004

Table 3.4 (continued)

yeasac 1026	0	0.02
Vit E 20.000	0	0.01
Sodium sesquicarbonate	0.49	0.49
OmnigenAF	0	0.12
celemanax	0	0.03
Biomos	0	0.02
ALIMET	0.04	0.03
AJI Pro L	0.02	0.02
Mepron	0.02	0.02
monodicalphos	0.19	0.17
Bovamine	0	0.004
Popionic acid	0.05	0.05
clarify 67%	0.02	0.02
Wheat Straw	1.34	1.07
Whole Cottonseed	2.76	2.76
Corn silage	19.95	14.49
NCSU dairy premix	0.20	0
Nutrients	**Holstein**	**Jersey**
NEI (Mcal/lb)	0.76	0.78
CP (%DM)	15.83	16.77
ADF (%DM)	22.26	20.43
NDF (%DM)	33.60	30.88
NFC (%DM)	38.20	38.77
RUP (%CP)	32.97	33.38
RDP (%CP)	67.03	66.62
Fat (%DM)	3.91	4.01
Met (%MP)	2.59	2.56
Lys (%MP)	7.66	7.53
Lys/Met Ratio	2.96	2.95
Ash (%DM)	8.47	9.58
Ca (%DM)	0.87	1.01
P (%DM)	0.38	0.40
Mg (%DM)	0.45	0.48
K (%DM)	0.94	0.97
Na (%DM)	0.47	0.51
Cl (%DM)	0.32	0.30
S (%DM)	0.25	0.28
Co (ppm)	0.25	0.27
Cu (ppm)	18.36	17.69
Fe (ppm)	217.50	203.19
I (ppm)	0.77	0.74
Mn (ppm)	43.11	41.50

Table 3.4 (continued)

Zn (ppm)	71.80	69.12
Se (ppm)	0.31	0.31
VitA (kIU/lb)	2.20	2.10
VitD(kIU/lb)	0.29	0.26
VitE (IU/lb)	14.70	18.89

FIGURES

Figure 1.1: Energy balance curve in dairy cows. (Iwersen et al., 2015)

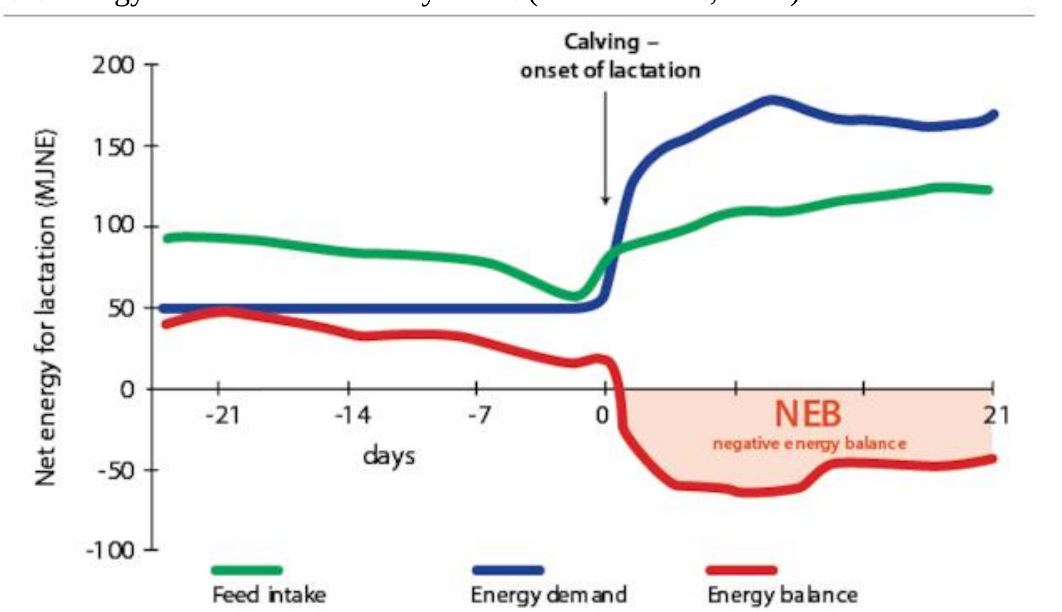


Figure 1.2: An example prototype BCS caliper with adjustable widths to 11.6, 16.5, 21.5, and 26.7 centimeters (Knauer and Baitinger, 2015).

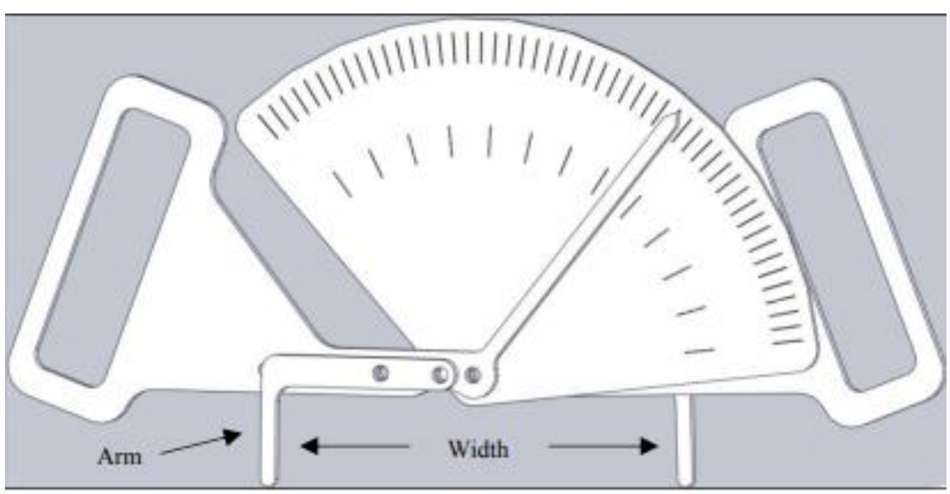
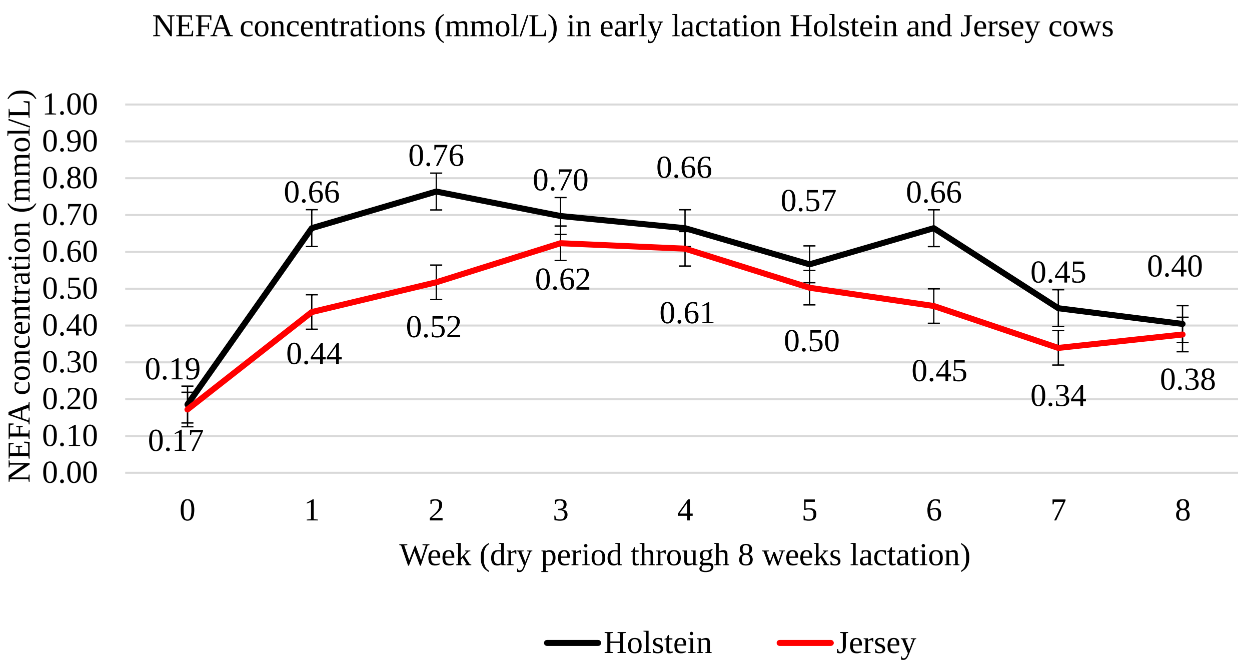
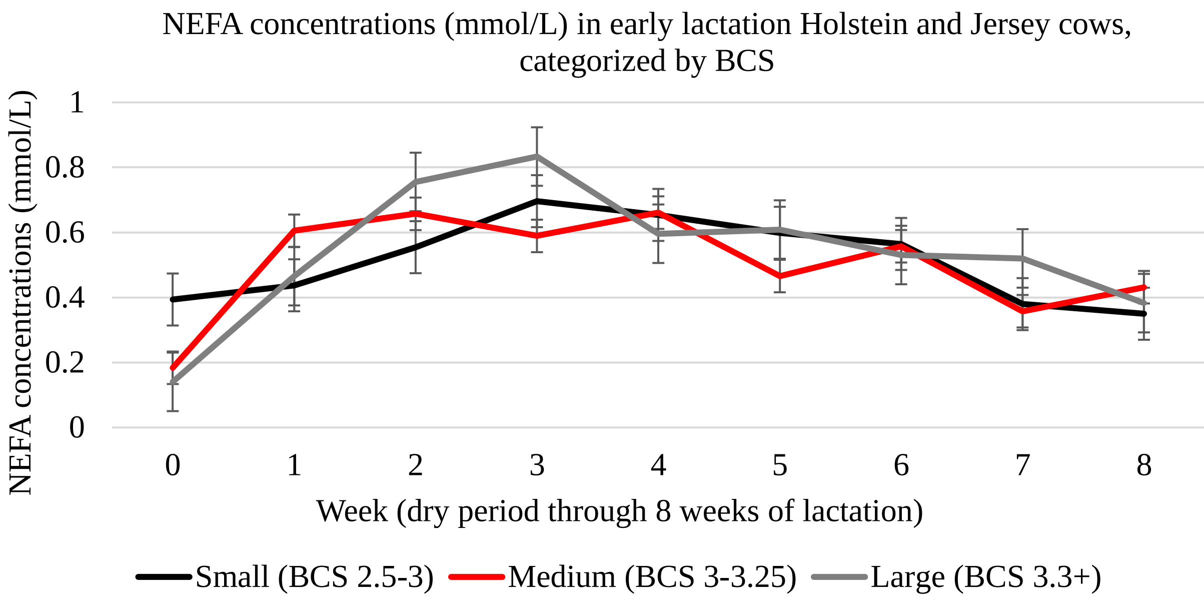


Figure 3.1. Least-squares means of average NEFA concentrations (mmol/L) across the first 8 weeks of lactation in Holstein (n=25) and Jersey (n=15) at the Lake Wheeler Dairy field lab.



Lsmeans, (SAS v. 9.4, Cary, NC). P=0.13.

Figure 3.2: Least- squares means of NEFA concentrations (mmol/L) categorized weekly by body condition score (BCS) across the first 8 weeks of lactation in Holstein (n=25) and Jersey (n=15) at the Lake Wheeler Dairy field lab.



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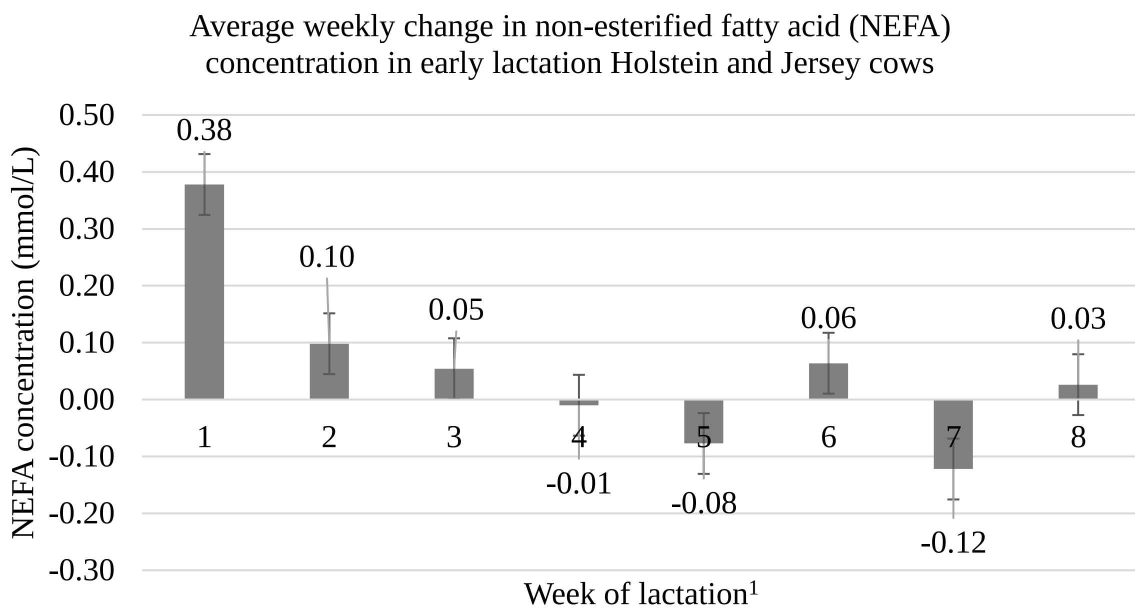
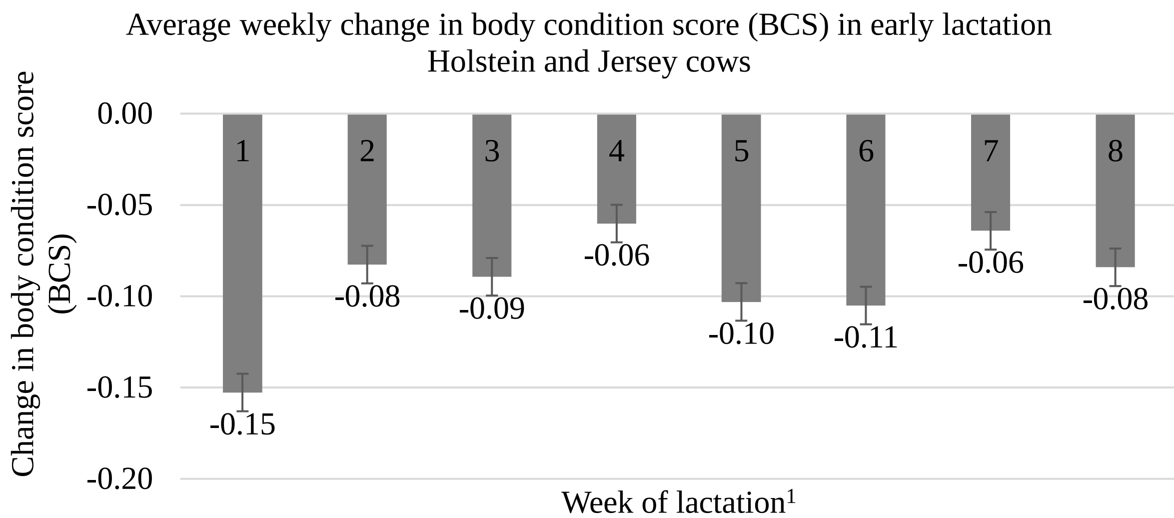
Lsmeans, (SAS v. 9.4, Cary, NC). P< 0.01

Figure 3.3: Least-squares means of average weekly change in NEFA concentrations (mmol/L) across the first 8 weeks of lactation in Holstein (n=25) and Jersey (n=15) at the Lake Wheeler Dairy field lab.



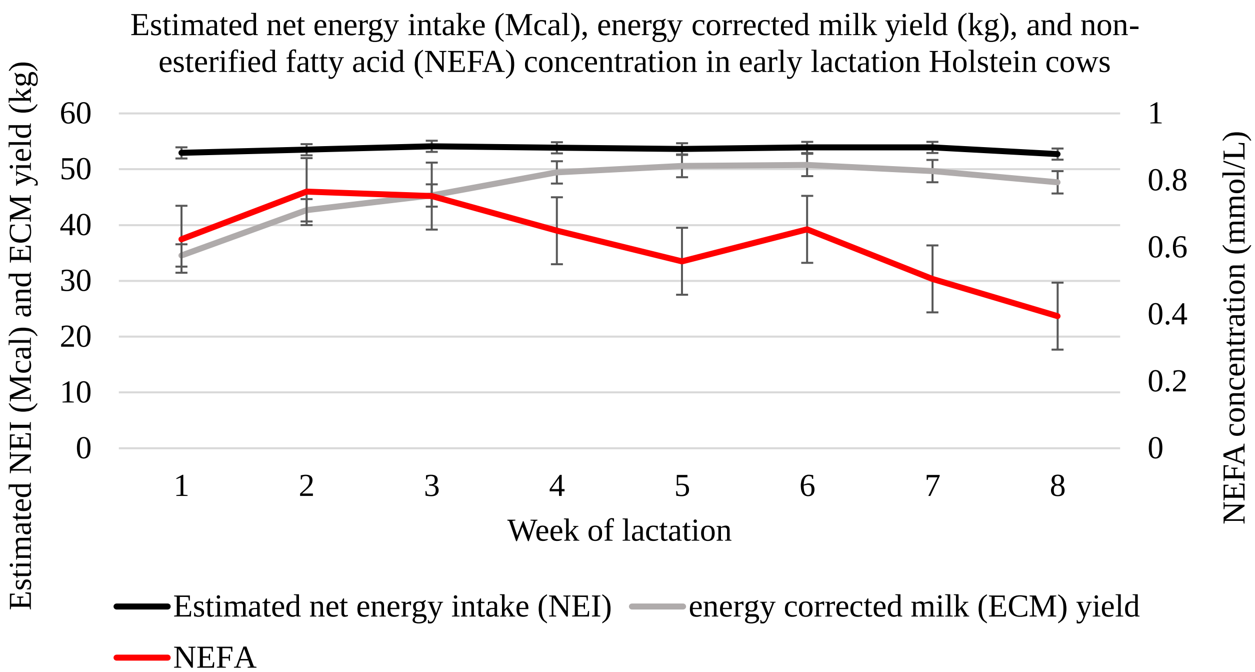
[1]1= week 2- week 1, etc. Lsmeans, (SAS v. 9.4, Cary, NC). P< 0.15.

Figure 3.4. Least-squares means of average weekly change in body condition score (BCS) across the first 8 weeks of lactation in Holstein (n=25) and Jersey (n=15) at the Lake Wheeler Dairy field lab.



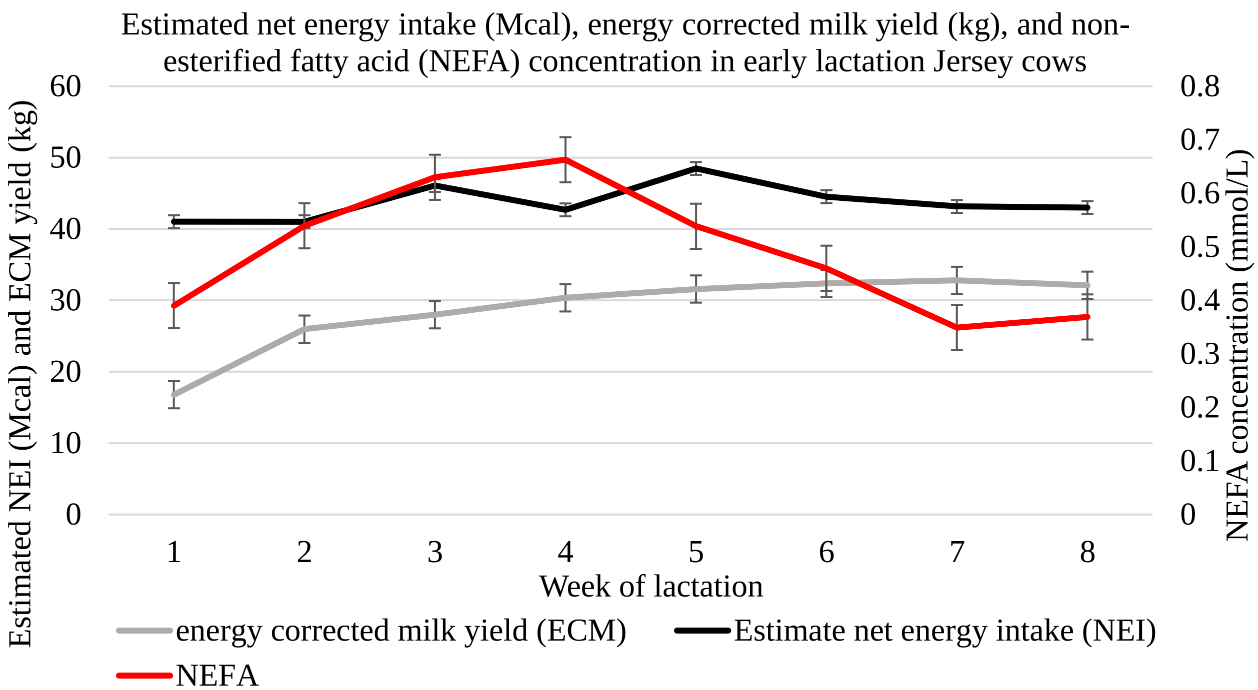
[1]1= week 2- week 1, etc. Lsmeans, (SAS v. 9.4, Cary, NC). P=0.53.

Figure 3.5. Least-squares means of estimated NEI (Mcal), energy corrected milk yield (ECM) and non-esterified fatty acid (NEFA) concentrations (mmol/L) in early lactation Holstein (n=25) cows at the Lake Wheeler Dairy field lab (Raleigh, NC, 2020).



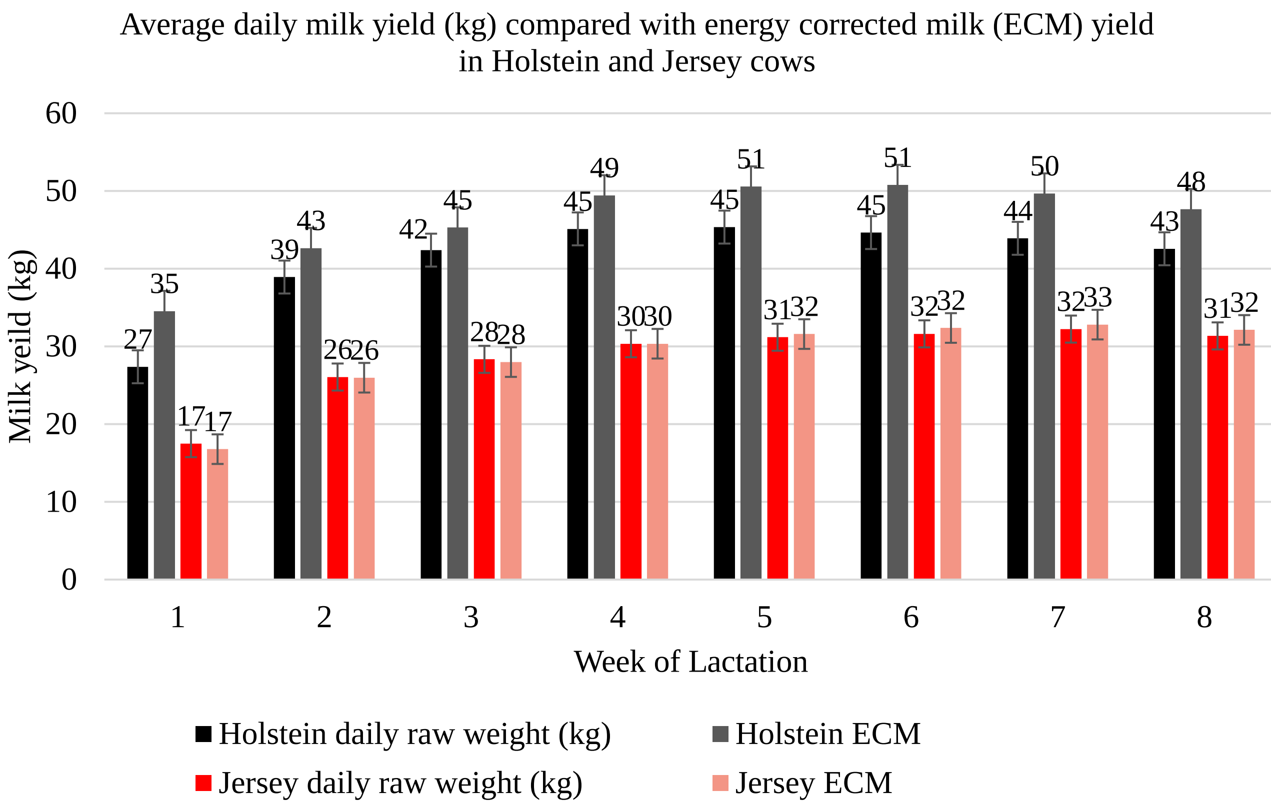
Lsmeans, (SAS v. 9.4, Cary, NC). NEI p=0.17, NEFA p=0.11.

Figure 3.6. Least-squares means of estimated NEI (Mcal), energy corrected milk (ECM) and non-esterified fatty acid (NEFA) concentrations (mmol/L) in early lactation Jersey (n=15) cows at the Lake Wheeler Dairy field lab (Raleigh, NC, 2020)



Lsmeans, (SAS v. 9.4, Cary, NC). NEI p=0.17, NEFA p=0.11

Figure 3.7. Least-squares means of average daily milk yield (kg) compared with energy corrected milk (ECM) yield across the first 8 weeks of lactation in Holstein (n=25) and Jersey (n=15) at the Lake Wheeler Dairy field lab.



Lsmeans, (SAS v. 9.4, Cary, NC), P=0.95.