THE EFFECTS OF ESTRUS ON DRY MATTER INTAKE AND FEEDING BEHAVIOR IN

BEEF HEIFERS OF DIVERGENT SIZES

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Title

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ABSTRACT

The biological process of estrus in cattle is known to initiate behavioral responses as a result of fluctuations in hormones, which may alter time budgeted for feeding. The current study aimed to quantify these variations that may exist in feed intake and behaviors in the days pre and post estrus. For this study, 517 estrous cycles in crossbred beef heifers of divergent sizes were used to analyze the impact of estrus behavior on feeding behavior with or without the presence of a bull. There were few differences in estrus behavior among heifers of divergent sizes, however the presence of a bull influenced all estrus activity parameters. Our findings indicate that feed intake and behaviors were sharply decreased on the day of estrus, but returned to baseline levels the following day. Thus, decreases in feed intake and behavior may serve as an additional tool indicating the onset of estrus.

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DEDICATION

To Brodie and Emily, never stop challenging yourself.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
AA	Aberdeen Angus
ADG	Average Daily Gain
BIF	Beef Improvement Federation
Ca	Calcium
СР	Crude protein
d	Day
DM	Dry matter
EE	Ether extract
g	Grams
h	Hours
ID	Identification
IEI	Inter estrus interval
kg	Kilogram
m	Minutes
mm	Millimeter
MLG	Moderate to large
N	Nitrogen
Р	Phosphorus
RA	Red Angus
s	Seconds
SAS	Statistical analysis system
SE	Standard Error
SMD	Small to moderate

TMR	
TRT	Treatment

CHAPTER 1. INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Reproductive efficiency in cattle requires accurate detection of estrus by either a trained AI technician or herd bull and proper timing of insemination. Although bulls with normal libido may detect the majority of cows and heifers in heat (McDonald et al. 1976), detection by technicians is contingent on the amount of time spent observing cattle as well as the opportunity to witness mounting behavior or other secondary signs of estrus. Standing to be mounted occurs 30-35 hours pre-ovulation (White et al., 2002), but only represents less than 1% of the estrus period (Senger, 1994). It is during this time frame that technicians must identify these animals and breed them accordingly. Other secondary signs of estrus may include hyperactivity, vocalization, and general restlessness (Lovendahl et al., 2010).

As females near estrus, there is an increase in number of steps they take, with primiparous females displaying a higher number of steps compared with multiparous females (Roelofs et al. 2005). In addition, the intensity of steps increases when three or more females are displaying behavioral estrus (Roelofs et al. 2005). Although these behavioral cues can be helpful in detecting estrus using visual observation, supplemental technology devices can aid in estrus detection success by providing continual monitoring of activity in the absence of the observer.

Estrus detection aids have been developed to supplement visual detection of estrus, as the improper detection of estrus has been reported to cost the dairy industry up to 300 million dollars annually (Senger, 1994; Roelofs et al. 2010). These detection aids, such as the electronic activity monitoring device Heatwatch (DDx Inc., Denver, CO), have proven to have an increased efficiency of accurately detecting the onset of estrus by 37 percent when compared with visual detection alone (Stevenson et al. 1996). Moreover, when used to detect estrus in peripubertal

beef heifers, the Heatwatch system tended to detect estrus in heifers with lower reproductive tract scores, shorter duration of estrus, and fewer standing events (Stevenson et al. 1996).

There are many variables that may affect an animal's daily intake, such as temperature (Mader, 2014), breed (Schenkel et al. 2004), frame size (Fox et al. 1988), and temperament (Bruno et al. 2016). Another variable that can impact intake is behavior associated with standing heat, which can reduce dry matter intake (DMI; Reith et al., 2014; Pahl et al., 2015). The use of electronic feeders such as the Insenctec system (Chapinal et al. 2007, Montanholi et al. 2010) can be used for measuring DMI and feeding behavior. These systems allow collection of information such as visits to troughs, meals, and time spent eating on an individual animal basis. By combining electronic estrus detection and feeding technologies, new avenues of research have assessed the effects of day of estrus on DMI and feeding behaviors (Zebari et al. 2018). The results of this research suggest that data collected from these systems may serve as an additional tool in detecting early onset of estrus events.

In this literature review, section 1 will discuss the overall topic of the mechanisms influencing the estrus cycle, estrus detection aids, estrus behavior, and the impact of bulls on estrus behavior. Section 2 will cover the drivers of feed intake as well as feeding behavior, and section 3 will combine these two topics in a discussion regarding the relationship between estrous activity and feed intake and behavior.

Section 1: Estrus

The estrus cycle in the bovine

Estrus, the external and observable indication of sexual receptivity (Roelofs et al., 2010), occurs approximately every 21 days in the bovine (Armstrong and Hansel, 1959; Pierson and Ginther, 1984). Estrous cycles in heifers are first initiated by puberty in the female, or the

attainment of reproductive competency as a result of maturation in the endocrine profiles and morphological structure of the reproductive system (Day and Anderson, 1998). Age at puberty is an important factor in the prospective longevity of a heifer to maintain productivity in a cow-calf production system (Ferrell, 1982).

In a study conducted by Wehrman et al. (1996), they found 25% of beef heifers exhibited precocious puberty, or puberty prior to 300 days of age. It is highly desired that beef heifers attain puberty by 12-15 months of age (Schillo et al. 1992) in order to calve by two years of age (Wehrman et al. 1996). This process of attaining puberty is contingent on genetic variation, heterosis, and size (Martin et al. 1992), as well as nutritional status, and environmental influences (Schillo et al. 1992).

Puberty in heifers may be immediately followed by a "silent heat" prior to a detectable estrus (Swanson et al. 1972). Silent heat is a phenomenon where ovulation occurs without behavioral estrus as a result of inadequate amounts of endogenous estrogen available to signal behavioral changes (Kyle et al. 1992). Although behavioral estrus may not be detectable in these cycles, they typically result in elevations of progesterone and regression of a luteal structure (Day et al.1998). Thus, functional endocrinology competency is achieved in succeeding cycles (Staigmiller et al. 1993).

Heifers are born with their lifetime supply of follicles, which are stored in their ovaries and established during embryonic development (Hansel and Convoy, 1983). Through a process of follicular waves and atresia of non-selected follicles, a dominant, or Graafian follicle containing an ovum will remain (Senger, 2012). As the Graafian follicle undergoes maturation and progressively produces increasing amounts of estradiol, a threshold of circulating amounts of estradiol in the blood is reached in the absence of progesterone from a corpus luteum, causing

behavioral estrus and a subsequent surge of luteinizing hormone (Roelofs et al., 2010). It is this surge of luteinizing hormone that will start the cascade of signals that ultimately culminate in ovulation (Lyimo et al. 2000). Ovulation, the process whereby the oocyte is released from the ovary (Richards, 2005) takes place approximately 30 hours after the onset of estrus (Roelofs et al. 2005), but can vary from female to female depending on ovarian function, parity (Saumande et al. 2005), and intensity and duration of estrus activity (Hockey et al. 2010). In a study conducted by White et al. (2002), utilizing the Heatwatch estrus detection system, the duration of estrus observed in crossbred cows ranged from 0.5 to 36.3 hours, with 70% of cows in estrus for 11-20 hours. These findings are similar to those of Stevenson et al. (1996), in which beef heifers had an average estrus duration of 14 ± 0.8 hours with a range from 2.6 to 26.2 hours.

Estrus behavior

The duration of estrus is directly correlated with the number of times a cow is mounted (r=0.32; P<0.01; White et al. 2002). Beef cows in estrus for less than 15 hours received approximately 34.3 ± 4.0 mounts; whereas cows in estrus for greater than 15 hours received 58.4 ± 4.0 mounts. Additionally, mounting occurred more frequently in the morning between 06:01 and 12:00 (3.2 ± 0.2 mounts/hour) than any other time of day (2.1 ± 0.2 mounts/hour; White et al. 2002). Hurnick et al. (1975) found that dairy cows were most active during the nocturnal period from 20:00 and 03:00, indicating an increase in behavioral estrous as human presence decreased.

Duration of estrus and mounting behavior is also more prevalent on dirt surfaces in comparison to concrete surfaces. Vailes and Britt (1990) found that duration increased from 9.4 to 13.8 hours and mount intensity increased from 3.2 to 7 when non-lactating Holstein cows were housed on dirt floors in comparison to concrete floors. There are many other variables that

can influence mounting behavior. Studies conducted on beef and dairy cows, and heifers demonstrate that there is an effect of number of females in estrous on corresponding behavior. In a study conducted by Hurnick et al. (1975), mounts in dairy cows increased from approximately 11 mounts per estrus when one cow was in heat to 53 mounts per estrus when three cows were in heat, and 50 mounts per estrus when four or more cows were in heat. Similarly, Floyd et al. (2009) found, in beef cows, that when only one cow was in estrus, $11 \pm$ 6.2 mounts were observed during 11.6 hours; however, when seven or more cows were in estrus at the same time 50.4 ± 3.2 mounts were observed during 17.3 hours.

Additionally, an increased number of Holstein-Friesian dairy cows displaying estrous behavior simultaneously can increase the probability of cows forming sexually active groups, and thus displaying a mixture of secondary estrus behaviors (Roelofs et al. 2005). Further secondary signs of estrus can include sniffing, chin resting, licking, rubbing, head butting, and an increase in steps (Roelofs et al. 2010). Studies have attempted to quantify these secondary signs of estrus utilizing a point system. When a cow displays an estrus symptom, a corresponding set number of points are assigned, and if 100 points is reached within a 24-hour period, the cow is considered to be in estrus (Van Eerdenburg et al. 1996). The use of this point system resulted in a detection rate of 74% (Van Eerdenburg et al. 1996). Compared with primiparous, multiparous Holstein cows displayed fewer secondary signs of estrus, such as repeated mounting, vaginal mucus, and uterine tone (Madureira et al. 2015). When monitoring walking activity during estrus using pedometers, F. Lopez-Gatius, (2005) found that activity decreased linearly in Holstein-Friesian dairy cows as they increased in lactation number.

Mounting behavior, however, has been shown to be intensified in cows of increased parity. In a study utilizing dairy cattle of divergent ages, it was found that heifers received 5.5

mounts per hour; whereas older cows received 7.9 mounts per hour on average (Gwazdauskas et al. 1983). These findings are in agreement with De Silva et al. (1981), in which older dairy cows exhibited more mounting activity than their younger counterparts.

Season of the year can also play a role in the duration and intensity of estrus expression. In a study by White et al. (2002) utilizing beef cows, duration of estrus was longer in summer than in winter or spring; however, cows were mounted more in winter than in summer or spring. Also, the interval between mounts was longer in summer than in winter or spring. On the contrary, Floyd et al. (2009) found that non-lactating beef cows had a longer duration of estrus in winter than in summer, but in agreement with White et al (2002), found a tendency for more mounts in winter. A decrease in mounting behavior and duration between mounts in the summer months may be due to an animal's unwillingness to participate in physical activity during high temperatures. Differences among studies in terms of duration of estrus could be related to variations in climates, breed, or management practices.

In conjunction with the outward visual signs of estrus, there are also important non-visual effects of estrus that take place simultaneously. These non-visual effects of estrus include changes in the reproductive tract including increased blood flow, genital swelling, increased mucosal secretion, and elevated myometrial tone as a result of increased estrogen (Senger, 2012). This highly secretory mucus will aid in sperm transport to the site of fertilization. The mucus changes from a highly viscous hostile environment to a less viscous, favorable environment approximately 8-12 hours after the onset of estrus (Roelofs et al. 2010). An increase in white blood cells in the uterine mucosa prior to estrus will aid in phagocytosis of dead sperm, bacteria, and cellular debris (Roelofs 2010).

The impact of bulls on estrus behavior

The presence of a mature bull results in behavioral and physiological effects on estrus expression. Tauk and Berardinelli (2007) found beef cows when exposed to bull urine tended to have shorter intervals to estrus as compared with cows exposed to steer urine, with greater AI pregnancy rates observed in cows exposed to bulls or bull urine. Data is inconsistent on whether the physiological process of puberty in the female can by induced by the presence of a bull. In studies conducted by Macmillan et al. (1979) and Wehrman et al. (1996), they concluded the presence of a bull did not initiate early puberty. Contrarily, Roberson et al. (1991) found that exposure of heifers to bulls increased the proportion of pubertal heifers by 14 months of age. These results are consistent with findings by Izard and Vandenbergh (1982), in which water (CON) or bull urine (TRT) was administered via intranasal and intraorally once weekly for 8 weeks resulting in earlier onset of puberty in treated heifers during the experiment at a rate of 67% versus 32%.

In a study conducted by Hornbuckle et al. (1995), beef cows grazing pasture were randomly allocated to either be exposed to a teaser bull or not 16 days post calving for a duration of 46 days. Cows pastured with a teaser bull had increased concentrations of progesterone on day 46 of the experiment as compared with the control group not exposed to a bull. Additionally, the cows exposed to bulls had increased cyclic activity (75% cycling) versus those not exposed to a bull (25% cycling). Similarly, Zalesky et al. (1984) analyzed concentrations of progesterone in blood serum from crossbred beef cows three to 85 days after calving and observed that the onset of estrus occurred three weeks earlier in cows exposed to bulls compared with cows not exposed to bulls. This is an important factor in reproductive efficiency, as the failure to rebreed after calving can prompt management decisions such as culling from the herd.

The libido of the bull, or willingness to seek out and mate with the female, is critical in their ability to detect and successfully breed cows. Bulls with normal libido have been reported to detect up to 100% of potential estrous cycles when placed in pens with 20 heifers for up to 2 months (McDonald et al. 1976). Additionally, when placed in groups of synchronized females, beef bulls averaged 55 services, ranging from 14 to 101 mounts within a 30-hour observation period (Chenoweth, 1983).

Bulls are most active at detecting estrus in the early morning hours, at a time when cows were most receptive to mounting behavior (Orihuela, 2000). However, in a study conducted by Orihuela et al. (1983), they found mounting between cows occurred mostly at night, whereas mounting behavior by the bull occurred both day and night. Although it is not known if differences in the distribution of mounts has a physiological or socially induced cause, it has been shown that this distribution is affected by the presence of the bull. The social ranking of bulls grouped together in pastures can influence the number of cows serviced per bull. In a study by Blockey (1979), bulls in mixed age groups had differences in number of cows serviced, with the two oldest of five bulls in the group servicing all cows and the three youngest of the five bulls servicing none.

Similar to these dominance effects, in the presence of a bull, female to female mounting declines, as does the participation of females in sexually active groups (Kilgour et al. 1977; Orihuela, 2000). Additionally, within 30 minutes of the herd bull lying down, cows reformed the sexually active group and proceeded with mounting activity (Kilgour et al. 1977). Bulls are attracted to these groups and the visible display of mounting activity serves as a visual cue that arouses their sexual interest (Chenoweth, 1983).

Bulls rely on not only their visual senses but olfactory stimuli as well. When evaluated for preference of olfactory or visual stimulus, bulls spent more time observing and occupying an adjacent space containing heifers displaying mounting behavior and less time observing and occupying a space near a heifer that was unable to be mounted by means of a physical barrier but in estrus or a heifer in diestrus. This study indicates that olfactory stimuli alone is insufficient and that bulls use visual observation as a key indicator of estrus (Geary and Reeves, 1992). *Estrus detection aids*

Although visual observation continues to be the preferred method of estrus detection by trained technicians, its efficiency ranges from only 50-70% (Rorie et al. 2002). In a study conducted by White et al. (2002), twice daily visual observation failed to detect 29% of confirmed estrous events that were determined via ultrasonography observation of dominant follicle disappearance. Dransfield et al. (1998) found that 24% of lactating dairy cows express low intensity (less than 1.5 standing events) and short duration (less than 7 hours) of estrus; therefore, even with twice daily observations these cows may not be observed displaying estrus.

Detection of estrus by visual observation alone remains problematic, as standing to be mounted represents less than 1% of the estrous period (Senger, 1994). Additionally, recent studies in dairy cattle show that standing to be mounted when one cow was in heat was only recorded in 65% of estrus cycles as determined by visual observation when cows were housed in straw covered stalls or on pasture, but increased to 78% when at least two cows were in heat (Cutullic et al. 2009), indicating a need to improve efficiency and accuracy in detecting estrus. Estrus detection aids have been developed to supplement visual detection of estrus, as the improper detection of estrus has been reported to cost the dairy industry up to 300 million dollars annually (Senger, 1994; Roelofs et al. 2010).

Various estrus detection aids exist including pedometers, mount patches, pressure sensing devices, and more recently ear tag-based accelerometers. Pedometers can be attached to animals on collars or as leg mounted devices. These devices quantify movement through the use of motion switches, and are most practical for dairy operations because of their limited transmission range (Rorie et al. 2002). In a study conducted by Madureira et al. (2015), the data derived from leg mounted pedometers on Holsteins correctly predicted 85.5% of estrus episodes, or 849 of 993 events.

Mount patches have proven to be a useful tool in both the dairy and beef industry. These one-time use patches are stuck to the tail head of the cow or heifer, and are activated by the friction of a mounting herd mate. These patches will scratch off from a silver color to a bright color (pink, orange, red) indicating an animal has been mounted. Patch scores (zero to two) can be assigned based on the degree of activation (not activated to 100% activated), aiding AI technicians in making management decisions on timing of insemination. For example, in a study conducted in dairy cows, scored Estrotect patches were used as an estrus detection aid and resulted in a 75% efficiency rate in detecting cows in estrus and 94% accuracy rate in detecting ovulation risk, or cows that subsequently ovulated as determined by transrectal ovarian ultrasonography (Sauls et al. 2017). Estrotect patches have also been utilized in studies using beef cows, in which patch scores were assigned in conjunction with an AI protocol and used as a tool in establishing breeding decisions. By delaying AI in cows with inactivated patches, greater pregnancy rates were achieved (Hill et al. 2016).

Electronic pressure sensing devices, such as the Heatwatch system, are comprised of a battery-operated radio-wave transmitter that is enclosed in a mesh pouch and attached to the tailhead of the cow. When activated by the weight of a mounting herd mate, the device will

transmit information to a computer, including date, time, and duration of the mount (Dransfield et al. 1998). This system uses mount activity information to categorize animals as either suspected of being in heat or in standing heat, defining estrus as three mounts within four hours with a duration of two seconds or greater (Rorie et al. 2002). This system has also been used in both dairy and beef experimental trials as an estrus detection aid and to validate its accuracy and efficiency.

Utilizing 255 Holstein cows housed in free stall barns during the stress of summer heat, the Heatwatch system was only 48% efficient in detecting estrus (Peralta et al. 2005). This low efficiency is likely due to the extreme temperatures at the time, resulting in physical lethargy in these cows and the inability of the system to capture three mounts within a 4-hour period. Conversely, when used to detect estrus in peripubertal beef heifers, the Heatwatch system tended to detect estrus in heifers with lower reproductive tract scores, and was successful in identifying heifers with shorter duration of estrus and fewer standing events (Stevenson et al. 1996), resulting in a 37% increase (100% overall) in estrus detection compared with visual observation (73%) alone.

Some have noted that the Heatwatch system can be labor intensive, had a high incidence of transmitter failure, and required frequent patch maintenance (Srivastava et al. 2016). When used to detect estrus in dairy heifers, 25% of the mesh patches required periodic regluing within the first 30-45 days of being implemented (Rorie et al. 2002). Likewise, when used in Brahman influenced beef cows, 36 of 102 cows (35%) lost their patches within the first 30 days of estrus detection (Flores et al. 2006).

Senger (1994) outlined the ideal estrus detection system to be accurate, provide continuous data, operate for the lifetime of the cow, and have minimized or no labor

requirements. The most recent development in estrous detection technology consists of accelerometers and RFID technology embedded in ear tags. Systems available to producers include the Allflex Sensehub beef tag system (Allflex livestock intelligence, Madison WI) and the Cowmanager system (Agis Automatisering, Harmelen, the Netherlands).

The Cowmanager system, developed primarily for the dairy industry, consists of a sensor that can be fitted around an electronic ID. This sensor collects information on activity, eating behavior, rumination, and temperature. These variables, with the exception of temperature, have been validated independently against visual observation by a trained technician (Bikker et al. 2014) and in detecting estrus events in dairy cattle that were also fitted with pedometers and collar mounted activity monitors (Dolecheck et al. 2015). The Cowmanager system was more accurate in detection of estrus (90.8%) in comparison to neck (48.4%) and leg mounted pedometers (81.7%), as well as three other systems, when evaluated in a precision dairy technology monitoring study when compared against progesterone levels and ovarian ultrasound (Mayo et al. 2018).

Section 2: Feed intake

Drivers of intake

There are many factors that influence the amount of feed consumed by cattle. Environmental factors, such as temperature and exposure to shade, biological factors such as breed, sex, size, age and temperament, as well as management factors including stocking rate and composition of the diet may all influence intake.

Temperature fluctuations, particularly in the summer months can be challenging for cattle consuming high energy diets, causing heat stress or even death (Mader, 2014). Additionally, when exposed to heat-stressed conditions, cattle consume less daily DM (St-Pierre et al. 2003).

The use of shade provisions can have beneficial effects on feed intake in feedlot cattle. In a study by Hagenmaier et al. (2016), cattle provided shade had greater DMI in comparison to those without shade provisions. *Bos Taurus* heifers, when exposed to prolonged heat and humidity, had an increased heart rate and core body temperature in addition to reduced feed intake. However, *Bos indicus* heifers did not have altered feed intake when exposed to similar conditions (Beatty et al. 2006).

These findings suggest that breed can also play a role in feed intake variation. When six breeds of purebred bulls of similar age and size were evaluated for intake across 140 days, differences were found in daily intake, resulting in variances in growth rate and body composition traits (Schenkel et al. 2004). Differences in daily intake can also be dependent on sex of the animal. Heifers ate more than steers when fed the same diet across 70 days in two years (Elzo et al. 2009).

Many studies have addressed correlations between an animal's size and feed efficiency. The Beef Improvement Federation has established criteria for assessing the frame size of beef cattle. These criteria can be used to determine nutrient requirements of all classes of cattle under differing feed and management strategies. Dry Matter Intake is directly correlated with frame size, with increasing frame scores aligned with increases in intake (Fox et al. 1988). Likewise, Walker et al. (2015) utilized crossbred beef cows of divergent sizes to determine differences in DMI during two stages of production, and observed that cows that were larger consumed more feed during both stages of production.

Age of animal can also impact its feed intake. Relative to size, younger animals will eat less as a result of their body capacity. Interestingly, when DMI was measured in dairy cows of varying ages in the days immediately pre-calving, younger cows consumed greater DMI than

older cows, indicating that younger cows were not as stressed by the onset of parturition as older cows were (Marquardt et al. 1977). The difference in DMI may also be due to the higher energy maintenance requirements of younger animals, driving them to consume more versus their older counterparts (NRC. 2001).

The temperament, or disposition of an animal can play key roles in influencing many production parameters throughout its productive lifetime, including the amount of feed consumed on a daily basis when housed in group scenarios. Beef heifers with a calm temperament, determined by exit velocity from a working facility, had a greater ADG and a greater daily DMI in comparison to heifers with an excitable temperament (Olson et al. 2019). Similarly, Bruno et al. (2016) found that steers, when measured for temperament utilizing both objective and subjective measures, had differences in DMI and ADG across a 58-day feeding period. Steers categorized as slow had a greater DMI and ADG in comparison to steers categorized as fast (Bruno et al. 2016).

Competition among animals at a feed station or bunk can also influence the amount of feed consumed per day. Cattle fed together in a group housing system will develop a social hierarchy, with dominant individuals having advantages in feed consumption over their subordinate counterparts. In a study conducted by Olofsson (1999) utilizing dairy cows, it was observed that antagonistic behaviors indicative of social order hierarchy had an effect on intake, with cows displaced by bunk competition eating less than those not in a group pen.

Management factors, such as the availability of fresh, clean water can cause alterations in feed intake. Water is an essential nutrient that aids cattle daily in digestion, metabolism, temperature regulation, and growth (NRC. 2000). Variations in temperature and season can influence the amount of water that cattle drink, which in turn is regulated also by DMI (Ahlberg

et al. 2018). Proper management of water stations is necessary to ensure fresh water availability, as poor water quality as a result of contamination will reduce intake (Zimmerman, et al. 2002).

Of all the variables mentioned above that may influence feed intake, the composition of the diet offered may be the greatest driver of variation in DMI. Diets are formulated or offered for specific situations, for specific classes of cattle, management practices, stage of lactation, or feed availability. Physical and chemical characteristics of the feed, such as particle size, fiber concentration, fermented ingredients, and ease of starch hydrolysis can all have impacts on DMI (Allen, 2000). Utilizing periparturient dairy cows, McNamara et al. (2003) found that by offering a higher concentrate allocation thereby increasing daily energy density, there was a subsequent increase in DMI. When accessing energy dense diets in feedlot cattle, Krehbiel et al. (2006) observed a correlation (R = 0.631) between a decrease in DMI as metabolizable energy increased. Likewise, there is a threshold limitation in which ruminal starch fermentation may result in a decrease in DMI (Allen, 2000).

Feeding behavior

Variations in the mechanisms controlling feed intake can be further understood with the addition of feeding behavior information (Tolkamp et al. 2000). Feeding behavior data can be collected across a variety of systems such as the Calan gate system (Mazaika et al. 1988, Ferris et al. 2006), Growsafe system (Mendes et al. 2011, Schwartzkopf-Genswein et al. 1999), or the Insentec system (Chapinal et al. 2007, Montanholi et al. 2010). These systems allow collection of information such as visits to troughs, meals, and time spent eating on an individual animal basis. These data can be used to help explain differences in intake among dietary treatments or to predict illness.

A visit can be defined as a singular appearance of an animal to a feeding station. When utilizing a system that is operated by use of radio frequency identification, the system will record the ID of the animal, weight of the trough, and time of day. Upon exit, the system will record these same parameters, therefore calculating quantifiable behavioral data (Chapinal et al. 2007). Visits can vary dramatically from one animal to another, but can also differ depending on feed composition (Rodenhuis et al. 2017), bunk space competition (Olofsson, 1999), or residual feed intake (RFI) treatment groups (Montanholi et al. 2010). RFI is a performance trait that has been used to reflect variation in metabolic processes in an attempt to quantify feed efficiency in cattle. When separated into RFI groups categorized as low, medium, or high, Montanholi et al. (2010) found that high RFI group steers had more visits to Insentec troughs as compared with medium and low RFI steers. This trait, among others, aided in identifying variables that can potentially be used to describe parameters related to beef steer efficiency. Additionally, visit to feeders have been used to assess the functionality of feed systems. In a study using the GrowSafe technology, visits to feeders as determined by the software was highly correlated with visual observation of feed attendance verified through video surveillance (Schwartzkopf-Genswein et al. 1999).

Meals are feeding bouts that are separated by specific intervals of time. These intervals vary throughout the literature from 2 minutes to 40 minutes (Tolkamp et al. 2000). Regardless of the interval, data quantified into meals can be useful in explaining differences found in varying intakes and dietary treatments. In a study examining the influence of dry rolled corn processing and increasing dried corn distillers grains with solubles (DDGS) inclusion on growth performance and feedlot behavior of finishing cattle, Swanson et al. (2014) found that meal size decreased and meal number increased with finer dry roll corn processing and with increasing inclusion rates of DDGS. These data suggest that cattle may have adapted to diets by altering

their feeding behavior due to changes in the ruminal environment including a decrease in ruminal pH. Meals have also been used to describe the interaction between temperament and feeding behavior. Beef heifers described as calm had greater meal duration and consumed meals that were larger in comparison to heifers described as excitable (Olson et al. 2019). This behavioral data can be used to make inferences about performance, efficiency, and can be useful in making management decisions (Olson et al. 2019).

The amount of time an animal spends eating per day can also be a useful feeding behavior variable. This component can be broken down further to describe time per visit, time per meal, or be used to calculate eating rate. Heifers of divergent RFI status (low or high), when acclimated to either a Calan gate system or the Growsafe system, had differences in time spent eating, with low RFI heifers spending less time at the bunk, and eating rates, with low RFI heifers tending to have a slower meal eating rate as compared to heifers classified as high RFI (Hafla et al. 2013). In a study comparing the effect of metabolizable protein (MP) on feeding behavior of finishing steers, there was a difference of time spent eating per day. As the MP content of the diet increased, the time spent eating also increased (Sitorski et al. 2019). In this study, this behavior may help in understanding how feeding behavior is affected by MP inclusion rates in finishing diets, leading to development of new feeding strategies.

Moreover, records of feeding behavior have been used to detect and monitor morbidity in feedlot cattle. By using the Growsafe system to collect individual animal feeding behavior, data revealed that steers identified as morbid spent less time and had less feeding bouts than steers considered to be healthy (Sowell et al. 1999). Similarly, when utilizing feed behavior data collected from feedlot steers, the Growsafe system was able to detect morbidity 4.1 days earlier than experienced pen riders (Quimby et al. 2001). The use of technology in detecting

fluctuations in feeding behavior can be a valuable tool in early detection and prevention of health associated problems (von Keyserlingk and Weary, 2010).

Section 3: The relationship between estrus activity and feed intake/behavior

Decreases in DMI and feeding behavior can be associated with health related issues, and as indicators of estrus. With advancements in feed technology systems that quantify feeding activity, these data can also be used as tools to determine day of estrus in cattle. Feeding behavior, such as amount of time ruminating, declines on the day of estrus. Utilizing a collar mounted rumination sensor, Reith and Hoy (2012) found that on the day of estrus across 265 estrus cycles, rumination time decreased on average by 17%, with primiparous cows having a more pronounced decline in comparison to multiparous cows. Likewise, when utilizing the Insentec system and collar mounted rumination sensors in primiparous and multiparous Holstein cattle, Pahl et al. (2015) found that rumination time decreased by 75 minutes, feeding time decreased by 58 minutes, DMI decreased by 8.75%, and feeding rate increased by 0.016 Kg/min on the day of estrus.

These data proved to be useful additions of early detection of estrus in this study. When examining the effects of estrus on feeding behaviors collected using the Insentec system, Zebari et al. (2018) found that DMI decreased by 12%, visits to feeders were reduced by 11%, and feeding duration was reduced by approximately one hour or 27%. Reith et al. (2014) found similar reductions in DMI in addition to a decrease in water intake and body weights on day of estrus. This study also assessed whether correlations existed between these variables on non-estrus days. It was observed that positive correlations existed between daily DMI and daily water intake, as well as between cow's daily body weight and daily water intake. Interestingly,

the cows in this study did not have a reduction in the amount of concentrate consumed on day of estrus (Reith et al. 2014).

The behavioral patterns of beef heifers in estrus are well established, and systems are available that accurately quantify feed intake and feeding behavior of individual animals. However, data regarding the impacts of estrus and associated behaviors on intake and feeding behavior in beef heifers is lacking. Therefore, the objectives of this study were to evaluate estrus activity among heifers of divergent sizes, determine the extent of differences the presence of a herd bull has on estrus behavior, and to quantify feeding intake and behavior in the 7 days pre and post estrus.

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CHAPTER 2. THE EFFECTS OF ESTRUS ON DRY MATTER INTAKE AND FEEDING BEHAVIOR IN BEEF HEIFERS OF DIVERGENT SIZES

Abstract

Crossbred beef heifers (n = 165) across two years (2016 and 2017) were used to evaluate the impacts of estrus on feed intake and feeding behavior in the days before, during, and after standing estrus. Estrus behaviors were also evaluated with or without the influence of a herd bull. Our hypothesis was that estrus activity would reduce DMI and alter feeding behavior. Heifers were acclimated to the Insentec feed system and were assigned to pen by frame score; small to moderate frame (SMD; frame \leq to 5.50; n = 112) and moderate to large frame (MLG; frame score greater than 5.50; n = 53). On d one of the study, heifers were fitted with Accubreed electronic pressure sensing heat detection devices to monitor estrus behavior. Heifers were fed a total mixed ration containing 78.5% grass hay, 16.5% corn silage, and 5% vitamin and mineral premix for ad libitum intake for 107 days in year 1 and 75 to 106 days in year 2. Heifers in year 2 were received on two different dates (June 6th and July 7th) resulting in differing days on feed. During the evaluation period, feed intake and behavior, and date, time, and duration of individual mount events were monitored. Upon completion of the study, feed intake data were aligned with estrus data for each of the 7 d before and after the first mount of an estrus. Each individual estrus event (n=517) for each heifer was used as experimental unit. Feed intake and behavior data were analyzed for the impact of treatment (SMD or MLG) and year (year 1 or year 2), day relative to estrus (d -7 to 7), and their respective interactions using the mixed procedures of SAS with individual heats within heifer ID used as repeated measures. Estrus data were analyzed for the impact of treatment (SMD or MLG) using the generalized linear model of SAS. Data were considered significant at P < 0.05. MLG heifers had a greater mount duration as compared to

SMD heifers (P=0.03), however there were no other differences among treatment and day relative to estrus for any of the other response variables measured, including mounts per estrus, time mounted, estrus duration, or inter estrus interval. Mounts per estrus, the total time a heifer was mounted, and estrus duration all decreased in the presence of a herd bull ($P \le 0.001$). Contrarily, mount duration increased (P=0.005) as did inter-estrus interval, (P<0.001) when a bull was present in the pen. There was a difference in DMI, DMI per visit, meals, and eating rate $(P \le 0.03)$ on the day of estrus (day 0) when compared with each of the seven days before and after. There was no statistical difference in time per visit among the 15 days of observation per estrus (P=0.15). There was an impact of year and day relative to estrus on time spent eating, DMI per meal, and time spent eating per meal ($P \le 0.008$). Total time spent eating, time spent eating per meal, and DMI per meal were all reduced on the day of estrus (P < 0.001). There was also an interaction of TRT group and day relative to estrus on number of visits to troughs and time spent eating per meal ($P \le 0.05$). Both treatment groups had a reduction (P<0.001) of visits and time spent eating per meal on the day of estrus. We observed that the change in feed intake and behavior relative to estrus is rapid, and returned to baseline levels within one day following standing heat.

Introduction

Reproductive efficiency in cattle is reliant on the success of the ability to conceive in the earliest estrus cycle possible, whether that be post calving or as a first-time heifer. Although bulls with normal libido may detect the majority of cows and heifers in heat (McDonald et al. 1976), the use of visual detection alone by trained technicians can be labor intensive, inefficient (Rorie et al. 2002) and ultimately reduce success in AI programs. There are known behaviors that are indicative of estrus, such as vocalization, hyperactivity (Lovendahl et al. 2010), chin

resting, an increase in steps (Roelofs et al. 2010) and standing to be mounted (Vailes and Britt, 1990, White et al. 2002, Floyd et al 2009). This period of behavioral estrus may last from 30 minutes to 17.7 hours (Flores et al. 2006), but standing to be mounted, indicating receptivity to breeding, accounts for less than 1% of this time frame (Senger, 1994). It is during this very short interval that technicians must visually identify cattle in standing heat and breed them accordingly.

Estrus detection aids, such as the Heatwatch system (DDx Inc., Denver, CO), have been developed to supplement visual detection of estrus and have proven effective in detecting short cycles or cycles with fewer standing events (Stevenson et al. 1996). The ability of this system to quantify timing and mounting behaviors can be advantageous to producers in properly scheduling inseminations, thereby increasing AI success (Dransfield et al. 1998).

Additionally, researchers have hypothesized that the behaviors associated with standing heat can have a negative effect on feed intake (Reith et al., 2014; Pahl et al., 2015). With feeding systems such as the Insentec system (Hokofarm Group B. V., the Netherlands), intake and feeding behaviors, such as visits to troughs, time spent eating, and meals consumed in a day can be quantified in the day's pre- and post-estrus. The use of feed intake and behavior information may be utilized as an additional tool in determining the onset of estrus.

Our hypothesis for this study was that behavioral estrus activity would reduce DMI and alter feeding behavior in heifers of divergent sizes. The objectives of this study were to 1) evaluate estrus activity among heifers of divergent sizes across two years; 2) determine the extent of differences the presence of a herd bull would have on estrus behavior; 3) quantify feed intake and behavior in the seven days pre- and post-estrus.

Materials and Methods

All animal procedures were conducted with approval of the Institutional Animal Care and Use Committee at North Dakota State University.

One hundred sixty-five cross bred-beef heifers from the NDSU Dickinson Research Extension Center were transported to the NDSU Beef Cattle Research Complex in Fargo, ND across a two-year period (2016-2017). Seventy-three heifers were used in year 1 and 92 heifers were used in year 2. Heifers were acclimated to the Insentec Roughage Intake Control (RIC) Feed system (Hokofarm Group B. V., the Netherlands) over approximately a 14 to18 day period. Frame score classifications were determined based on frame score of the heifers, with frame score calculated as a function of hip height and age per BIF guidelines (Beef Improvement Federation, 2016). Frame score classifications were 1) small to moderate (SMD) with a frame score ≤ 5.50 ; or, 2) moderate to large (MLG) with a frame score greater than 5.50. Year 1 consisted of 44 SMD heifers and 29 MLG heifers, and year 2 consisted of 68 SMD heifers and 24 MLG heifers. Overall there were 112 SMD heifers and 53 MLG heifers. Heifers were assigned to pens by treatment and designated either a Red Angus or Aberdeen Angus bull at breeding. Small to moderate frame heifers had an overall average start weight of 325 ± 3.6 Kg, whereas MLG heifers had an overall average start weight of 382 ± 4.3 Kg.

Heifers were fed a total mixed ration (TMR) delivered once or twice daily consisting of 78.5% grass hay, 16.5% corn silage, and 5% premix on a dry matter basis formulated for ad libitum intake and a targeted growth rate of 0.68 Kg per day (NRC. 2000). Diet TMR samples were collected weekly and dried in a 55°C oven and ground to pass a 1-mm screen. Samples were analyzed for dry matter (DM), crude protein (CP), ash, N (Kjehldahl method), Ca, P and ether extract (EE) by standard procedures (AOAC, 1990) at the North Dakota State University

Nutrition Laboratory. Crude protein was determined by multiplying N by 6.25. The TMR contained 11.79% crude protein in year 1 and 12.38% crude protein in year 2. The TMR was provided for the 107-day experimental trial in year 1 and 75 to 106 days in year 2. Heifers in year 2 were received on two different dates (June 6th and July 7th) resulting in differing days on feed.

All heifers were fitted with radio frequency identification tags in their ear to operate the Insentec system. Data collected by the Insentec system for each individual included visits to troughs, time spent per visit, and intake per visit. These data were used to further calculate feed intake (grams; per meal, per day), time spent eating (minutes; per meal, per day), total visits to feeders per day, and number of meals per day. A visit was defined as each time the Insentec system detected a heifer at the bunk and a meal was defined as a distinct feeding event that consists of breaks no longer than 7 minutes (Montanholi 2010). Calculations were made to determine kilograms DMI per individual visit and meal based on weekly TMR dry matter analysis.

Each heifer also received an Accubreed heat detection device (Estrotect, Denver, CO) at the initiation of the evaluation period. The Accubreed system consisted of a battery powered radiotelemetry device that was inserted into a mesh pouch, then affixed to the tail head of each heifer. When a heifer received a mount, a pressure switch was activated on the transmitter that sent a signal to the main station buffer, which then transmitted the data to a computer. Data recorded for each mounting event included animal ID, date, time, and duration of the mount. A heifer was considered in standing heat (day 0) when three mounts were received with a duration greater than or equal to two seconds within a four-hour period as defined by the Accubreed system. The end of an estrus event was defined as the last mount with no subsequent mounts

within 12 hours (White et al. 2002). Breeding bulls were placed in pens with heifers and estrus evaluation continued for the duration of the 45-d breeding season; beginning August 1st and concluding September 15th. Accubreed data was summarized for each individual estrus event per heifer and included mounts (no.), mount duration (s), time mounted (s), estrus duration (m and h), inter-estrus interval (d), and presence of bull (Y or N). Pregnancy status was determined post bull removal and again approximately 28 days later via transrectal ultrasonography (Aloka SSD-500, Corometrics Medical Systems, Wallingford CT, equipped with a 5-MHz linear array transrectal transducer).

Upon completion of the evaluation period, data from the Insentec feed system was combined with data from the Accubreed system. Feed intake (DM basis) and behavior was summarized by day for each individual heifer and aligned with day of each respective estrus (d 0) as defined by the Accubreed system. All feed and behavioral estrous data were compiled for seven days prior (d -7) and seven days post-estrus (d 7). Summary of variables evaluated by day relative to estrus is found in **Table 1**.

Each individual estrus event (n=517) was used as experimental unit. Estrus data including mounts, mount duration, time mounted, estrus duration in minutes and hours, and inter-estrus interval were analyzed for the impact of treatment (SMD or MLG) using the generalized linear model of SAS. The model for feed intake and behavior data included treatment (SMD or MLG), year (year 1 or year 2), day relative to estrus (d -7 to 7), and their respective interactions using the mixed procedures of SAS (9.4, SAS Inst. Inc., Cary, N. C.) with individual heats within heifer used as repeated measures. The covariance structure used was unstructured, selected based on lowest AIC/BIC values. The Kenward-Roger approximation was used to determine the denominator degrees of freedom for the tests of fixed effects. Pregnancy

data were analyzed for the impact of year and TRT and their interaction using the GLM

procedure of SAS. Results are reported as least square means and data were considered

significant at $P \leq 0.05$.

Table 1. Summary of response variables used to evaluate the impact of day relative to estrus on feeding behavior

Term	Definition
Dry matter intake	A heifers total intake on an as fed basis multiplied by the dry matter percentage of the TMR measured in Kg
Time eating	Total time a heifer spent eating per day measured in seconds
Visits	Total number of visits to troughs per heifer per day
Meals	The sum of all distinct feeding events that consists of breaks no longer than 7 minutes per heifer per day
Time per visit	The total time a heifer ate per day divided by the total number of visits to troughs in that day measured in seconds
Time per meal	The total time a heifer ate per day divided by the total number of meals in that day measured in seconds
Intake per visit	The total DMI divided by the total number of visits to troughs per heifer per day measured in Kg
Intake per meal	The total DMI divided by the total number of meals per heifer per day measured in Kg
Eating rate	The total DMI in grams divided by the total eating time in minutes per heifer per day

Results

Estrus behavior

Overall, 517 estrous cycles were observed with a mean duration of 10.1 ± 0.29 hours (Table 1). The number of mounts per estrus ranged from three to 101 mounts for a single cycle with a duration ranging from two to 12.25 seconds. The total time a heifer stood for mounting behavior (sum of all individual events) ranged from six to 424 seconds, with estrus cycles occurring every 19.97 ± 0.12 days (**Table 2**).

Item	Mean ± SE	Range
Mounts	17.3 ± 0.71	3 to 101
Mount duration, s ¹	3.93 ± 0.06	2 to 12.25
Time mounted, s ¹	66.2 ± 2.74	6 to 424
Estrus duration, h ²	10.1 ± 0.29	0.02 to 37.72
Estrus duration, min ³	606.5 ± 17.68	1 to 2263.00
Inter-estrus interval, d ⁴	19.97 ± 0.12	16.06 to 32.25

Table 2. Summary of behavioral estrus activity from 517 recorded estrus events in crossbred beef heifers

¹Seconds

²Duration of estrus defined as the time in hours from the first mount of a confirmed estrus to the last mount ³Duration of estrus defined as the time in minutes from the first mount of a confirmed estrus to the last mount ⁴Inter-estrus interval defined as the number of days in between 2 sequential estrus cycles

There were no differences among treatment for the response variables mounts per estrus,

time mounted, estrus duration, or inter-estrus interval; however, MLG heifers had a greater

mount duration as compared with SMD heifers (*P*=0.03; Table 3).

Item	MLG ¹	SE	SMD ²	SE	<i>P</i> -value
Mounts per estrus	16.23	1.24	17.79	0.86	0.30
Mount duration, s ³	4.12	0.10	3.84	0.07	0.03
Time mounted, s ³	62.10	4.82	68.13	3.33	0.30
Estrus duration, h ⁴	9.43	0.52	10.43	0.36	0.11
Inter-estrus interval, d ⁵	19.96	0.22	19.97	0.15	0.96

Table 3. Comparison of estrus behavior among crossbred beef heifers of divergent frame sizes

¹Treatment: Moderate to large frame heifers = frame greater than 5.50

²Treatment: Small to moderate frame heifers = frame \leq to 5.50

³Seconds

⁴Duration of estrus defined as the time in hours from the first mount of a confirmed estrus to the last mount ⁵Inter-estrus interval defined as the number of days in between 2 sequential estrus cycles

Pregnancy rate was similar (P = 0.38) among heifers of divergent frame sizes and was not

impacted (P = 0.76) by year, with 87.5 % of SMD heifers becoming pregnant and 92.5 % of

MLG heifers becoming pregnant. The summary of pregnancy rates per pen can be found for

both years in Table 4. The overall pregnancy rate for year 1 was 90.4% and 86.9% in year 2.

Total number of heifers per pen varied per year and breed of bull was allocated evenly among

TRT. Pregnancy rates varied among pen and year, with a Red Angus bull in year 2, pen four

having the lowest pregnancy rate of 60%.

Year	Pen	TRT	Bull breed	Total heifers	Pregnant	Preg. rate
1	3	SMD^2	AA ³	22	20	91%
1	4	SMD^2	RA^4	22	19	86%
1	5	MLG^1	AA ³	14	14	100%
1	6	MLG^1	RA^4	15	13	87%
2	2	SMD ² /MLG ¹	RA^4	20	20	100%
2	3	SMD^2	AA ³	20	18	90%
2	4	SMD^2	RA^4	20	12	60%
2	5	SMD ² /MLG ¹	AA ³	19	18	95%
2	6	SMD ² /MLG ¹	RA^4	20	18	90%

Table 4. Summary of pregnancy rates in crossbred beef heifers by pen in year 1 (2016) and year 2 (2017)

¹Treatment: Moderate to large frame heifers = frame greater than 5.50

²Treatment: Small to moderate frame heifers = frame \leq to 5.50

³Bull breed = Aberdeen Angus

⁴Bull breed = Red Angus

The presence of a herd bull influenced estrus behavior. Mounts per estrus, the total time a heifer was mounted, and estrus duration all decreased in the presence of a herd bull (P < 0.001; **Table 5**. During the 45-day breeding season, mounts decreased by 38% or 7.62 mounts when a bull was present with heifers, the total time a heifer was mounted decreased by 33% or 24.5 seconds, and estrus duration decreased by 24% or 2.61 hours. Contrarily, mount duration increased by 0.34 seconds (P = 0.0049) as did inter-estrus interval, which increased by 2.91 days (P < 0.001) when a bull was present in the pen (**Table 5**).

Item	No bull ¹	SE	Bull ²	SE	<i>P</i> -value
Mounts per estrus, no.	20.10	0.86	12.48	1.13	<.0001
Mount duration, s ³	3.81	0.07	4.15	0.10	0.0049
Time mounted, s ³	75.23	3.39	50.73	4.43	< 0.001
Estrus duration, h ⁴	11.07	0.36	8.46	0.48	< 0.001
Inter-estrus interval d ⁵	20.27	0.53	23.18	0.50	< 0.001

Table 5. Comparison of estrus behavior of crossbred beef heifers with or without the exposure of a bull

¹No bull defined as the period of estrus activity in the absence of a herd bull

²Bull defined as the 45-day period of active bull breeding with the heifers

³Seconds

⁴Duration of estrus defined as the time in hours from the first mount of a confirmed estrus to the last mount ⁵Inter-estrus interval defined as the number of days in between two sequential estrus cycles

Relationship between estrus and feeding behavior

Feed intake and behavior of MLG and SMD heifers was previously reported by Fontoura, (2017). When separated into low or high RFI groups utilizing the Koch determination model there were no differences among heifers for feeding behaviors including meals (P = 0.14), time spent eating (P = 0.10), time per visit (P = 0.40), or eating rate per meal (P = 0.51). However, low RFI heifers had fewer visits to troughs (P = 0.01) and a greater eating rate per visit (P = 0.01) (Fontoura 2017). Heifers across both years had an average daily gain of 0.57 ± 0.01 Kg per day, with SMD heifers averaging 0.53 ± 0.02 and MLG heifers averaging 0.64 ± 0.02 Kg per day. Eight of 9 feeding behaviors evaluated were influenced by day relative to estrus ($P \le 0.02$) or a day × year interaction ($P \le 0.002$).

Table 6 depicts the impact of day on feeding behavior of all heifers. There was an impact ($P \le 0.002$) of day of estrus relative to the 7 d before and 7 d after on DMI (17.13% reduction), DMI per visit (6% increase), and eating rate (5% increase). Time per visit was not impacted (P = 0.15) by day over the 15 days of observation period.

Table 6. Impact of day relative to standing estrus on feeding behavior in crossbred beef heifers.

Day Relative to Estrus																	
Item	-7	-6	-5	-4	-3	-2	-1	01	1	2	3	4	5	6	7	SE	P-value
DMI, Kg ²	8.25	8.21	8.19	8.26	8.33	8.41	8.39	6.95	8.67	8.41	8.49	8.41	8.48	8.49	8.49	0.085	< 0.001
DMI per visit, Kg ³	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.003	<0.001
Time per visit, seconds	127.99	129.37	128.70	123.75	129.77	127.06	129.54	130.25	132.28	127.04	124.82	124.49	128.88	127.83	127.28	4.433	0.15
Meals, no. ⁴	9.97	10.16	10.16	10.21	10.10	9.87	9.84	9.82	9.69	9.61	9.95	9.91	10.10	10.03	10.16	0.175	0.03
Eating rate, g/min ⁵	55.55	55.34	56.27	57.15	56.11	55.77	57.58	60.67	58.52	58.66	58.16	58.32	58.41	58.61	58.67	1.206	0.002

¹Day 0 defined as the day of estrus

²Dry Matter Intake

³A visit was defined as each time the Insentec system (Hokofarm Group B.V., the Netherlands detected a heifer at a bunk.

⁴A meal was defined as eating periods that might include short breaks separated by intervals not longer than seven minutes (Montanholi et al., 2010; Swanson et al., 2014).

⁵Eating rate calculated by dividing the total eating time in minutes per heifer per day by the total DMI in grams

Day × year interactions were present ($P \le 0.0078$) for time spent eating, DMI per meal, and time spent eating per meal. In year 1, heifers had a 19.8% reduction in time spent eating and a 26.8% reduction in year 2 on the day of estrus (P = 0.0005; **Figure 1**). Heifers in year 2 consumed more in the seven days before and after day of estrus (d 0) but had a similar reduction in time spent eating on day of estrus.



Figure 1. Impact of year and day relative to estrus on time spent eating for crossbred beef heifers: Year 1=2016, Year 2=2017. Day of estrus=day 0.

Heifers also had a 14.2% reduction in DMI per meal in year 1 and a 21.2% reduction in year 2 on the day of estrus (P = 0.0078; Figure 2). Time spent eating per meal was reduced in year 1 by 16.9% and 26.1% in year 2 on the day relative to estrus (P=0.0018; Figure 3).

There was also an interaction of TRT group and day relative to estrus. Moderate to large heifers had a 19% reduction in visits to troughs on day of estrus, whereas SMD heifers had a 21.75% reduction in visits to troughs. (P = 0.05; Figure 4). Similarly, time spent eating per meal was reduced by 24.69% in MLG heifers and 17.81% in SMD heifers on day of estrus (P = 0.01; Figure 5).



Figure 2. Impact of year and day relative to estrus on DMI per meal for crossbred beef heifers: Year 1=2016, Year 2=2017. Day of estrus = day 0.



Figure 3. Impact of year and day relative to estrus on time spent eating per meal for crossbred beef heifers: Year 1=2016, Year 2=2017. Day of estrus=day 0.



Figure 4. Impact of frame size group and day relative to estrus on visits to troughs for crossbred beef heifers: MLG=moderate to large frame heifers, SMD=small to moderate frame heifers. Day of estrus=day 0.



Figure 5. Impact of frame size group and day relative to estrus on time spent eating per meal for crossbred beef heifers: MLG=moderate to large frame heifers, SMDI=small to moderate frame heifers. Day of estrus=day 0.

Discussion

When proposing a new management strategy to beef cattle producers, considerations need to be made to ensure differences do not exist in reproductive efficiencies in animals of divergent frame sizes. Wireless heat detection technology, such as Accubreed, is available to help quantify estrus parameters and assist in verifying any differences that may exist (Rorie et al. 2002).

The number of mounts per estrus cycle ranged from three to 101 with an average of 17.3 \pm 0.7038. Stevenson et al. (1996) observed an average of 50.1 \pm 6.4 mounts when employing the Heatwatch system in crossbred yearling beef heifers. The greater average number of mounts in that study could be attributed to the fact that the heifers were estrus synchronized utilizing MGA supplementation followed by a PGF_{2a} injection; whereas the heifers utilized in this study had natural estrus cycles and were bull bred. When a greater number of females express behavioral estrus, the corresponding number of mounts received increases (Hurnick et al. 1975, Floyd et al. 2009). The average mount duration was 3.93 seconds with the total time a heifer stood to be mounted during an estrous cycle ranging from 6 to 424 seconds. The average estrus duration was 10.11 hours, similar to that of Roelofs et al. (2005) in which Holstein-Friesian dairy cows had a duration of estrus that ranged from 10.8-13.6 hours. In a study utilizing dairy cattle the duration of estrus across 17 herds ranged from 33 minutes to 35.8 hours (Dransfield et al. 1998). This duration of estrus is similar to the present study in which duration of estrus ranged from one minute to 37.7 hours.

The inter-estrus interval observed among the 165 heifers was 20.0 days for all estrus events. When analyzing estrus events outside of the breeding season, the inter-estrus interval was 20.3 days, similar to that of Armstrong and Hansel (1959) and Pierson and Ginther (1984).

A shorter inter-estrus interval inclusive of all estrus events may have been the result of short cycles similar to those described by Odde et al. (1980) in which of 198 estrus cycles less than 17 days, 86% of these were between the first and second detected estrus, with seven to ten day cycles occurring most frequently. In the current study, mounts per estrus, time mounted, estrus duration, and inter-estrus interval did not differ among frame score groups on day relative to estrus. However, MLG heifers had a greater mount duration compared with SMD heifers. This may be due to the relative size of the SMD heifers and their ability to support the weight of their herd mates, particularly in year 2 in which three of five pens had a mix of SMD and MLG heifers.

Pregnancy rates did not differ among treatments, year, or the TRT × year interaction. The presence of a bull during the breeding season impacted estrus behavior. Mounts per estrus, time mounted, and estrus duration decreased while mount duration increased when a bull was present. Orihuela (2000) also showed duration of estrus was reduced when a bull was present while Kilgour et al. (1977) reported female to female mounting declined as a result of bull dominance. The participation of females in sexually active groups also has been shown to be reduced when in the presence of a herd bull (Kilgour et al. 1977; Orihuela, 2000). Additionally, within 30 minutes of the herd bull lying down, cows reformed the sexually active group and proceeded with mounting activity (Kilgour et al. 1977).

Feeding behavior was affected by day and year in the present study. Time spent eating, DMI per meal, and time spent eating per meal were all reduced on the day of estrus. In a study utilizing dairy cows and the Insentec system, Pahl et al. (2015) also found a reduction in time spent eating on the day corresponding to estrus. There is no current studies found in the literature that describe changes in meal behavior on day of estrus; however, the reductions

observed in this study correspond to decreases found in DMI and time spent eating on day of estrus. Year was also significant for these 3 variables. Heifers in Year 1 spent less time eating, spent less time eating per meal, and had a greater DMI per meal. These results suggest differences based on competition within a pen (Olofsson, 1999) as a consequence of stocking rate. In year 1, 60% of heifers were in pens that were stocked heavier than any of the five pens in year 2. There was also an interaction of treatment and day of estrus on visits to troughs and time spent eating per meal. Moderate to large frame heifers had a greater number of visits to troughs and spent more time eating per meal as compared to SMD heifers, with both treatments experiencing a reduction on day of estrus. Although there is no current literature that compares these variables in animals of divergent sizes on day of estrus, it is forthright to assume that larger animals will require a greater amount of time and visits to attain the intake necessary to achieve satiety. Walker et al. (2015) also found that beef cows of a larger body weight and higher RFI value consumed more DMI during lactation and post-weaning when compared to their lower body weight counterparts.

Both treatment groups experienced approximately a 17% reduction in DMI on the day of estrus, similar to that of Zebari et al., (2018), who observed a 12% reduction in DMI on day of estrus in dairy cows. Consequently, DMI per visit was also reduced on the day of estrus. Eating rate was greater for both treatment groups on day of estrus. This corresponds with data observed by Pahl et al. (2015), in which eating rate increased by 0.016 Kg/minute on the day of estrus in Holstein cattle.

Feed intake and behavior were altered by behavioral estrous activities. The observed alterations were rapid, and returned to baseline levels within one day following standing heat.

These findings suggest that the onset of estrus and the subsequent reduction in DMI and alterations in feeding behavior may not have major performance impacts on individual heifers.

Although the short term effects of estrus may not alter performance in individual heifers, the extent to which altered feeding behavior as a result of estrus in individual heifers plays a role in the social environment and feeding behavior of other heifers in a pen is yet to be determined.

Implications

Behavioral estrus activity varied between animals but was unaffected by frame score group, with the exception of mount duration. The presence of a herd bull had an impact on all estrous behavior variables measured, and pregnancy risk did not differ among frame size groups. Feed intake and behavior was altered on the day of standing heat, but returned to baseline values one day post estrus. Based on the current data, feed intake and behavior may serve as a useful tool in predicting the onset of estrus.

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CHAPTER 3. OVERALL CONCLUSIONS

Behavioral estrus activity varied between animals but was unaffected by treatment, with the exception of mount duration. The presence of a herd bull had an impact on all estrous behavior variables measured; however, pregnancy rate did not differ among treatment groups. Feed intake and behavior was altered on the day of standing heat, but returned to baseline values one day post estrus. Based on the current data, feed intake and behavior may serve as a useful tool in predicting the onset of estrus. Future studies could potentially focus on an hourly aspect to determine more specific timing as it pertains to the onset of estrus.