DOES CALCIUM PROPIONATE ELICIT SIMILAR GROWTH AND REPRODUCTIVE RESPONSES AS MONENSIN IN DEVELOPING HEIFERS?

by

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DEDICATION

To my grandfather, Daryl, for his excitement and encouragement of my career working with animals.

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ABSTRACT

Replacement beef heifer development is critical for the continued production of beef and for ranch success. Objectives of this research were to investigate the growth and reproductive responses of developing heifers fed similar basal diets supplemented with pellets containing different feed additives. Pellet treatments consisted of 2.27 kg·heifer^{-1}·d^{-1} of: 1) control with no feed-additive (CON), 2) 200 mg·heifer^{-1}·d^{-1} monensin (MON), or 3) 40 g·heifer^{-1}·d^{-1} calcium propionate (PRO). Experiment 1 utilized one hundred and ninety-eight heifers (254 ± 3.8 kg) stratified by BW and randomly assigned to one of six pens (n = 2 pens per treatment). Experiment 2 utilized fifty-eight heifers (304 ± 3.4 kg) stratified by BW and randomly assigned to one of 12 pens (4 pens per treatment). Heifers were fed a grass hay basal diet in both experiments (Exp. 1: 65% TDN and 11% CP; Exp. 2: 62% TDN and 15% CP, DM basis). Experiment 1 was 129 d and Experiment 2 was 122 d. Body weights were collected at the beginning and end of each experiment, with interim BW collected every 30 d. Serum samples were collected via coccygeal venipuncture and analyzed for progesterone to determine pubertal status. Overall pregnancy rates and pregnancy rate from AI were determined. Experiment 1 observed no differences for initial BW, final BW, overall ADG, pregnancy rate from AI, or overall pregnancy rate ($P \geq 0.16$). Fewer CON and PRO heifers were pubertal at experiment start than MON heifers ($P \leq 0.01$) in Experiment 1, which was unexpected. Differences in puberty achievement were diminished at the end of the experiment. Experiment 2 observed no differences for initial BW, final BW, overall ADG, pregnancy rate from AI, or overall pregnancy rate ($P \geq 0.19$). Treatment had no effect on puberty achievement at beginning or end of experiment ($P \geq 0.09$). A period effect was observed for ADG in both experiments ($P \leq 0.01$) which may be related to cold stress. Neither calcium propionate nor monensin resulted in improved performance compared to no feed additive. Further research is warranted to elucidate the impact of calcium propionate on heifer development.

CHAPTER ONE

LITERATURE REVIEW

The United States of America consumed 11,678 tons of beef in 2016 (USDA, 2017). This number is estimated to rise throughout 2017 and into 2018. In July 2017, there were 103 million reported head of dairy and beef cows and calves. Of that, 4.70 million head were beef replacement heifers (USDA, 2017).

In 2017, it was estimated that 12.8 million head of cattle and calves were on feed for slaughter, with the number of calves being produced continuing to rise (USDA, 2017). In order to continue filling the demand for beef in the U.S., ranchers must look to the future for what they can produce. This will include both cattle produced for beef and cattle produced to maintain or expand herd size. An integral piece of this future is development of replacement heifers, one of the major programs of a beef cattle operation (Roberts et al., 2009). It is critical for a rancher to develop heifers efficiently and economically in order to be a successful operation; therefore, management of the heifer development program is key. A common goal for developing replacement heifers is to achieve optimal growth rates in order to attain puberty at an early age and be pubertal for her first breeding season. This will allow the animal to become a productive member of the herd by calving earlier and resulting in increased lifetime productivity as well as increased longevity in the herd (Lynch et al., 1997; Cushman et al., 2013; McNeel and Cushman, 2015).

Often, ranchers will incorporate a feed additive, such as an ionophore, into their heifer development program to promote growth and therefore obtain puberty earlier in the life of the heifer. Ionophores are reported to have a positive cost to benefit ratio and have been estimated to save the cattle industry approximately $1 billion a year through improved gain and health benefits (Callaway et al., 2003). Monensin is one of the most commonly used ionophores and has been shown to improve feed efficiency in growing cattle (Duffield et al., 2012). However, monensin is required to be fed daily. Therefore, researching other feed additives that may promote growth and early puberty attainment may be useful for ranchers with more extensive operations. Developing replacement heifers is crucial for maintaining a productive cow herd and sustaining stocker operations (Funston et al., 2012). Researching management strategies to improve replacement heifer development is important for the sustainability and success of the beef cattle industry. In addition, successful management strategies will aid in growth and efficiency of the industry. The remainder of this literature review will encompass an overview of heifer development including endocrinology of puberty, age at puberty, target weight concept, ionophore function, monensin research, and calcium propionate research.

Endocrinology of Puberty

Growth occurs in specific waves; as an animal grows, the focus is first on neural tissue, then bone, muscle, and lastly adipose tissue (Owens et al., 1993). The reproductive system is the last to develop in a young beef female making the factors that affect puberty critical (Patterson et al., 1992). Contrary to how it is often described, puberty is not an event, but rather a cascade of events occurring over time (Schillo et al., 1992, Senger,

2012). Fundamentally, it is the secretion of gonadotropin releasing hormone (GnRH) from hypothalamic neurons at the appropriate frequency and quantities in order to stimulate gonadotropin release by the pituitary. Development of the hypothalamic GnRH neurons are impacted by development of a threshold body size and exposure to certain environments, such as season of birth and housing density. A female animal's hypothalamus has both a surge and tonic center which are groups of hypothalamic nuclei that influence reproduction. The heifer's hypothalamus is the center for neural control of reproductive hormones. Onset of puberty is limited by failure of the hypothalamus to secrete sufficient amounts of GnRH (Day and Anderson, 1998, Senger, 2012).

Hypothalamus development occurs gradually with the animal as it grows. Contained within the hypothalamus are the surge and tonic center. These centers control the release of GnRH through feedback loops from gonadal steroids such as estradiol (E_2) and progesterone (P_4). Negative feedback suppresses GnRH neurons while positive feedback stimulates GnRH neurons. In a mature female, positive feedback from gonadal E_2 will stimulate GnRH release. The surge center will not be able to release large amounts of GnRH to stimulate ovulation until there is positive feedback by E_2 (Day et al., 1987; Senger, 2012). On the other hand, progesterone exerts negative feedback at the hypothalamus; inhibiting GnRH neurons and causing GnRH to be secreted at basal levels. This allows sufficient follicular development in non-pregnant females and cessation of cycles in pregnant females.

However, pre-pubertal females lack sufficient amounts of gonadal estradiol to stimulate the surge center to secrete high amplitude pulses of GnRH. The neurons present

in the pre-pubertal animal are sensitive to estradiol, but because estradiol is too low, they cannot secrete much GnRH. In order to have ovulation, the surge center must reach full neural activity resulting in sudden bursts of GnRH known as the preovulatory GnRH surge. This preovulatory surge of GnRH is a series of rapid, high amplitude pulses. Inability of the surge center to reach full neural activity will result in ovulation failure (Senger, 2012).

The tonic center regulates GnRH pulse frequency. In a mature female, these pulses stimulate the anterior lobe of the pituitary to release follicle stimulating hormone (FSH) and luteinizing hormone (LH) at high levels. A pre-pubertal female is characterized by having a lack of gonadal estradiol to stimulate the surge center as well as low frequency GnRH pulses from the tonic center. At low concentrations of estradiol, the tonic center has a high sensitivity to negative feedback and as a result does not secrete high levels of GnRH and gonadotropins remain low. Consequently, follicular development cannot result in high circulating levels of estradiol (Senger, 2012).

The pubertal transition for a female occurs when the negative feedback sensitivity to estradiol is decreased in the tonic center. Consequently, this results in higher amounts of GnRH being secreted. This causes an increase in LH pulse frequency, which stimulates the ovary to secrete more estradiol. Estradiol has an activity threshold and once it is reached it causes a large discharge of GnRH from the surge center. This positive feedback then allows ovulation to take place and puberty follows (Perry, 2016). It is important to note that the sensitivity of the surge center to positive feedback does not change very much and remains high even after birth. Change occurs with decreased

sensitivity to negative feedback in the tonic center that triggers the onset of puberty. The decreased sensitivity to negative feedback by the tonic center means that smaller and smaller quantities of estradiol are required to stimulate the release of GnRH, resulting in the secretion of FSH and LH (Day et al., 1987). These gonadotropins then stimulate more follicles and more estradiol is secreted until the surge center releases the preovulatory surge of GnRH (Moran et al., 1989; Senger, 2012). Figure 1 illustrates how the release of GnRH (represented by subsequent and corresponding LH concentration) builds slowly as the animal grows until the appropriate thresholds are reached and puberty occurs.

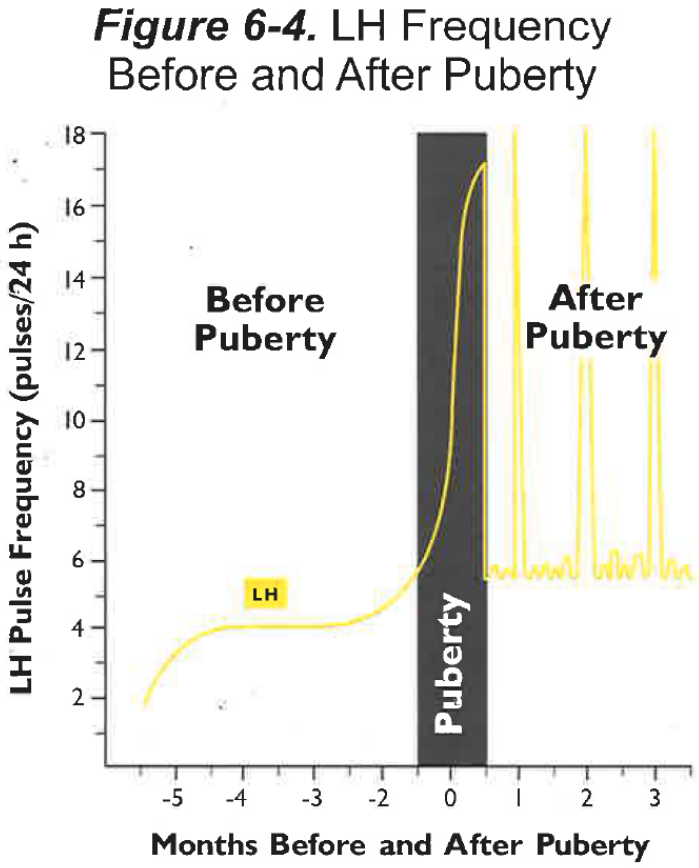

Figure 1. LH pulses over time and puberty achievement.
LH pulses as a reflection of GnRH pulses in heifers prior to the onset of puberty. Note the time required for the pulse frequency to become high enough for puberty to be achieved. (Adapted from Senger, 2012).

After puberty has been achieved, females enter reproductive cyclicity. Estrous cycles are a series of predictable reproductive events that begin at estrus and end at the subsequent estrus. This cycle will continue throughout the animal's adult life and can only be interrupted by pregnancy, lactation, or inadequate nutrition. Cessation of this cycle is known as anestrus; a period when cyclicity stops. This can also be caused by pathologic conditions of the reproductive system such as uterine infection, persistent corpus lutea (CL), or a mummified fetus.

Estrous cycles give females opportunities to become pregnant. Cycles are divided into two distinct phases, the follicular phase and luteal phase. The follicular phase is the time from regression of the corpus luteum (CL) to ovulation. It is dominated by large follicles that secrete E_2. The luteal phase is the time from ovulation until CL regression. The dominant structures are CL and the primary hormone is P_4. In this phase, follicles grow and regress but aren't producing high levels of E_2.

Included within the two phases of the estrous cycle are four stages. The follicular phase is made up of the proestrus and estrus stages. The luteal phase consists of metestrus and diestrus; each stage accomplishes specific reproductive steps. Proestrus includes formation of ovulatory follicles and E_2 secretion. Estrus is where sexual receptivity is exhibited along with peak E_2 secretion. Metestrus consists of CL formation and the beginning of P_4 secretion. The final stage, diestrus, is where luteal secretion of P_4 is sustained.

Metestrus is the time between ovulation and the formation of a functional CL. During early metestrus both E_2 and P_4 are low. Then the follicle undergoes luteinization and P_4 secretion increases after ovulation. The CL is fully functional in the diestrus stage; during this time, P_4 secretion is high. These high levels of P_4 tell the uterus to prepare for early embryo development and prepare the reproductive tract for initiation and maintenance of pregnancy (Senger, 2012). Most of progesterone's effects are exerted by regulating transcription of genes through specific nuclear receptors. After ovulation, the CL is developing and high circulating concentrations of P_4 are restricting the secretion of

LH (Niswender et al., 2000). This continuous cycle and alternating peaks of P_4 and E_2 are illustrated in Figure 2.

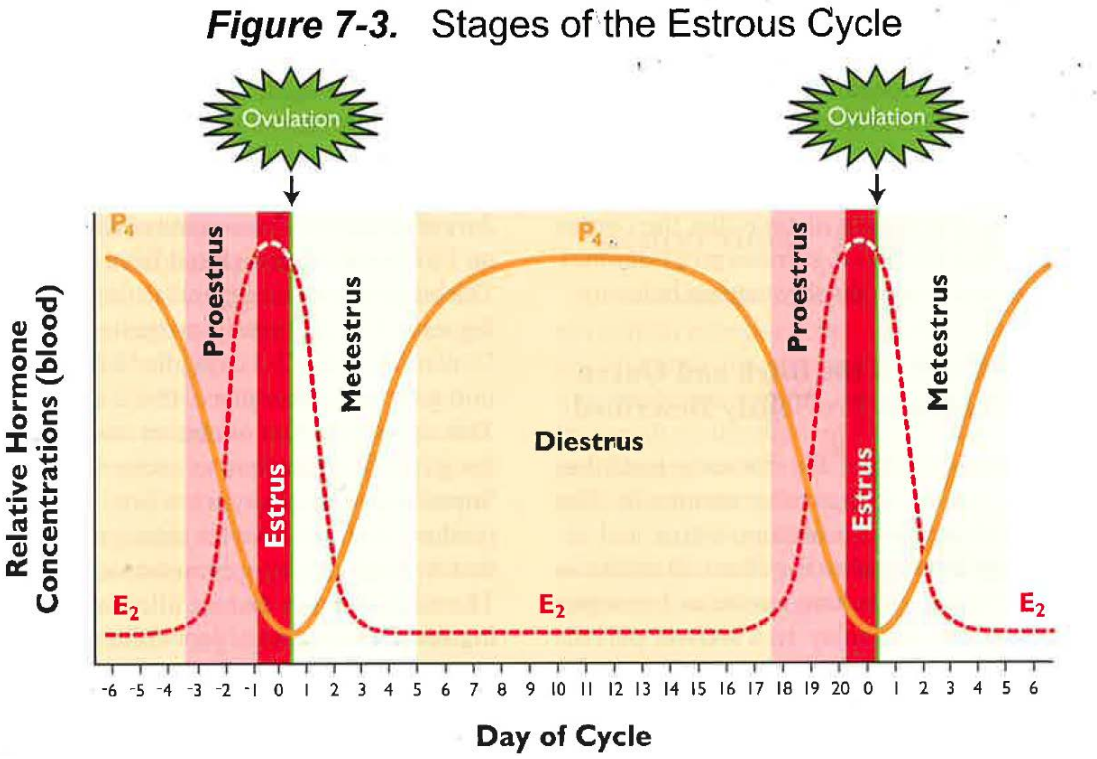

Figure 2. Stages of the estrous cycle in a beef female.
Note the different stages and alternating peak levels of progesterone and estrogen.
(Adapted from Senger, 2012).

Heifers attain puberty at a pre-determined weight that is unique to each animal (Funston and Deutscher, 2004). The actual weight cannot be predicted, but it does appear that age at puberty is directly related to growth rate (Roberson et al., 1991; Patterson et al., 1992). Although growth is a primary factor in age at puberty, there are other aspects that are important. These can include genetic and environmental variables such as breed, forage availability, and adaptation of a breed to certain environmental conditions

(Wiltbank et al., 1966; Patterson et al., 1992; Hall et al., 1995). Pregnancy cannot be achieved until the heifer reaches this weight threshold and begins cycling (Sprott et al., 1988; Patterson et al., 1992).This is where heifer development methods become important to a rancher. In addition, it is important to consider the breed of heifer being used, a supply of affordable feed ingredients, and a focus on herd longevity (Endecott et al., 2013).

Age at Puberty

Decreasing age to puberty is ideal for increased conception rates and decreased costs. In addition, having heifers calve early in their first breeding season can impact longevity (Lesmeister et al., 1973; Endecott et al., 2013). Along with longevity, calving heifers earlier in the calving season results in heavier weaned calves and allows the heifers a longer interval to rebreeding (Cushman et al., 2013). These observations were based on a 20-year study evaluating Angus crossbred heifers where calf birth date, weight, gender, and calving ease score were all recorded. The study found that roughly 80% of heifers who calved in the first 21 days of their first calving season stayed in the herd to produce a fifth calf compared to 70% for heifers who calved at day 22 or later of the season. This resulted in longer average herd life for early-calving heifers compared to heifers that calved after the first 21 days. The authors concluded that heifers that calve early had increased cumulative calf weaning weight that amounted to an extra calf in their lifetime (Cushman et al., 2013).

These goals for heifer development are well established; there has been much research and proposed ideas on how to achieve these goals. An early thought was that

exposure to a bull would decrease days to puberty as it was theorized that bull urine contained a priming pheromone (Izard and Vandenbergh, 1982). However, there have been inconsistencies with the data on this subject. One study looked to clarify and evaluate the exposure of pre-pubertal heifers to bulls on age at puberty. Treatments consisted of exposure to bulls or isolation from bulls. Results indicated that bull exposure may be beneficial in terms of social cues, but research has not shown any definite advantages (Roberson et al., 1991). Puberty cannot be accelerated in animals that have not reached that threshold weight to trigger the hypothalamus' response to E_2 (Senger, 2012).

In the early 1990's, a theory arose that using recombinant bovine somatotropin (bST) on developing heifers could alter age at puberty. Hall et al. (1994) conducted a study where Angus heifers were injected with bST to evaluate the effects of dietary energy on LH release, follicular development, and the onset of puberty. Treatments consisted of high dietary energy and bST, high dietary energy and vehicle injections (medium without bST), moderate dietary energy and bST, and moderate dietary energy with vehicle injections. These authors found that bST treatment altered intermediary metabolism towards the accretion of lean tissue and decreased fat deposition. There was a tendency for more high energy diet heifers to be pubertal at experiment end compared to the moderate energy diet heifers; however, body weight at puberty did not differ among treatment groups. It was concluded that secretion of LH was not affected by bST and that chronic treatment of bST did not alter age at puberty (Hall et al., 1994).

There has also been research conducted on precocious, or early developing puberty in beef heifers. A study by Wehrman (1996) examined the incidence of precocious puberty and exposure to a bull on the incidence of precocious puberty. Heifers were considered to have shown precocious puberty if the onset of luteal function was before 300 days of age. Results of this study found that there was no effect of bull exposure on the incidence of precocious puberty. Precocious puberty can occur in as many as 25% of developing heifers, but bull exposure seems to play no role in its onset (Wehrman et al., 1996).

Target Weight Concept

Historically, it was recommended that heifers be developed to 60-66% of their mature body weight by 13 months of age for successful reproduction (Patterson et al., 1992; Funston and Deutscher, 2004; Perry, 2016). However, this perceived rule has been challenged and more recent research has examined developing heifers to a lighter body weight (BW). A 3-year study conducted by Funston and Deutscher (2004) developed spring-born heifers to 55 or 60% mature BW by approximately 13-14 months of age. The study focused on reproduction and calf responses. Heifers were from the MARC II herd in Nebraska and were assigned to either a low-gain (55% mature BW at breeding) or high-gain treatment (60% mature BW at breeding). Target weights were calculated from the average weights of the breeding cow herd. Heifers were fed ad libitum hay (10% CP) and a pellet (20% CP) containing monensin (88 mg/kg) as needed to achieve the desired gain. The study found that heifers on the high-gain diet gained 0.14 kg/d more, but cost $22/heifer more than the low-gain group over the development period. The high-gain

group ultimately achieved 58% of their mature BW at breeding and the low-gain achieved 53%. Calf birth date, calf weight and calving difficulty were similar across both groups. It was concluded that developing heifers to 53% mature BW had no adverse effects compared to 58% mature BW. However, costs were increased to develop heifers to the heavier weight (Funston and Deutscher, 2004), highlighting opportunities for ranchers to reduce heifer development costs.

The concept of developing heifers to a pre-breeding target BW of 50 or 55% of mature BW was further investigated by Martin et al. (2008). A 3-year study utilized 261 MARC II heifers randomly assigned to either intensive (INT) or relaxed (RLX) development systems. Heifers in the INT group were developed to 55% mature BW before a 45-d breeding season while those in RLX group were developed to 50% mature BW before a 60-d breeding season. Mature BW were calculated from the cow herd. At the conclusion of the study, it was found that heifers in the INT group weighed 57% of mature BW and RLX weighed 51% of mature BW. Feeding to a pre-breeding BW of 51% of mature BW was more cost effective than 57%, even though heifers were given a 15-d longer breeding season. Despite the longer breeding season, conception rates were similar between groups, achieving 87% or better, and a similar proportion of heifers calved in the first 45 days of the calving season. In addition, second-calf production between the two development groups were similar, indicating that heifers given 15-d extension on their first breeding season rebred with similar efficiency as those with the 45-d season. The authors concluded that heifers can be developed to lighter than

traditional target BW without incurring negative effects on profitability or productivity (Martin et al., 2008).

Often heifers are often developed in a dry lot setting; however, developing to a lighter target BW on winter range can be both effective and reduce development costs (Funston and Larson, 2011). Two hundred ninety-nine heifers were utilized in a 3-year study comparing traditional post-weaning dry lot to extensive winter grazing using corn residue (CR) and winter range. Heifers grazing CR and winter range gained 0.40 kg of BW/d less than heifers in the dry lot and were consuming 8% less crude protein (CP). However, once CR grazing was depleted (roughly 100 days for each year), heifers were moved back to a dry lot where they experienced compensatory gain. They ultimately had greater ADG than those originally in dry lots. Though the CR heifers had lower BW at pregnancy, their compensatory gain could have contributed to reproductive improvement as evidenced by similar final pregnancy rates. In addition, the cost of winter grazing CR was $0.46 heifer/d compared to $0.70 heifer/d for the dry lot system. Utilizing winter grazing for at least 135 d post weaning to limit dry lot time reduced overall heifer feed cost by $42 compared to dry lot development for the entire pre-breeding period. It was concluded that other forms of development, other than the traditional dry lot, can be suitable for achieving mature BW less than 65% and still have acceptable reproduction and calf production, while potentially reducing development costs (Funston and Larson, 2011).

Developing heifers with the right nutrition is critical, especially during post-weaning. If a heifer gains too little after weaning it can delay puberty resulting in poor

conception rates (Wiltbank et al., 1966; Clanton et al., 1983; Funston et al., 2015). One study concluded that heifers should gain 100 kg between weaning and breeding, but it was unclear if the majority of that weight gain should be uniform over time or majority at the beginning or end of a feeding period (Clanton et al., 1983). To investigate, Angus/Hereford cross heifers were assigned to one of three treatments to either gain heavily in the beginning of the trial, gain heavily at the end or gain steadily throughout. Results indicated no difference in overall growth, age at puberty, conception rate, or calf production based on timing of gain (Clanton et al., 1983). This study highlights the fact that while heifers need to gain adequate weight between weaning and breeding, there is room to adjust how much and when that weight is gained. Another study investigated the effects of delaying weight gain until the last third of a heifer's developmental period. Angus-Hereford cross heifers were randomly assigned to either an even gain group or late gain group. The experiment was conducted twice in two consecutive years and lasted approximately five months. Results indicated no detrimental effects on age at puberty or pregnancy rate from delaying the majority of weight gain and that utilizing compensatory gain could potentially decrease development costs (Lynch et al., 1997). However, it is important to provide adequate nutrition without under or over-feeding. If heifers lack energy and protein, age at puberty can be delayed resulting in poor conception rates. If overfeeding occurs, heifers may exhibit weak estrous, reduced conception rates, and increased embryonic mortality (Patterson, et al., 1992).

Ionophores

Method of Action

Developing heifers in a way that reduces age to puberty is greatly enhanced through the use of ionophores. Ionophores were originally developed as an anticoccidial for poultry, but it was soon discovered that they can depress feed intake without decreasing weight gain in cattle, improving feed to gain conversion (Bergen and Bates, 1984).

Ionophores are carboxylic polyether compounds that are toxic to many ruminal microorganisms. These compounds function by binding to cations and facilitating their movement across cell membranes. This results in disruption of ionic gradients and a decrease in intracellular pH. The proton pump expels protons and results in a depletion of adenosine tri-phosphate (ATP; Russel and Strobel, 1989; NRC, 2016). There are many types of ionophores that have been used including monensin, lasalocid, salinomycin, and narasin. However, monensin has probably been the most commonly used and researched form (Schelling et al., 1984, Callaway et al., 2003).

Gram-positive bacteria are affected by monensin and gram-negative bacteria have shown resistance. This could be related to the impermeability of the bacterial membrane of gram-negative bacteria (NRC, 2016). Shifts in the ruminal population toward more gram-negative bacteria also results in a shift in volatile fatty acids (VFA) produced in the rumen toward increased amounts of propionate and an associated decrease in acetate and butyrate (Bergen and Bates, 1984; NRC, 2016). Propionate has a higher enthalpy than acetate and can be readily oxidized by the animal. This results in more available feed

energy for productive purposes (Nagaraja et al., 1982; Russel and Strobel, 1989). Cattle fed diets high in readily fermentable carbohydrates with ionophores exhibited depressed feed intake, but increased feed conversion (feed/gain) without decreasing BW gain. When the diet consists of considerable amounts of β-linked carbohydrates (roughages), ionophores were found to improve BW gain without depressing intake, while still resulting in improved feed conversion (Bergen and Bates, 1984). In addition to this, a decline in methane production is often seen (Bergen and Bates, 1984, Russel et al., 1989). This results in enhanced animal performance due to improved retention of carbon and energy during rumen fermentation (Richardson et al., 1976; Bergen and Bates, 1984).

Monensin has been shown to improve feed efficiency through improved nitrogen metabolism and increased energy metabolism in the rumen. Monensin has an amino acid-sparing effect by decreasing amino acid-fermenting bacteria. This results in decreased deamination of amino acids and ammonia concentration in the rumen which leads to increased flow of dietary amino acids to the abomasum. This is thought to increase the amount of dietary protein escaping ruminal degradation and result in more availability for digestion in the small intestine (Bergen and Bates, 1984; Russel and Strobel, 1989).

While ruminants have the ability to transform dietary protein and non-protein nitrogen (NPN) into high quality meat protein, their efficiency of feed nitrogen use for growth is low (10-20%). Therefore, a large portion of dietary nitrogen is excreted in the urine and feces (NRC, 2016). Ionophores, like monensin, increase the digestible energy content of a diet as well as increasing the digestibility of nitrogen (Spears, 1990). This is

thought to be from a protein sparing effect that decreases ruminal ammonia nitrogen (Richardson et al., 1976; Randel, 1990).

History and Early Trials

Monensin was discovered at Eli Lilly Laboratories in 1974 and is a compound produced by *Streptomyces cinnamonensis*. Early studies found that when the compound was fed to cattle it decreased ruminal acetic and butyric acid while increasing propionic acid without altering total volatile fatty acid production (Haney and Hoehn, 1967; Moseley et al., 1977; Goodrich et al., 1984). This response indicates an improvement in the capture of feed energy during ruminal fermentation (NRC, 2016). One of the earliest studies evaluated the impact of monensin on the growth rates of yearling heifers fed either 200 mg or 0 mg of monensin per head per day. The study found that 92% of the monensin-fed heifers achieved puberty by breeding compared to 58% of the 0 mg monensin-fed group. It was concluded that monensin had no negative effects on performance or reproduction. The authors promoted monensin stating the data suggested heifers fed monensin achieved puberty earlier than those fed no monensin (Moseley et al., 1977).

Another study evaluated the nutritional and reproductive responses of first-calf heifers fed monensin. Heifers were fed either 200 mg or 0 mg of monensin per head per day. The study followed 32 Angus and Hereford heifers from the start of monensin treatment at 180 d post-weaning through the birth of their first calf. Results found monensin supplemented heifers gained more weight during gestation and lost 5.5 kg less weight during lactation than the non-monensin group. In addition, the monensin group

had decreased postpartum interval to first estrus by 13 days. Heifers in the monensin group were also in better body condition at the time of calving and produced calves that were 5.9 kg heavier at birth. The authors concluded that feeding 200 mg monensin per head per day had no detrimental effects and improved feed efficiency (Hixon et al., 1982).

RUP and Propionate Salts

Recently, the area of heifer development has begun researching other feed additives using propionate. Propionate supplementation is often used in dairy cattle to assist with the transition from late gestation to early lactation. During this time, the cow's liver must adapt from a minimal glucose demand to an exceedingly increased demand. Ruminal propionate is a precursor for gluconeogenesis and a product of rumen fermentation. Propionate is largely absorbed across the ruminal wall and supplies a part of the ruminant's energy requirements. It is estimated that propionate provides 32 to 73% of the demand for glucose (DeFrain et al., 2005; NRC, 2016). Dairy cattle are often challenged to intake sufficient amounts of a diet to meet the amino acid and energy needs during this transition period. McNamara et al. (2005) found dairy cattle supplemented with calcium propionate ate 1.2 kg/d more and in vitro incubations showed increased adipose tissue lipogenesis compared to cows fed a control diet. While this data was unable to prove there were direct effects of calcium propionate on glucose entry or glucose transporter activity in adipose tissue, the results are consistent with the theory.

However, research on propionate supplementation in beef cattle is still new. Waterman et al. (2014) fed developing heifers a diet of 908 g per day of control

supplement with 130 g of rumen undegradable protein (LRUP), 908 g per day of LRUP with 170 g of rumen undegradable protein (HRUP), or 1814 g of a protein and energy diet with 120 g of rumen undegradable protein and 100 g of propionate salt (LRUP+E). Eighty-four heifers were divided into two weight treatments consisting of a heavy group (230 ± 2.0 kg) and a lighter group (206 ± 2.6 kg). The objective of this study was to observe if light weight heifers could increase body weight gain and conceive by 15 months of age when supplemented with energy and propionate salt. Initial BW was greater for the LRUP and HRUP groups than for the LRUP+E group, as intended by study design. Results showed that although the LRUP+E lightweight heifers began the study 24 kg lighter, they only differed from the other groups by 15 kg at breeding with significant BW gains made from days 125 to 159. In addition, pregnancy rates were similar across groups. The authors concluded that lightweight heifers receiving a diet with additional energy and propionate salts can reach a critical BW before puberty (Waterman et al., 2014). However, it must be noted that the data from this study is confounded by the different energy levels and initial heifer body weight, warranting further research.

Another study evaluated responses from young postpartum beef cows when fed supplements that either met or exceeded metabolizable protein (MP) requirements. Protein is often limited when cattle graze mature vegetation. An idea arose that providing RUP supplements once degradable protein needs have been satisfied will lead to repartioning of nutrients. In theory, it would transition nutrients away from sinks like lactation and towards maintenance, growth, or reproduction (Waterman et al., 2006). The

study utilized lactating 2-year old primparous cows. Cows were assigned to one of three treatments: 261 g of MP from RUP calculated to meet the MP requirement, 292 g of MP from RUP calculated to supply 31 g of MP in excess, or 297 g of MP from RUP with 100 g of propionate salt calculated to supply 36 g of MP in excess plus propionate. The supplements were designed to include increasing levels of MP from RUP and so it was hypothesized that increasing MP and propionate salt would increase the glucogenic potential. The study found as MP from RUP with or without propionate increased, a 4 d decrease was observed in postpartum interval, but pregnancy percentage did not differ among treatments

Glucose tolerance tests were conducted and showed that cows fed RUP propionate salts had greater rates of glucose clearance which could indicate improved nutrient incorporation. This could have influenced the abbreviation of postpartum interval that was observed. In addition, when supplements with increased glucogenic potential were fed, it was thought that glucose availability would also increase. This could impact tissue response to insulin as evident by the increase in glucose disappearance. Milk production is reliant on glucose which is then converted to lactose. The data shows a numerical decrease in milk production of 520 g/d from the third diet containing increased MP from RUP along with propionate salts. In theory, if the cows were more sensitive to insulin, they could partition nutrients away from milk production. The authors suggested that the data indicates not just vegetative quality, duration of lactation, and season of grazing as impacting a young postpartum beef cow's ability to respond and incorporate nutrients in insulin-sensitive tissue but type of supplementation may also play a role

(Waterman et al., 2006). It could be concluded that using ruminally degradable and undegradable protein with or without propionate salt could provide a method to alter metabolic function and decrease duration of postpartum anestrus in young postpartum beef cows.

Mulliniks et al. (2011) also investigated the use of ruminally undegradable protein and propionate salts on young range beef cows. This study utilized 2- and 3-year old range cows fed one of three supplements all of which were formulated to provide 36% CP on as as-fed basis. It was hypothesized that providing additional RUP could reduce days to first estrus and BW loss. The treatments included 328 g of CP from 110 g of RUP with 0 g of propionate salts (PS); 328 g of CP from 157 g of RUP with 40 g of PS; or 329 g of CP from 158 g of RUP with 80 g of PS (Mulliniks et al., 2011).. The results showed a quadratic response to glucogenic potential where the 40 g of propionate salts resulted in the shortest days to first estrus. Calf weaned per cow exposed to bulls increased quadratically, but there was no difference in milk yield. Even though the propionate salt treatments cost more, there were increased profits from cows fed 40 g of PS compared to those fed 0 g and 80 g (Mulliniks et al., 2011).

Nutrient Partitioning

Nutrient partitioning was evaluated further with a study examining responses of post-partum beef cows fed protein supplements containing propionate salt (Endecott et al., 2012). Young (2- and 3-year-old) postpartum cows were fed supplements containing either 142 g ruminally undegradable protein or 151 g of ruminally undegradable protein with 80 g of propionate salt. Cows fed the diet without propionate salt took 13 d longer to

return to estrus and produced more milk in the second year compared to the first year of the experiment, but cows fed a supplement with calcium propionate had consistent return to estrus and produced the same amount of milk regardless of year. The authors concluded that cows supplemented with glucogenic precursors (GP) from ruminally undegradable protein and propionate partitioned nutrients away from milk production (Endecott et al., 2012).

Monensin's label indication is for daily feeding, whereas calcium propionate can be fed less frequently. This could potentially be useful to producers and help reduce associated costs with feeding a product every day such as monensin. These studies highlight using propionate as a feed additive in developing heifers and postpartum cows. Propionate is not an ionophore, but rather the VFA itself, and may have different modes of action in the rumen compared to an ionophore like monensin. Therefore, further research into the use of propionate for developing heifers is needed. The objectives of this study shall be to investigate if calcium propionate elicits similar growth and reproductive responses as monensin in developing heifers.

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CHAPTER THREE

DOES CALCIUM PROPIONATE ELICIT SIMILAR FEED EFFICIENCY AND REPRODUCTIVE RESPONSES AS MONENSIN IN DEVELOPING HEIFERS?

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Manuscript Information Page

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Abstract

Replacement beef heifer development is critical for the continued production of beef and for ranch success. Objectives of this research were to investigate the growth and reproductive responses of developing heifers fed similar basal diets supplemented with pellets containing different feed additives. Pellet treatments consisted of 2.27 kg·heifer^{-1}·d^{-1} of: 1) control with no feed-additive (CON), 2) 200 mg·heifer^{-1}·d^{-1} monensin (MON), or 3) 40 g·heifer^{-1}·d^{-1} calcium propionate (PRO). Experiment 1 utilized one hundred and ninety-eight heifers (254 ± 3.8 kg) stratified by BW and randomly assigned to one of six pens (n = 2 pens per treatment). Experiment 2 utilized fifty-eight heifers (304 ± 3.4 kg) stratified by BW and randomly assigned to one of 12 pens (4 pens per treatment). Heifers were fed a grass hay basal diet in both experiments (Exp. 1: 65% TDN and 11% CP; Exp. 2: 62% TDN and 15% CP, DM basis). Experiment 1 was 129 d and Experiment 2 was 122 d. Body weights were collected at the beginning and end of each experiment, with interim BW collected every 30 d. Serum samples were collected via coccygeal venipuncture and analyzed for progesterone to determine pubertal status. Overall pregnancy rates and pregnancy rate from AI were determined. Experiment 1observed no differences for initial BW, final BW, overall ADG, pregnancy rate from AI, or overall pregnancy rate ($P \geq 0.16$). Fewer CON and PRO heifers were pubertal at experiment start than MON heifers ($P \leq 0.01$) in Experiment 1, which was unexpected. Differences in puberty achievement were diminished at the end of the experiment. Experiment 2 observed no differences for initial BW, final BW, overall ADG, pregnancy rate from AI, or overall pregnancy rate ($P \geq 0.19$). Treatment had no effect on puberty achievement at beginning or end of experiment ($P \geq$

0.09). A period effect was observed for ADG in both experiments ($P \leq 0.01$) which may be related to cold stress. Neither calcium propionate nor monensin resulted in improved performance compared to no feed additive. Further research is warranted to elucidate the impact of calcium propionate on heifer development.

Key words: heifer development, monensin, calcium propionate, puberty

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Introduction

Developing replacement heifers is an important program in a beef cattle operation that can influence cow longevity and lifetime productivity (Roberts et al., 2009). Heifers attain puberty at a genetically pre-determined weight that is unique to each animal (Funston and Deutscher, 2004). Although this weight cannot be predicted, it is directly related to growth (Roberson et al., 1999), making management of heifer development critical. Heifers that obtain puberty at an early age and calve early in their first calving season have increased herd longevity and a longer interval to rebreeding (Endecott et al., 2013).One method of influencing growth during heifer development is utilization of ionophores. It is well established that feeding ionophores to developing heifers improves feed efficiency and decreases age at puberty (Bergen and Bates, 1984). It has been shown that ionophores improve gains and feed efficiency without detrimental effects to other areas of performance, such as fertility or milk production (Goodrich et. al., 1984).

Although ionophores do not alter fertility, they can lead to decreased age at puberty in heifers. Sprott et al. (1988) suggested that this change in reproductive performance from feeding monensin was likely due to an increased propionate to acetate ratio in the rumen. Propionate is metabolized to generate around 27 to 54% of the glucose present in a ruminant (NRC, 2016) and represents a large portion of the energy required for weight gain.

Propionate salts have been used as supplement ingredients in past research (Mulliniks et al., 2011; Endecott et al., 2012; Waterman et al., 2014). It has been suggested that young postpartum cows fed glucogenic precursors, including propionate salts, have improved reproductive efficiency (Mulliniks et al., 2011). Young postpartum range cows fed protein supplements containing calcium propionate altered nutrient partitioning away from milk production and toward body weight gain, resulting in shorter days to first estrus (Endecott et al., 2012). Young postpartum range cows fed a protein supplement containing increased ruminally undegradable protein (RUP) and calcium propionate returned to estrus earlier than cows fed cottonseed meal supplement with no added RUP or propionate. These changes occurred independently of measurable changes in BW dynamics, suggesting improved efficiency of nutrient use (Waterman et al., 2006).

Limited data exists on the use of calcium propionate as a feed additive in heifer development diets. Waterman et al. (2014) included propionate salt in a high energy supplement fed to lightweight replacement heifers to investigate if their performance could equal heavier replacement heifers fed one of two lower-energy protein supplements. Heavier heifers were fed 908 g/d supplement with either low RUP or high

RUP and lightweight heifers were fed 1814 g/d of the low RUP supplement with 100 g/d of calcium propionate. The low and high RUP supplements were fed twice per week and the calcium propionate supplement was fed three times per week. Although not statistically significant, the lightweight heifers started out 24 kg lighter than their herdmates, but only differed by 15 kg at breeding. Additionally, more lightweight heifers were pubertal prior to breeding compared to the heavier heifers but all heifers had similar pregnancy rates at the end of the experiment. However, these data were confounded due to the different supplement energy levels and initial heifer body weight, warranting further research into the use of calcium propionate as a feed additive for developing heifers.

The objectives of the study were to investigate the growth and reproductive responses of developing heifers fed similar basal diets supplemented with pellets containing no feed additive, monensin, or calcium propionate.

Materials and Methods

This two-year project was approved by the Montana Agricultural Experiment Station Agricultural Animal Care and Use Committee (AACUC #2015-AA07).

Experiment 1

Experiment 1 took place at the Bair Ranch near Martinsdale, MT from January 6, 2016 through May 4, 2016. Bair Ranch Angus/Simmental replacement heifers (n = 198; 254 ± 3.8 kg) were stratified by body weight (BW) and randomly assigned to 1 of 6 pens. Treatments were randomly assigned to pens (2 pens/treatment). Treatments consisted of

2.27 kg·heifer^{-1}·d^{-1} pellets containing 1) control, no feed-additive (CON), 2) 200 mg·heifer^{-1}·d^{-1} monensin (Rumensin®, Elanco; MON), or 3) 40 g·heifer^{-1}·d^{-1} calcium propionate (NutroCAL™, Kemin Industries, Inc.; PRO). Pellets were milled by CHS Nutrition, Great Falls, MT, and were top dressed on the basal diet of grass hay each day. Grass hay harvested at Bair Ranch (65% TDN, 11% CP, DM basis; Table 1) was fed once daily to yield bunks where scattered feed was present and most of the bunk was visible 24 h after feeding. Nutrient composition of hay and pellets is detailed in Table 1 and ingredient composition of pellets is detailed in Table 2.

Body weights were collected at the beginning and end of the study, with interim BW collected every 30 d. Heifer ADG was calculated for each period (period = approximately 30 d) of the experiment and for the entirety of the experiment. A total of 4 serum samples were collected via coccygeal venipuncture at the beginning (n = 2 samples, 10 days apart) and end (n = 2 samples, 10 days apart) of the experiment for progesterone analysis. Corvac™ (Monoject™ Covidien Ag, Neuhausen Am Rheinfall, Switzerland) serum separator tubes (16 mm × 100 mm) were used for blood collection. Samples were collected at days 0, 10, 79, and 89. The samples were spun in a centrifuge at 3800 × *g* for 30 min at 4° C to separate the serum from the blood. Serum was decanted into plastic vials and stored at -20° C for later analysis.

Heifers were synchronized beginning May 4, 2016, using a Select Synch protocol. Heifers received a GnRH injection on May 4, and estrus detection and artificial insemination (AI) began May 10. Heifers not detected in estrus by May 11 were given an injection of $PGF_2\alpha$, and AI continued until May 15. Heifers who did not express estrus

were not time-bred. All heifers were turned out with cleanup bulls on range according to Bair Ranch protocol. Heifers were then gathered for pregnancy detection in August; however, 11 heifers were missing in the gather and are not included in the pregnancy data (n = 187). Pregnancy was detected on August 19, 2016 via rectal ultrasonography by the ranch veterinarian. Pregnant heifers' fetuses were aged by the veterinarian and designated as either AI- or cleanup bull-sired.

Serum samples were analyzed for progesterone using an enzyme-linked immunoassay assay (ELISA; Enzo Life Sciences, Farmingdale, NY, USA) validated for beef cattle serum. Serum samples (n = 796) were randomized by beginning and end collection dates (days 0 and 10, and days 79 and 89) due to variation in serum tube size, resulting in 6 assays per collection date. Samples were assayed with beef male samples for standardization. The optical density from the ELISA was used to determine progesterone concentrations. Intra- and inter- assay coefficient values (CVs) for a pooled sample that contain 0.30 ng/mL of P4 were 6.1 and 4.6, respectively. The sensitivity of the assay was 4.9 pg/mL. Heifers were deemed pubertal at a given time point (e.g., beginning or end of experiment) if one or both progesterone concentrations from the respective 10-day period were ≥ 1 ng/mL as described by Waterman et al., (2006).

Body weights and ADG were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with fixed effects of treatment, period, their interaction and the random effect of pen. Pen was the experimental unit. Puberty and pregnancy rate were analyzed using the GENMOD procedure of SAS. Significance parameters were set at $P \leq 0.05$.

Experiment 2

Experiment 2 utilized Montana State University Bozeman Agricultural Research and Teaching (BART) Farm Red Angus and Angus cross replacement heifers and was conducted from January 3, 2017 through May 5, 2017. Heifers (n = 58; 304 ± 3.4 kg) were stratified by BW and breed type and randomly assigned to one of 12 pens, 6 on the north side and 6 on the south side of the drylot. Treatments were randomly assigned to pens by side so that each treatment was replicated on both sides of the drylot (4 pens/treatment). Treatments were identical to the previous study and were top dressed over BART Farm grass hay (62% TDN, 15% CP, DM basis; Table 1). Grass hay was fed once daily at a rate of 9.1 kg per heifer and bunks were monitored daily to adjust amounts as needed. If bunks were slick, feed was increased by 2.27 kg per heifer.

Body weights were collected every 30 days. Heifer ADG was calculated for each period of the experiment for the entirety of the experiment. A total of 6 serum samples were collected via coccygeal venipuncture at the beginning (n = 2 samples, 10 days apart), middle (n = 2 samples, 10 days apart) and end (n = 2 samples 10 days apart) of the experiment for progesterone analysis. Samples were collected on days 0, 10, 48, 58, 97, and 107. Serum was collected and processed as described previously.

Heifers started synchronization on April 25, 2017 when CIDRS were placed and each heifer was given an injection of GnRH. A week later on May 2, CIDRS were removed, and heifers received an injection of $PGF_2\alpha$ and an Estrotect™ heat watch patch on the tail head. Estrus detection and artificial insemination occurred for 72 hours with heifers being checked 4-5 times a day. Heifers not detected to be in estrus were given an injection of

GnRH on May 5 and time bred via AI. Heifers were turned out with cleanup bulls on May 12, 2017. Pregnancy was detected on September 21, 2017, via rectal ultrasonography by local veterinarian. Pregnant heifers' fetuses were aged by the veterinarian and designated as either AI- or cleanup bull-sired.

Serum samples for 2017 were analyzed as previously described using ELISA assays. Samples (n = 348) were randomized by beginning/middle and end due to variation in serum tube size, resulting in 7 assays per collection date. Samples were assayed with pregnant cow samples for standardization. The optical density from the ELISA was used to determine progesterone concentrations. Intra- and inter- assay coefficient values (CVs) for a pooled sample that contain 0.30 ng/mL of P4 were 8.0 and 11.33, respectively. The sensitivity of the assay was 4.6 pg/mL. Criteria for puberty attainment were as previously described.

Body weights and ADG were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with fixed effects of treatment, period, their interaction and the random effect of pen. Pen was the experimental unit and block (side of the drylot) was included in the model. Puberty and pregnancy rate were analyzed using the GENMOD procedure of SAS.

Results

Experiment 1

No treatment by period interactions were detected ($P = 0.73$). Heifer initial BW were similar among treatments ($P = 0.94$; Table 3) as intended by study design. Heifers did not exhibit significant differences in overall ADG ($P = 0.73$) due to treatment and this

resulted in similar final BW ($P = 0.43$). A period effect was observed for ADG ($P \leq 0.01$; Table 4). Heifers gained the least in period 1 and the most in period 4, while periods 2 and 3 were intermediate.

Serum samples were analyzed for progesterone (P4) content to determine puberty status. As previously described, heifers were deemed pubertal at a given time point if one or both P4 concentrations from the 10-day period were ≥ 1 ng/mL. At the start of the experiment, more MON heifers were pubertal compared to CON or PRO heifers ($P \leq 0.01$; Table 3). At the end of the study, the differences between the treatments were diminished. Ultimately, the MON group achieved 100% puberty at the end of the experiment compared to 98% for CON and 93% for PRO ($P < 0.01$; Table 3).

At the conclusion of the feeding trial, heifers were artificially inseminated (AI) and then turned out with a clean-up bull. Pregnancy rate to AI and overall pregnancy rate were collected to further evaluate the effect of treatment on conception rates. There were no treatment differences for pregnancy to AI ($P = 0.16$) nor for overall pregnancy rate ($P = 0.32$). Heifers achieved overall pregnancy rates of 83% or above (Table 3).

Experiment 2

Period by treatment interaction was not significant ($P = 0.23$). Heifer initial BW were similar among treatments ($P = 0.96$; Table 5) as intended by study design. Heifers did not exhibit significant differences in overall ADG ($P = 0.98$) and this resulted in similar final BW ($P = 0.98$). A period effect was observed for ADG ($P \leq 0.01$; Table 6). Heifers gained the least in period 1 and the most in period 4, while periods 2 and 3 were intermediate.

Serum samples were analyzed for P4 content and criteria for puberty were identical as previously described. There were no significant differences for achievement of puberty at the start of the experiment ($P = 0.27$; Table 4). Additionally, there were no treatment effects on puberty achievement at the middle ($P = 0.15$) or at the end of the experiment ($P = 0.09$). Heifers in the MON treatment had 100% puberty achievement compared to 84% for CON and 95% for PRO.

At the end of the feeding trial heifers were bred AI and then turned out with a clean-up bull. There were no treatment differences for pregnancy to AI ($P = 0.32$) nor for overall pregnancy rate ($P = 0.15$; Table 5). Heifers in the PRO group had a pregnancy rate of 79% compared to 95% for both CON and MON (Table 5).

Discussion

Average daily gains were similar for all treatments in both experiments. This is contrary to previous literature where feeding monensin increased ADG compared to a control diet. Utley et al. (1976) reported heifers fed 200 $mg \cdot heifer^{-1} \cdot d^{-1}$ of monensin gained 0.76 kg/d compared to heifers fed the control diet that gained 0.68 kg/d. In addition, Mosely et al. (1977) found heifers fed monensin gained 0.36 kg/d more than heifers fed a control diet. Additionally, Waterman et al. (2014) found heifers fed a protein supplement with calcium propionate gained 0.33 kg/d compared to heifers fed protein supplements without calcium propionate who gained 0.14 kg/d; however, this data is confounded by initial heifer BW and supplement energy density.

Both experiments reflected period effects for ADG where period 1 had the lowest gain. One explanation for this could be attributed to weather differences between periods. Heifers were exposed to temperatures outside their thermoneutral zones (TNZ) for periods during both years. Cattle experience a range of temperatures described as the TNZ where energy is not expended to maintain body temperature (Field, 2007). When air temperatures are below the lower critical temperature (LCT) of the TNZ, animals can experience cold stress that could impact performance (Webster et al., 1970; Birkelo et al., 1991; Houseal and Olson, 1995; NRC, 2016). The LCT for cattle with a heavy winter coat is -7.77° C (Field, 2007). In 2016, Harlowtown, MT (approximately 45.06 km from Martinsdale) experienced 10 d in January that the minimum temperature was below this LCT compared to 2 d in February. In 2017, Bozeman MT experienced 29 d in January below this LCT and 28 d in February (Underground, 2016). Wind data taken from the nearest weather station recording wind speed (approximately 72 km from Martinsdale) recorded average wind speeds of 19 and 21 kmh for January and February, respectively. Average wind speeds for Experiment 2 in Bozeman were recorded at 6 and 10 kmh for January and February, respectively (National Climatic Data Center, 2016, 2017). Cold stress could have impacted heifer performance in these experiments as reflected in the period effects observed for ADG.

In both experiments, heifers were stratified by BW then assigned to treatments. Since heifers attain puberty at a genetically pre-determined weight that is related to growth (Patterson et al., 1992; Funston and Deutscher, 2004), it would be expected that stratifying by BW would remove variation in puberty achievement. This was not

observed in Experiment 1 where fewer CON and PRO heifers were pubertal compared to MON at experiment start. While it would be difficult to attribute this observation to a treatment effect where progesterone concentrations decreased for CON and PRO and/or increased for MON after the first 10 d of feeding, it cannot be ruled out. This anomaly was not observed in Experiment 2, as puberty achievement at the start of the experiment was similar for all treatments as intended by study design.

More monensin fed-heifers achieved puberty compared to calcium propionate and control at experiment end in Experiment 1. This is similar to previous literature where monensin-fed heifers had increased puberty rates over control-fed heifers. Mosely et al. (1977) found that 92% of heifers fed 200 mg·heifer^{-1}·d^{-1} monensin achieved puberty compared to 58% fed a control diet. Similarly, it was found that heifers supplemented with monensin achieved puberty 8 days earlier compared to control fed heifers (Purvis and Whittier, 1996). This was not observed in Experiment 2 where puberty achievement at experiment end was similar across all treatments, contrary to previous literature. Additionally, our observation of no differences in puberty achievement between PRO and the other treatments is dissimilar from previous research. Waterman et al. (2014) found that heifers fed a protein supplement with calcium propionate had higher puberty rates of 57% compared to 30% for heifers fed other protein supplement treatments without calcium propionate. However, it's important to note that these animals were fed on range in an extensive situation and the calcium propionate heifers received a more energy-dense diet. Furthermore, protein supplements without calcium propionate were fed twice a week while the protein supplement with calcium propionate was fed three times per

week. Supplements containing calcium propionate were also fed infrequently (2x per week) in previous research with postpartum cows (Waterman et al., 2006; Mulliniks et al., 2011; Endecott et al., 2012). It could be theorized that calcium propionate might have more benefit when fed less frequently, where the feed additive is introduced into the rumen in pulses rather than a constant supply. This warrants further research on frequency of calcium propionate supplementation.

There were no differences in either experiment for pregnancy to AI or overall pregnancy rates. This could indicate that treatments ultimately resulted in equivalent reproductive proficiency similarly to Purvis and Whittier (1996).Waterman et al. (2014) found that lightweight heifers fed a low RUP diet supplemented with calcium propionate achieved comparable pregnancy rates of 65% compared to 74% for their heavier counterparts.

Neither monensin nor calcium propionate as feed additives for developing heifers resulted in improved performance compared to no feed additive. The performance of MON and PRO heifers did not reflect previous observations in the literature.

Much of the research utilizing monensin as a feed additive in heifer development occurred during the 1970's and 1980's. During this same period, the beef cattle industry made a shift from developing heifers to have their first calf at 3 years of age to developing heifers to have their first calf at 2 years of age (Funston et al., 2012). This placed greater selection pressure for puberty achievement and fertility on yearling heifers than previous management strategies. Furthermore, the discovery of the association between increased sire scrotal circumference and decreased age at puberty in their

daughters was made during this time period (Brinks et al., 1978). Breed association sire summaries show substantial scrotal circumference increases since that time and a similar response for decreasing heifer age at puberty would be expected. Utilizing monensin as feed additive to develop heifers during this era might have brought more advantages during a time period of increasing selection pressure for heifers to achieve puberty at a younger age.

As mentioned previously, calcium propionate supplements in previous research were fed less frequently (2-3 x per week) than in this experiment, where it was fed every day. Perhaps calcium propionate would have more benefit if fed less frequently, as would be more common on an extensively managed operation. Additionally, previous research developed heifers in an extensive situation where the basal diet consisted of lower quality or dormant forage (Waterman et al., 2014). In that situation, a feed additive such as monensin or calcium propionate might have more benefit for heifer gain and reproductive performance compared to an intensive system where heifers are provided with more nutrient-dense diets.

Conclusions

Growth and reproductive responses in developing beef heifers fed different feed additives were examined in two experiments. The performance of monensin and calcium propionate did not reflect previous observations in research.

The bulk of monensin research was conducted in the 1970's and 1980's. This time period also encompasses the beef industry's shift to having heifers calve at 2 years of age instead of 3, as well as the discovery of the association between scrotal circumference in

bulls and age at puberty in their daughters. These production changes led to increased selection pressure for early heifer puberty. Monensin research saw large advantages from its use during this industry change that may not be as noticeable in today's cow herd.

Calcium propionate supplements in previous research were fed less frequently (2-3 x per week) than in this experiment where it was fed every day. Perhaps calcium propionate would have more benefit if fed less frequently, as would be more common on an extensively managed operation. Additionally, previous research developed heifers in an extensive situation where the basal diet consisted of lower quality or dormant forage. In that situation, a feed additive such as monensin or calcium propionate, might have more benefit for heifer gain compared to an intensive system where heifers were provided with more nutrient dense diets.

Neither monensin nor calcium propionate as feed additives for developing heifers resulted in improved performance compared to no feed additive. Heifer genetic potential, energy density of basal diet, and frequency of supplementation could have all played a role in the performance of heifers in these experiments. Therefore, further research is warranted to elucidate the impact of feed additives such as monensin and calcium propionate in more extensive heifer development systems using lower quality forage and to elucidate feeding frequency impacts of calcium propionate on growth and puberty characteristics of developing heifers.

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Table 1. Nutrient composition of hay and pellet treatments

			Pellet Treatments[a]		
Item, % DM basis	Experiment 1 Hay	Experiment 2 Hay	CON	MON	PRO
TDN	65.5	61.7	70.4	70.3	69.7
ADF	34.1	39.6	9.9	9.9	9.7
CP	11.0	14.9	22.1	22.1	22.1
Fat	3.2	1.3	3.4	3.4	3.4

[a]CON = no feed-additive, MON = 200 mg·heifer^{-1}·d^{-1} monensin, PRO = 40 g·heifer^{-1}·d^{-1} calcium propionate. Treatments were fed at 2.27 kg·heifer^{-1}·d^{-1}

Table 2. Ingredient composition of pellet treatments

	Pellet Treatments [a], %		
Item, as fed basis	CON	MON	PRO
Wheat middlings	36.3	36.3	34.8
Ground corn	20.0	20.0	20.0
Canola meal	20.0	20.0	20.0
Soybean meal	7.0	7.0	7.5
Dried distillers grain	5.0	5.0	5.0
Malt sprouts	5.0	5.0	5.0
Cane molasses	5.0	5.0	5.0
Calcium carbonate	0.9	0.9	0.1
Salt	0.2	0.2	0.2
Bonding agent	0.1	0.1	0.1
Trace mineral premix	0.5	0.5	0.5
Vitamin premix	0.01	0.01	0.01

Rumensin®	--	200 mg/d	--
NutroCAL™	--	--	40 g/d

[a]CON = no feed additive; MON = 200 mg·heifer^{-1}·d^{-1} monensin; PRO = 40 g·heifer^{-1}·d^{-1} calcium propionate. Treatments were fed at 2.27 kg·heifer^{-1}·d^{-1}

Table 3. Experiment 1: Effects of no feed-additive (CON), monensin (MON) or calcium propionate (PRO) treatments on growth and reproductive traits of developing heifers

	Treatment[a]				
	CON	MON	PRO	SE	*P*-value
Initial BW, kg	252	253	254	3.7	0.94
Final BW, kg	381	385	378	3.7	0.43
Overall ADG, kg/d	1.21	1.17	1.18	0.1	0.73
Percent pubertal at experiment start	80 (58/66)[b]	98 (65/66)[c]	77 (51/66)[b]	--	≤ 0.01
Percent pubertal at experiment end	98 (65/66)	100 (66/66)	93 (62/66)	--	0.05
Pregnancy rate to AI, % (pregnant/inseminated)	61 (37/60)	61 (38/61)	46 (60/64)	--	0.16
Overall pregnancy rate, %	90 (54/60)	83 (52/62)	92 (59/64)	--	0.32

[a]CON = no feed additive; MON = 200 mg·heifer^{-1}·d^{-1} monensin; PRO = 40 g·heifer^{-1}·d^{-1} calcium propionate. Treatments were fed at 2.27 kg·heifer^{-1}·d^{-1}

[b,c] Means without a common superscript differ ($P \leq 0.01$)

Table 4. Experiment 1: Period effect ($P \leq 0.01$) for ADG

Period (30 d)	ADG	SE
P1	0.88[b]	0.08
P2	1.30[a,c]	0.08
P3	0.98[a,b]	0.08
P4	1.59[c]	0.08

[a,b,c] Means without a common superscript differ ($P \leq 0.01$)

Table 5. Experiment 2: Effects of no feed additive (CON), monensin (MON) or calcium propionate (PRO) treatments on growth and reproductive traits of developing heifers

	Treatment[a]				
	CON	MON	PRO	SE	*P*-value
Initial BW, kg	307	310	309	5.9	0.96
Final BW, kg	437	436	437	6.5	0.98
Overall ADG, kg/d	1.21	1.17	1.19	0.06	0.68
Percent pubertal at experiment start	63 (12/19)	85 (17/20)	79 (15/19)	--	0.27
Percent pubertal at experiment middle	73 (14/19)	95 (18/19)	95 (18/19)	--	0.16
Percent pubertal at experiment end	84 (16/19)	100 (20/20)	95 (18/19)	--	0.09
Pregnancy rate to AI, % (pregnant/inseminated)	53 (10/19)	53 (10/19)	31 (6/19)	--	0.33
Overall pregnancy rate, %	95 (18/19)	95 (19/20)	79 (15/19)	--	0.15

[a]CON = no feed additive; MON = 200 mg·heifer^{-1}·d^{-1} monensin; PRO = 40 g·heifer^{-1}·d^{-1} calcium propionate. Treatments were fed at 2.27 kg·heifer^{-1}·d^{-1}

Table 6. Experiment 2: Period effect ($P \leq 0.01$) for ADG

Period (30 d)	ADG	SE
P1	0.89[a]	0.05
P2	1.29[b]	0.05
P3	0.97[a]	0.05
P4	1.59[c]	0.05

[a,b,c] Means without a common superscript differ ($P = <0.01$)

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