THESIS

EFFECTS OF MONENSIN SODIUM AND PLANT EXTRACTS CONTAINING CINNAMALDEHYDE, CAPSICUM OLEORESIN, AND EUGENOL ON DAYS TO PUBERTY, GAIN, PREGNANCY RATE, AND FEED EFFICIENCY IN DEVELOPING BEEF HEIFERS RECEIVING A HIGH ROUGHAGE DIET

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ABSTRACT

EFFECTS OF MONENSIN SODIUM AND PLANT EXTRACTS CONTAINING CINNAMALDEHYDE, CAPSICUM OLEORESIN, AND EUGENOL ON DAYS TO PUBERTY, GAIN, PREGNANCY RATE, AND FEED EFFICIENCY IN DEVELOPING BEEF HEIFERS RECEIVING A HIGH ROUGHAGE DIET

The objectives of the studies were to determine the effect of monensin sodium vs. a combination of plant extracts containing cinnamaldehdye, capsicum oleoresin, and eugenol on ADG, feed efficiency, pubertal onset, subsequent conception rate following AI, and differences in docility among yearling beef heifers.

During exp. 1, Angus heifers (n = 105; initial BW 347.4 ± 29.5 kg) were utilized in a completely randomized block design for a 72 d experiment. Heifers were randomly distributed within 5 weight blocks**,** and assigned to one of 3 treatments: monensin sodium (**MON**); combination of plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol (**CCE**); or no feed additive (**CON**). Supplement consisted of a dried distillers grain base, and was administered at a rate of 0.32 $kg \cdot hd^{-1} \cdot d^{-1}$ as a top dress on a high forage ration. The MON premix was fed to supply 200 $mg \cdot hd^{-1} \cdot d^{-1}$ monensin sodium, and the CCE premix was fed to supply 1400 $mg \cdot hd^{-1} \cdot d^{-1}$ of the plant extracts; cinnamaldehyde, capsicum, and eugenol. These dosage levels were recommended by ADM Alliance (Archer Daniels Midland Inc; Quincy, Illinois). All heifers were observed daily to document behavioral estrus to define age at puberty. Pubertal onset was defined as the onset of behavioral estrus and was measured in d during the 72-d feeding period. Body weight was collected on 2 consecutive d at the beginning and end of the trial to determine

initial BW, final BW, and ADG. Ort samples were collected and weighed every other d to calculate feed intake and feed efficiency. Performance measurements included ADG, DMI, G:F, and pubertal onset. There was a tendency (P = 0.06) for CON (0.53 kg·hd^{-1}·d^{-1}) and MON (0.47 kg·hd^{-1}·d^{-1}) heifers to have greater ADG compared to CCE (0.41 kg·hd^{-1}·d^{-1}). There was a tendency (P = 0.09) for CON heifers to have greater AI pregnancy rate than females that received MON (59.8 vs. 44.2%). There were no differences (P > 0.10) for DMI, G:F, or pubertal onset between treatments.

During exp. 2, of the study, Angus heifers (n = 107; initial BW of 318.4 ± 26.1 kg) were utilized in a balanced randomized block design using a 2 X 2 factorial arrangement of treatments. Females were stratified according to initial BW and randomly assigned to pens. Pens were randomly assigned to 1 of 4 treatments: 1) monensin sodium (**MON**); 2) combination of plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol (**CCE**); 3) no feed additive (**CON**); or a combination of both monensin sodium and a combination of plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol (**COMB**). The MON premix was fed to supply 200 mg·hd^{-1}·d^{-1} monensin sodium, the CCE premix was fed to supply 12,000 mg·hd^{-1}·d^{-1} of the plant extracts, and the COMB premix was fed to supply 12,000 mg·hd^{-1}·d^{-1} of the plant extracts as well as 200 mg·hd^{-1}·d^{-1} monensin sodium. During exp. 2, blood samples were collected via jugular venipuncture at 11 d intervals for progesterone concentration via RIA analysis to determine time of pubertal onset. Once a blood sample reached a progesterone concentration ≥1 ng/mL, the heifer was considered to be pubertal. Also during exp. 2, subjective chute scores (**CS**) and objective exit velocity (**EV**) measurements were taken at 11 d intervals for each heifer. Weight for each heifer was also collected at 11 d intervals to calculate ADG. Ort samples were collected and weighed every other d to calculate feed intake and feed efficiency. Performance

measurements included ADG, DMI, G:F, initial BW, 60 d BW, and final BW. Behavioral measurements take on d 0, 60, and 120 included EV and CS. Reproductive performance measurements included age at puberty, pregnancy rate to AI, BCS, and BW at breeding.

Heifers in the COMB (1.1 $kg \cdot hd^{-1} \cdot d^{-1}$) and CCE (0.8 $kg \cdot hd^{-1} \cdot d^{-1}$) treatment group that received a combination of plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol along with monensin sodium had a greater ADG ($P = 0.04$) than the CON (0.6 $kg \cdot hd^{-1} \cdot d^{-1}$) and the MON (0.7 $kg \cdot hd^{-1} \cdot d^{-1}$) heifers. These data explain that DMI was reduced ($P = 0.02$) for heifers in the MON (9.80 kg ± 0.57) and the COMB (9.16 kg ± 0.57) treatments when compared to females in both the CON (10.55 kg ± 0.57) and CCE (11.36 kg ± 0.57) treatment groups. There were no differences ($P > 0.05$) between treatments for G:F, initial BW, 60 d BW, or final BW.

Differences in behavior were measured between treatments. Final EV was greater ($P = 0.05$) for heifers in the CCE (0.66 m/s) as well as the COMB (0.73 m/s) treatment groups when compared to females in the CON (0.71 m/s) and MON 1.19 (m/s) treatments. There was no difference ($P > 0.05$) between treatments for initial, midpoint, or final CS; initial and midpoint EV were also not affected ($P > 0.05$) by treatment.

Receiving monensin sodium (347.1 d), a combination of plant extracts (342.5 d), or a combination of the 2 (355.4 d) decreased ($P = 0.03$) age at puberty when compared to the controls (370.6 d). Breeding weight was collected the last d of supplementation, heifers in the CCE (440.06 kg) exhibited the greatest BW at breeding compared to MON (422.6 kg), COMB (422.8 kg), or CON (429.71 kg) There was a tendency ($P = 0.06$) for CON (0.53 $kg \cdot hd^{-1} \cdot d^{-1}$) and MON (0.47 $kg \cdot hd^{-1} \cdot d^{-1}$) heifers to have greater ADG compared to CCE (0.41 $kg \cdot hd^{-1} \cdot d^{-1}$). Body

condition scores, response to estrus synchronization, and pregnancy rate were not ($P > 0.05$) different by treatment.

Key Words: Average daily gain, Beef heifers, Monensin, Plant extracts, Puberty

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CHAPTER I

REVIEW OF LITERATURE

Monensin

Monensin sodium is an ionophore or compound produced by *Streptomyces cinnaraonensis.* Monensin sodium has been defined as a substance capable of interacting stoichmetrically with metal ions, consequently serving as a carrier of ions being transported across a bimolecular lipid membrane (Bergen and Bates, 1984). Supplementing Monensin increases feed efficiency in cattle (Depenbusch et al., 2008), and alters rumen fermentation making propionic acid a greater proportion of ruminal volatile fatty acid production (Davis and Erhart., 1976). Not to mention this compound will decrease feed intake, while increasing ruminal pH (Erickson et al., 2003). Monensin has shown to reduce feed intake, and Moseley et al., (1977) reported that heifers fed monensin required 382 g/d less feed than heifers fed a control. In vitro studies have indicated less microbial methane production in conjunction with monensin (Bartley et al., 1979). Lastly, numerous studies have shown that monensin sodium reduces rumen degradation of protein, allowing for a greater proportion of protein to "bypass" the rumen and be absorbed by the body (Chen et al., 1991).

Ionophores such as monensin have been labeled ion bearers. The transport cycle initiates when an anionic form of an ionophore directly associates with a cell membrane in a stabilized environment. Monensin is a negatively charged anion capable of pairing ions with a metal cation, which are positively charged. A lipophilic cycle cation is the direct result of this pairing and will be diffused through the membrane wall. Monensin disrupts the sodium (Na+) Potassium (K+) pump. The Na+/K+ pump will normally transport 3 Na+ out of the cell and two K+ into the cell,

maintaining the charge differential across the cell membrane. Monensin has a high affinity (10 times) for Na+ than for K. Monensin can play a key role within the rumen, by interfering with normal ion flux (Bergan and Bates, 1984). Monensin sodium is a valuable tool for cattle producers, and has an array of effects on the ruminant animal.

Effect of Monensin on Volatile Fatty Acid Production

Numerous studies have well documented the effects of monensin sodium on VFA production within the ruminant. Monensin sodium has been shown to increase production of propionic acid, which is the main source of blood glucose. Carbohydrates are broken down in the rumen into volatile fatty acids or VFAs. These VFAs include acetate, propionate, and butyrate. Acetate and butyrate have an even number of carbons; acetate having four carbons and butyrate having two carbons. Because they have an even number of carbons, they can go directly into fat storage or can be oxidized to generate ATP. However, propionate has an odd number of carbons (three carbons); therefore it is utilized in the liver pairing with another three-carbon compound to form a six-carbon compound which yields the greatest ATP. Studies have shown that monensin has increased molar percentage of propionic acid as much as 52%, with no effect of total volatile fatty acid production (Richardson et al., 1974). Another study found that ruminal moles/100g propionic acid was increased by 30.9% in monensin sodium fed heifers compared to a control group (Moseley et al., 1977). To support these results, Lamenager et al., (1980) found a significant increase in the molar percentage of propionic acid in the rumen, with a decrease in molar percentage of acetate while feeding cattle a high roughage diet along with 200 mg of monensin sodium. A study was conducted by Smith et al., (2010), which stated monensin sodium linearly increased the molar proportion of propionate while linearly decreasing the molar proportions of acetate, butyrate, and isovalerate with a linear decrease in the A:P ratio. By

affecting the A:P ratio as well as increasing the molar proportion of propionate and decreasing the molar percentage of acetate, monensin sodium plays a critical role in allowing for more feed efficient cattle and has proven to be a valuable tool to cattleman.

Decrease Methane Production

In vitro and In vivo studies have concluded less microbial methane (CH_4) production by utilizing monensin sodium (Bartley et al., 1979). This becomes very important concerning the regulation of CH_4, especially when considering enteric CH^4 production results in a loss of feed energy equivalent to 1 L of CH_4 to 39.5 kJ of feed energy (Guan et al., 2006). Smith et al., (2010) explained that total gas production was decreased by up to 9% in cattle being fed monensin sodium in combination with a diet comprised of high roughage. These researchers also conducted two in vitro experiments analyzing the effects of monensin sodium on CH_4 production, and found a decrease of CH_4 production by 13 and 15% when monensin sodium was added to the in vitro cultures. It has not been proven that monensin sodium has any effect on methanogens, which are microorganisms responsible for the production of methane in both the ruminant and human. Monensin sodium aids in the decreased metabolism of formate to carbon dioxide and hydrogen leading to the decreased production of methane, or inhibiting bacteria in the rumen that aids in the production of CH_4 (Smith et al., 2010). Conflicting research has been discussed, which explained the reason for a decrease in CH_4 is due to a decrease in DMI rather than a direct effect of ionophores such as monensin on CH^4 production (Johnson and Johnson., 1995). However, the majority of literature states that ionophores do directly affect CH_4 production, with consistent results throughout entire feeding periods (Smith et al., 2010). Methane production accounts for an extensive loss of energy, by limiting the production of methane we can allow for cattle to more effectively convert feed intake to pounds of gain.

Protein bypass

Monensin sodium has a “protein sparing” effect (Chen et al., 1991). It is accepted that monensin sodium has a protein sparing effect which causes a reduction in degradation of protein, peptides, and amino acids (Bergen and Bates., 1984). Studies have indicated a decreased concentration of ammonia, and an increase in non-ammonia, non-protein nitrogen. These data suggest that monensin sodium has a direct effect toward deamination (Chen et al., 1991). Deamination is the removal of and amine group from a certain molecule, and the process of breaking down amino acids when protein is readily available. It is possible that monensin sodium reduces protein degradation; however, it appears to increase deamination of amino acids within the ruminant (Russell and Strobel., 1989). Protein synthesis and turnover is extremely energy expensive. Several ATPs are required to generate one peptide bond, which is the coupling of one amino acid to another. When protein escapes rumen degradation, it is referred to as “protein bypass.” With the increase of protein bypass, more protein passes to the small intestine allowing for a greater proportion of dietary protein to be absorbed and utilized. Monensin sodium causes a decreased breakdown of dietary protein within the rumen, which is proven by a decrease in rumen ammonia levels (Whetstone et al., 1981). This study also explained a decrease in ammonia nitrogen levels, with an increase of both alpha-amino nitrogen and peptide levels when monensin was supplied.

Effects of Monensin on Pubertal Onset and Pregnancy Rate

Puberty has been defined as ovulation accompanied by signs of estrus and the occurrence of normal luteal function (Perry et al., 2012). It is essential to have females reach puberty prior to the breeding season due to pregnancy success during the breeding season has been correlated

with the percent of heifers that had reached puberty before or perhaps early in the breeding season (Perry et al,. 2012). By changing dietary intake and utilizing feed additives such as an ionophore like monensin sodium, age and weight at the time of pubertal onset can be manipulated independent of body weight gain, thus reducing feed cost while developing beef heifers as well as overall cost of production (Lalman et al., 1993). Studies have been conducted analyzing the effect of monensin sodium on decreasing age at puberty as well as its effect on pregnancy rate. It has been shown that ionophores such as monensin sodium have decreased both the age and weight of females at puberty (Purvis and Whittier 1996). These results also state that there was a trend for heifers that received an ionophore to be more conducive to become pregnant following artificial insemination, possibly due the increased percentage of female that were pubertal prior to estrus synchronization. It has been show that altering rumen fermentation by changing dietary energy will allow females to reach puberty at a younger age (Moseley et al., 1977) Heifers that conceive earlier in the breeding season and calve earlier in the calving season, yield a greater number of heavier calves throughout a more productive lifetime (Lesmeister et al., 1973). Not to mention females who calve earlier in the calving season are allowed a longer postpartum period, reducing the likelihood of them falling out of the herd due to reproductive failure. Attainment of early puberty should be an important managerial tool, and the ability to achieve early puberty with minimal inputs and high compensatory gain has an economic advantage (Lalman et al., 1993). A study conducted by Moseley et al. (1977) reported 92% of heifers that were fed monensin sodium reached puberty compared with 58% of heifers fed control and was not due to an increase in average daily gain or body weight. Lalman et al., (1993) found that diet composition changed the age and weight at puberty even though ADG was similar, and heifers who received monensin sodium were on average 5 d younger than control

females, with similar dry matter intake between the treatment groups. Also, at the end of the breeding season the pregnancy rate was not different between heifers fed monensin sodium when compared to the control group (Lalman et al., 1993, McCartor el al., 1979, Moseley et al., 1977). Purvis and Whittier (1996) state that heifers who received the ionophore monensin sodium were 8 d younger, and 10 kg lighter at puberty when compared to the control group which received no feed additive or growth promotant. However, these results showed no significant improvement in pregnancy rate comparing the heifers who received an ionophore to the heifers that did not.

Plant Extracts: Cinnamaldehyde, Capsicum oleoresin, & Eugenol

There is a growing demand for naturally fed cattle, and with this growing demand the use of plant extracts has risen. Because of this growing demand, plant extracts are more commonly being fed as natural alternatives (Cordozo et al., 2006). Unfortunately, few studies have been conducted analyzing the effects of these plant extracts on feedlot performance, and of the studies reported to date, results have been conflicting. Perhaps due to the lack of explanation entailing the mechanism of how essential oil mixtures affect rumen fermentation through modification of microorganisms (Meyer et al., 2009). The majority of data which analyzes these plant extracts differ between species, diet, and level at which the plant extract is administered. Plant extracts such as cinnamaldehyde, capsicum oleoresin, and eugenol occur naturally and may be extracted using organic solvents (Calsamiglia et al., 2005). These plant extracts have been shown to improve metabolism in ruminants. In vitro studies have shown that these plant extracts may inhibit methane production, while concurrently decreasing production of ammonia. (McIntosh et al.,2003). Administration of a combination of plant extracts has also been shown to reduce DMI while increasing ADG. Importantly, combining these natural plant extracts, studies show a

synergistic effect by enhancing ruminal fermentation as each natural plant extract may affect different mechanisms of rumen fermentation and digestion (Calsamiglia et al. 2005).

Cinnamaldehyde is a plant extract known as Cinnamomum cassia, and is a phenylpropanoid that has antimicrobial activity and is the major active ingredient of cinnamon oil (Yang et al., 2010). Eugenol is an aromatic aldehyde, which naturally occurs in cloves. Capsicum oleoresin (capsaicin) is a derivative of chili peppers. Studies have shown that cinnamaldehyde has reduced acidosis and alleviated stress in growing feedlot cattle (Yang et al., 2010). Extensive research has been conducted analyzing the molecular proportion of acetate to propionate, favoring an increase of propionic acid within the rumen when utilizing these plant extracts (Yang et al., 2010, Cardozo et al., 2006)

It has been found that cinnamaldehyde is dosage dependent (Busquet et al., 2005). Cardozo et al., 2006 found that cattle fed a low (200 mg) and medium (800 mg) dosage level had a greater reduction in both water and feed intake than cattle fed a high (1600 mg) dosage level. This author used monensin sodium as a positive control, and found cinnamaldehyde and monensin sodium had a positive effect only at the beginning of the feeding period. The author reported that neither monensin nor cinnamaldehyde improved long term performance throughout the entire feeding period.

Cinnamaldehyde was found to reduce the molar proportion of acetate, while increasing propionate (Busquet et al., 2005). Volatile fatty acid concentrations were affected up to 20% when compared to monensin treated steers and 15% when compared to control steers receiving no essential oil or ionophore (Meyer et al., 2009). A mixture of cinnamaldehyde and eugenol has shown to reduce acetate levels and increase propionate, while reducing the levels of ammonia

(Cardoza et al., 2006). When examined *in vitro* (Cardozo et al. 2006) found cinnamaldehyde and capsicum oleoresin reduced NH3-N concentrations, while capsicum oleoresin and eugenol concurrently decreased branched chain volatile fatty acid concentrations.

Previously stated, by combining these plant extracts, studies have shown a synergistic effect by enhancing ruminal fermentation as each natural plant extract may affect different mechanisms of rumen fermentation and digestion (Calsamiglia et al. 2005). Several studies have been conducted utilizing several different mixtures of plant extracts. Lourenco et al., (2008) combined eugenol, cinnamaldehyde, and saponins. The results of this study indicated a decrease in total VFA production by approximately 1/3 when compared to a control receiving none. It is reported that the eugenol in this experiment caused a slight inhibition of bio hydrogenation, and cinnamaldehyde caused an inhibition of microbial biomass activity. The inhibition of the microbial biomass and activity is likely due to the large dosage (1800 mg) that was utilized for this study, as cinnamaldehyde is extremely dosage dependent. A combination of cinnamaldehyde and eugenol was utilized in combination and was administered to developing beef heifers (Cardozo et al., 2006). No reduction in total DMI of barley straw was seen, nor a significant reduction in water intake when compared to a control. Also, no effect was seen on ruminal pH levels in heifers fed the plant extracts, and no difference was seen for total VFA concentrations. Cinnamaldehyde and eugenol did have a significant effect by increasing the molar proportion of propionate and decreasing the molar proportion of acetate. The concentration of branched-chain VFA, such as acetate and butyrate, were found to be significantly lower in heifers who received the plant extracts when compared to a control group. The cinnamaldehyde in this study appeared to increase the proportion of propionic acid, and the eugenol in this study appeared to effectively decrease the molar proportion of acetate. The cinnamaldehyde in this study decreased the acetate

to propionate ratio. The author explained that the effects of the plant extracts appeared to be dependent on the diet and pH level in terms of the ruminal microbial fermentation. Numerous studies have been conducted which use a mixture of plant extracts, further research must be conducted analyzing appropriate dosage levels of mixtures and the effects of these plant extracts on ruminal fermentation.

Fixed Time Artificial Insemination

Developing replacement heifers is a large economic investment for beef operations, and costs associated with developing heifers cannot be recovered if the females do not conceive and remain productive in the herd; therefore it is imperative that heifers conceive early in the breeding season (Perry.,2012). Artificial insemination and estrous synchronization can be valuable tools for the beef cattle industry due to the ability to increase productivity and reproductive efficiency. Optimizing productivity and efficiency of each beef cow is essential to beef cow herds. Productivity of the beef cow is highly dependent on reproductive efficiency, and is commonly measured by the number of calves the female produces throughout her lifetime (Dziuk et al., 1983). Artificial insemination and estrous synchronization are underutilized by the beef cow industry, which allow for reproductive efficiency and overall productivity to be increased. Artificial insemination allows for the utilization of elite genetics for more rapid progress within the herd. Unfortunately, only 10% of beef cows receive AI each year (NAHMS, 1997). By conceiving to a synchronized estrus, females weaned calves 13 d older and 9.5 kg heavier on average than heifers who did not conceive via estrous synchronization (Schafer et al., 1990). Also, heifers that calve early in the calving season tend to be more productive over their lifetimes than do females who calve late in the season. These calves grew significantly faster and

were heavier at weaning while having a higher average calf production when compared to females who were born late in the calving period (Lesmeister et al., 1973).

Although AI and estrous synchronization proves to be beneficial, the utilization of these techniques remains low. The proportion of females who breed early in the breeding season produce more uniform calf crops and the calving season is shortened (Dziuk and Bellows, 1983). Artificial insemination and synchronized estrus are valuable tools that increase profitability and overall productivity of an operation. Estrus synchronization protocols that reduce cost and labor, while increasing synchrony of the cow herd as well as pregnancy rate are needed to improve usage of these techniques within the beef industry.

The Utilization of Intravaginal Progesterone Inserts to Synchronize Estrus

There are proven limitations to the success of estrus synchronization protocols, some of these limitations include anestrous cattle or prepubertal heifers, as well as time and labor associated with an estrus synchronization protocol (Short et al., 1990; Patterson et al., 1992; Lucy et al., 2001). However, estrus synchronization protocols resulting in a highly synchronized estrus result in a reduction of both time and labor associated with estrus detection, making estrus synchronization more applicable to the producer (Mallory et al., 2011). By utilizing progesterone within an estrus synchronization protocol, a producer can increase cyclicity and ovulation in prepubertal heifers and anestrous cows (Lucy et al., 2001). A common method to improve synchrony of estrus is to administer progesterone for an extended period followed by prostaglandin (Lucy et al., 2001). Research has been conducted using a controlled internal drug release (**CIDR)** insert 7 d prior to administration of prostaglandin, which ensured that the corpus luteum will regress in response to prostaglandin because all cattle will have a corpus luteum that

has developed for at least 7 d (Roche et al., 1999). Also, by administering exogenous progesterone, estrus will be delayed in cattle that naturally undergo luteolysis and regression of the corpus luteum during a progestin and prostaglandin treatment (Roche et al., 1999). Wilson et al., (2010) synchronized females using both a 5-d CO-Synch + CIDR and 7-d CO-Synch + CIDR and compared pregnancy rates to females who were synchronized by utilizing a 5-d CO-Synch and 7-d CO-Synch without a CIDR. The results show that the females that were synchronized with a CIDR were, respectively, 1.87 and 2.04 times more likely to become pregnant to a FTAI. Not to mention, by using a CIDR as part of the Select Synch protocol, pregnancy rates were improved when compared to cows who received a Select Synch with no CIDR (Lamb et al., 2001). The GnRH-PG protocol is ineffective in synchronizing estrus prior to FTAI, because 5 to 15% of females will exhibit estrus prior to administration of prostaglandin (Kojima et al., 2000; Schafer et al., 2007). Because of the ineffectiveness, estrus synchronization protocols that utilize a progestin have been used which will aid to minimize the likelihood of females exhibiting premature estrus (Schafer et al., 2007). With the utilization of a progestin, producers have been supplied a tool that will allow for an increased synchrony of estrus, while also serving to induce cyclycity in both anestrous cows and prepubertal heifers. By improving synchrony and limiting the percent of prepubertal heifers and anestrous cows, pregnancy rates following a synchronized estrous and artificial insemination can be maximized.

The use of Gonadotropin Releasing Hormone to Synchronize Follicular Waves

Exogenous gonadotropin releasing hormone (**GnRH**) has been used to cause ovulation of the dominant follicle as well as manipulate follicular waves in both beef cows and heifers (Bo et al., 1995). Gonadotropin releasing hormone can cause ovulation in follicles > 10 mm in diameter (Ryan et al., 1998). Establishment of pregnancy is related to ovulation to the first injection of

GnRH in an estrus synchronization protocol (Vasconcelos et al., 2001). A new follicular wave at the beginning of a TAI protocol will yield an increase in grade 1 and 2 embryos, number of blastomeres, as well as number of live blastomeres when compared to females who do now ovulate and begin a new follicular wave in response to GnRH at the beginning of an estrus synchronization protocol (Cerri et al., 2009; Perry et al., 2007). Yet, induced ovulation of small primordial follicles at the time of artificial insemination will decrease pregnancy rate due to an infertile or incompetent oocyte, inadequate uterine environment, or both. Not to mention an increase in early embryonic mortality has been seen when small follicles (<11 mm) are induced to ovulate, primarily due to a lack of progesterone from the corpus luteum (Busch et al., 2007). It has also been stated that during synchronization of yearling beef heifers, administration of GnRH at the time of insertion of a CIDR holds limited value (Lamb et al., 2006). The ability of a single inject of GnRH to induce ovulation is dependent on the day and stage of the estrous cycle in which it is administered, and only about 66% of beef cows will respond (Geary et al., 2000). Also, pregnancy rates to TAI is possible in beef cows, yet results in beef heifers are inconsistent (Patterson et al., 2007; Mallory et al., 2011), this may be due to the inability to effectively synchronize follicular waves (Lamb et al., 2006; Mallory et al., 2011). Consistent control of follicular waves as well as follicular development is critical for developing an estrous synchronization protocol that yields and increase in pregnancy rate without heat detection, or by appointment breeding (Perry et al., 2012). By effectively controlling follicular development, it is possible to optimize the follicular diameter and consequently estradiol exposure which plays a large role in terms of achieving desirable pregnancy success (Perry et al., 2012).

Follicular turnover is dependent on the stage of follicular development at the time in which exogenous GnRH is administered (Geary et al., 2000; Atkins et al. 2008). By

administering GnRH at random stages of the estrus cycle 45% to 60% of beef heifers will ovulate, and have recruitment of a new follicular wave (Moreira et al., 2000; Atkins et al.,2008). Low pregnancy rates from TAI may result from small immature dominant follicles at the time of a GnRH-induced ovulation (Atkins et al.,2008). In addition, Atkins et al., (2005) used transrectal ultrasonography to analyze follicular sizes as well as the day of the estrus cycle in which administration of exogenous GnRH had the greatest ovulatory response. Transrectal ultrasonography was conducted on d 2, 5, 10, 15, and 18. The author reported the mean diameter of the largest follicle at the time of the initial GnRH injection was greatest in the d 10 group followed by d 18, 15, 5 and 2. The ovulatory response following GnRH was 42% among all heifers, and ovulation in response to GnRH was affected by the day of the cycle. When ovulatory response was evaluated for each day, the results illustrate that females in the d 5 and 10 groups had the greatest proportion of females ovulate, followed by the d 2, 18 and 15 groups. It was also reported that females in the d 18 and 15 groups had a significantly higher proportion of females undergoing luteolysis and exhibiting estrous prior to administration of prostaglandin and receiving artificial insemination. Moreira et al., (2000), conducted a similar study in which they results were slightly contrasting. This researcher reported that heifers at d 10 had the largest diameter of the dominant follicle, followed by d 18, 15, 10, and 2. The mean diameter of the largest corpus luteum was the greatest on d 10 followed by d 15, 10, 18, and 2. When ovulatory response following administration of GnRH was measured, the results illustrated the greatest ovulatory response was seen from heifers on d 5, followed d 18, 15, 10, and 2. The increase in ovulatory response to GnRH on d 18 may be due to these females already having experienced luteolysis causing regression of the corpus luteum and entering the follicular phase of the estrus cycle.

Effect of a presynchronized estrus on pregnancy rate following Timed-artificial insemination

Presynchronization protocols are utilized in order to more effectively synchronize follicular waves, due to the inconsistent response of GnRH to effectively cause follicular turnover at the beginning of an estrus synchronization protocol specifically in yearling heifers. Research has stated that ovulation in response to GnRH in beef heifers is influenced by the day of the estrous cycle in which it is administered, and presynchronization that uses a progestin prior to administration of GnRH and Prostaglandin may aid to increase the proportion of dominant follicles large enough in diameter to respond to GnRH, thus improving the synchronization of follicular waves (Busch et al., 2007). Also, these results reported an increase in pregnancy rates following TAI, estrous response, and improved synchronization of estrus in beef heifers that were presynchronized by using a prolonged (14-d) CIDR protocol when compared to a control group which received a 7-d CO-Synch + CIDR protocol. Synchronizing estrus by using a presynchronization protocol has been well researched and documented. The majority of results from these studies have proven to more effectively manipulate and synchronize follicular waves, however some of the literature is conflicting. Zuluaga et al., (2010) presynchronized females by administering a single injection of GnRH 7 d prior to initiation of a 7 d CO-Synch + CIDR protocol, and discussed that pregnancy rate and fertility were not significantly improved when compared to the control group receiving a 7 d CO-Synch + CIDR protocol. Ovulatory response and follicular dynamics were also analyzed in this study. Heifers that were presynchronized were two-fold more likely to ovulate to GnRH at the beginning of the estrus synchronization protocol when compared to the non presynchronized group. However, neither emergence of a new follicular wave after administering GnRH at the beginning of the 7-d CO-Synch + CIDR or ovulation in response to GnRH-2 was different among the

presynchronized heifers vs. those who were not presynchronized. Mean follicle sizes were measured for both treatments, and the sizes of the dominant follicles were similar at each respective stage of synchronization for each of the treatments. DeJarnette et al., (2004) conducted a similar study, presynchronizing females using melengesterol acetate (MGA) for a prolonged 14 d feeding period, as well as a short term MGA feeding period prior to administration of GnRH or prostaglandin. Females in the short term MGA feeding period (**STMGA**) had a decrease pregnancy rate compared to long term presynchronization, and implied that follicular development and concurrent estrogenic activity may perhaps be delayed by feeding MGA for a short term feeding period. In addition, by presynchronizing females with a short term progestin decreased the overall estrous response while delaying the interval to estrus, as >60% of females in the long term presynchronization exhibited estrus whereas 30% of females in the STMGA exhibited estrus. Finally, Perry et al., (2012) presynchronized females by administering a single injection of prostanglandin 3 d prior to insertion of a CIDR and a GnRH injection. Heifers that received prostaglandin 3 d prior to receiving a CIDR, exhibited a decrease in progesterone concentrations. The author states that by decreasing circulation of progesterone concentrations will allow for an increase in LH pulse frequency allowing for an increase in follicular growth rates. Likewise heifers that received PG prior to CIDR insertion exhibited an increase in follicular turnover following GnRH on d 0, as well as a decrease in variation of follicular diameter. Heifers that were pretreated with prostaglandin had an increase in pregnancy rate following a Fixed-time AI. By causing luteal regression 3 d prior to administration of GnRH and a progestin, this study found an increase in pregnancy rate, as well as ovulation following GnRH on d 0 where 88% of pretreated females ovulated compared to 67% of heifers who were not pretreated.

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CHAPTER II

EFFECTS OF MONENSIN SODIUM AND PLANT EXTRACTS CONTAINING CINNAMALDEHYDE, CAPSICUM OLEORESIN, AND EUGENOL ON DAYS TO PUBERTY, GAIN, PREGNANCY RATE, AND FEED EFFICIENCY IN DEVELOPING BEEF HEIFERS RECEIVING A HIGH ROUGHAGE DIET

B.J. Bigler

Summary: Our objective was to determine the effect of monensin sodium vs. a combination of plant extracts containing cinnamaldehyde, capsicum oleoresin, and eugenol on ADG, feed efficiency, pubertal onset, and subsequent conception rate following AI in yearling beef heifers. Two-hundred-twelve Angus heifers were used in 2 experiments. During Exp.1, heifers were randomly distributed within 5 weight blocks, and assigned to one of 3 treatments: monensin sodium (**MON**); combination of plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol (**CCE**); or no feed additive (**CON**). For Exp. 2, heifers were randomly distributed among 4 weight blocks, and assigned to one of 4 treatments: MON, CCE, CON, or a combination of monensin sodium in addition to the natural feed additives cinnamaldehyde, capsicum oleoresin, and eugenol (**COMB**). Supplement consisted of a dried distillers grain base, and was administered at a rate of 0.32 $kg \cdot hd^{-1} \cdot d^{-1}$ as a top dress on a high forage ration. The MON premix was fed to supply 200 $mg \cdot hd^{-1} \cdot d^{-1}$ monensin sodium, and the CCE premix was fed to supply 1,400 $mg \cdot hd^{-1} \cdot d^{-1}$ of the plant extracts for Exp. 1, and 1,100 $mg \cdot hd^{-1} \cdot d^{-1}$ for Exp. 2. The COMB premix was fed to supply 200 $mg \cdot hd^{-1} \cdot d^{-1}$ of monensin sodium, as well as 1,100 $mg \cdot hd^{-1} \cdot d^{-1}$ of plant extracts. All heifers were observed daily to document behavioral estrus to define age at

puberty. For Exp. 1, BW were collected on 2 consecutive d at the beginning and end of the trial to determine initial BW, final BW, and ADG. Ort samples were collected and weighed every other d to calculate feed intake and feed efficiency. During Exp. 2, blood samples and BW were collected at 11 d intervals, while ort samples were collected weekly. Performance measurements included ADG, DMI, G:F, and pubertal onset. There was a tendency (P = 0.06) for CON (0.53 $kg \cdot hd^{-1} \cdot d^{-1}$) and MON (0.47 $kg \cdot hd^{-1} \cdot d^{-1}$) heifers to have greater ADG compared to CCE (0.41 $kg \cdot hd^{-1} \cdot d^{-1}$). There was a tendency (P = 0.09) for CON heifers to have greater AI pregnancy rate than females that received MON (59.8 vs. 44.2%). There were no differences (P > 0.05) for DMI, G:F, or pubertal onset between treatments. These data suggest no improvement in ADG, feed efficiency, AI pregnancy rate, or d to puberty in yearling beef heifers supplemented with monensin sodium or a combination of plant extracts; capsicum oleoresin, eugenol, and cinnamaldehyde. Results from Exp. 2 of the study suggested that receiving monensin sodium (347.1 d), a combination of plant extracts (342.5 d), or a combination of the two (355.4 d) significantly (P = 0.03) decreased age at puberty when compared to the controls (370.6 d). Heifers in the CCE (440.06 kg) exhibited the greatest weight at breeding when compared to MON (422.6 kg), COMB (422.8 kg) or CON (429.71 kg). There was a tendency (P = 0.06) for CON (0.53 $kg \cdot hd^{-1} \cdot d^{-1}$) and MON (0.47 $kg \cdot hd^{-1} \cdot d^{-1}$) heifers to have a greater ADG compared to CCE (0.41 $kg \cdot hd^{-1} \cdot d^{-1}$). Body condition scores, response to estrus synchronization, pregnancy rate, or percent of females that calved within the first 21 d of the calving season were not (P > 0.05) affected by treatments. Heifers in the COMB (1.1 $kg \cdot hd^{-1} \cdot d^{-1}$) and CCE (0.8 $kg \cdot hd^{-1} \cdot d^{-1}$) treatment group that received a combination of plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol had a greater ADG (P = 0.04) than the CON (0.6 $kg \cdot hd^{-1} \cdot d^{-1}$) and the MON ($kg \cdot hd^{-1} \cdot d^{-1}$) heifers who did not. Dry matter intake was reduced (P = 0.02) for heifers in

the MON (9.80 kg ± 0.57) and the COMB (9.16 kg ± 0.57) treatments when compared to females in both the CON (10.55 kg ± 0.57) and CCE (11.36 kg ± 0.57) treatment groups. There were no differences ($P > 0.05$) between treatments for G:F, initial BW, 60 d BW, or final BW. Differences in behavior were measured between treatments. Final exit velocity (EV) was higher ($P = 0.05$) for heifers in the CCE (0.66 m/s) as well as the COMB (0.73 m/s) treatment groups when compared to females in the CON (0.71 m/s) and MON 1.19 (m/s) treatments. There was no difference between treatments for initial, midpoint, or final CS; initial and midpoint EV were also not affected by treatment.

Introduction

It is economically important to develop beef heifers cost effectively. By using an antibiotic or ionophore such as monensin this may be done. However, social acceptance of ionophores has decreased. Therefore, it may be beneficial to identify alternatives to develop beef heifers that will increase feed efficiency and ADG. Supplementing monensin increases feed efficiency in cattle (Depenbusch et al., 2008), and alters rumen fermentation making propionic acid a greater proportion of ruminal volatile fatty acid production (Davis and Erhart., 1976). A study conducted by Moseley et al. 1977 found that 77% of heifers that received monensin reached puberty vs. 47% of control heifers with similar ADG. Few studies have been conducted analyzing the effects of plant extracts such as cinnamaldehyde, capsicum oleoresin, and eugenol on feedlot performance, and of these studies results have been conflicting. A mixture of cinnamaldehyde and eugenol has shown to reduce acetate levels and increase propionate (Cardoza et al., 2006), and capsicum oleoresin and eugenol decreased branched chain volatile fatty acid concentrations. To stimulate the utilization of these plant extracts, long term-data documenting performance and age at pubertal onset and pregnancy rates are required. Limited literature has been published

examining the effects of monensin and these 3 plant extracts on pregnancy rates and pubertal onset in beef heifers. These observations led to the hypothesis that monensin and a combination of these plant extracts will increase feed efficiency and gain in beef heifers, allowing females to reach puberty at a younger age and increase pregnancy rates following TAI compared to females receiving no feed additive. Our objective was to determine the effect of monensin sodium vs. a combination of plant extracts containing cinnamaldehyde, capsicum oleoresin, and eugenol on ADG, feed efficiency, pubertal onset, and subsequent pregnancy rate following AI in yearling beef heifers.

Materials and Methods

Animals and Diets

This project was approved by the Institutional Animal Care and Use Committee at Colorado State University. Two-hundred-twelve Angus heifers were used in two experiments. Heifers used in Exp. 1 had an initial BW of 347.4 ± 29.5 kg and were used in a 72-d randomized complete-block design experiment. Body weight was collected on 2 consecutive d at the beginning and end of the feeding period to determine initial BW, final BW, and ADG. Based on the initial BW, heifers were blocked into 5 different weight categories: light (273 kg), mid light (297 kg), medium (313 kg), mid heavy (327 kg), and heavy (354 kg). Pens were then randomly assigned to 1 of 3 treatments: monensin sodium (**MON**); combination of plant extracts cinnamaldehyde, Capsicum oleoresin, and eugenol (**CCE**); or no feed additive (**CON**). Each treatment consisted of 5 pens, with exactly 10 heifers per pen.

Supplement consisted of a dried distillers grain base, and was administered at a rate of 0.32 $kg \cdot hd^{-1} \cdot d^{-1}$ as a top dress on a high forage ration. The MON premix was fed to supply 200

mg·hd^{-1}·d^{-1} monensin sodium, and the CCE premix was fed to supply 1,400 mg·hd^{-1}·d^{-1} of the plant extracts. Heifers received supplementation from d 1 to 72 of the feeding period, and the supplement was administered immediately after receiving the basal diet (Table 2.1).

Heifers for Exp. 2 had an initial BW of 318.4 ± 26.1 kg, and were on study for a 140 d feeding trial in a 2 X 2 factorial design. Females were blocked according to the initial BW into 4 different weight categories: light (294.16 kg), medium (309.86 kg), mid heavy (329.53 kg), and heavy (338.33 kg). Pens were randomly assigned using a random number generator to 1 of 4 treatments: monensin sodium (**MON**); combination of plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol (**CCE**); no feed additive (**CON**); or a combination of both monensin sodium as well as a combination of plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol (**COMB**). Each treatment consisted of 4 pens, with 6-7 heifers per pen.

The type of supplement and rate of administration was similar between Exp. 1 and Exp. 2. During Exp. 2 the MON premix was fed to supply 200 mg·hd^{-1}·d^{-1} monensin sodium, the CCE premix was fed to supply 1,100 mg·hd^{-1}·d^{-1} of the plant extracts, and the COMB premix was fed to supply 1,100 mg·hd^{-1}·d^{-1} of the plant extracts as well as 200 mg·hd^{-1}·d^{-1} monensin sodium. During Exp. 2, heifers received supplementation from d 1 to 140 of the feeding period, and supplement was administered by top dressing into fed bunks, directly after receiving the basal diet (Table 2.1). The high forage diet was formulated for a target rate of gain 0.68 kg/hd/d, and the ration was re-formulated accordingly until the target rate of gain was achieved.

Data Collection

Orts and as fed samples of the ration were collected every other d. Feed was then adjusted accordingly to minimize refusals. Orts along with as fed samples of the ration were

composited by both treatment and month for analysis of DM and nutrient content. The DM of these samples was determined by drying at 60°C for 48 h in a forced-air oven. Nutrient analysis was determined by SDK (SDK Laboratories, Hutchinson, KS). All heifers were observed daily to document behavioral estrus to define age at puberty. For this experiment, pubertal onset was defined as the onset of behavioral estrus and was measured in d during the 72 d feeding period. Estrus detection aids (Estrotect Heat Detector, Rockway Inc., Spring Valley, WI) were applied to all females at the beginning of the trial and were used secondarily to visual heat detection. Heat patches were replaced periodically if they had been lost.

For Exp. 2, orts and as fed samples of the ration were collected once weekly, and feed refusals were determined by a trained bunk reader. Feed was then adjusted accordingly to minimize refusals. Analysis of DM and nutrient content of the orts and as fed samples were conducted the same for Exp. 1 and Exp. 2. All heifers were observed daily to document behavioral estrus to define age at puberty for the duration of the 140 d feeding period. Estrus detection aids (Estrotect Heat Detector, Rockway Inc.,) were applied to all females at the beginning of the trial and were used secondarily to visual heat detection. If an estrus detection aid was lost, it was immediately reapplied.

Blood Collection and RIA

Samples of blood (10 mL) were collected from a subset (n = 75) of heifers via jugular venipuncture every 11 d starting on d 0 of the experiment. Blood was centrifuged at 2,500 x g at 4°C for 20 min, the serum was then recovered and frozen until RIA. When a blood sample had a concentration of progesterone of ≥1 ng/mL, the heifer was considered to be pubertal. Intra and interassay CV for progesterone assays were 5.4 and 8.9% respectively.

Behavioral Measurements

During Exp.2 of the study, subjective and objective behavioral measurements were taken at 11 d intervals starting on d 0 of the experiment. Exit velocity (**EV**) values were collected by utilizing an infrared sensor timing system (FarmTek Inc., North Wylie, TX). The EV was measured in m/s, and was collected starting at 1.892 m from the head catch of the chute and ending 1.892 m beyond that point.

In addition, a subjective chute score (**CS**) was recorded for each animal upon restraint. The CS was determined by a trained scorer, which marked a 15 cm long line scale which has been described by Gruber et al., (2010). The marks were then used to determine numerical values ranging from 0 to 5, 0 = calm, and 5 = aggressive.

Pregnancy

During Exp. 1, heifers were stratified among treatments to 1 of 2 estrus synchronization protocols: 1) 5-d Pre-Synch + 7-d CO-Synch + CIDR, or 2) 7-d CO-Synch + CIDR. All injections were given intramuscularly at 100 µg of GnRH and 25 mg prostaglandinF2α (PGF). On d –14, the 5-d Pre-Synch heifers were administered GnRH and Controlled Internal Drug Release (CIDR), 7 d later the CIDR was removed and heifers received an injection of PGF. On d 0, all heifers received a CIDR and GnRH, followed 7 d later by PGF and CIDR removal. All heifers received timed-artificial insemination (TAI) and GnRH 54 ± 2 h after PGF. Pregnancy was diagnosed by rectal ultrasonography 55 d after TAI. Ultrasonography was conducted using a 7.5 MHz transducer.

For Exp. 2, all heifers received a single estrus synchronization protocol. All injections were given intramuscularly at a dosage of 100 µg of GnRH and 25 mg PGF. On d 0 of treatment

heifers received a CIDR, 14 d later the CIDR was removed, and then followed by a single injection of PGF 16 d later. Heifers received TAI and GnRH 72 ± 2 h after receiving PGF injection.

Statistical analysis

For both experiments, data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC). Pen was the experimental unit, and the fixed effects included treatment as well as BW block. Response variables included DMI, ADG, pubertal onset, and pregnancy rate to TAI. For Exp. 1 of the experiment females were arranged as a complete randomized block design, whereas heifers Exp. 2 were arranged as a 2 x 2 factorial with a complete block design. Treatment effects were significant at $P < 0.05$, while any trends were defined at $P \leq 0.10$ as a complete randomized block design.

Results and Discussion

During Exp, 1, there was no difference for initial BW (347.4 ± 29.5 kg), or final BW (424.9 ± 25.1 kg; Table 2.2) between treatments. There was a tendency ($P = 0.06$) for CON (0.53 $kg \cdot hd^{-1} \cdot d^{-1}$) and MON (0.47 $kg \cdot hd^{-1} \cdot d^{-1}$) heifers to have greater ADG compared to CCE (0.41 $kg \cdot hd^{-1} \cdot d^{-1}$). Dry matter and nutrient intakes were not different between treatments. These data also illustrate no difference ($P > 0.05$) between treatments for gain to feed. The lack of difference in G:F is due to the lack of difference in both ADG and DMI. Dry matter intake and the G:F ratio show that CCE or MON heifers were not ($P < 0.05$) more feed efficient than CON females.

No difference ($P = 0.91$) for pubertal onset was found between treatments (Table 2.3). Yet, numerically MON (88.6%) and CCE (88.6%) had a greater percent of pubertal females at the end of the study than CON (71.4%). We found no difference ($P = 0.18$) for pregnancy rates

to TAI between treatments. However, a tendency (P = 0.09) was found for CON heifers to have a greater pregnancy rate than females that received MON.

During Exp. 2, there was no difference for initial BW (318.4 ± 26.1 kg), or final BW (413.4 ± 25.7 kg; Table 2.5) between treatments. Heifers in the COMB (1.1 kg·hd^{-1}·d^{-1}) and CCE (0.8 kg·hd^{-1}·d^{-1}) treatment group that received a combination of plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol exhibited a greater ADG (P = 0.04) than the CON (0.6 kg·hd^{-1}·d^{-1}) and the MON (0.72 kg·hd^{-1}·d^{-1}) heifers who did not (Table 2.5). Not to mention, these data explain that DMI was significantly reduced (P = 0.02) for heifers in the MON (9.80 kg ± 0.57) and the COMB (9.16 kg ± 0.57) treatments when compared to females in both the CON (10.55 kg ± 0.57) and CCE (11.36 kg ± 0.57) treatment groups. However, there were no differences (P > 0.05) between treatments for gain to feed, nor was a difference found for 60 d BW (Table 2.5)

Final EV was significantly faster (P = 0.05) for heifers in the CCE (1.52 m/s), COMB (1.37 m/s), and CON (1.43 m/s) treated females when compared to females in the MON (.84 m/s) treatment group (Table 2.7). There was no difference between treatments for initial, midpoint, or final CS, initial and midpoint EV were also not affected by treatment (Table 2.7).

Receiving monensin sodium (347.1 d), a combination of plant extracts (342.5 d), or a combination of the 2 (355.4 d) decreased (P = 0.03) decreased age at puberty when compared to the controls (370.6 d; Table 2.6). Heifers in the CCE (440.06 kg) exhibited the greatest weight at breeding (P = 0.09) when compared to MON (422.6 kg), COMB (422.8 kg) or CON (429.71 kg; Table 2.6). Body condition scores, response to estrus synchronization, pregnancy rate, nor proportion of females that calved within the first 21 d of the calving season were not (P > 0.05) affected between treatment (Table 2.6).

Results differ from Exp.1 to Exp. 2, however it is evident that the impact of monensin on feed efficiency which includes ADG and DMI have not been consistent. Notably, it has been stated that few studies have analyzed the effects of these plant extracts on feedlot performance, and of these studies results have been conflicting (Cordozo et al., 2006). The variation between experiments, is likely differences in animal response as has been observed in other studies.

When ADG was analyzed for Exp,1 , the group receiving the plant extracts performed the lowest when compared to the MON and CON heifers, while females receiving MON did not differ from the CON heifers. It has been found that cinnamaldehyde and plant extracts are dosage dependent (Busquet et al., 2005). Cattle fed a low (200 mg) or medium (800 mg) dosage level had a greater reduction in both water and feed intake than cattle fed a high (1,600 mg) dosage level (Cardoza et al., 2006). These data analyzing BW gain are supported by Raun et al.,(1976) and; Moseley et al. (1977), which reported that gains in body weight were not increased by feeding monensin. The recommended dosage level of CCE was 1,100 mg, which was recommended to us by ADM Alliance. Yet, the CCE group received a higher than recommended dosage level (1,400 mg) of plant extracts, and may have had an adverse effect. This would explain the lack of differences for ADG and nutrient intake which was seen in the CCE treatment group in the current study. The recommended dosage level to analyze pubertal onset and long term feedlot performance was 1,100 mg of these plant extracts.

Our results during Exp.1, for DMI are in agreeance with results reported by Cardoza (2006), which used monensin as a positive control, and found cinnamaldehyde and monensin had a positive effect only at the beginning of the feeding period, and reported that neither monensin nor the plant extract cinnamaldehyde improved long term performance throughout the entire feeding period. These data are also consistent with other research, which found that DMI was not

affected between heifers fed monensin when compared to a control (Lalman et al., 1993). In that, weights were collected at the beginning and the end of the feeding period. Therefore, we cannot draw conclusion about the effects that MON and CCE had at different periods of the feeding trial. Unlike other research, these data do not suggest an increase in nutrient utilization. This may also be attributed to a high amount of refusals due to elevated levels of roughage in the diet.

After analyzing ADG for Exp. 2, these data suggest heifers in the CCE and COMB group outperformed the females in the MON and CON group. The CCE group performed better than the MON and CON group, perhaps due to the high DMI rate. The increased performance of ADG seen in the CCE heifers is consistent with other research performed by Geraci et al., (2012) which explained that steers fed plant extracts had a higher ADG, especially toward the end of the feeding period when compared to cattle who received monensin sodium. The COMB group exhibited the greatest ADG when compared to the other treatments. The combination of the plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol in addition to monensin sodium was developed because the indication that this combination may favorably affect DMI, ADG, and consequently feed efficiency; however literature analyzing this combination was not found which made any comparison difficult.

Our results from Exp. 2, show that females who received monensin sodium, and specifically the COMB group who received both the monensin sodium as well as the plant extracts, exhibited a decrease of DMI. This reduction in DMI is consistent with Erickson et al., (2003) who explained that monensin sodium showed a decrease in feed intake, and concurrently increased the ruminal pH. It has also been stated that by administering heifers monensin sodium, feed intake will be reduced allowing these heifers to require 382 g/d less than heifers fed a control supplementation (Moseley et al., 1977). In a meta-analysis, results indicated that

monensin improved feed efficiency and reduced DMI, and heifers who received monensin had a daily intake of 8.6 kg, and an ADG of 1.15 kg, which allowed these cattle to be 6.4% more feed efficient than cattle who received no monensin (Duffield et al., 2012). This was also noted by Stock et al. (1995) explaining that cattle fed monensin exhibited a reduction of intake along with an increase of BW gain consequently increasing the feed efficiency of cattle who received monensin. These effects of monensin sodium may certainly have caused heifers in the COMB treatment to have a reduced intake of DM; while also receiving capsicum oleoresin which is known to cause reduced DMI it is no surprise these females would have a significantly lower DMI. Unfortunately, the reduction of DMI did not significantly improve feed efficiency in heifers that received only monensin sodium. A reduction of DMI was not seen in heifers receiving the plant extracts; however it has been found that with the addition of capsicum oleoresin, DM intake is favorably affected, and that cinnamaldehyde and eugenol will alter VFA profiles while reducing protein degradation (Cardozo et al.,2006). It is also known that these plant extracts are dosage dependent, therefore it is tempting to hypothesize that a different dosage level, these plant extracts could garner more favorable results for DMI and feed efficiency.

Heifers in the COMB group had the greatest reduction of DMI, while having the greatest ADG which would suggest that they are the most feed efficient treatment group. Perhaps with a greater number of replicates a significant difference could have been found. Geraci et al (2012) explained that the mode of action for the plant extracts is different than for monensin sodium. Perhaps by administering the 2 together, several pathways are activated allowing these females to be more feed efficient than those who received the plant extracts or monensin alone. Although not measured in this study, it is tempting to hypothesize that these females were more feed

efficient due to the effect of monensin sodium and the plant extracts on VFA production within the rumen. A significant increase in the molar percentage of propionic acid in the rumen, and a decrease in molar percentage of acetate has been observed, while feeding cattle a high roughage diet along with 200 mg of monensin sodium. A study was conducted by Smith et al (2010), which stated monensin sodium linearly increased the molar proportion of propionate while linearly decreasing the molar proportions of acetate, butyrate, and isovalerate with a linear decrease in the Acetate : Propionate (A:P) ratio. Not to mention, cinnamaldehyde was found to reduce the molar proportion of acetate, while increasing propionate (Busquet et al., 2005), and a mixture of cinnamaldehyde and eugenol has shown to reduce acetate levels and increase propionate, while reducing the levels of ammonia (Cardoza et al., 2006). Perhaps by feeding these two additives together the pathways that cause these cattle to become more feed efficient after receiving either monensin sodium or the plant extracts could be enhanced.

Females in the CCE, CON, as well as the COMB treatment group exited the chute at a significantly faster rate than heifers in the MON treatment. It is unknown why females in the MON group would exit the chute at a slower pace. However, other factors must be analyzed before conclusions can be drawn about the effects that plant extracts and monensin have on docility; such factors must include chute order and time in chute. These factors perhaps affected the EV of these animals, and unfortunately were not collected.

Results for pubertal onset are consistent with previous research stating that the percentage of heifers pubertal at the end of the feeding period was similar for each treatment group, when comparing monensin to a control (Lalman et al., 1993). Moseley et al (1982) stated that monensin decreased pubertal onset independent of ADG or increased body weight, and it is known that body weight is a key threshold pertaining to the occurrence of pubertal onset in beef

heifers (Wiltbank et al., 1966). Therefore, it has been hypothesized that there is a relationship between energy metabolism and responsiveness of the endocrine system with heifers fed monensin (Moseley et al., 1982). Receiving MON (285.39 d) or CCE (281.84 d) did not decrease ($P = 0.95$) age at puberty when compared to heifers receiving CON (284.09 d; Table 2.3). It is important to note that the heifers utilized for this study were weaned in October and fed a high nutrient ration until December, leading to hypothesize that a percentage of females were nearing an appropriate weight and age to become pubertal prior to the Exp.1. If this hypothesis is correct we may not have seen the statistically significant difference. In future experimentation, females should start treatment directly following a short warm up period and weaning.

However, during Exp. 2, receiving MON and CCE did decrease age at puberty when compared to heifers who received the CON supplementation. These data agree with research that has been conducted stating ionophores such as monensin have decreased both the age and weight of females at puberty (Purvis and Whittier, 1996). These results also state that there was a trend for heifers that received an ionophore to be more conducive to become pregnant following AI, possibly due the increased percentage of females that were pubertal prior to estrus synchronization. It has been shown that by altering rumen fermentation by changing dietary energy will allow females to reach puberty at a younger age (Moseley et al., 1977). By changing dietary intake and utilizing feed additives such as an ionophore like monensin sodium, age and weight at the time of pubertal onset can be manipulated independent of body weight gain, thus reducing feed cost while developing beef heifers as well as overall cost of production (Lalman et al., 1993). Heifers who receive the plant extracts would undergo the same ruminal effects, altering rumen fermentation by changing dietary energy and composition. These results support research performed by (Purvis and Whittier 1996) which explain that heifers who received an

ionophore may have had an altered hormonal status and perhaps could have an affected physiological maturity, and that certain pathways are being utilized to enhance pubertal onset in heifers fed ionophores. From these results it certainly is tempting to support the hypothesis stated by (Moseley et al., 1982) which explained that there is a relationship between energy metabolism and responsiveness of the endocrine system with heifers fed monensin. These hypotheses seem likely for heifers who received monensin sodium; however, it seems likely that pubertal onset was hastened due to an increase in ADG that was seen in females who received the plant extracts. By increasing ADG of these heifers perhaps we allowed them to be at a more appropriate BW to become pubertal and at a younger age when compared to the controls. This becomes a critical factor when considering that BW is a key threshold pertaining to the occurrence of pubertal onset in beef heifers (Wiltbank et al., 1966). By increasing ADG we can have a greater proportion of females who are pubertal and who have attained an appropriate weight prior to the breeding season. Body condition scores were taken on breeding day, no difference (P = 0.80) was found between treatments (Table 2.3). After the feeding period heifers were housed on grass pasture until breeding without receiving a TMR or any of the three experimental treatment supplements, this may account for the small variation and lack of difference in BCS.

Pregnancy rate following fixed-TAI was not improved in heifers for Exp. 1 or for heifers in Exp. 2 of the experiment. Our results are consistent with research conducted by Moseley et al., (1977), and McCartor et al., (1979) which illustrate no improvement in pregnancy rate in heifers fed monensin sodium. Not to mention, results explained by Purvis and Whittier (1996), which illustrate that there is no significant improvement in pregnancy rate comparing the heifers who received an ionophore to the heifers that did not.

Implications

Developing heifers is a costly factor in cattle production. Beef producers must continue to seek methods to allow heifers to achieve pubertal onset with minimal inputs and increased efficiency. By administering females monensin and the plant extracts age at pubertal onset can be decreased. It also appears that by combining these two feed additives females can reach puberty at a younger age, while being more feed efficient. Thus, allowing the producer to develop heifers as efficient and cost effective as possible, which may be an extremely valuable tool to the beef industry.

Table 2.1 Percent Dry Matter of Basal Diet heifers received for Exp 1

Ingredient	% DM[3]
Grass hay	47.24
Corn silage	33.96
Whole corn	14.88
Dried distillers grain	3.90
Premix[1]	
Composition[2]	
Crude protein	9.79
Crude fiber	23.85
NEG	0.40
NEM	0.73
TDN	65.40
Fat	2.68
NFE	57.21

[1]Premix-dried distillers based supplement, mixed to supply 200 mg of monensin sodium, 14,000 mg of the plant extracts cinnamaldehyde, Capsicum oleoresin, and eugenol, or no feed additive or ionophore.

[2]Composition-composition of the ration with a target rate of gain 0.68 kg/hd/d. NEG = Net Energy-Gain, NEM = Net energy-maintenance, TDN = Total digestible nutrients, NFE = Nitrogen free extract

[3]Percent dry matter of items comprising the basal ration

Table 2.2. The effects of monensin sodium and plant extracts cinnamaldehyde, capsicum and eugenol on performance in beef heifers during Exp. 1

	Treatment[1]			
Item	MON	CCE	CON	SEM[2]
G:F	0.07	0.07	0.07	0.03
Total DMI kg/d	16.50	16.10	16.94	2.21
Initial BW, kg	364.62	366.80	349.67	28.70
Final BW kg	431.30	421.00	422.40	25.11
ADG, kg/d	0.47	0.41	0.47	0.39

[1]Treatments: CON = Control receiving no monensin or plant extract, MON = monensin group receiving 200mg/hd/d, CCE = group receiving a combination of plant extracts: cinnamaldehyde, capsicum oleoresin, and eugenol.
[2]Standard error of the mean.

Table 2.3. The effect of monensin and plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol on reproductive performance in yearling beef heifers during YR 1

	Treatment[1]			
Item	MON	CCE	CON	SEM[2]
Age at puberty, d	285.39	281.84	284.09	32.02
Pubertal heifers at the end of the feeding period, %	88.56	88.56	71.42	7.29
BCS at breeding	4.5	4.5	4.5	0.41
Pregnancy rate to TAI, %	45.66	59.70	62.82	14.80
Calved within the first 21 d, %	58.62	77.80	59.26	13.90
Calved within the second 21 d, %	27.60	18.52	25.93	14.40
Calved within the third 21 d, %	13.80	3.70	7.41	14.40

[1]Treatments:CON = Control receiving no monensin or plant extract, MON = monensin group receiving 200mg/hd/d, CCE = group receiving a combination of plant extracts: cinnamaldehyde, Capsicum oleoresin, and eugenol.

[2]Standard error of the mean.

[cd]Means within a row lacking a common superscript differ ($P < 0.05$)

Table 2.4 Percent Dry Matter of Basal Diet heifers received for Exp 2

	Rations[1]		
Ingredient[2]	R1[5]	R2[6]	R3[7]
Alfalfa Hay	19.1	20.7	33.6
Corn Silage	35.8	29.9	30.8
Wheat Straw	31.8	28.3	17.3
Whole Corn	13.3	21.1	18.3
Grass Hay			4.8
Premix[3]			
Composition[4]			
Crude Protein	9.7	10.2	10.0
NEG	0.4	0.5	0.4
NEM	0.7	0.8	0.7
TDN	63.9	68.9	62.4
Fat	3.6	3.6	2.9

[1]Percent dry matter of items comprising the basal ration
[2]Specific ingredients in which the basal ration was comprised of
[3]Premix-dried distillers based supplement, mixed to supply 200 mg of monensin sodium, 1100 mg of the plant extracts cinnamaldehyde, Capsicum oleoresin, and eugenol, 1,100 mg of the plants extracts in addition to 200 mg of monensin sodium, or no feed additive or ionophore.
[4]Composition-composition of the ration with a target rate of gain 0.68 kg/hd/d. NEG = Net Energy-Gain, NEM = Net energy-maintenance, TDN = total digestible nutrients
[5]R1-First ration heifers received, females received this ration for 59 d of feeding period.
[6]R2-Second ration formulated in order to achieve target rate of gain, females received this ration for 43 d of feeding period
[7]R3-Third ration formulated in order to achieve target rate of gain, females received this ration for 38 d of feeding period

Table 2.5 The effect of monensin and a combination of the plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol on feedlot performance in developing beef heifers during Exp 2

	Treatments[1]				SEM[2]	*P* =		
Item	MON	CCE	CON	COMB		Interaction	MON	CCE
ADG, kg/d	0.7	0.8	0.6	1.1	0.13	0.38	0.19	0.04
DMI, kg/d	9.80	11.36	10.55	9.16	0.57	0.19	0.02	0.85
G:F	0.003	0.002	0.002	0.0003	0.0006	0.51	0.11	0.35
Initial BW, kg	319.4	318.3	317.1	320.41	10.2	0.96	0.28	0.58
60 d BW, kg	354.4	362.9	354.5	352.7	10.4	0.15	0.13	0.31
Final BW, kg	407.6	416.5	397.9	431.6	13.0	0.84	0.38	0.14

[1]Treatments: CON = Control receiving no monensin or plant extract, MON = monensin group receiving 200 mg/hd/d, CCE= group receiving 1,100 mg/hd/d of a combination of plant extracts: cinnamaldehyde, Capsicum oleoresin, and eugenol. COMB = Combination group receiving monensin sodium at a rate of 200mg/hd/d, as well as 12,000 mg/hd/d of a combination of plant extracts: cinnamaldehyde, Capsicum oleoresin, and eugenol.
[2]Standard error of the mean.
[ab]Means within a row lacking a common superscript differ ($P < 0.05$)

Table 2.6 Effect of monensin and a combination of plant extracts cinnamaldehyde, capsicum oleoresin, and eugenol on reproductive performance in yearling beef heifers during year 2

	Treatments[1]				SEM[2]	Contrasts *P* =		
Item	MON	CCE	CON	COMB		Interaction	MON	CCE
BCS[3]	5.24	5.45	5.56	5.40	0.13	0.36	0.17	0.87
Weight at breeding, kg	422.57[b]	440.06[a]	429.71[b]	422.82[b]	13.56	0.09	0.0013	0.08
Estrus, %[4]	72.60	80.35	76.20	72.63	4.15	0.73	0.36	0.73
Age at pubertal onset, d	347.11[a]	342.47[a]	370.61[b]	355.36[ab]	11.74	0.03	0.46	0.18
Pregnancy rate following TAI, %[5]	64.28	62.50	54.22	61.25	5.48	0.49	0.58	0.74

[1]Treatments: CON = Control receiving no monensin or plant extract, MON = monensin group receiving 200mg/hd/d, CCE = group receiving 12,000 mg/hd/d of a combination of plant extracts: cinnamaldehyde, capsicum oleoresin, and eugenol. COMB = Combination group receiving monensin sodium at a rate of 200mg/hd/d, as well as 1100 mg/hd/d of a combination of plant extracts: cinnamaldehyde, Capsicum oleoresin, and eugenol.
[2]Standard error of the mean.
[3] Body Condition scores were collected and analyzed using the 9-point scale (1 = thin, 9 = obese; Richards et al., 1986)
[4] Percent of heifers who exhibited estrus following synchronization prior to artificial insemination.
[5]Proportion of heifers who conceived to a fixed-TAI
[ab]Means within a row lacking a common superscript differ ($P < 0.05$)

Table 2.7 Effects of Monensin and the plant extracts cinnamaldehyde, Capsicum oleoresin, and eugenol on behavioral differences in developing beef heifers during year 2

	Treatments[1]				SEM[2]	*P* =		
Item	MON	CCE	CON	COMB		Interaction	MON	CCE
Initial, EV[3] m/s	1.89	1.72	1.89	1.96	0.02	0.19	0.22	0.51
Midpoint EV, m/s	1.82	1.61	1.67	1.67	0.03	0.47	0.13	0.71
Final EV, m/s	.84[a]	1.52[b]	1.43[b]	1.43[b]	0.09	.13	0.05	0.05
Initial CS[4]	2.21	1.81	2.33	1.83	0.26	0.47	0.70	0.05
Midpoint CS	1.96	2.15	2.40	2.16	0.09	0.14	0.19	0.98
Final CS	1.53	1.49	1.29	1.37	0.07	0.13	0.59	0.91

[1]Treatments:CON = Control receiving no monensin or plant extract, MON = monensin group receiving 200mg/hd/d, CCE = group receiving 12,000 mg/hd/d of a combination of plant extracts: cinnamaldehyde, Capsicum oleoresin, and eugenol. COMB = Combination group receiving monensin sodium at a rate of 200mg/hd/d, as well as 12,000 mg/hd/d of a combination of plant extracts: cinnamaldehyde, capsicum oleoresin, and eugenol.
[2] Standard error of the mean.
[3] Exit velocity leaving the chute was collected as the velocity exhibited from 1.892 m to 3.784 m beyond the head catch of the chute and utilized an electronic infrared laser system
[4]Subjective chute scores (CS) were determined consistent with Gruber et al. (2010)
[ab]Means within a row lacking a common superscript differ ($P < 0.05$)

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CHAPTER III

THE EFFECT OF A 7-D CIDR-BASED PRE-SYNCHRONIZATION PROTOCOL ON RESPONSE TO GNRH AT THE START OF 7-D CO-SYNCH AND PREGNANCY RATE FOLLOWING TIMED-AI IN YEARLING BEEF HEIFERS

Summary: Our objectives were to evaluate: 1) the effect of using a 7-d controlled internal drug release (**CIDR**) based pre-synchronization protocol on pregnancy rates following fixed-time AI, and 2) ovulation and follicular dynamics after administration of GnRH at the start of CO-Synch + CIDR. Yearling beef heifers (n = 609) at 3 locations were randomly assigned to: 1) 5-d Pre-Synch + 7-d CO-Synch + CIDR, or 2) 7-d CO-Synch + CIDR. All injections were given intramuscularly at 100 μg of GnRH analogue and 25 mg prostaglandinF2α (**PGF**). On d –14, the 5-d Pre-Synch heifers were administered GnRH and CIDR. The CIDR was removed on d -7 and heifers received an injection of PGF. On d 0, all heifers received a CIDR and GnRH. The CIDR was removed and heifers received PGF on d 7. All heifers received Time Artificial Insemination (TAI) and GnRH 54 ± 2 h after PGF. Follicle presence and size were determined in a subset (n = 135) of heifers on d 0 and 3 at 2 locations to determine ovulatory response following GnRH on d 0 via ultrasonography. All heifers were observed for behavioral estrus for 54 hrs following PGF. Pregnancy was determined through ultrasonography 50 ± 5 d after TAI. No treatment x location interaction (P = 0.45) was observed for pregnancy to TAI, and a greater (P = 0.01) proportion of 7-d CO-Synch + CIDR vs. 5-d Pre-Synch heifers were pregnant to TAI (52.0 vs. 33.5 ± 7.3%, respectively). No difference (P = 0.56) was observed for ovulatory response after administration of GnRH on d 0 between 7-d CO-Synch + CIDR (48.2 ± 0.32%) and 5-d Pre-Synch + 7 d CO-Synch + CIDR (54.6 ± 0.31%) heifers. Using a 7-d CIDR based pre-synchronization protocol did

not increase synchrony of a new follicular wave or ovulation after GnRH administration on d 0, and decreased pregnancy rates to TAI.

Introduction

Developing an estrus synchronization protocol that increases synchrony of follicular waves and pregnancy rate following a fixed-TAI is needed. Establishment of pregnancy is related to ovulation to the first injection of GnRH in an estrus synchronization protocol (Vasconcelos et al., 2001). Also, follicular turnover is dependent on the stage of follicular development at the time exogenous GnRH is administered (Geary et al., 2000; Atkins et al. 2008). By administering GnRH at random stages of the estrous cycle 45 to 60% of heifers will ovulate, and have recruitment of a new follicular wave (Moreira et al., 2000; Atkins et al., 2008). Low pregnancy rates to TAI may result from small immature dominant follicles at the time of a GnRH-induced ovulation (Atkins et al., 2008). Pre-synchronization prior to administration of an estrus synchronization protocol that uses GnRH and PG may assist in improving synchrony of follicular waves in heifers (Moreira et al., 2000; Busch et al., 2007). In addition, Atkins et al. (2005) explained that ovulatory response to GnRH is dependent on the day of the estrous cycle in which it is administered, and found the greatest ovulatory response following GnRH was on d 5 of the estrous cycle. Our hypothesis was that a 7-d controlled internal drug release (**CIDR**) based pre-synchronization protocol would increase pregnancy rates following fixed-time AI, and these females would exhibit a greater ovulatory response after administration of GnRH analogue at the beginning of CO-Synch + CIDR. The objectives of this study were to evaluate: 1) the effect of using a 7-d CIDR based pre-synchronization protocol on pregnancy rates following TAI, and 2) ovulation and follicular dynamics after administration of GnRH at the start of CO-Synch + CIDR.

Materials and Methods

Animals

This project was approved by the Institutional Animal Care and Use Committee at Colorado State University. Six hundred nine beef heifers were used at 3 locations (location 1, n = 25; location 2, n = 110; location 3, n = 474). Heifers were randomly assigned within each location to 1 of 2 treatments: 1) 5-d Pre-Synch + 7-d CO-Synch + CIDR, or 2) 7-d CO-Synch + CIDR in a pairwise comparison arrangement. Heifers were assigned to treatment by either chute order, ear tag, or random number generator. All injections were given intramuscularly at 100 µg of GnRH analogue and 25 mg prostaglandinF2α (**PGF**). On d –14, the 5-d Pre-Synch heifers were administered GnRH and CIDR. The CIDR was removed on d -7 and heifers received an injection of PGF. On d 0, all heifers received a CIDR and GnRH. The CIDR was removed and heifers received PGF on d 7. All heifers received TAI and GnRH 54 ± 2 h after PGF (Figure 3.1).

Heifers were exposed to bulls for natural service 10 d after TAI until the end of the breeding season which varied in length by location, but averaged 45 d. Pregnancy was determined through ultrasonography at all 3 locations using a 5.0-MHz sector array transducer 50 ± 5 d after TAI. Rectal palpation was used to determine final pregnancy on d 120 ± 20. Pregnancy loss was defined as a heifer having a viable fetus at the first ultrasound, with no viable fetus at the final pregnancy diagnosis. Females who exhibited a viable fetus at d 50 ± 5 were considered to have conceived to TAI for this study.

Ovarian Transrectal Ultrasonography

Heifers at 2 locations (location 1, n = 25; location 2, n = 110) were used as a subset to examine ovarian structures. Transrectal ultrasonography was performed using a 5.0-MHz sector array transducer in order to determine response of ovarian structures following administration of exogenous GnRH analogue on d 0 of synchronization. On d 3 of treatment, all heifers were ultrasounded again in order to analyze response of GnRH analogue on the ovarian structures. The diameter of each follicle was measured on both ovaries, and the number of follicles from each ovary were recorded, along with the diameter and number of corpora lutea. Follicles were then classified according to their diameter: Class I (2 to 5 mm), Class II (6 to 9 mm), or Class III (>9 mm); (Moreira et al., 2000, Atkins et al., 2008). A dominant follicle was one that of which was at least 2 mm greater than other follicles (Sirois and Fortune, 1990; Moreira et al., 2000).

Statistical Analysis

A generalized linear model (GLIMMIX procedure, SAS Inst. Inc., Cary, NC) was used to analyze differences in TAI pregnancy rate. Available factors included BCS, sire, AI technician, location, and treatment. Factors were defined significant at $P < 0.05$, while any trends were defined at $P \leq 0.10$. Factors that were included in the final model were location, treatment, and treatment X location interaction.

A linear regression model (LOGISTIC procedure in SAS), was used to analyze ovarian response and follicle sizes. Available factors included size of dominant follicle on d 0 and 3, ovulatory response following GnRH, treatment, location, and treatment X location, and all were included within the final model.

Results and Discussion

Body condition scores were collected at all locations. No difference ($P > 0.05$) was found for BCS between locations (Table 3.3). At breeding, females at locations 2 and 3 received only pasture forage and heifers at location 2 received a high forage diet. This may explain the lack of variation in BCS among locations.

Overall pregnancy rate to TAI at location 2 was significantly higher (P = 0.0001; Table 3.1) than at locations 1 and 3. No treatment x location interaction ($P = 0.45$) was observed for pregnancy to TAI, therefore these data were pooled across locations. At location 1, there was a tendency ($P = 0.08$) between treatments. These results were similar at location 3 where the 7-d CO-Synch + CIDR control treatment group outperformed ($P = 0.003$) the 5 d pre-synch + CIDR females. At Location 2 however, both of the treatment groups performed similarly, and no difference ($P = 0.45$) was found. It is inexplicable why the 5 d pre-synch + CIDR performed well at location 2, and low at both locations 1 and 3. Although, Small et al. (2009) pre-synchronized females by utilizing a CIDR for 15 d, and found that the dominant follicular size as well as ovulation following GnRH and removal of the CIDR was increased. Yet, pregnancy rates were not consistently improved. Dahlen et al., (2003) administered a single injection of GnRH 6 d prior to initiation of the CO-Synch protocol causing the dominant follicle to ovulate, and recruitment of a new follicular wave. This failed to improve pregnancy rates when compared to heifers that received a CO-Synch + CIDR protocol. Prior to our research, Zuluaga et al., (2010) compared pregnancy rates following TAI in beef heifers that were pre-synchronized to a group that received no pre-synchronization and found that TAI pregnancy rates did not differ ($P = 0.18$) between treatments. This research also illustrated that only ovulation after administration of GnRH at the beginning of an estrous synchronization protocol was benefitted by pre-

synchronization, and failed to increase fixed-TAI pregnancy rate when compared to females that were not pre-synchronized. Zuluaga et al., (2010) explained that pre-synchronization did in fact increase the proportion of females ovulating to GnRH at the initiation of an estrous synchronization protocol, but at the synchrony of new follicular wave emergence was also increased for the treatment group that was not pre-synchronized. Perhaps, because of the drought year, heifers were not on an increasing plane of nutrition during the synchronization process and breeding, which may have also had a negative impact on becoming pregnant to TAI.

Based upon ultrasound data collected on d 0 and 3 of treatment, no difference ($P = 0.78$) was observed for overall ovulatory response and emergence of a new follicular wave after administration of GnRH between the 7-d CO-Synch + CIDR and 5-d Pre-Synch + 7 d CO-Synch + CIDR heifers. Yet, a tendency ($P = 0.07$) was found for a treatment X location interaction between locations 1 and 2. There was also a tendency ($P = 0.07$) for 5-d Pre-Synch heifers to have an increased ovulatory response and follicular turnover following GnRH based upon the d 0 and d 3 ultrasound of ovarian structures at location 1. No difference ($P = 0.77$) was found for ovulatory response following administration of GnRH at location 2 (Table 3.2). The results at location 1 are consistent with Moreira et al. (2000) stating that synchrony of the emergence of a new follicular wave following administration of GnRH was improved on d 5 of the estrous cycle followed by d 10, 15, and 18. These results are also supported by Atkins et al. (2008), which also explained that the GnRH induced LH surge had the greatest efficacy on d 18 followed by d 5, 15, 10, and 2. Unfortunately, we did not measure exactly when the heifers ovulated via transrectal ultrasonography or blood collection. So it is possible that not all females were effectively synchronized to d 5 of the estrous cycle. Perhaps there may have been a lack of ability to detect any differences at location 1 and not location 2 due to the increased sample size at location 2.

It has been stated that establishment of pregnancy is related to ovulation in response to the first injection of GnRH at the beginning of an estrus synchronization protocol (Vasconcelos et al., 2001). However, it is unknown why the greatest ovulatory response for the 5 d pre-synch treatment group was seen at location 1, and yet the pregnancy rate for this treatment group was the lowest. Examination of ovarian structures was not collected at breeding, yet it is possible that some dominant follicles that ovulated at the time of AI were not competent or produced an oocyte that was infertile. When diameter of the dominant follicle was measured on d 0, it was seen that location 2 exhibited a greater diameter ($P = 0.03$) of the dominant follicle than location 2. However, no difference was seen between treatments for diameter of the dominant follicle at each location (Table 3.4). It has been shown that the size of the ovulating dominant follicle affects the fertility of the oocyte produced from an induced ovulation in cattle with a synchronized estrus (Perry et al., 2005). Females who exhibit an ovulatory follicle > 12 mm at the time of GnRH-induced ovulation have been shown to have a greater pregnancy rate when compared to females induced to ovulate a dominant follicle ≤ 12 mm (Lamb et al., 2001). By utilizing GnRH to induce ovulation of follicles that are ≤ 11 mm, has been proven to decrease pregnancy rate while increasing the occurrence of late embryonic or fetal mortality (Perry et al., 2005).

Implications

Although synchrony of an emergence of a new follicular wave was seen at one location, the 5-d Pre-Synch treatment group failed to improve overall pregnancy rates at all three locations. Further research must be conducted analyzing the optimal day to initiate treatment that will increase synchrony as well as pregnancy rates following TAI. Also, pre-synchronizing females did not sufficiently or consistently increase pregnancy rate following TAI or ovulatory

response following administration of GnRH. Pre-synchronization must consistently and considerably increase the likelihood of females that become pregnant to TAI to outweigh the added cost and labor associated with the protocol to become an effective management tool for cattlemen.

Table 3.1 Pregnancy rates to timed AI (TAI) in beef heifers by estrous synchronization treatment by location as well as overall

	Treatment			
	5-d Pre-Synch[1]	7-d CO-Synch + CIDR[2]		
Location	%	%	SEM[3]	*P* - Value
1	15.38	50.00	19.5	0.08
2	51.85	59.26	4.5	0.43
3	33.19	46.88	9.3	0.003
Combined[4]	33.5	52.0	7.3	0.01

[1] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) on d -14, and 100 µg GnRH analogue (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im, d – 7 CIDR removed and 25 mg PGF (Lutalyse, Pfizer Animal Health) im. On d 0 heifers received a CIDR and 100 µg GnRH analogue, d 7 CIDR removed and PGF 25 mg. Heifers received TAI and 100 µg GnRH analogue 54 ± 2 h following PGF.
[2]Heifers received a CIDR and 100 µg GnRH analogue i.m. on d 0, on d 7 the CIDR was removed and 25 mg PGF. Heifers received TAI and 100 µg GnRH analogue 54 ±2 h following PGF.
[3]Standard Error of the Mean
[4] No treatment x location interaction ($P = 0.45$) was observed for pregnancy to TAI, therefore these data were pooled across locations.

Table 3.2 Ovulatory response among beef heifers to exogenous GnRH for a pre-synchronized estrous cycle vs. no pre-synchronization

	Treatment			
	5-d Pre-Synch	7-d CO-Synch + CIDR[2]		
Location	%[4]	%	SEM[3]	*P* - Value
1	92.3	58.3	1.19	0.07
2	70.4	67.9	0.41	0.77
Combined[5]	75.0	66.2	0.63	0.11

[1] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) on d -14, and 100 µg GnRH analogue (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im, d – 7 CIDR removed and 25 mg PGF (Lutalyse, Pfizer Animal Health)im. On d 0 heifers received a CIDR and 100 µg GnRH analogue, d 7 CIDR removed and PGF 25 mg. Heifers received TAI and 100 µg GnRH analogue 54 ±2 h following PGF.
[2]Heifers received a CIDR and 100 µg GnRH analogue i.m. on d 0, on d 7 the CIDR was removed and 25 mg PGF. Heifers received TAI and 100 µg GnRH analogue 54 ±2 h following PGF.
[3]Standard Error of the Mean
[4]Percent of females who ovulated following exogenous GnRH analogue. Heifers were ultrasounded on d 0 of treatment and sizes of all follicles and corpus lutea were recorded. Heifers were ultrasounded again on d 3 of treatment, and each female was considered to have ovulated if a reduction of the dominant follicle was seen.
[5] A tendency ($P = 0.07$) was found for a treatment X location interaction between locations 1 and 2

Table 3.3 Body condition score as well as overall pregnancy rate following timed-AI (TAI) of beef heifers by location

Location	n =	BCS[1]	Overall percent pregnant to TAI
1	25	5.5 ± 0.70	29.87[a]
2	110	4.8 ± 0.60	55.59[b]
3	474	5.1 ± 0.52	39.83[a]

[1] Body Condition scores were collected and analyzed using the 9-point scale (1 = thin, 9 = obese; Richards et al., 1986)
[ab] means within a column, lacking a common superscript differ ($P < 0.05$)

Table 3.4 Diameter of dominant follicle among beef heifers on d 0 of synchronization for a pre-synchronized estrous cycle vs. no pre-synchronization

	Treatment			
	5-d Pre-Synch	7-d CO-Synch + CIDR[2]		
Location			SEM[3]	*P* - Value
1	8.10	8.90	.66	0.41
2	11.31	10.60	1.43	0.71
Combined[4]	9.70	9.73	0.76	0.98

[1] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) on d -14, and 100 µg GnRH analogue (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im, d – 7 CIDR removed and 25 mg PGF (Lutalyse, Pfizer Animal Health)im. On d 0 heifers received a CIDR and 100 µg GnRH analogue, d 7 CIDR removed and PGF 25 mg. Heifers received TAI and 100 µg GnRH analogue 54 ±2 h following PGF.
[2]Heifers received a CIDR and 100 µg GnRH analogue i.m. on d 0, on d 7 the CIDR was removed and 25 mg PGF. Heifers received TAI and 100 µg GnRH analogue 54 ±2 h following PGF.
[3]Standard Error of the Mean
[4] No treatment x location interaction ($P = 0.50$) was found between locations 1 and 2 therefore the data was pooled.

Figure 3.1 Estrous synchronization treatments administered to beef heifers

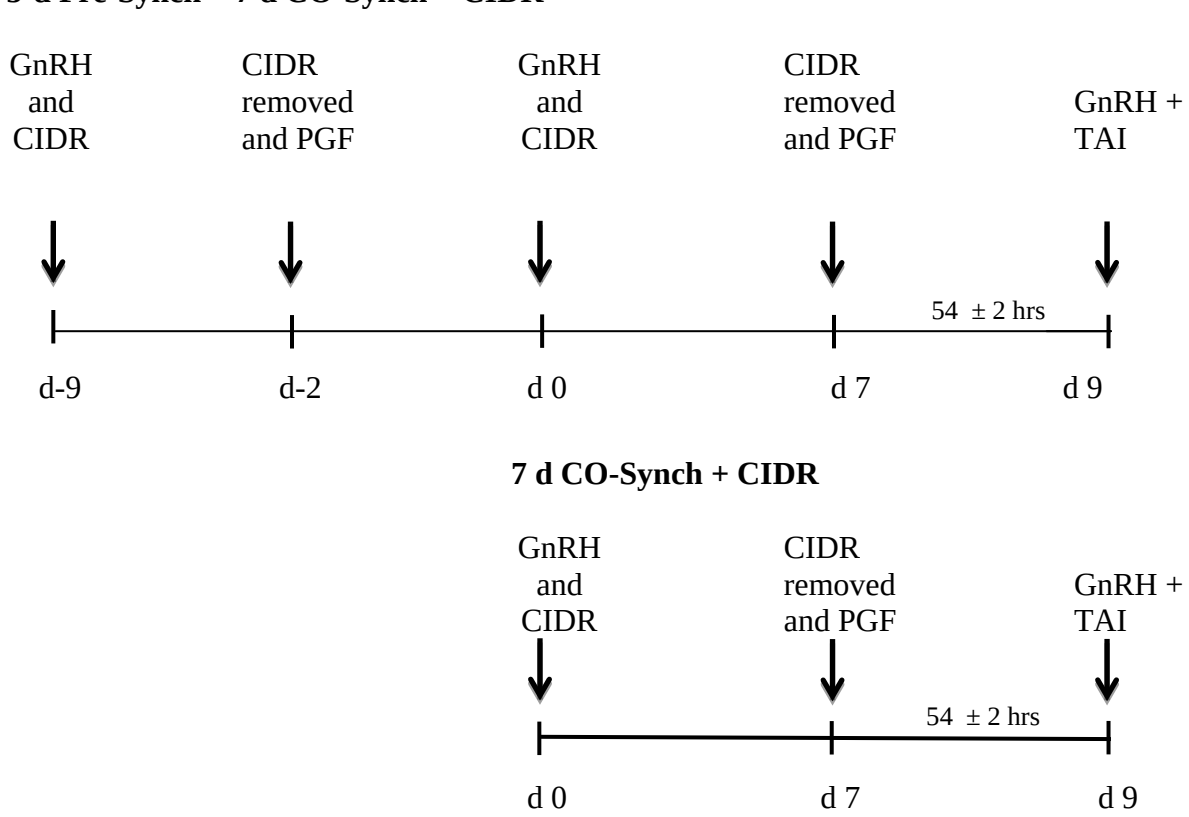

Figure 3.1 Illustration of the 5-d Pre-Synch + 7 d CO-Synch + CIDR and 7 d CO-Synch + CIDR protocols that heifers received. Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) on d -14, and 100 µg GnRH analogue (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im, d – 7 CIDR removed and 25 mg PGF (Lutalyse, Pfizer Animal Health)im. On d 0 heifers received a CIDR and 100 µg GnRH analogue, d 7 CIDR removed and PGF 25 mg. Heifers
received TAI and 100 µg GnRH analogue 54 ±2 h following PGF.

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APPENDIX

SAS Code for Chapter II

Code for analyzing feed lot performance and pubertal onset during Y1 of experiment

```
title1 "DMI";
proc mixed;
class  trt;
model DMI= trt/ddfm=kr;
random pen;
lsmeans trt/pdiff;
run;
title1 "ADG";
proc mixed;
class  trt;
model ADG= trt/ddfm=kr;
random pen;
lsmeans trt/pdiff;
run;
title1 "H24";
proc mixed;
class  trt;
model H24= trt/ddfm=kr;
random pen;
lsmeans trt/pdiff;
run;
title1 "H48";
proc mixed;
class  trt;
model H48= trt/ddfm=kr;
random pen;
lsmeans trt/pdiff;
run;
title1 "H72";
proc mixed;
class  trt;
model H72= trt/ddfm=kr;
random pen;
lsmeans trt/pdiff;
run;
title1 "GF";
proc mixed;
class  trt;
model GF= trt/ddfm=kr;
random pen;
lsmeans trt/pdiff;
```

run;

SAS Code for Chapter II

Code for analyzing feed lot performance and pubertal onset during Y1 of experiment

```
PROC IMPORT OUT= WORK.heifer
            DATAFILE= "X:\My Documents 2013\Bigler\Heifer Development YR
2\YR2SASREADY.xlsx"
            DBMS=EXCEL REPLACE;     RANGE="Sheet1$";
RUN;
proc print data=heifer(obs=5);run;

%macro aov (Y);
title "Y=&Y.";
proc mixed; class BWBLOCK Rumensin Rumenext ;
 model &y.= Rumensin|Rumenext ;
 random BWBLOCK;
 lsmeans Rumensin|Rumenext / adj=tukey;run;
 %mend;

%aov(STARTEV);%aov(MIDEV);%aov(FINALEV);%aov(STARTCS);%aov(MIDCS);%aov(FINALC
S);
 %aov(DMI);%aov(GF);%aov(FG);%aov(ADG);
 %aov(INITIALBW);%aov(SIXTYBW);%aov(FINALBW);
 %aov(BREEDINGWT);%aov(PREG);%aov(ESTRUS);%aov(BCS);
 %aov(DTP);

 title"Y=MIDEV x=STARTEV";
proc mixed; class BWBLOCK Rumensin Rumenext ;
 model MIDEV= Rumensin|Rumenext STARTEV;
 random BWBLOCK;
 lsmeans Rumensin|Rumenext / adj=tukey;run;
 title"Y=FINALEV x=STARTEV";
proc mixed; class BWBLOCK Rumensin Rumenext ;
 model FINALEV= Rumensin|Rumenext STARTEV;
 random BWBLOCK;
 lsmeans Rumensin|Rumenext / adj=tukey;run;


 title"Y=MIDCS x=STARTCS";
proc mixed; class BWBLOCK Rumensin Rumenext ;
 model MIDCS= Rumensin|Rumenext STARTCS;
 random BWBLOCK;
 lsmeans Rumensin|Rumenext / adj=tukey;run;
 title"Y=FINALCS x=STARTCS";
proc mixed; class BWBLOCK Rumensin Rumenext ;
 model FINALCS= Rumensin|Rumenext STARTCS;
 random BWBLOCK;
 lsmeans Rumensin|Rumenext / adj=tukey;run;


 title"Y=SIXTYBW x=INITIALBW";
proc mixed; class BWBLOCK Rumensin Rumenext ;
 model SIXTYBW= Rumensin|Rumenext INITIALBW;
```

```
 random BWBLOCK;
 lsmeans Rumensin|Rumenext / adj=tukey;run;
 title"Y=FINALBW x=INITIALBW";
proc mixed; class BWBLOCK Rumensin Rumenext ;
 model FINALBW= Rumensin|Rumenext INITIALBW;
 random BWBLOCK;
 lsmeans Rumensin|Rumenext / adj=tukey;run;
```

SAS Code for Chapter III

Code for analyzing pregnancy following Fixed-TAI

```
proc print;

proc means;
by trt;

proc sort;
by loc;

proc means;
by loc;

*visual trt bcs tech sire preg loc;

proc glimmix;
class  trt  loc;
model preg (reference=first)= trt|loc /dist=binary;
lsmeans trt|loc/pdiff ilink cl;

run;


proc glimmix;
class  trt  loc;
model bcs = trt|loc ;
lsmeans trt|loc/pdiff  cl;

run;

PROC IMPORT OUT= WORK.ardec
            DATAFILE= "E:\Synchronization Project\sync.xlsx"
            DBMS=EXCEL REPLACE;     RANGE="ARDEC$";
RUN;
proc print data=ardec(obs=5);run;



PROC IMPORT OUT= WORK.ecrc
            DATAFILE= "E:\Synchronization Project\sync.xlsx"
            DBMS=EXCEL REPLACE;     RANGE="ECRC$";
RUN;
data ecrc; set ecrc;
if visual;
time=(AI-(CIDR+12*360))/360;/*??*/
run;
proc print data=ecrc(obs=5);run;
```

```
PROC IMPORT OUT= WORK.rabbit
            DATAFILE= "E:\Synchronization Project\sync.xlsx"
            DBMS=EXCEL REPLACE;     RANGE="Rabbit Creek$";
RUN;
proc print data=rabbit(obs=5);run;


title'test for sire/tech';
proc glimmix data=ardec; class trt sire tech;/*test for sire/tech*/
 model pregnancy= trt/distribution=MULT ;
  random sire tech;
*lsmeans trt/ilink;
run;


proc glimmix data=ecrc; class trt  tech;/*test for sire/tech*/
 model pregnancy= trt/distribution=MULT ;
  random  tech;
*lsmeans trt/ilink;
run;

proc glimmix data=rabbit; class trt  tech sire;/*test for sire/tech*/
 model pregnancy= trt/distribution=MULT ;
  random  tech sire;
*lsmeans trt/ilink;
run;

data allloc; set ardec(in=in1) ecrc(in=in2) rabbit(in=in3);
if in1 then location='ARDEC';
if in2 then location='ECRC';
if in3 then location='Rabbit';
bcs2=bcs*bcs;
run;
proc print data=allloc(obs=5);run;

proc glimmix data=allloc; class trt location sire tech;/*test for
sire/tech*/
 model pregnancy= trt|location/distribution=MULT ;
  random sire tech;
*lsmeans trt/ilink;
run;

title' loc|trt for 3 locations';
proc logistic data=allloc  ; class trt location/param = glm;
 model pregnancy(ref=first)=trt|location ;
 lsmeans trt|location/pdiff ilink;
 oddsratio 'trt1 vs trt2' trt /;
 run;

title' loc|trt for 3 locations & BCS';
```

```
proc logistic data=allloc  ; class trt location bcs/param = glm;
 model pregnancy(ref=first)=trt|location bcs;
 lsmeans trt|location bcs/ ilink pdiff;
 run;


title' Y=patch  with loc|trt for 3 locations for ECRC';
proc logistic data=allloc  ; class trt /param = glm;
 model patch=trt ;/* prob of a lower score*/
 lsmeans trt/ ilink pdiff;
 oddsratio 'trt1 vs trt2' trt /at (location=all);
 run;


%macro ov(Y);
title" Y=&Y.  with loc|trt for 2 locations for ARDEC & Rabbit";
proc logistic data=allloc  ; class trt location/param = glm;
 model &Y.=trt|location ;/* prob of a lower score*/
 lsmeans trt|location/ ilink pdiff;
 oddsratio 'trt1 vs trt2' trt /at (location=all);
 run;
 %mend;
%ov(LOCL0);%ov(LOFollicle0);%ov(D_0CL);%ov(ROCL0);%ov(ROFollicle0);
%ov(LOCL3);%ov(LOFollicles3);%ov(D_3CL);
%ov(ROCL3);%ov(ROFollicle3);

title'means for bcs by location';
proc sort data=ecrc;run;
proc glimmix data=ecrc ;
      class  trt bcs;
      model bcs = trt/ solution;
      lsmeans ecrc /ilink pdiff;
run;

title' loc|trt for 3 locations & BCS';
proc logistic data=allloc  ; class trt location bcs/param = glm;
 model bcs=location;
 lsmeans location / ilink pdiff;
 run;


PROC IMPORT OUT= WORK.rc
        DATAFILE= "X:\My Documents 2013\Bigler\Ovary\SyncSASREADY.xlsx"
        DBMS=EXCEL REPLACE;     RANGE="'Rabbit Creek$'";
RUN;PROC IMPORT OUT= WORK.ARDEC
        DATAFILE= "X:\My Documents 2013\Bigler\Ovary\SyncSASREADY.xlsx"
        DBMS=EXCEL REPLACE;     sheet="ARDEC";
RUN;
*Proc Freq for Ovulation Chisquare tests;
proc freq data=ARDEC;
```

```
table trt*ovulate / chisq;
run;
proc freq data=rc;
table trt*ovulate / chisq;
run;

*Merge data from BOTH (2) locations;
data allloc;
set ARDEC (in=ina) rc(in=inrc);
if ina then location='ARDEC';
if inrc then location='RC';
run;

title'OVULATE: loc|trt for 2 locations';
proc logistic data=allloc  ; class trt location/param = glm;
model ovulate(ref=first)=trt|location ;
lsmeans trt|location/pdiff ilink;
oddsratio 'trt1 vs trt2' trt /;
run;
*Look only at cows that ovulated;
data ovulated;
set allloc;
IF ovulate=1;
run;

*What class of follicle had highest chance of ovulating;


title 'Ovulation vs Follicle0 size';
proc sort data=allloc;
by location trt;
proc freq data=allloc;
by location;
table follicle0*ovulate /chisq fisher;
run;

title 'Ovulation vs Follicle0 size BY LOCATION';
proc logistic data=allloc;
by location;
class follicle0/param = glm;
model ovulate(ref=first)=follicle0 ;
lsmeans follicle0/pdiff ilink;
*oddsratio 'trt1 vs trt2' trt /;
run;
```

```
title'follicle0: loc|trt for 2 locations';
proc glm data=allloc  ; class trt location;
model follicle0=trt|location ;
lsmeans trt|location/pdiff ;
run;


title'follicle3: loc|trt for 2 locations';
proc glm data=allloc  ; class trt location;
model follicle3=trt|location ;
lsmeans trt|location/pdiff ;
run;

title'follicle0: trt for 2 locations';
proc freq data=allloc; tables trt*follicle0/chisq;run;


title'follicle3: trt for 2 locations';
proc freq data=allloc; tables trt*follicle3/chisq;run;
```