

Supplementation of Chromium Propionate Positively Impacts Reproductive Performance of Beef
Females

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Abstract

Return to estrus following the postpartum interval to achieve pregnancy success on time is a considerable obstacle for beef females. Chromium supplementation increases available glucose and insulin sensitivity within cells. Two experiments were conducted to investigate the effects of supplementing Chromium propionate (CrP) during the peripartum until weaning on productive and reproductive performance in *Bos taurus* beef cows. In Exp. 1, 62 Angus-based beef cows were stratified by predicted calving date, body weight (BW) and randomly assigned to one of two treatments: 1) CON, (n=30) supplementation of corn gluten, soy hull pellet feed (50:50) with a mineral pack at 1 kg⁻¹hd⁻¹d; or 2) TRT, (n=32) supplementation at 1 kg⁻¹hd⁻¹d of corn gluten, soy hull pellet feed (50:50) with a mineral pack containing 1.4 g of Chromium Propionate (KemTRACE® Chromium 0.4%, Kemin Industries, Inc., Des Moines, IA). Cows remained on a single pasture equipped with SmartFeed trailers for individual supplement intake (SmartFeed®, C-lock Inc., Rapid City, SD). The experiment lasted 98 days, starting 63 days pre-breeding to 35 post-fixed-time artificial insemination (TAI). Ovarian ultrasonography was performed on days -10, -3, 0 (TAI Day), and 7 to determine the diameter of the largest follicle and corpus luteum (CL) volume. Age, days postpartum (DPP), initial and final BW, and supplement intake were similar ($P>0.05$) between treatments. However, TRT cows had a larger follicle ($P=0.028$) on d 0, increased CL volume ($P=0.038$), and increased ($P=0.0213$) circulating progesterone (P4) on day 7. In Exp. 2, 953 beef cows across nine locations were assigned to one of two treatments: 1)

CON, supplementation of a mineral product at 113 g⁻¹hd⁻¹d (n=464 cows; 16 experimental units); or 2) TRT, supplementation of mineral product at 113 g⁻¹hd⁻¹d containing 1.4 g of CrP (n=489 cows; 16 experimental units). Supplementation started approximately 37 days pre-calving and continued until weaning for 345 days. Age, DPP, d-10 body condition score (BCS), initial and final BW, BCS, calf birth and weaning weight, and mineral disappearance were similar ($P>0.05$) between treatments. However, CrP cows tended ($P=0.081$) to have greater estrus expression (68.3 and 60.2 ± 3.1 %, for CrP and CON, respectively) and greater ($P=0.045$) TAI pregnancy rates (55.2% vs. 49.9% ± 2, for CrP and CON, respectively). We conclude that supplementation of CrP to beef cows during the peripartum through weaning did not affect BW or BCS, but increased ovulatory follicle diameter, estrus expression, CL volume, and P4 concentration, and one or more of these positive effects of CrP likely contributed to the improvement in TAI pregnancy rate.

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General Audience Abstract

Reproductive efficiency in beef cow-calf operations relies heavily on pregnancy success as early in the breeding season as possible. Several factors influence the return to cyclicity after calving, including days post-calving, body condition, and nutritional status of the dam. Chromium is an essential trace mineral that can be added to cattle diets to promote glucose uptake by cells and mitigate the negative impacts of the stressful postpartum period. Two studies were designed to evaluate the effect of chromium propionate supplementation to beef cattle during the pre-calving through weaning period on reproductive parameters and overall cow performance. In experiment one, 62 cows were assigned to two treatment groups: control (feed containing a standard mineral pack) and treatment (feed containing 1.4g of chromium propionate supplementation). The second experiment included 953 cows assigned to one of two treatment groups: control (supplementation of free choice mineral product) and treatment (supplementation of free choice mineral containing 1.4 grams of chromium propionate). Both groups in experiment two had the same target feed intake. In both experiments, beef females were exposed to estrous synchronization and timed artificial insemination and were exposed to a bull for natural service in the next estrous cycle. Cows receiving the chromium supplementation did not experience any changes in body condition score, weight change, or average daily gain in either experiment. However, reproductive performance was affected by chromium-supplemented cows had an increased dominant follicle size on the day of timed-artificial insemination (TAI), larger corpus

luteum volume 7 days post artificial insemination, increased progesterone concentrations, and increased progesterone to corpus luteum ratio in experiment 1. Supplementation of Cr in experiment 2 tended to increase estrus expression and showed an increase in the pregnancy rate of TAI. Calf performance was not impacted by chromium propionate supplementation. These results indicate that chromium propionate supplementation could benefit the reproductive performance of beef cows.

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Abbreviations

ADG: Average Daily Gain

AI: Artificial Insemination

BCS: Body Condition Score

BW: Body Weight

CIDR: Controlled Internal Drug Release

Cr: Chromium

CrP: Chromium Propionate

DMI: Dry Matter Intake

DPP: Days Postpartum

E₂: Estradiol

FE: Feed Efficiency

FSH: Follicle Stimulating Hormone

GnRH: Gonadotropin-Releasing Hormone

LH: Luteinizing Hormone

NEB: Negative Energy Balance

NEFA: Non-Esterified Fatty Acid

P₄: Progesterone

PGF_{2α}: Prostaglandin F 2 Alpha

PG: Prostaglandin

SBM: Soybean Meal

TAI: Timed-Artificial Insemination

Chapter One: Introduction

Introduction

The global population is projected to reach 9.8 billion by 2050, and improvements in efficiency and sustainability in global agriculture are essential. Concurrently, the available agricultural land is anticipated to diminish as urbanization and infrastructure development expand. The current beef production system encompasses multiple sectors that collectively contribute to producing high-quality protein for the food supply and meeting the increased beef demand. By 2030, global meat consumption is expected to rise by 14% compared to 2020, with beef accounting for a substantial share of this increase (Font-i-Furnols, 2023). The American Heart Association (AHA) and the Dietary Guidelines for Americans recommend limiting red meat intake to no more than 6 ounces per day to support cardiovascular health (Ichinose, 2024).

Nevertheless, red meat remains a vital component of a balanced diet, providing high-quality protein, iron, zinc, and vitamin B12, essential for muscle function, energy production, and overall health (McNeill and Van Elswyk, 2012; Sharma et al., 2013). Across the United States, beef remains a popular choice, with many Americans regularly including it in their meals due to its nutritional value and cultural significance. Therefore, ensuring sustainability in the beef cattle industry is critical. The beef industry demonstrates a capacity for technological adaption and actively employs various management strategies, including genetic selection tools, reproductive technologies, vaccination protocols, and nutritional interventions, to enhance efficiency while minimizing costs.

Despite a relatively slow adoption rate of advanced technologies across the industry, the utilization is prevalent among larger producers (USDA NAHMS, 2017). In response to these

challenges, the adoption and implementation of advanced reproductive technologies such as estrous synchronization, genetic testing, and embryo transfer are just three of the numerous technologies available for enhancing productivity within the beef cattle industry. Nonetheless, producing one healthy calf from each cow each year is complicated by various factors, including fertilization issues, early pregnancy loss, disease, and environmental stressors. Reproductive performance is the primary limiting factor in beef cattle production, with substantial calf crop losses resulting from the failure of cows to conceive (Lauderdale, 2009). Pregnancy failure among beef females is estimated to cause an economic loss of approximately \$2.8 billion annually in the United States (Mercadante et al., 2020). This loss equates to roughly \$6.25 per every cow exposed and for every one percent decrease in pregnancy rate. Beef females must return to estrus within 80-85 days following calving to maintain a yearly calving interval. However, that depends on whether female beef undergoes uterine involution, proper follicular development, resumption of estrus, and successful ovulation events (Velazquez et al., 2008). Several factors influence pregnancy success in beef cattle, including the presence of a suckling calf, inadequate body condition, postpartum anestrus period, days postpartum, and parity (Stevenson et al., 2003; Perry et al., 2004).

Nutrition heavily contributes to cyclicity status and resumption of estrus in suckling beef cows. The nutritional status and BCS at the time of calving are the most critical factors associated with the resumption of estrus and fertile ovulation (DeRouen et al., 1994; Hess et al., 2005a; D'Occhio et al., 2019). The calving period in beef cattle refers to a specific timeframe within the year, typically lasting 45 to 90 days, during which all calves within the herd are expected to be born (Rhinehart, 2020). Ensuring proper nutrition through the calving period to meet the dam's energy requirements will impact her ability to return to cyclicity and conceive

the following breeding season. Several metabolic nutritional mediators are essential for the return to estrus following parturition, with glucose being arguably the most critical metabolic substrate for reproductive function in beef cows (Short and Adams, 1988). Glucose is a primary metabolic component used by the central nervous system, which releases GnRH from the hypothalamus. Decreased glucose levels impair GnRH secretion, inhibit LH secretion, and prevent the surge needed for ovulation (Hess et al., 2005b). Evidence indicates that Cr supplementation will impact the resumption of estrus by facilitating glucose uptake and utilization in the brain.

Chromium increases insulin receptor sensitivity, enhancing the efficiency of glucose uptake into cells. The improved glucose metabolism supports various critical mechanisms, including energy production, muscle growth, immune function, and reproductive performance. By optimizing glucose partitioning, Cr supplementation can help meet the metabolic demands of beef cattle during various stages of production (Anderson, 2003). In dairy cows, Cr supplementation increased milk production and DMI, increasing glucose clearance and insulin responsiveness postpartum (Hayirli et al., 2001). The increase in DMI of beef cattle during the postpartum interval, specifically for the first 71 days following calving, has decreased. the interval from calving to first ovulation, followed by supporting a normal, shorter luteal phase of 100 days. The shorter luteal phase indicates an earlier resumption of cyclicity in beef females. The increase in feed intake promotes fat deposition, which is required to re-establish ovarian function (Guzmán et al., 2012; Diskin and Kenny, 2016). Supplementation of Cr in beef cows can positively impact reproductive performance by increasing the available glucose for cyclicity and mitigating harmful effects during stressful production periods, specifically the postpartum period.

Therefore, we hypothesized that beef females supplemented with CrP during the postpartum interval would enhance reproductive performance. Specifically, this supplementation would promote an earlier return to estrus, improve follicular development, and support the hormonal regulation necessary for ovulation. By optimizing insulin sensitivity and energy partitioning, CrP supplementation may help mitigate the adverse effects of postpartum anestrus, reduce the time to first service, and ultimately improve AI conception rates. These improvements in reproductive efficiency could contribute to increased productivity and more consistent calving intervals, benefitting both the health of beef cows and females and the overall profitability of beef production systems.

Chapter Two: Review of Literature

The Bovine Estrous Cycle

The bovine estrous cycle consists of two distinct phases: the follicular and luteal phases. Moreover, those two distinct phases can be divided into four periods: proestrus, estrus, metestrus, and diestrus. A standard estrous cycle of a bovine female lasts, on average, 21 days but can range from 17 to 24 days. The estrous cycle is a complex endocrine process that drives estrus and ovulation, regulated by various hormones responsible for physiological changes.

Follicular phase

Proestrus lasts 2-5 days and immediately follows the decline of progesterone and regression of the CL, typically referred to as luteolysis. At this point, LH pulse frequencies increase along with follicular estradiol secretion. The primary hormone influence is estrogen, produced by developing follicles. As E₂ levels rise, a preovulatory GnRH surge occurs, triggering the preovulatory LH surge. The LH surge is essential for finalizing the dominant follicle maturation and stimulating granulosa cells to begin to synthesize prostaglandins to aid in weakening the wall of the ovary to allow for the rupture of the follicle (Duffy and Stouffer, 2003; Binelli et al., 2014).

Estrus is characterized as the time when the female is receptive to breeding. Standing estrus is a shorter period, ranging from 6-24 hours in beef cattle, with an average of 15 hours. During this time, E₂ reaches its highest concentrations within the body, with the production coming from the developing follicles preparing for ovulation. Ovulation typically occurs 23-30 hours after the onset of estrus (Roelofs et al., 2010). The primary sign of estrus is the willingness to allow other herd mates to mount the female. Secondary signs of estrus include increased movement, vocalization, and clear discharge (Perry, 2004a). Luteinizing hormone spikes to

initiate ovulation, and progesterone levels are low. The ovulation event initiates the transition from the follicular phase to the luteal phase (Larson and Randle, 2008).

Luteal Phase

Metestrus typically lasts 3-5 days. At the beginning of metestrus, ovulation occurs approximately 24 to 32 hours following the onset of estrus (Perry, 2004). During this time, there is a hormonal shift as progesterone begins to be secreted by the early-developing CL. The cow is no longer receptive to the bull or other females mounting her. Visual indicators of the female being in metestrus are bleeding as a physical rupture of the ovary to release the follicle happens.

Diestrus is the longest period bovine estrous cycle, typically 12-14 days. The primary hormone is P4, secreted from the corpus luteum during this time (Larson and Randle, 2008). Progesterone acts to prime the uterus and create a favorable environment for a pregnancy. The P4 levels will continue to rise until they reach a certain level, which will remain constant (Lamb et al., 2010b). Towards the end of diestrus, PGF2a will lyse the CL and allow the female to return to estrus.

Follicle Development

Folliculogenesis is the formation of Graafian follicles from a pool of primordial follicles (Spicer and Echtenkamp, 1995). In the bovine, primordial follicle development begins around day 90 of gestation. Furthermore, primary and secondary follicle formation starts around 140 and 210 days in gestation. Before activation, primordial follicles comprise a single layer of granulosa cells. After activation, primary follicles have a single layer of cuboidal granulosa cells and the growing oocyte (Yang and Fortune, 2008). Development of antral follicles occurs in two stages; the first stage is identified as the 'slow' growth phase. The slow growth phase takes around 30

days, from antrum acquisition at 300 μm to a small 3-5mm follicle size. This growth phase is completed when the follicle is around 3mm. The second growth phase is identified as the 'fast' phase and is entirely gonadotropin dependent, lasting 5-7 days (Lussier et al., 1987). During this fast growth phase, the cohort of follicles grows and goes through dominant follicle selection and dominant follicle growth (Mihm and Bleach, 2003).

Androgens stimulated by LH produced by theca cells collect at the basement membrane and converse with local granulosa cells, with aromatase by the influence of FSH, converting them to estrogens. It is well documented that a certain threshold and ability to produce androgens is required for the preovulatory follicle (Drummond and Findlay, 1999). Estrogen is necessary for the proliferation of granulosa cells in the follicle and influences the actions of FSH and LH. The increase in follicle size is due to the rise in granulosa cells (Goldenberg et al., 1972). Another role E_2 plays in the follicle is differentiating granulosa cells to establish proper FSH, LH, and prolactin receptors (Drummond and Findlay, 1999). The emergence of a new follicle wave is caused by the rise and peak of FSH production.

The activation of primordial follicles is then when the follicle is classified as primary. During this phase, granulosa cells are cuboidal in shape instead of the initially flattened arrangement, and granulosa cell numbers rapidly increase. Granulosa cells acquiring LH receptors is thought to be one of the main contributing factors to the follicle developing to the capacity of ovulation. Previous research has indicated that dominant follicles have increased levels of mRNA for the LH receptor (LHr) in the theca and granulosa cells when compared to recruited follicles that do not reach dominance (Bao and Garverick, 1998).

It has been well-documented that the bovine female has two to three follicular waves per estrous cycle. The emergence of cohorts of follicles arises around day 8-10 of the cycle in the

late luteal phase of cycles that include three follicular waves and results in one large (>10 mm) dominant follicle (Ginther et al., 1997). Follicle development starts with a cohort of follicles that begin to emerge under the strong influence of FSH. Follicles that develop LH receptors have a better fate of becoming dominant follicles vs those that become atretic. Two stages of growth occur in follicle development and are referred to as the slow growth and fast growth processes.

Follicle Recruitment

A gonadotrophin surge occurs with the rise in FSH, which aids in stimulating the cohort of follicles to develop beyond 4 mm in diameter (Lamb et al., 2010b). Following a decrease in FSH production, approximately 2-3 days later, a smaller cohort of follicles continues developing until one follicle remains and becomes dominant. The remaining subordinate follicles will undergo spontaneous atresia via apoptosis. Once the follicles reach 5 mm in diameter, they produce E₂ and inhibin, both of which act as inhibitors of FSH. As FSH production declines, inhibin-A and E₂ levels increase (Mihm and Bleach, 2003). Additionally, other follicle secretions, such as follistatin, further inhibit FSH levels from rising again (Mihm and Bleach, 2003).

Follicle Selection and Dominance

Follicle selection occurs when a single follicle is recruited from the original cohort of follicles and continues growth, while the other follicles from the same cohort undergo atresia. The decline of FSH is thought to be controlled by the increasing concentrations of E₂ produced from the cohort of follicles. Selection is complete once the largest follicle reaches 8mm in size,

around 2.7 days after the initiation of the follicle wave or around 61 hours after the LH surge (Ginther et al., 1999).

While dominance occurs, antral follicles in the slow growth phase are prevented from entering the faster growth FSH-dependent growth phase by the dominant follicle, which is secreting high levels of E_2 (Fortune et al., 2004). Following ovulation, a rise in circulating concentrations of FSH is initiated, and a new follicular wave begins (Adams et al., 1992).

Corpus Luteum Development

The first accurate diagram of the corpus luteum was in 1672 by Regnier de Graaf (Thiery, 2009). Historically, the corpus luteal was referred to as a gland by Malpighi de Graaf. Moreover, this gland was initially called an endocrine gland in 1898 (Donaldson and Hansel, 1965). In 1903, Frankel was experimenting with removing the ovaries from pregnant rabbits, which resulted in the termination of the pregnancies, further solidifying the theory that CL is required for implantation. In 1934, several research groups purified P4 from the secretions of the CL. Willard M. Allen was one of the most influential people in the purification process of P4. In 1935, at the Second International Conference on the Standardization of Sex Hormones in London, the official name of P4 was decided on (Di Renzo et al., 2020).

Corpus luteum formation begins at the activation of the LH receptors in the follicular cells before the preovulatory LH surge, which causes rapid differentiation of the follicles into the CL formation. Corpora lutea cell types include small luteal cells, large luteal cells, fibroblasts, endothelial cells, and pericytes (O'Shea et al., 1989). Before ovulation, the basement membrane of the follicle begins to disassociate with theca and granulosa cells. Granulosa cells are differentiated into large luteal cells, whereas theca cells are differentiated into small luteal cells

(Medeiros et al., 2021). Although there are differences in size, both large and small luteal cells are responsible for producing P4. However, large luteal cells secrete approximately 80% of the P4 in the CL (Perry et al., 2005). The vascularization must be adequate for the CL to secrete P4 at high levels. The factors influencing the vascularization of the CL begin in the follicle development stages. Numerous angiogenic factors are responsible for the vascularization of the CL, such as vascular endothelial growth factor A (VEGFA) and basic fibroblast growth factor (FGF2), insulin-like growth factors (IGF-1 and IGF-2), and angiopoietins (ANPT-1 and ANPT-2) (Schams and Berisha, 2004; Shirasuna et al., 2012). The vascularization of the CL is believed to be a driver of luteal function and proper secretion of P4, the central role of the CL.

Progesterone production

Progesterone is a regulator for a multitude of physiological functions, such as preparing the uterus for pregnancy, maintaining early pregnancy, and preventing the cow from the ability to ovulate and express signs of estrus (Perry, 2004b). In the presence of successful fertilization, P4 secretion will continue to support and maintain the pregnancy. Notably, inadequate P4 concentrations following fertilization are thought to be a reason for reduced fertility and, therefore, contribute to pregnancy loss (Wiltbank et al., 2011). On the contrary, increased P4 levels following a successful fertilization event have been associated with increased interferon-tau production and, consequently, increased circulating conceptus size on days 13 and 16 (Clemente et al., 2009). Retrospectively, dairy cows classified as pregnant had increased plasma P4 concentrations compared to nonpregnant cows on days 4 and 5 (Butler et al., 1996). Elevated

P4 concentrations were found to be related to pregnancy retention when analyzed on week 7 or 9 of pregnancy (Starbuck et al., 2004).

Luteolysis

If the oocyte is not adequately fertilized, or the early developing embryo fails to progress correctly through development, the uterus releases the luteolytic hormone PGF_{2a} around days 16-20 of the estrous cycle. A vascular countercurrent exchange system of PGF_{2a} ensures that luteolysis will reach the ovary and cause the regression of the CL. A rapid decrease in P4 is accompanied by negative feedback on the pituitary LH secretion. The pulse-like secretions of PGF_{2a} are transported to the CL via a counter-current exchange mechanism. Luteal cells possess PGF_{2a} receptors on the plasma membrane and have an inhibitory effect on P4 secretion. Prostaglandin _{2a} is also effective in reducing blood floods due to its vasoconstrictor activity and depletes the environment of nutrients needed to continue steroidogenesis (Pharriss, 1971).

Historical Reproductive Management of Beef Cattle

The physiology of reproduction has been evident since the 1600s with the description of AI was first implemented in cattle in the early 1900s. The use of AI was desirable because of the ability to increase genetic variation by utilizing a variety of sires with diverse genetic potential. Additionally, the decrease in transmission of diseases between animals and the ability to evaluate sperm production and quality were also appealing to producers. In 1899, a Russian vet, Dr. Ivanovich Ivanov, was the first to implement artificial insemination techniques in mares. In 1914, the artificial vagina was designed for semen collection in dogs, and it was later modified in the 1930s for use in bulls. Early semen collection techniques involved retrieving semen from the vagina of a fresh ejaculate using a sponge or syringe (Ivanoff, 1922). During the 1930s,

electroejaculation technology was introduced and eventually adopted for bull semen collection in 1954 (Dziuk et al., 1954). Finally, in 1937, Danish veterinarians developed the rectovaginal AI method, which remains in use today. This method revolutionized AI by reducing the quantity of sperm required per insemination, improving biosecurity, and enhancing fertility outcomes. Today, AI combined with estrous synchronization is the most implemented technology in beef operations across the United States.

History of Estrous Synchronization

Synchronization of the estrous cycle of ruminants began in the 1920s with F.F. McKenzie at the University of Missouri using sheep as a model (McKENZIE et al.). In 1943, Casida and others reported that a subcutaneous injection of follicle-stimulating extracts followed by I.V. injection of luteinizing extracts from the sheep pituitary resulted in a lengthening of the estrual period (WARWICK and CASIDA, 1943). In 1956, a group at the University of California ground crystalline P4 in a starch emulsion and injected beef heifers on various days of the estrous cycle. This resulted in heifers not exhibiting estrus and inhibited CL formation. Two additional studies were performed on beef heifers administering P4 and equine gonadotropin, followed by TAI (Nellor and Cole, 1956). The first estrous synchronization strategy focused on regressing the CL with an injection of PG and detecting estrus between 18 and 80 hours following the injection.

Moreover, in 1972, PGF_{2a} was reported to be luteolytic in cattle by several scientists who reported injecting heifers with 30 mg of PGF_{2a} tromethamine salt resulted in a return to estrus in 2 to 4 days. If injected between days 6 to 9 and 13 to 16 but not days 2 to 4 of the estrous cycle, which aided in understanding critical responsiveness times (Lauderdale et al., 1974). After

establishing that the follicular growth pattern is wave-like, GnRH injections were utilized to cause the LH surge and initiate the ovulation of the large dominant follicle.

Current Estrous Synchronization Strategies

Estrous synchronization has been a reproductive management strategy in practice for over 30 years. The benefits of estrous synchronization include shortening the calving season, increasing calf uniformity, and using AI. Furthermore, using AI allows for integrating new and progressive genetics of sires into the herd. Estrous synchronization utilizes three main hormones to control the estrous cycle of the bovine female: GnRH, P4, and PGF_{2a}. The Ovsynch protocol was one of the first protocols coined to assist in simplifying synchronization protocols and improving AI pregnancy rates (Nowicki et al., 2017). Implementing FTAI protocols on beef operations appeals to producers because of two factors: eliminating estrus detection and limiting cattle handling events (Lamb et al., 2010). A CIDR, GnRH, and PGF_{2a} can achieve a more synchronized breeding window.

Gonadotropin-Releasing Hormone

Research was conducted to develop gonadotropin-releasing hormone analogs to aid in synchronizing follicular waves. The primary hormone that controls the start of the cascade of other hormone release is gonadotrophin-releasing hormone (GnRH) and causes the release of Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH) from the pituitary. Analogs for GnRH are available to help induce the release of pituitary hormones into the blood to act on the ovary. After administering GnRH, ovulation will occur 24-48 hours (Kanitz, 2003).

Progesterone

Progesterone aids in regulating the duration of the estrous cycle and assists in maintaining pregnancy in the bovine female (Spencer et al., 2006). Progesterone analogs have been used to keep P4 levels elevated within the estrous cycle. The most common form of P4 utilized is an intravaginal progesterone insert or controlled internal drug release device (CIDR) (Lamb et al., 2010b). The CIDR was first developed in New Zealand in the 1980s (Wheaton et al., 1993). Estrus expression following the withdrawal of a CIDR was highest compared to control cows or cows treated with MGA (Perry et al., 2004). Within estrous synchronization programs, P4 is used to suppress estrus and prevent ovulation by having a negative feedback effect on the hypothalamus, which inhibits FSH and LH production. Supplementing P4 with a CIDR has been shown to improve the fertility of cows without a CL at the beginning of an FTAI protocol (Bisinotto et al., 2015).

Prostaglandin F_{2a}

Prostaglandin F_{2a} is a naturally occurring luteolytic hormone widely used to synchronize the estrus of cattle (Lamb et al., 2010). The uterus of the cow produces PGF_{2a} and causes regression of the present CL (Diaz et al., 2002). The mechanism of PGF_{2a} works to secrete the luteolytic hormone in a pulsatile manner via a counter-current exchange mechanism. The initial studies with PGF_{2a} were conducted to investigate cattle fertility following an injection of PGF_{2a} (Lauderdale et al., 1974). In brief, cattle were assigned to one of three treatments: 1) control, receiving no PGF_{2a}; 2) cows receiving 30 mg of PGF_{2a} and observed for estrus twice daily and inseminated 12 hours after the onset of estrus 7 days after the injection, or 3) cows receiving 30

mg of PGF_{2a} and inseminated 72 and 90 hours after PGF_{2a} without estrus detection time. Cows were palpated from days 35 to 60 after AI to determine pregnancy status. About 50% of the cattle were detected in estrus within 7 days after the PG injection. After the injection, 88% of cows were detected on days 2, 3, and 4. Pregnancy rates were similar between groups, regardless of whether the cows were bred off estrus detection. However, the luteolytic effect of PGF_{2a} is only successful if the CL has acquired the receptors to regress the CL present.

Hormones Influencing Reproduction

IGF-1

Insulin-like growth Factor 1 has a strong relationship to the reproductive performance of beef cows. The majority of the IGF-1 measured in the blood of cattle is produced by the liver (Yakar et al., 1999; Fenwick et al., 2008; Velazquez et al., 2008). Reproductive success can be assessed by circulating IGF-1 levels (Velazquez et al., 2008). Research in ewes indicated that an infusion of IGF-1 increased E₂ secretion in the follicular phase, suggesting that IGF-1 is a simulator of steroidogenesis (Scaramuzzi et al., 1999). Due to plasma IGF-1 concentrations being directly related to energy status, it is known to be a hormonal mediator of nutritional control of fertility (Zulu et al., 2002). Patton et al. (2007) reported that increased plasma IGF-1 during the first 2 weeks of lactation in dairy cows was associated with an earlier return to ovulation. Overall, IGF-1 is a crucial regulator of the reproductive axis and has strong interactions with the hypothalamus and pituitary function to aid in ovarian steroidogenesis and corpus luteum function (Spicer et al., 1993).

Leptin

Leptin plays a considerable role in the estrous cycle of the bovine female. The presence of adipose tissue impacts reproductive performance through the hormone leptin. Leptin works through the GPR54 receptor on kisspeptin (KISS1) neurons in the hypothalamus (Tena-Sempere, 2006). Previous research has indicated that females with elevated leptin levels before ovulation are more fertile than females who did not exhibit increased leptin levels. In addition to playing a virtual role in regulating adipose tissue, leptin is known to interact at the level of the hypothalamus to regulate gonadotropin-releasing hormone (GnRH) activity and secretion (Hausman et al., 2012; Odle et al., 2018).

Cortisol

Under stress conditions, cortisol is released to meet the body's increased demand for energy and to distribute glucose to tissues with a higher demand for glucose, primarily organs such as the liver and the brain (Kegley, Spears 1995). Cortisol inhibits the entry of glucose into peripheral tissues to spare it for tissues of higher demand. One of the initial studies with Cr supplementation indicated reduced cortisol levels in stressed calves (Chang and Mowat, 1992a).

Heat stress is a common issue associated with a negative impact on reproductive outcomes of bovine females. Previous research with growing pigs, laying hens, and dairy cows has indicated that Cr supplementation aided in the reduction of harmful effects that are evident in heat-stressed animals (Sahin et al., 2010; Soltan, 2010; Bin-Jumah et al., 2020).

Mineral Supplementation Strategies

Beef cattle require many minerals for regular maintenance, growth, and reproduction. Minerals levels required by cattle can be broken up into two categories: macrominerals > 100 ppm in a diet and trace minerals, which are needed in amounts of <100 ppm in a diet. Macrominerals include calcium, phosphorus, magnesium, potassium, sodium, chlorine, and sulfur. Essential trace minerals, or microminerals, include iron, zinc, manganese, copper, iodine, cobalt, and selenium. Mineral consumption by cattle typically occurs when consuming forages; however, different regions across the United States lack minerals, making supplementation necessary. The required mineral level depends on age, sex, size, and physiological state.

Trace mineral supplementation is essential for optimizing cattle health, immune function, and overall performance (Palomares, 2022). Additionally, vitamins A, D, and E are integral to mineral metabolism and absorption processes, enhancing the efficacy of trace mineral utilization within the bovine. Numerous options exist for delivering minerals to cattle, including adding minerals to the water source, oral drenching, injection, ruminal boluses, offering protein or energy feeds, and free-choice supplementation (Greene, 1999).

Chromium overview

Currently, there is no requirement for dietary chromium supplementation in beef cattle. However, it is suggested that Cr requirements for beef cattle range from 0.6 to 0.75mg/Cr per day. Symptoms of chromium deficiency include impaired glucose tolerance, insulin resistance, and elevated serum insulin concentrations . Moreover, similar symptoms can be identified in animals, along with impaired reproduction function, decreased longevity, and impaired growth (Chang and Mowat, 1992b; Vincent, 2004). Although research has indicated that Cr

supplementation does not directly affect blood concentrations of essential hormones such as growth hormone, insulin, and IGF-1, it directly reduces blood cortisol levels in animals under stress.

Blood glucose levels within the body and production in the liver are maintained by insulin. A family of five transmembrane proteins (GLUT) transport glucose via facilitated diffusion across the cell plasma membrane (Vargas et al., 2024). The primary GLUT transporter responsible for most glucose uptake into the skeletal muscle and adipose tissue is GLUT4. Glucose tolerance tests (GTT) are commonly used to evaluate the glucose-insulin signaling pathway and measure the ability of the body to clear glucose from the blood (Nickles et al., 2022). The glucose tolerance factor stabilizes the insulin molecule or facilitates insulin receptor binding on tissues, which is required to metabolize carbohydrates, proteins, and lipids (Jones, 2014). The Cr³⁺ atom in the GTF facilitates the interactions between insulin and the insulin receptors on specific tissues such as muscle and fat. After binding to its receptor, insulin initiates cellular glucose uptake and aids in clearing it from the blood (Anderson, 1981; Anderson, 2003; Hua et al., 2012)

One unique feature of GLUT4 is the ability to move between the intracellular domain and the plasma membrane (Huang and Czech, 2007). The GLUT4 has been deemed as an insulin-dependent transporter protein. In the absence of insulin, 90% of GLUT4 remains intracellular. However, in the presence of insulin, GLUT4 storage vesicles undergo exocytosis to the plasma membrane and the sarcolemma and T-tubules of skeletal muscles. In the plasma membrane, GLUT4 is the most efficient in facilitating glucose uptake into the cell. After completing the necessary mobilization of glucose, GLUT4 returns to the cell by budding vesicles on the plasma

membrane containing clathrin. Once internalized, GLUT4 is a part of early endosomes and stored back into intracellular vesicles.

Chromium Forms

Chromium can naturally be detected in the oxidation states of 2-, 0, 2+, 3+, and 6+. However, the most stable form of chromium is in the trivalent state (3+) (Anderson, 1981). Both inorganic and organic forms of Cr exist; the inorganic forms, such as 6+, are often reduced to Cr³⁺ and absorbed in the small intestine (Lashkari et al., 2018). Many forms of organic Cr exist but are generally found as Cr-methionine, Cr-picolinate, Cr- nicotinic acid complex, and Cr-yeast, Cr chloride, and Cr propionate (Sahin et al., 2010; Spears, 2019). The Cr bioavailability rate is critical in the absorption process. It has been well documented that inorganic sources of Cr have a bioavailability rate between 1 and 3%, whereas organic sources of Cr supplementation offer a bioavailability rate of 15 to 30% (Prasad, 2013). Chromium propionate, the only organic source of Cr supplementation available for livestock diets, is highly absorbable.

Chromium is found in numerous tissues within the body but at the highest levels in the spleen, liver, and kidney; however, lower levels of Cr can be found in the pancreas, heart, muscles, lungs, brain, and bones (Vincent, 2001; Zarczynska and Krzebietke, 2020). Chromium absorption works through passive diffusion into the intestines, and absorption occurs in the proximal aspect of the jejunum (Sahin et al., 2010). The delivery of Cr to cells is facilitated by binding to the transferrin receptor after forming an endosome within the cell (Zarczynska and Krzebietke, 2020). Chromium then binds to a low molecular weight Cr-binding substance referred to as chromodulin (Vincent, 2004). Chromodulin works to bind with high affinity to the insulin receptor and activates the tyrosine kinase function. Furthermore, stimulated insulin

binding by Cr is also associated with the phosphorylation of insulin receptor substrate- 1 (IRS-1) and phosphatidylinositol 3-kinase (PI 3-kinase). As a result of Cr supplementation, post-activation of these insulin-signaling pathways allows and leads to translocation of glucose transporters (GLUT), particularly GLUT4, from the intracellular space to the plasma membrane (Chen et al., 2006; Volek et al., 2006). Glucose uptake is mediated by GLUT across the bovine system (Bentley et al., 2012). Glucose transporters can be found in various cell types, but GLUT1 and 4 are mainly found in skeletal muscle, adipocytes, and cardiomyocytes (Vargas et al., 2024).

The translocation of insulin-regulated GLUT4 translocation is mediated by the lipid kinase phosphatidylinositol 3-kinase (PI3K). Upon insulin binding to its receptor on the cell surface, the receptor undergoes a conformational change, activating its tyrosine-kinase domain and initiating downstream phosphorylation of insulin receptor substrates (IRS) and c-CBL, a proto-oncoprotein (Bryant et al., 2002). A necessary step to stimulate insulin is the phosphorylation of Try896, which helps translocate GLUT4 into different cells (Stuart et al., 2014). A recent study indicated that Cr improves the response to insulin in adipocytes and increases the IRS1 phosphorylation, which results in enhanced insulin function (Chirivi et al., 2024). Research has demonstrated that granulosa cells and theca interna layers from follicles had considerable levels of GLUT, specifically GLUT1, GLUT3, and GLUT4. Expression levels of mRNA for GLUT4 were also found in the CL in the bovine (Nishimoto et al., 2006).

History of Chromium Supplementation

The first significant observations on Cr-immune system interactions were made from studies including growing feedlot calves, completed in the late 1980s and early 1990s by Dr.

D.N. Mot at the University of Guelph. In 1992 (Chang and Mowat, 1992a), the effects of Cr supplementation were investigated through two phases of treatment. The first phase supplemented Cr through a high Cr-yeast method, where 2 mg of Cr per gram of yeast was offered to steers with or without injectable long-acting oxytetracycline (LAOTC) on performance and morbidity of 108 Charolais-crossed stressed feeder calves. The second phase began 28 days later, and steers were vaccinated with infectious bovine rhinotracheitis and parainfluenza. Two weeks later, 96 steers from the previous phase were rerandomized within Cr treatment and supplemented with either soybean meal (SBM) or urea-corn supplementation of corn silage. Chromium inclusion was reduced to .2 ppm of total DM. Including LAOTC increased DMI, and CR without LAOTC tended to follow the same pattern.

Furthermore, the Cr-supplemented group of calves without LAOTC observed a 30% increase in ADG and a 27% increase in feed efficiency (FE). In the next phase of the growing period, steers supplemented with Cr had an increase in serum protein and ureal levels. A significant decrease in serum cortisol levels was noted in both Cr and SBM-supplemented calves. Increased stress is often associated with elevated glucose utilization, which increases the mobilization of Cr. Once Cr is mobilized, it cannot be reabsorbed and is excreted in the urine. Increased urinary excretion is associated with depleting Cr stores (Anderson, 1981). Decreased cortisol levels in steers fed Cr could suggest that stressors were alleviated more easily with the support of Cr, resulting in improved performance.

Mechanisms affected by Chromium

Several pathways and mechanisms within the body are affected when Chromium is supplemented. Under the influence of heat stress, the cows' normal functions are compromised.

Heat stress directly affects lipid, protein, and carbohydrate metabolism. Furthermore, numerous other adverse effects are evident: body and rectal temperatures increase, reduction in feed intake, decreased cellular energy bioavailability, and overall impaired reproductive and endocrine function (Bin-Jumah et al., 2020). The transition period of dairy cows is from 21 days prepartum to around 21 days postpartum, and insulin resistance and a decrease in DMI often occur during this time (Mezzetti et al., 2021). A consequence of the decreased DMI, while the animal is experiencing heat stress, can ultimately impact the milk production level.

Energy Balance

Numerous metabolites and metabolic hormones such as NEFA, insulin, glucose, cortisol, and IGF-1 have been indicators affecting stereognosis, follicular dynamics, and in vitro oocyte development. A negative energy balance (NEB) is associated with metabolic changes during elevated energy demands. The energy demands of a beef cow are drastically increased by lactation and restoration of body condition during the early postpartum period. Changes in the regulation of nutrient partitioning and glucose metabolism at the end of the pregnancy and early lactation drive the cow to suffer from an NEB. An NEB can negatively impact reproduction through a delayed resumption of cyclicity and lower conception rates.

Non-esterified Fatty Acid (NEFA)

Non-esterified Fatty acid (NEFA) concentrations in the blood directly reflect the energy status of the animal. High serum NEFA concentrations indicate a metabolic disease in cattle. Towards the end of pregnancy, the dam partitions nutrients for the final stages of fetal growth. During this time, the dam will develop peripheral insulin resistance, which allows for an increase

in nutrients available for placental transfer. Expected changes in NEFA concentrations occur in early postpartum cattle while experiencing stressful metabolic demands of partitioning energy towards lactation and restoring body conditions. Higher blood NEFA concentrations are associated with a longer return to estrus period and delayed ovulation (Giuliodori et al., 2011). Increases in NEFA concentrations are evident five days before calving in dairy cattle. However, it is known that there is a standard range of increase that naturally occurs within the females' body. Kia et al. (2023), found that dairy cows with elevated NEFA levels prior to calving were at an increased risk of having milk fever and incidences of dystocia. Grimard et al. (1995), performed a study feeding multiparous suckled cows a 100% (CE) or 70% (LE) energy requirement diet, and found that females in the low energy group had decreased follicle size and increased NEFA concentrations.

On the contrary, lower concentrations of NEFAs in dairy cattle were associated with a higher probability of becoming pregnant during the first two insemination services in the subsequent breeding period (Kia et al., 2023). However, numerous research groups reported no difference in NEFA concentrations prior to calving and the association with subsequent fertility to first or second insemination services (Patton et al., 2007; Chapinal et al., 2012; Garverick et al., 2013; Rodríguez et al., 2020). Specifically, when supplementing Cr during the periods females may experience a negative energy balance state, positive effects of reducing NEFA concentrations were observed in dairy cows supplemented with Cr-methionine 0.03 mg/kg of BW (Smith et al., 2008).

Beta-hydroxybutyrate

Beta-hydroxybutyrate (BHB) is a ketone within the body that plays a significant role in energy metabolism, especially during periods when glucose is limited. BHB in cattle indicates metabolic status during periods of high energy demand. Elevated concentrations of BHB indicate metabolic dysfunction, a result of poor adaptation to the NEB (Herdt, 2000). Furthermore, increased BHB levels can negatively affect cattle reproductive performance, leading to delayed ovulation and reduced conception rates. Mulliniks et al. (2013), investigated the association of serum metabolites to days to conception date and found that cows classified as an early breeder, conceiving within the first 15 days of breeding, had a lower concentration of BHB compared to cows classified as LATE, conceiving during the last 45 days of breeding.

Body Condition Score

The BCS of a beef cow ranges from 1-9, with 1 being extremely emaciated and 9 being obese (Kunkle et al., 1998). Body condition of these females during the postpartum interval influences returning to cyclicity. It is well documented that females who lose more than 1 BCS following calving will have a prolonged interval to ovulation (Crowe, 2008).

The number of days to first estrus and ovulation was significantly decreased in cows that calved at a body condition score of 5 or greater (Richards et al., 1986). A relationship between ovarian activity and postpartum energy balance is defined as changes in the BCS of cows. Beef cows that calve in a lower BCS have more difficulty restoring their body weight and experience a prolonged NEG during the postpartum period (Houghton et al., 1990). Furthermore, cows in lower BCS at the time of breeding experience lower conception rates than those in adequate BCS

at the time of breeding. Moreover, cows that conceive in a BCS of 5 have increased pregnancy outcomes compared to cows that conceive in a BCS of less than 5 (Stevenson et al., 2015)

Summary and Research Goals

Enhancing efficiency in beef cow-calf operations is essential to meet the growing demands of the beef industry. This requires the adoption of advanced technologies and a deeper understanding of effective reproductive management practices to maximize productivity. The research in the subsequent chapters aims to expand the current knowledge surrounding strategies that support reproductive success in beef cows. Specifically, this research focuses on chromium supplementation as a management solution to mitigate the effects of the postpartum interval, a critical period that influences subsequent reproductive performance. By addressing the physiological changes during this interval, chromium propionate supplementation may offer a practical solution to improving reproductive performance.

Chapter Three Investigating the Effects of Chromium Propionate Supplementation on Reproductive Performance of Beef Cows

Introduction

The economic success of beef cow-calf operations hinges on producing one healthy calf per cow annually. Ensuring pregnancy success requires effective management strategies and the implementation of advanced reproductive technologies. Over several decades, research has focused on improving fertility, increasing the number of cycling females at the start of the breeding season, and ultimately shortening the calving season to produce a more uniform calf crop (Lamb et al., 2010a). However, achieving this goal involves overcoming numerous challenges. The nutritional status of beef females during breeding is essential to pregnancy success. In the postpartum period, the cow must balance nutrient allocation between lactation demands and recovery of body condition to ensure she is ready for the subsequent breeding season (Larson, 2020). Implementing effective nutritional strategies is vital for optimizing reproductive outcomes.

Chromium is vital in carbohydrate, lipid, and protein metabolism and is considered an essential nutrient for humans and animals (Nutrition et al., 1997; Vincent, 2004). Chromium activates the body's glucose tolerance factor (GTF), enhancing insulin activity by improving the interaction between insulin and its receptor and facilitating increased glucose uptake (Mertz, 1992; Vincent, 2001; Arif et al., 2019). Organic and inorganic sources of Cr supplements are available, with organic forms recognized as having greater bioavailability (Pierce et al., 2009). Chromium propionate (CrP) has been identified as one of the most bioavailable organic forms (Matthews et al., 2001). In 2009, the Food and Drug Administration Center for Veterinary

Medicine issued regulatory guidance allowing CrP supplementation in cattle diets up to 0.5 mg/Cr/kg dietary dry matter (**DM**) (Kemin Industries, Inc., Des Moines, IA); CrP is still the only organic Cr source approved for cattle in the United States (CFR - Code of Federal Regulations Title 21). Chromium functions by binding to a low-molecular-weight chromium-binding substance oligopeptide, chromodulin. Chromodulin comprises four amino acid residues: glycine, cysteine, glutamate, and aspartate (Yamamoto et al., 1987; Davis et al., 1997). In the presence of insulin, chromodulin stimulates the tyrosine kinase activity, amplifying the insulin receptor (Vincent, 2000).

The glucose tolerance factor is a biologically active form of Cr, known to regulate carbohydrate metabolism (Anderson, 1981). Cows supplemented with CrP had an increased glucose clearance when measured with the glucose tolerance test (Sumner et al., 2007). This indicated that cows that consumed CrP had a faster glucose uptake rate by insulin into skeletal muscle and adipose tissues. Similarly, Bunting et al. (1994), found that CrP-supplemented cattle cleared glucose from the blood 40% faster in steers and 27% faster in heifers when compared to control cattle.

Overall performance in bovine females is evident through multiple research projects. Specifically, dairy cattle supplemented with CrP were found to have increased milk production, BCS, feed intake, and CL to P4 ratio (Smith et al., 2005; Soffa et al., 2023). In beef cattle, in feedlot settings, CrP supplementation has positively impacted feed efficiency, including increased dry matter intake (DMI), average daily gain (ADG), gain- to feed- ratio (G:F), and BW of steers during the finishing period. Additionally, carcass weight was improved, with steers receiving CrP harvesting heavier hot carcass weights than controls (Bernhard et al., 2012; Baggerman et al., 2020). However, using CrP supplementation in the beef cow-calf sector is

relatively novel, necessitating further research. These experiments investigated the effects of CrP supplementation from the peripartum period through weaning on the productive and reproductive performance of *Bos Taurus* beef cows. We hypothesized that CrP-supplemented cows would have improved nutritional status during the peripartum and pre-breeding, directly impacting ovulatory follicle growth, subsequent CL volume, P4 concentration, and ultimately increasing pregnancy rates to TAI.

Materials and Methods

All animals utilized were cared for in accordance with practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS. 2010), and experimental protocols were reviewed and approved by the Virginia Tech Institutional Animal Care and Use Committee (#22-065).

Experiment 1

This experiment lasted for 98 days and was conducted at the Virginia Tech- Kentland Beef Unit, with 62 lactating, multiparous Angus-based commercial cows (age = 7.2 ± 0.61 yr., DPP = 80.8 ± 4.61 d, and initial BW = 528.6 ± 16.21 kg). Approximately 63 days pre-breeding, cows were stratified by calving date, BW, and randomly assigned to one of the following treatments: 1) CON (n= 30) supplementation of a corn gluten feed, soybean hull pellet mix (50:50) with a mineral pack at $1\text{kg}^{-1}\text{hd}^{-1}\text{d}$; or 2) TRT (CrP) (n= 32) supplementation at $1\text{kg}^{-1}\text{hd}^{-1}\text{d}$ of corn gluten feed and soybean hull pellet mix (50:50) with a mineral pack containing 1.4 g CrP (KemTRACE® Chromium 0.4%, Kemin Industries, Inc., Des Moines, IA). Cows remained on a

single pasture equipped with feed bunks capable of monitoring individual supplement intake (SmartFeed[®], C-lock Inc., Rapid City, SD).

All cows were enrolled in the 7-day CO-Synch + CIDR TAI protocol (Larson et al., 2006) from day -10 to 0. More specifically, cows received 100 ug of gonadotropin-releasing hormone (Factrel; Zoetis, Florham Park, NJ) plus a controlled internal drug release (**CIDR**) containing 1.38 g of progesterone (**P4**; Zoetis) on day -10, 25 mg of prostaglandin F2a (Lutalyse; Zoetis) and CIDR removal on day -3, followed in 66 h by a second 100-ug injection of gonadotropin-releasing hormone and AI (day 0). All injections were given intramuscularly in the neck region of the cows. Estrus detection patches (Estroject; Rockway Inc., Spring Valley, WI) were applied on day -3 to all cows, and the occurrence of estrus was recorded at TAI. Estrus was defined as the removal of >50% of the rub-off coating on the patch. All cows were inseminated on day 0 by the same technician, using semen from multiple *Bos Taurus* sires (n=3) equally distributed between treatments. Cow BW and BCS (Wagner et al., 1988) were recorded at the beginning of the experiment, at the start of the TAI protocol, and at pregnancy diagnosis on d 35.

Transrectal ultrasonography (5.0 MHz linear transducer Ibex Pro, E.I. Medical Imaging, Loveland, CO) was performed concurrently with blood sampling and synchronization protocol working periods on days -17, -10, -7, and 0 relative to TAI to measure the diameter of the largest follicle and CL volume (day 7). The vertical and perpendicular horizontal diameters of the largest follicle on each ovary and all CL were measured and recorded. The average value of the vertical and horizontal diameter measurements was used to calculate follicle diameter. CL volume (V) was estimated using the formula $V = 4/3 \times \pi r^3$, where r = one-half of the overall value of the vertical and horizontal diameters. In the presence of a CL fluid-filled cavity, the volume of the cavity was subtracted from the total volume of the CL, resulting in a value that

depicted the volume of the luteal tissue present (Mercadante et al., 2015). Cow pregnancy rates to TAI were assessed with transrectal ultrasonography by the presence of a viable fetus (Easi-Scan Veterinary Ultrasound Scanner) and were determined 35 days post-TAI.

Blood samples from the jugular vein were collected from each cow on days -17, -10, -7, 0, and 7 into blood collection tubes containing freeze-dried sodium heparin (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ). Plasma samples were centrifuged at 2,400 g for 15 minutes at 4°C and analyzed for P4 using radioimmunoassay (RIA) kits (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA). The assay kit was previously validated for bovine serum (Kirby et al., 1997) using an assay volume of 100 μ l. The intraassay coefficient of variation was 0.67%. All samples were run as singletons in a single assay with a 0.1 ng/mL sensitivity.

Experiment 2

The second experiment lasted for a total of 348 days and was conducted on cow-calf operations (n=9) managed by the Virginia Department of Corrections, with a total of 953 suckled, lactating, primiparous, and multiparous Angus-based cows (age = 5.3 ± 0.61 yr, DPP = 79.9 ± 4.61 d, and [BCS] = 5.1 ± 0.05). Within the location, cows were sorted by BCS, age, and DPP on day -92 and allocated to a total of 26 pastures/groups so that average BCS, age, and DPP were similar among groups. Groups were then assigned to one of the following treatments: 1) CON (n= 13), 484 beef females supplemented with free choice mineral; or 2) TRT (CrP) (n=13), 471 beef females supplemented with free choice mineral product containing 1.4g of CrP (KemTRACE® Chromium 0.4%, Kemin Industries, Inc., Des Moines, IA). Groups were maintained in tall- fescue-dominated pastures with ad libitum access to forage, free choice

mineral supplement, and water throughout the experimental period. Groups were enrolled in the same TAI protocol described in Exp.1 (Larson et al., 2006) from day -10 to 0. Multiple experienced AI technicians (n=8) and semen from different *Bos Taurus* sires (n=5) were used across locations and groups but balanced between treatments within each location. Estrus detection patches (Estroject; Rockway Inc., Spring Valley, WI) were applied on day -3 to all cows, and the occurrence of estrus was recorded at TAI. Estrus was defined as the removal of >50% of the rub-off coating on the patch. Cows were exposed to natural service > 10 d after timed AI. Cow pregnancy rates to TAI were assessed with transrectal ultrasonography by the presence of a viable fetus (Easi-Scan Veterinary Ultrasound Scanner) and were determined between days 55 and 65 after TAI. A second pregnancy diagnosis was performed between days 85 and 95 to determine a final pregnancy rate. Cow BW and BCS (Wagner et al., 1988) were recorded at the beginning of the experiment, at the beginning of the TAI protocol, at both pregnancy diagnoses, and at the end of the experiment, when calves were weaned.

Feed Sampling and nutritional analysis

Samples of forage, feed, and mineral products were collected at the beginning of the experiment, at the first pregnancy diagnosis, and at the end of the experiment, then pooled together for nutritional analysis by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Prior to submitting grass samples for nutritional analysis, the samples were dried for 48 h at 55°C in forced-air ovens for DM calculation. All samples were analyzed using wet chemistry procedures for CP (method 984.13; AOAC, 2006) and ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp., Fairport, NY; AOAC, 2006) NDF (Van Soest et al., 1991). Results of the nutritional analysis of minerals offered in Exp. 2 are

depicted in Table 3-4. Forage nutritional analysis for each location is in Table 3-5 for Exp. 2. Water was offered ad libitum for the entirety of both experiments. At the beginning of the TAI protocol, all cows were vaccinated against respiratory viruses (Bovilis Vista 5 L5; Merck; Animal Health; Rahway, NJ). At the final pregnancy diagnosis, beef females deemed pregnant received a booster vaccine to prevent *Leptospira Hardjo Bacterin* (Spirovac; Zoetis Animal Health.; Parsippany Troy Hills, NJ).

Statistical Analyses

Quantitative and binary data were analyzed respectively with the MIXED and GLIMMIX procedures of SAS (version 9.3 SAS/STAT; SAS Inst., Inc., Cary, NC). Data from Exp. 1 used cow as the experimental unit. Estrus expression and TAI pregnancy rate were analyzed using the GLIMMIX procedure of SAS with the effects of treatment, and all interactions. BCS, BW, Weight change, follicle size, CL volume, and P4 concentration with the fixed effect of treatment were analyzed using the MIXED procedures of SAS. Results are reported as least square means and separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and $P \leq 0.10$. Data from Exp. 2 were analyzed using group/pasture as the experimental unit, whereas model statements contained the effect of treatment and included group (treatment x location), cow (group), and location as random variables.

Results

Experiment 1

The initial cow age, DPP, initial and final BW, and ADG were similar ($P > 0.1$) between treatments (Table 3-1). Supplement feed intake was similar ($P > 0.1$) between CrP and CON

cows, with both reaching the target intake of $1\text{kg}^{-1}\text{hd}^{-1}\text{d}$ (Table 3-1). Estrus expression did not differ among the CON and CrP cows ($P>0.1$). Although not statistically significant, there was a numerical increase in TAI pregnancy rate in CrP-supplemented cows compared to CON cows (81.2% vs $55.1\% \pm 20.1$ $P=0.685$). Follicle diameter was similar on day -10 and -3 and significantly ($P=0.028$) larger on day 0 (TAI) for CrP cows (Figure 3-1). In addition, the volume of the CL was increased on day 7 post-TAI in cows that consumed CrP compared to CON cows (Figure 3-2; $P=0.03$). Circulating P4 concentration was increased ($P=0.02$) on day 7 post-TAI (Figure 3-3). Furthermore, the ratio of CL volume to circulating P4 was increased (Figure 3-4; $P=0.001$) in CrP cows compared to CON cows.

Experiment 2

For experiment two, cow age, DPP, and BCS were similar between treatments ($P > 0.1$; Table 3-2). Mineral disappearance was similar between treatments (165.5 vs 152.9 $\text{kg}^{-1}\text{hd}^{-1}\text{d}$, for CON and CrP, respectively). There was no difference between BW, weight change, and BCS between groups throughout the experiment. The reproductive performance of beef cows enrolled in Exp.2 is summarized in Table 3-3. CrP-supplemented cows tended ($P=0.081$) to have a greater estrus expression than CON cows. Additionally, cows that consumed CrP had an increased ($P=0.045$) TAI pregnancy rate compared to CON cows (55.2% vs $49.9\% \pm 2$, for CrP and CON, respectively). However, final pregnancy rate at the end of the breeding season was not different ($P>0.922$) between groups.

Calf performance was analyzed by BW, collected at birth, on day 95 of the experiment, and at the end of the experiment, which was the time of weaning. No differences were observed between the groups at any time weights were recorded of the calves. Furthermore, calf ADG was

similar ($P > 0.05$) between groups. These results indicate that CrP supplementation supports follicle and CL development and post-AI hormonal changes in beef cows. Additionally, more CrP-supplemented cows tended to express estrus before breeding and had an increase in TAI pregnancy rate.

Discussion

Follicle development and dominant follicle size are crucial for increased oocyte competence and pregnancy success. Lopes et al. (2007), found that larger ovulatory follicles are associated with subsequent increased pregnancy rates in beef females. In Exp.1, CrP supplementation was associated with increased ovulatory follicle size. Previous studies have shown that heifers ovulating follicles smaller than 10.7mm in diameter have reduced pregnancy rates compared to those with 12.88 mm or larger follicles (Perry et al., 2007). Larger follicles produce more estradiol and contain more TCA cycle intermediates than smaller follicles (Hessock et al., 2023). Consequently, larger follicles are associated with developing superior oocytes, better suited for producing viable embryos (Labrecque et al., 2016).

Estradiol concentrations are crucial in regulating estrus behavior in cattle and creating the optimal environment to support sperm transport and conceptus survival (Perry and Perry, 2008). Furthermore, expressing estrus before TAI has been associated with increased accessory sperm, indicative of improved embryo viability and quality (Larimore et al., 2015). Moreover, cows exhibiting estrus indicated a 27% improvement in AI conception rates compared to others not detected in estrus (Richardson et al., 2016). Increasing the availability of insulin and glucose directly increases the estradiol production from the first postpartum dominant follicle in dairy cattle (Butler et al., 2004). Therefore, the increase in circulating concentrations of estradiol initiates the behavioral estrus signs (Allrich, 1994; Richardson et al., 2016). Although estradiol-

17B concentration was not measured in the present study, the enhanced glucose availability from CrP supplementation may contribute to increased estradiol production by dominant follicles. The increased dominant follicle size in the CrP cows could be associated with increased estradiol production and greater estrus expression, as we observed in Exp.2.

Morphological changes in granulosa and theca cells of the dominant follicle are crucial for their transformation into P4-secreting luteal cells of the CL following ovulation (Donaldson and Hansel, 1965). As a result, ovulation from a larger dominant follicle often leads to an increase in CL volume (Vasconcelos et al., 2001). In the present experiment, CrP supplementation resulted in cows ovulating larger dominant follicles at TAI and greater CL volume 7 days later. A larger CL volume is associated with greater circulating P4 concentrations (Vasconcelos et al., 2001). These findings are consistent with results observed in dairy cattle supplemented with CrP in a short-duration, high-dose strategy, which increased the P4 produced per average unit of CL volume compared to CON cows (Soffa et al., 2023).

The present study also showed increased circulating P4 on day 7 in the CrP group, corresponding with greater CL volumes. These results align with previous reports correlating CL volume and circulating P4 concentrations (Gómez-Seco et al., 2017). Previous research has shown that a rapid increase in circulating P4 levels post-ovulation is critical for embryo survival and pregnancy maintenance (Perry et al., 2005; Lopes et al., 2007). I could argue that the increased glucose availability in CrP-supplemented cows could potentially enhance CL development and P4 secretion by providing more energy to support luteal function, leading to more P4 production during the early stages of pregnancy and could be related to the numerical increase in AI pregnancy rate was observed in cows supplemented with CrP. However, the experiment was not designed to detect differences in pregnancy rates and had fewer animals. A

statistical difference in TAI pregnancy rate was found in Exp. 2, which was designed with a more significant number of animals. A previous study with dairy cattle showed a tendency to increase the TAI pregnancy rate at day 28 in females supplemented with Cr-met (Bryan et al., 2004). To our knowledge, this is the first report of an increase in pregnancy rate to TAI in beef females supplemented with CrP.

Despite the lack of significant differences in overall pregnancy rate for the breeding season, CrP supplementation was associated with increased TAI pregnancies. This increase in TAI pregnancy rate will lead to more calves being born at the beginning of the calving season. Numerous studies have documented the timing of conception as critical for reproductive success and longevity, especially in heifers (Cushman et al., 2013). Females who conceive early in the breeding season benefit from a longer interval between calving and returning to estrus before the subsequent breeding season. This early conception allows for increased time for the calves to gain weight before weaning, resulting in more kg of calf weaned per cow exposed and overall herd productivity and profitability (Rodgers et al., 2012).

In beef cows, BCS before breeding is strongly correlated with the resumption of estrus and subsequent pregnancy success (Spitzer et al., 1995; Stevenson et al., 2015; D'Occhio et al., 2019). Throughout the experiments, all cows maintained an acceptable nutritional status based on BCS, and no significant changes in BW or BCS were observed between treatment groups, consistent with previous studies on Cr supplementation in the form of either CrP or Cr-Met (Hayirli et al., 2001; Trojan et al., 2023). In contrast, Smith et al. (2005) reported an increase in BW in dairy cows supplemented with chromium-methionine (Cr-Met) during the postpartum period. The diets of dairy and beef cows differ significantly due to their distinct energy demands. Dairy cows have substantially greater energy requirements primarily driven by lactation,

especially during early lactation when their metabolic demand peaks, often resulting in an NEB because they cannot meet the high energy demands of milk production (Collard et al., 2000; Walsh et al., 2024). While beef cows produce enough milk to nourish their calves, their lactation energy demands are lower. Therefore, it is understandable that dairy cows facing greater energy demands during the postpartum period show an increase in BW when supplemented with Cr-Met.

Additionally, the duration of the postpartum interval directly impacts pregnancy success (Short et al., 1990; Stevenson et al., 2015). In these studies, no significant differences were found in DPP during breeding across both experiments and treatment groups. The postpartum period is a time of considerable stress for beef females. Although the exact requirement for Cr is not well-established, it is known that Cr reserves can become depleted under stressful conditions (Anderson, 2003). Animal stress is often assessed by measuring hair or serum cortisol levels (Latif et al., 2017; Heimbürge et al., 2019). Chang and Mowat (1992), reported that Cr supplementation in feeder steers led to reduced serum cortisol concentration compared to non-supplemented steers. Although cortisol concentration was not measured in these studies, it is plausible that CrP supplementation aided recovery during the postpartum period, potentially facilitating the return to cyclicity by the start of the TAI protocol.

Conclusions

The findings from these studies indicate that although overall cow nutritional status was not significantly affected by CrP supplementation during the peripartum period through weaning, reproductive performance was improved. Specifically, CrP enhanced ovulatory follicle diameter

at TAI, and subsequent CL volume and greater concentration of P4. Ultimately, these positive effects contributed to greater TAI pregnancy rates of beef cows supplemented with CrP.

Table 3- 1. Overall performance of beef cows enrolled in Exp. 1 that did (CrP) or did not (CON) receive chromium propionate supplementation.

Item²	CON	TRT	SEM	P-value
n	30	32		
Age, yrs	7.4	7.2	0.61	0.907
Days Postpartum, d	81.3	80.8	4.61	0.951
Supplement Intake, kg/d	1.22	1.03	0.21	0.534
BW day 0, kg	526.2	528.6	16.21	0.895
BW day 125, kg	548.5	551.4	13.50	0.641
BW day 165, kg	562.8	574.6	11.41	0.289
ADG, kg/d	0.221	0.278	0.03	0.524

CON, (n=30) supplementation of corn gluten, soy hull pellet feed (50:50) with a mineral pack at 1 kg⁻¹hd⁻¹d; or 2) TRT, (n=32) supplementation at 1 kg⁻¹hd⁻¹d of corn gluten, soy hull pellet feed (50:50) with a mineral pack containing 1.4 g of Chromium Propionate (KemTRACE[®] Chromium 0.4%, Kemin Industries, Inc., Des Moines, IA).

² BW= body weight; ADG = average daily gain.

Table 3- 2. Performance of beef cows in Exp. 2 supplemented with chromium propionate during the peripartum period through breeding and weaning.

Item	Treatments		SEM	P-Value		
	CON	TRT		Trt	Loc	Trt*Loc
<i>Cow parameters</i>						
Age, yrs	5.4	5.3	0.61	0.917	0.448	0.999
Days postpartum, d	79.2	79.9	4.61	0.910	0.578	0.999
Mineral intake, g ⁻¹ hd ⁻¹ d	165.5	152.9	22.62	0.698	0.153	0.999
BW day 0, kg	515.7	515.6	15.11	0.995	0.419	0.969
BW day 65, kg	558.5	551.4	14.50	0.734	0.422	0.894
BW day 95, kg	580.8	594.6	13.51	0.489	0.227	0.986
BW day 285, kg	574.2	570.9	14.32	0.872	0.406	0.817
BW change, kg	57.8	53.7	7.56	0.711	0.280	0.751
ADG, kg/d	0.199	0.182	0.02	0.640	0.305	0.708
BCS day 0	5.1	5.1	0.05	0.351	0.172	0.137
BCS day 65	5.4	5.4	0.09	0.828	0.257	0.633
BCS day 95	5.3	5.3	0.10	0.963	0.416	0.404
BCS day 285	5.1	5.2	0.10	0.735	0.276	0.386
<i>Calf parameters</i>						
Birth weight, kg	33.1	32.8	0.65	0.769	0.346	0.989
BW day 95, kg	102.5	102.1	4.52	0.952	0.520	0.928
Weaning weight, kg	268.1	271.9	6.74	0.691	0.625	0.934

CON supplementation of a mineral product at 113 g⁻¹hd⁻¹d (n=464 cows; 16 experimental units); TRT, supplementation of mineral product at 113 g⁻¹hd⁻¹d containing 1.4 g of CrP (n=489 cows; 16 experimental units).

Table 3- 3. Reproductive performance of beef cows supplemented with chromium propionate during the peripartum period, through breeding and weaning in Exp. 2.

Item	Treatment		SEM	<i>P</i> -value		
	CON	TRT		Trt	Loc	Trt*Loc
Estrus, %	60.2	68.3	3.1	0.081	0.077	0.352
TAI Pregnancy rate, %	49.9	55.2	2.1	0.045	0.621	0.175
Final Pregnancy rate, %	89.9	90.2	2.0	0.917	0.956	0.618

CON supplementation of a mineral product at 113 g⁻¹hd⁻¹d (n=464 cows; 16 experimental units); TRT, supplementation of mineral product at 113 g⁻¹hd⁻¹d containing 1.4 g of CrP (n=489 cows; 16 experimental units). Estrus was determined based on the activation of a breeding indicator (Estroject) on the day of fixed-time artificial insemination (TAI), the final pregnancy rate after one TAI, and a 70-day breeding season with natural service sires.

Table 3- 4. Nutritional analysis of supplements offered to beef cows

Item ¹	CON	TRT
Dry Matter, %	98.30	98.22
Crude Protein, %	7.39	7.11
Calcium, %	12.28	11.07
Phosphorus, %	2.19	3.15
Magnesium, %	8.38	8.70
Potassium, %	0.43	0.59
Sodium, %	7.17	7.39
Iron, PPM	2314.50	2712.22
Zinc, PPM	3232.00	3307.78
Copper, PPM	580.50	874.11
Manganese, PPM	1367.90	1646.33
Molybdenum, PPM	1.70	1.90
Sulfur, %	0.70	0.77
Chloride Ion, %	10.11	10.75
Cobalt, PPM	25.69	24.98
Chromium, PPM	15.38	42.53

CON (n=30), supplementation of corn gluten, soy hull pellet feed (50:50) with a mineral pack at 1 kg ⁻¹hd⁻¹d; or 2) TRT, (n=32) supplementation at 1 kg ⁻¹hd⁻¹d of corn gluten, soy hull pellet feed (50:50) with a mineral pack containing 1.4 g of chromium propionate (KemTRACE[®] Chromium 0.4%, Kemin Industries, Inc., Des Moines, IA).

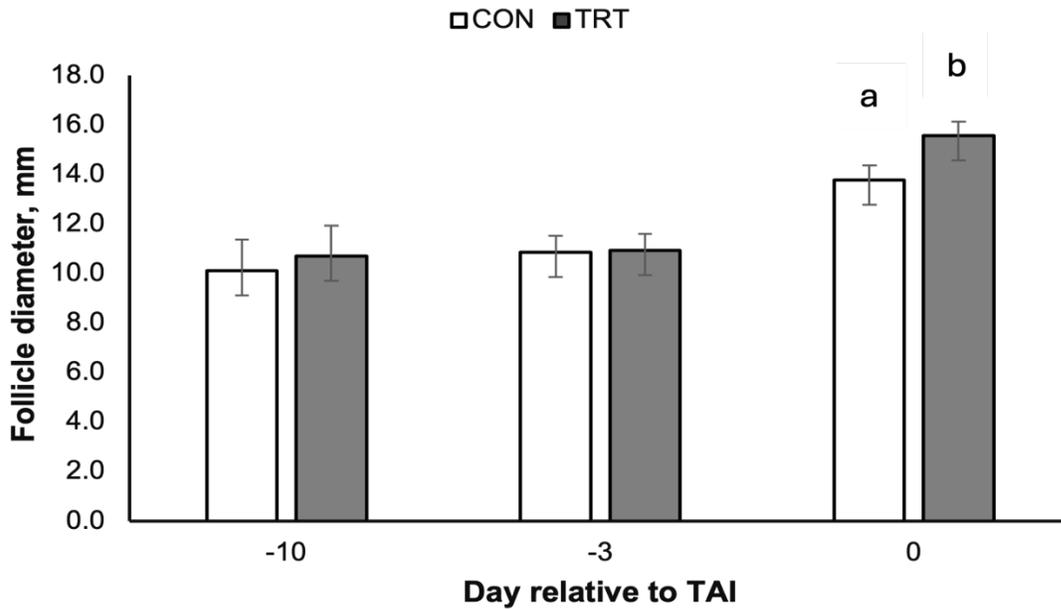
¹ Values obtained via wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

Table 3- 5. Nutritional analysis of pastures grazed by cows receiving a mineral supplement with or without chromium propionate in Exp. 2.

Item ¹	CON	TRT
Dry Matter, %	27.51	30.22
Crude Protein, %	20.09	18.11
Adjusted Crude Protein, %	19.94	18.11
ADF, %	30.03	29.97
aNDF, %	56.03	57.10
NFC, %	14.37	15.11
TDN, %	63.71	63.29
ME, Mcal/kg	2.50	2.46
Chromium, %	0.98	0.78

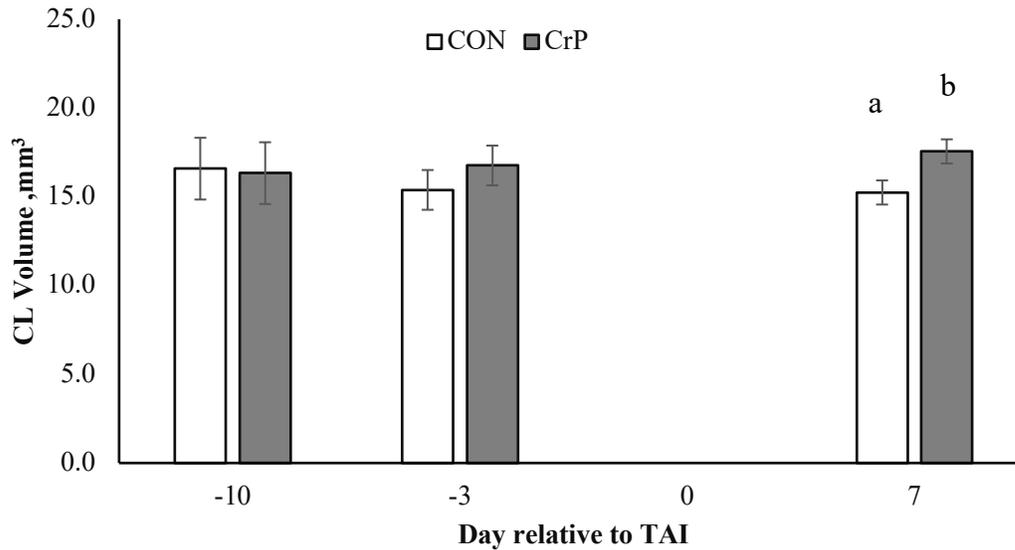
¹ Values obtained via wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY). CON supplementation of a mineral product at 113 g ⁻¹hd ⁻¹d (n=464 cows; 16 experimental units); TRT, supplementation of mineral product at 113 g ⁻¹hd ⁻¹d containing 1.4 g of CrP (n=489 cows; 16 experimental units).

Figure 3- 1. Diameter of largest follicle on days relative to TAI of beef cows in Exp.1.



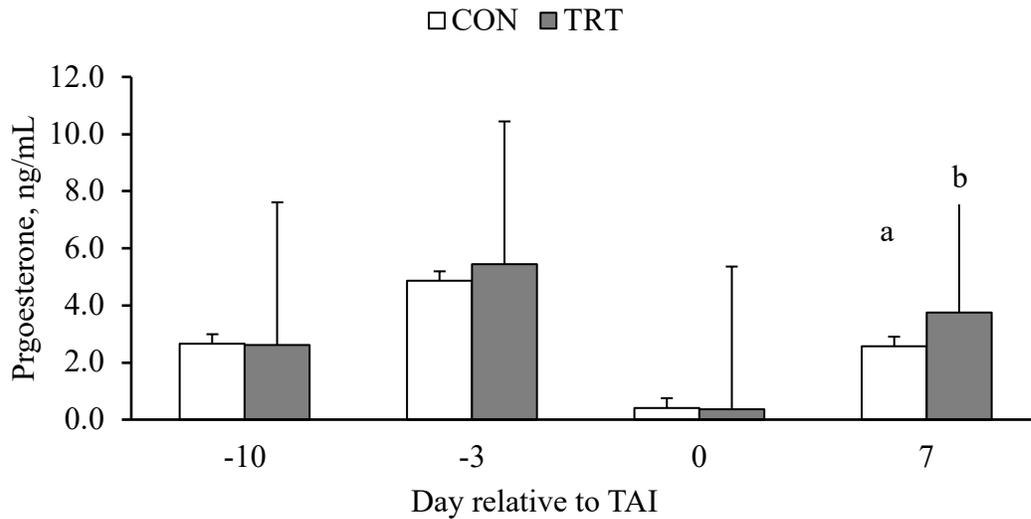
Diameter of the largest follicle on days relative to fixed-time artificial insemination (TAI) of beef cows in Exp. 1 exposed to the 7-day CO-Synch+CIDR synchronization protocol and receiving a chromium propionate supplement. CON (n=30), supplementation of corn gluten, soy hull pellet feed (50:50) with a mineral pack at 1 kg⁻¹hd⁻¹d; or 2) TRT, (n=32) supplementation at 1 kg⁻¹hd⁻¹d of corn gluten, soy hull pellet feed (50:50) with a mineral pack containing 1.4 g of chromium propionate (KemTRACE® Chromium 0.4%, Kemin Industries, Inc., Des Moines, IA). ^{a,b} different superscripts denote the difference between treatment groups; Trt*day *P* = 0.028.

Figure 3- 2. The volume of CL present on days relative to TAI of beef cows in Exp.1



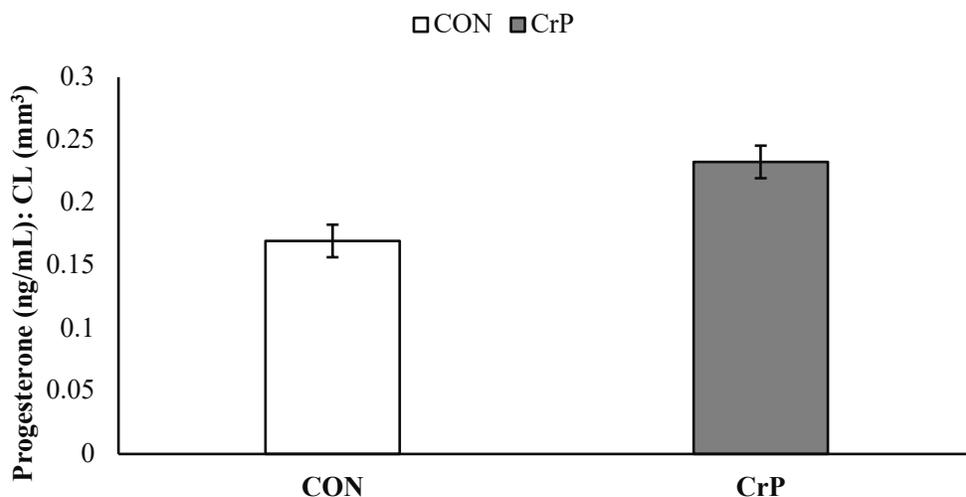
The volume of Corpus Luteum (CL) present on days relative to fixed-time artificial insemination (TAI) of beef cows in Exp. 1 exposed to the 7-day CO-Synch+CIDR synchronization protocol and receiving a chromium propionate supplement. CON (n=30), supplementation of corn gluten, soy hull pellet feed (50:50) with a mineral pack at 1 kg⁻¹hd⁻¹d; or 2) TRT, (n=32) supplementation at 1 kg⁻¹hd⁻¹d of corn gluten, soy hull pellet feed (50:50) with a mineral pack containing 1.4 g of chromium propionate (KemTRACE® Chromium 0.4%, Kemin Industries, Inc., Des Moines, IA). ^{a,b} different superscripts denote the difference between treatment groups; Trt*day *P* = 0.038.

Figure 3- 3. The concentration of progesterone on days relative to TAI of beef cows in Exp. 1.



The concentration of progesterone on days relative to fixed-time artificial insemination (TAI) of beef cows in Exp. 1 exposed to the 7-day CO-Synch+CIDR synchronization protocol and receiving a chromium propionate supplement. CON (n=30), supplementation of corn gluten, soy hull pellet feed (50:50) with a mineral pack at 1 kg⁻¹hd⁻¹d; or 2) TRT, (n=32) supplementation at 1 kg⁻¹hd⁻¹d of corn gluten, soy hull pellet feed (50:50) with a mineral pack containing 1.4 g of chromium propionate (KemTRACE® Chromium 0.4%, Kemin Industries, Inc., Des Moines, IA).^{a,b} different superscripts denote the difference between treatment groups; Trt*day *P* = 0.021.

Figure 3- 4. The progesterone ratio to average CL volume (mm³) of beef cows exposed to the 7-day CO-Synch+CIDR synchronization protocol and receiving a chromium propionate supplement in Exp.1.



The progesterone ratio to average corpus luteum (CL) volume (mm³) of beef cows exposed to the 7-day CO-Synch+CIDR synchronization protocol and receiving a chromium propionate supplement in Exp.1. CON (n=30), supplementation of corn gluten, soy hull pellet feed (50:50) with a mineral pack at 1 kg⁻¹hd⁻¹d; or 2) TRT, (n=32) supplementation at 1 kg⁻¹hd⁻¹d of corn gluten, soy hull pellet feed (50:50) with a mineral pack containing 1.4 g of chromium propionate (KemTRACE[®] Chromium 0.4%, Kemin Industries, Inc., Des Moines, IA). Significance ($P=0.002$) between treatment groups.

Chapter Four Survey on Reproductive Technologies Utilized by Virginia Cow-calf Producers

Introduction

Reproductive efficiency is a critical factor influencing the productivity and profitability of beef cow-calf operations. Effective reproductive management can increase calf crop percentage, improve herd genetics, and optimize resource utilization, all contributing to efficient beef cow-calf operations. Advancements in reproductive technologies and management strategies have provided producers various methods to enhance reproductive outcomes. However, adopting and implementing these management practices can vary widely among producers due to differences in operation size, management goals, resource availability, and producer knowledge. In Virginia, beef cow-calf operations represent a significant portion of the state's agriculture industry, contributing to the national beef supply. Despite their importance, there is limited data on the specific reproductive management practices utilized by Virginia producers and how they align with national averages.

This survey assessed the reproductive management practices used by Virginia beef cow-calf producers. By gathering insights on standard reproductive management practices, adoption of various technologies, and producer demographics, it aims to investigate the reproductive management strategies of beef cow-calf operations in Virginia. The findings may serve as a foundation for developing targeted extension programs to support producers in improving their herd reproductive performance and overall productivity.

Materials and Methods

A web-based survey designed by QuestionPro™ Software was utilized to develop a 26-question survey for beef cow-calf producers across Virginia. The questions were designed to be similar to those in the United States Department of Agriculture National Animal Health Monitoring System (USDA NAHMS) survey for seamless comparison.

Data collection

Beef cow-calf producers across Virginia were the target population for this survey. The survey software created a Quick-Response (QR) code and a web address (URL). The survey link and QR code directed participants to an introduction page of the survey, including information about the objective, the investigators' contact information, and a statement informing participants that their responses were anonymous, with no identifiable information needed for participation. For assessment, the survey was submitted to the Virginia Tech Institutional Review Board (VT-IRB) for the Protection of Human Subjects. The study was deemed “Not Human Subjects Research” by the VT-IRB due to the anonymity of the respondents; this exempted the study from the requirements for IRB approval.

The survey was divided into four sections: 1) producer demographic information, 2) breeding season information, 3) standard reproductive management practices, and 4) veterinarian and extension involvement. The survey was shared on social media and presented at several conferences. Additional participants were obtained from advertising this survey at the Virginia Beef Expo. Producers were asked to scan a QR code or visit the link to participate in the survey.

Results and Discussion

The results of this study describe the reproductive management practices utilized by beef cow-calf producers across Virginia, which may provide insight into their strengths and

weaknesses. The USDA NAHMS report categorizes producers based on region; however, individual state reports are unavailable.

The completion rate was 83.9%, with 109 out of 130 Virginia beef cow-calf producers that started the survey completing it. Demographic details are presented in Table 4-1. Most producers (61.4%) are over 46 years of age, aligning with the national average of 58.1 years (Rossi, 2024). Additionally, 78% of respondents indicated that their operation is a second source of income. This is comparable to findings from a 2021 survey of Mississippi cow-calf producers, where 88% reported a secondary income from their operations (Gunderson et al., 2024), and the 2017 USDA NAHMS Beef study reported that 81.3% of U.S. cow-calf operations were secondary income sources (USDA NAHMS, 2017).

The USDA categorizes cattle producers into three groups based on herd size: small producers with 1-49 head, medium producers with 50-199 head, and large producers with 200 or more head. In the current study, 51.72% of producers identified as medium-sized, while 39.66% had 1-49 head of cattle. In contrast, the USDA NAHMS report indicates that 60.2% of producers were categorized as large, owning more than 200 head. The smaller herd sizes observed among Virginia producers may stem from larger operations requiring more labor and time, which can be challenging to manage when the beef operation is not the primary source of income.

Descriptive results of herd size and basic management practices are in Table 4-2. A significant 87.8% of producers reported to have a defined breeding season. Among these, 42.6% indicated that they have both a fall and spring breeding season. When asked about the duration of the breeding season, 65.5% of producers state that it lasts between 50-90 days. In contrast, data from the USDA NAHMS Beef Study revealed that a majority (58.7%) of producers did not have a defined breeding season. Among those with a defined breeding season, 73.3% completed their

breeding season within 105 days or fewer. This suggests that Virginia producers are more aggressive in having a shorter breeding season when compared to the national average.

Assessing the body condition of females within a producer's operation indicates the herd's nutritional status. In the present study, 55.4% of respondents indicated they perform yearly body condition scores on their herd.

Understanding how producers market their calves, and their utilization of quality assurance programs is essential in assessing how Virginia beef cattle enter the production system. These quality assurance programs add value to cattle, help producers meet industry standards, and increase marketability and financial returns (Adam et al., 2016). Moreover, understanding the impact of extension personnel and veterinarians on a beef operation is vital in indicating areas across the state that may require additional resources. Extension specialists and veterinarians provide a wealth of knowledge and services, ranging from herd health management, disease prevention, nutritional guidance, and reproductive management support. Responses of producers and their interactions with extension personnel and veterinarians are presented in Table 4-3

In Tables 4-5 and 4-6, the utilization of reproductive management strategies is presented from the USDA NAHMS survey in 2017 and the results from this current study. The survey was designed to have similar responses to compare national averages and Virginia producers. Pregnancy diagnosis is a highly profitable tool for producers, usually with a veterinarian on site; 46.9% of producers indicated their primary method for pregnancy diagnosis was rectal palpation. When producers were asked about overall breeding practices on an operation, the number one technology utilized was artificial insemination, with 15.7% of producers utilizing this

reproductive technology. Surprisingly, compared to the USDA NAHMS report, only 11.6% of producers indicated implementing this technology within their herd.

Conclusion

The survey investigating Virginia beef producers provides valuable insight into adopting and utilizing reproductive technologies within the state's beef industry. Although advanced reproductive technologies such as estrous synchronization, AI, and genetic selection offer benefits for improving herd productivity and efficiency, their adoption remains poor. Results indicate that larger producers are more likely to implement these technologies, while smaller producers may face financial and logistical barriers that limit their use. This survey revealed that Virginia cow-calf producers are slightly behind in adopting and utilizing various reproductive technologies and management strategies compared to national averages. Moving forward, extension programs' targeted outreach and education efforts could help address barriers that limit adoption of such management practices. Virginia's beef industry can enhance its productivity and sustainability by supporting producers in implementing reproductive technologies.

Table 4- 1. Descriptive results for respondent demographic information.

Question	Number of responses	Percent¹
Are you a cow-calf producer in VA?	128	
Yes	116	90.6
No	12	9.40%
What is your age group?	127	
Under 18	2	1.57%
18-25	16	12.60%
26-35	14	11.02%
36-45	15	11.81%
46-55	28	22.05%
56-65	20	15.75%
66-75	24	18.90%
75 or older	6	4.72%
Prefer not to answer	2	1.57%
What is the highest level of school you have completed or the highest degree you have received?	125	
Elementary	1	0.80%
High school degree or equivalent (e.g., GED)	22	17.60%
Associate degree	17	13.60%
Bachelor's degree	53	42.40%
Master's degree	28	22.40%
PhD	2	1.60%
Prefer not to answer	2	1.60%
What is your gender?	127	
Female	47	37.01%
Male	78	61.42%
Prefer not to answer	1	0.79%
Other	1	0.79%
Is your beef operation your primary source of income?	116	
Yes	26	22.41%
No	90	77.59%

¹ Response total out of 100%

Table 4- 2. Summary of Survey Responses on Herd Size, Breeding Season, and Reproductive Management Practices

Question	Number of responses	Percent¹
What is your herd size?	116	
Small (1-49)	46	39.66%
Medium (50-199)	60	51.72%
Large (200 or more)	10	8.62%
Does your herd have a defined breeding season?	115	
Yes	101	87.83%
No	14	12.17%
When is your breeding season?	115	
Fall	37	32.17%
Spring	29	25.22%
Fall and Spring	49	42.61%
How many days is your breeding season?	113	
Less than 50 days	22	19.47%
50 to 70 days	38	33.63%
70 to 90 days	36	31.86%
100 to 120 days	7	6.19%
More than 120 days	10	8.85%
Do you perform body condition scoring on your cows every year?	112	
Yes	62	55.36%
No	50	44.64%
Do you perform pregnancy diagnosis every year?	112	
Yes	72	64.29%
No	40	35.71%
Do you perform a breeding soundness exam (semen test) on your bull every year?	112	
Yes	56	50.00%
No	56	50.00%

¹ Response total out of 100%

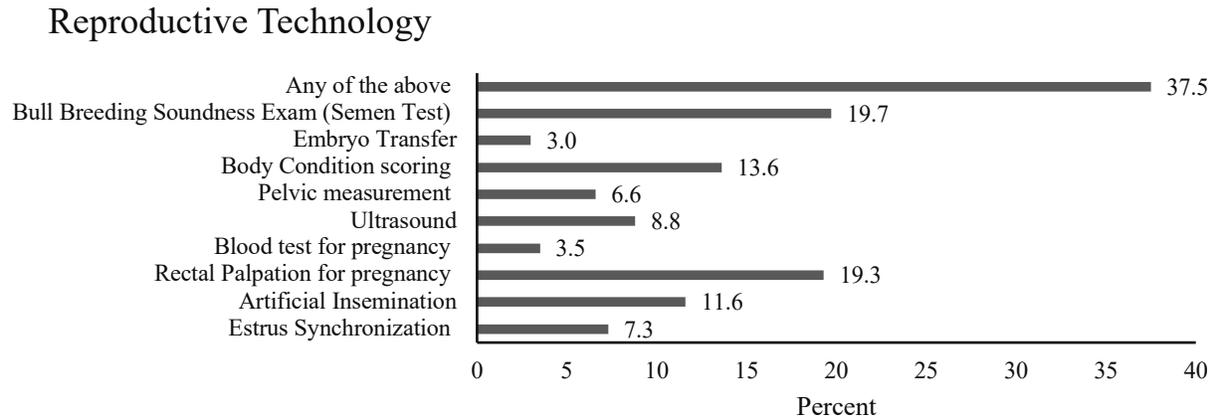
Table 4- 3. Extension, Veterinarian, and Marketing questions regarding operation.

Question	Number of responses	Percent
Where do you primarily get your information about cattle management practices?	109	
Veterinarian	57	52.29%
Internet	22	20.18%
Extension Agent	17	15.60%
Other	13	11.93%
Where do you primarily market your calves?	109	
Local Sale barn	31	28.44%
Private Treaty	30	27.52%
Virginia Feeder Calf Association Sales	33	30.28%
Online auction	3	2.75%
Annual production sale	3	2.75%
Other	9	8.26%
Do you consider Virginia Cooperative Extension as a resource that adds value to your beef operation?	109	
Yes	89	81.65%
No	20	18.35%
Do you feel like your area is adequately serviced by a large animal veterinarian?	109	
Yes	68	62.39%
No	41	37.61%

Table 4- 4. Reproductive management questions

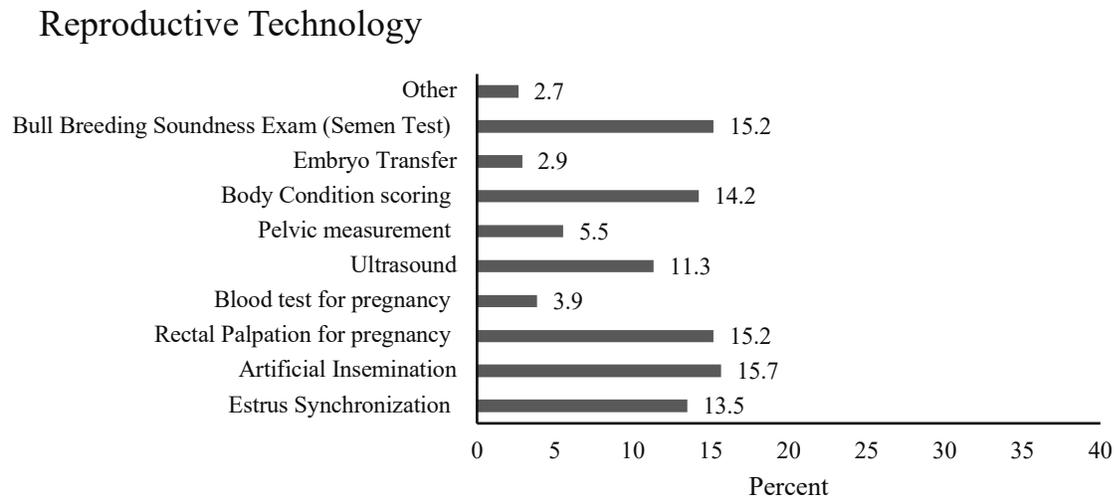
Question	Number of responses	Percent
What is your intended breeding method for your cows?	112	
Exposed only to bulls	51	45.54%
Only artificially insemination	7	6.25%
Brought on as bred females	1	0.89%
Artificially inseminated then exposed to the bull	52	46.43%
Embryo Transfer	1	0.89%
What is your intended breeding method for your heifers?	112	
Exposed only to bulls	45	40.18%
Only artificially insemination	12	10.71%
Brought on as bred females	5	4.46%
Artificially inseminated then exposed to the bull	50	44.64%
What is your 21-day calving rate? (% of calves born the first 21 days of your calving)	112	
Less than 20%	4	3.57%
20% to 40%	21	18.75%
40% to 60%	47	41.96%
More than 60%	40	35.71%
What is your primary method of pregnancy diagnosis?	111	
Blood test	10	9.01%
Rectal palpation	52	46.85%
Ultrasonography	33	29.73%
Other	16	14.41%
What is your approximate bull to cow ratio during the breeding season?	109	
1 bull to 70 cows	5	4.59%
1 bull to 50 cows	16	14.68%
1 bull to 25 cows	71	65.14%
1 bull to 15 cows	17	15.60%
Do you perform genomic testing on your herd?	109	
Yes, only to make replacement decisions	23	21.10%
Yes, on all animals	13	11.93%
No	70	64.22%
Other	3	2.75%

Table 4- 5. 2017 Beef Cow-calf Reproductive Management Practices USDA NAHMS Report



For the USDA NAHMS report, producers were asked if they used any of the following reproductive technologies in 2017. The figure above includes all sizes of operations across the United States. Respondents could select more than one answer.

Table 4- 6. Reproductive Technologies utilized by Virginia Beef Cow-calf producers



For this current survey, producers were asked if they used any of the following reproductive technologies: The figure above includes all sizes of operations across Virginia. Respondents could select more than one answer.

Overall Summary and Conclusions

Reproductive efficiency is a critical determinant of productivity, and profitability in beef cow-calf operations is essential for productivity and profitability. Various factors influence management practices and outcomes. The combined findings provide a perspective on reproductive efficiency in beef cow-calf operations, mainly focusing on adopting reproductive technologies and nutritional interventions like CrP supplementation. As global demand for beef escalates, adopting a multifaceted approach that ingratiate innovative technologies with optimized management practices is vital for maximizing reproductive outputs.

The survey conducted among Virginia beef cow-calf producers reveals valuable insight into the utilization of reproducible technologies within the state. Technologies like estrous synchronization, AI, and genetic selection have demonstrated benefits for improving herd productivity. However, adoption of these practices needs to be more consistent across producers. Larger producers, with more resources and labor capacity, are more likely to implement these technologies, whereas smaller producers face financial and logistic barriers. Compared to national averages, Virginia's producers are slightly behind in adopting these strategies, highlighting the need for targeted extension programs and education to bridge this gap and allow producers to understand the gain in utilizing such practices.

In addition to technological interventions, nutritional strategies such as CrP supplementation have shown promising results in improving reproductive performance in both beef and dairy cattle. While CrP did not significantly affect overall cow nutritional status during the peripartum period through weaning, it was associated with improved key reproductive parameters. These physiological improvements contributed to higher pregnancy rates following TAI, suggesting that CrP may be beneficial in mitigating the postpartum stressors affecting

reproductive outcomes. In conclusion, it is imperative to enhance beef producers understanding of the substantial impact of targeted reproductive management strategies on pregnancy success. Increasing awareness and knowledge regarding the benefits of these practices can lead to greater adoption across the United States beef industry. Significant improvement in reproductive efficiency can be achieved by promoting the utilization of advanced reproductive technologies and nutritional interventions. This will contribute to beef cow-calf operations' sustainability and overall productivity, ensuring the industry can meet the rising global demand for beef.

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